



REVIEW ARTICLE

REVIEW ON BIOFERTILIZERS

T.SIVAKUMAR

Department of Microbiology, Kanchi shri Krishna college of Arts and Science, Tamil Nadu, India

*Corresponding author e-mail: shiva.fungi@gmail.com

Abstract

In recent years, use of microbial inoculants as a source of biofertilizer has become a hope for most of the countries, as far as economical and environmental view points are concerned. Therefore, in developing countries like India, it can solve the problem of high cost of fertilizers and help in saving the economy of the country. The term "Biofertilizers" is a popular misnomer. It refers to living organisms, which augment plant nutrient supplies in one way or the other. In the strictest sense, real Biofertilizers are the green manure and organics (materials of biological origin which are added to deliver the nutrients contained them). Biofertilizers are 1. Carrier based inoculants containing cells of efficient strains of specific microorganisms (mainly bacteria) used by farmers for enhancing the productivity of the soil either by fixing atmospheric N or by solubilizing soil P or by stimulating the plant growth through synthesis of growth promoting substance 2. Blue Green Algae or Cyanobacteria and 3. Mycorrhizae.

Keywords: biofertilizer, Blue Green Algae Cyanobacteria, Mycorrhizae.

Introduction

The population of our country is increasing in geometric progression while food production is increasing in arithmetic progression. To meet the needs of people, several methods are being adapted to increase the food production; one such thing is supplementing the deficient nutrients to a plant and to increase its yield by addition of chemicals and biofertilizers. Biofertilizers are becoming more and more popular, since it is cheap and renewable one, and will not cause any pollution. One of the major concerns in today's world is pollution and contamination free environment. The use of chemical fertilizers and pesticides has caused tremendous effect to the environment. Biofertilizers will help to solve such problems as increased salinity of soil and chemical run off from the agricultural field.

Nitrogenous fertilizers produced in industry by Haber-Bosch process consume high energy (about 13,500 K Cal/Kg N fixed). In such industries fossil fuel is the source of energy. In recent years, due to Gulf Crisis, the cost of crude oil increased about three fold within a years. Therefore, fossil fuel based method of farming more expensive accordingly. To combat with this problem, however, it is necessary to develop an alternative method of supply nutrients to crop plants.

The Term "Biofertilizer" denotes all the nutrients inputs of biological origin should be referred to as Microbiological process synthesizing complex compound and their further release into only medium to the close vicinity of plant root which are again take up plants. Therefore the appropriate

form of biofertilizer should be "microbial inoculants" as suggested by Subba Rao (1922).

3.1. Bacterial biofertilizers

There increased the crop yield about 10 – 20 % when seeds were bacterization of bacteria was reported by copper (1959). This also demonstrated the increase in crop yield such as wheat, Barly, Maize, Sugar, Beet, Carrot, Cabbage and Potato.

Moreover information's gathered on associate symbiosis (i.e. Symbiosis between roots of glasses of *Azopirillum* has increased the interest on this bacterium to be used seed inoculants for cereals (Dewan and subba Rao, 1979).

Bacterization is a technique of seed dressing with bacteria (as water suspension) for example *Azotobacter*, *Bacillus*, *Rhizobium*, etc., it has been proved that bacteria can successfully be established in root region of plant which in turn improve the growth of hosts.

Bacterial biotertilizers named "Azotobacteria" (Containing cells of *Azotobacter Chroococcum*) and Phosphobacteria (Containing cells of *B.megaterium* var *posohobacterium*) have been used in erstwhile USSR and East European countries respectively.

In Rhizosphere bacteria secrete growth substance and secondary metabolics which contribute to seed germination and plant growth. (Subba Raw, 1982) Dwivedi *et al.*, 1989)

Effect of Rhizobial inoculants on crop yield

Legume crop yet benefit from Rhizobial symbiosis in addition certain amount of is left over in soil which is taken by the other plants Subba Rao and Tilak (1977) studied the effect of residual nitrogen of many legumes in the field crops of wheat and rice.

Gaur and Agarwdi (1989) studied that the interaction of the Nitrogen fixing bacteria and phosphorus solubilizing bacteria (*B.megaterium* and *Ps.striata*) and their effect on sorghum and Rice crops in a green house experiments by dual inoculation of sorghum with *A.brasilanse* and *Ps.striata*. They found a significant increase in root nitrogenase activity dry matter and seed yield as compound to single inoculation of both organism and control. Similarly nutrient uptake and yield of

rice increased when inoculated together with *A.chroococcum* and *Ps.striata* as compound to uninoculated control or single inoculated plants.

Carriers used for *Azospirillum* and *Azotobacter* are the same as for *Rhizobium* soil, farmyard manure (1.1) gave the good results as for as survival of Rhizobial cells. Seed inoculation with *A.brasilanse* increased the grain yield of sorghum, peal millet, finger millet and rice over the uninoculated central. Even in those crops, this saves the nitrogen to about 20-30 kg/ha (Tilak, 1991).

Tilak, (1991) have attributed that increase the plant growth in *Azospirillum* inoculated plants due to the production on indole acetic acid, Gibberellins and cytokinin like substances.

3.3. Benefits from biofertilizers

The production of *Rhizobium* inoculant in India was started in 1934, its commercial production was taken up is 1964 when Soya been was introduced in India. Encouraged by the series of inoculants application of Soya been, a great demand for biofertilizes for other legumes also emerged.

On application of biofertilizers, increase in rice yield ranges between 10 – 45 % kg of Nitrogen is left over in the soil which in turn is used took subsequent crops (Venkatraman, 1972), moreover benefits from algalization is about 25 – 30 KgN/ha Maize plans (Subba Rao, 1993)

Mishustin and Skilnikova (1969) studied that the effect of *Azotobacter* inoculants on cotton, Barley, Oat, Potato, Sorghum and Sugar Beat plants which showed in increase in yield over the in inoculated control. Effect of *A.chroococcum* inoculants on cotton Maize and sorghum was increased the crop yield in 0.7-26.2, 36.5-71.7 and 9.3-38 respectively (Shende and Apte, 1982)

Subba Rao and Tilak (1977) studied the effect residual Nitrogen of many legumes on the yield of subsequent crops of wheat or rice. They always found more yield of subsequent crop in *Rhizobium* inoculated fields than in uninoculated control.

Moreover information guttered on associate symbiosis (i.e) Symbiosis between roots of grasses and *Azospirillum* has increased the in terse on this bacterium to be used as seed inoculants for cereals (Dewan and Subba Rao, 1979) in recent years tree

living bacteria (*Azotobacteria*). In recent years, tree living bacteria (*Azotobacter*), associate (*Azospirillum*) and symbiotic (*Rhizobium*) bacteria and phosphate solubilizing ones, (e.g. *Bacillus megaterium*, *B.polymyxa* and *Pseudomonas striata*) are gaining much popularity. Such practices are being encouraged to save the chemical fertilizers natural economy and environmental.

Tien *et al.*, (1979) have attributed that the increased yield in pear millet obtained by inoculation of *Azospirillum* was due to the production of indole acetic acid, gibberellins and cytokinins like substances by the bacterium and their subsequent effect on the plant.

Field experiments conducted at IARI, New Delhi, have shown that upon the seed inoculation of sorghum and cotton by *A.chroococcum*, the yield was increased by 38% - 27 % respectively whereas increase in yield of wheat was 105% at the same conditions.

Biofertilizer play a main key role for selective absorption of immobile (P, Zn, and Cu, and mobile (C, S, Ca, K, Mn, CL, Br and N) elements to plants. These are all available to plant in less amount was separated by Tinker (1984).

Rewari (1984, 1985) reported that the increase in their grain yield over the control of Cajanes cajan, Cchickpea and Black gram plants. Subba Rao and Tilak (1977) studied that the increase the yield over the control of wheat and Rice plants.

Subba Rao and Singh (1985) reported that the increased the grain yield over the control with the treatments of *Azospirillum brasilense*, urea (alone) and Urea +*A.brasilense*. Tilak and Subba Rao (1987) studied the effect of seed inoculation with *A.brasilense* in near millet plants.

Streeter (1988) reported that the fertilizers Nitrogen on Nitrate Nitrogen obtained through Nitrification process in the soil, when present in excess in known to inhibit nodule formation and function.

Ghai and Thomas (1989) have defined green maturing as family practice where a leguminous plant which has derived enough benefits from the association with appropriate species of is ploughed into the soil and then a non-legume is grown and allowed to take the benefits of the already fixed Nitrogen.

Effect of seed inoculation with *A.brasilense* on Sorghum var CSHS was studied by Tilak (1991). In this experiments, the grain yield was increased in urea and *A.brasilense* treated plants over the control plants.

Carrier used for *Azopirillum* and *Azotobacter* are the as for *Rhizobium* soil and farmyard manure gave the good results as far as survival of bacterial cells are concerned. Bacterial cells survived for about 31 weeks. Seed inoculation with *A.brasilense* increased the grain yield of sorghum, pear millet, finger millet and Rice over the uninoculated control. Even these crops, this saves the Nitrogen about 20 - 30 kg/h (Tilak, 1991).

Biotertilizers and Biopesticide include many types of bacteria and fungi. One group that has been extensively investigated is plant growth promoting rhizobacteria (PGPR) PGPR are root colonizing bacteria beneficial to various agricultural crops. The beneficial effects include plant growth promotion, biological control of various disease and the activation of the defense responses of the hot plant, which is termed as induced systems Resistance (ISR) were reported by Glick and Bashan (1997). Glick (1995), Tang (1994) Frommel *et al.*, (1991); Lambert and Joss (1989); Kloepper *et al.*, (1989), Van peer and Schipper (1989), Kloepper *et al.*, (1980) Granamanickam *et al.*, (2002).

Bacteria associated with plants can be harmful and beneficial. Plant growth promoting (PGP) bacteria may promote growth directly, eg. By fixing of atmospheric N₂, solubilization of minerals such as phosphorus, production of siderophores a that solubilize and sequester iron or production of plant growth regulators (hormones) (Kloepper, 1997).

Symbiosis between leguminous plants and soil bacteria commonly referred as Rhizobia is of considerable environmental and agricultural importance, since they are response for an estimated 180 tones per year of biological Nitrogen fixation in worldwide (Postgate, 1998) Pulse breeding is generally done under high fertility condition and without *Rhizobium* inoculation. Many breeders have adapted the approach of adding large amounts of N- fertilizers to help reduce variability due to environmental factors in their selection plots, rather than ensuring that the plants are effectively modulated. This approach Nitrogen fixation while selecting for other desired traits. (Giller, 2001).

Field experiments carried out by the Indian Agricultural research institute different parts of India have revealed that seed inoculation of sorghum, Bajra and Ragi (Subba Rao, 2002).

Biological N-fixation (BNF) is an important attribute of symbiotic association of legume host with rhizobia, Bacterial – laxyonomy is in the evolutionary phase with the advent and use of molecular tools in the characterization and identification of then isolates (Manvika sahal and Johri, 2003).

Plant – species – specific selective enrichment of microflora in the rhizosphere milieu has been exploited in legumes from the point of view of N₂- fixation under Nitrogen limited conditions (Continno *et al.*, 1999; Gualitieri and Bisseling, 2000; Lafay and Burdon, 2000; Sessitsch *et al.*, 2002).

3.4. N₂ – Fixing Bacteria

The ability to reduce and siphon out such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and Archea. (Young, 1992) These include, a) symbiotic N₂ fixing forms, Viz, *Rhizobium*, the obligate symbionts in leguminous plants and Frankie in non-leguminous trees, and b) Non-symbiotic (Free – living, associative or endophytic) N₂ – fixing forms such as cyanobacteria, *Azospirillum*, *Azotobacter*, *Acetobacter diazotrophicus*, *Azoarcus* etc.

Biological nitrogen fixation is estimated to contribute 180 10⁶ metric tons/year globally (Postage, 1998), of which 80% comes from symbiotic associations and the rest from free-living or associative systems (raham, 1988)

3.5.Symbiotic N₂ – fixers

Two groups of N₂ fixing bacteria, i.e. rhizobia and Frankia have been studied extensively.

Rhizobium:

3.5.1. Classification of rhizobia

The classification of root nodulation rhizobia has been modified since 1984 and is likely to change further with more detailed studies on large number of *Rhizobium* strains from a wide variety of leguminous plants. At present only about 8-9% of the 14,000 or so known species of leguminous

plants have been examined for nodulation, and less than 0.5% have been studied relative to their symbiotic relationship with nodule bacteria.

An extensive survey of modulation status of legumes viz. chick pea, Pigeonpea, moongbean, Soybean, and groundnut with naïve rhizobia during 1967 – 72 (Sundara Rao *et al.*, 1962; Subba Rao *et al.*, 1972) and in 1977 – 80 (Tewari, 1979) under the all India co-ordinated pulse improvement programme has belied this assumption since except for groundnut, most legumes nodulated poorly at more than 50% of the places surveyed. There was a deficiency of specific *Rhizobium* even in traditional legume growing areas.

Field trial conducted in India showed that nearly 50% of nitrogenous fertilizer can be saved through rhizobial inoculants with considerable increase in yield depending on the legume, soil and agro- climatic conditions (Rewari and Tilak 1988; Tilak, 1993).

3.5.2. Non-Symbiotic N₂ fixers

Non-symbiotic N₂ fixation is known to be of great agronomic significance. The main limitation to non-symbiotic N₂ fixation is the availability of carbon and energy source for the energy intensive N₂ fixation process. This limitation can be compensated by moving closer to or inside the plants, viz in diazotrophs present in rhizosphere, rhizoplane those growing endophytically.

3.5.2. a. *Azospirillum*

Members of genus *Azospirillum* fix-nitrogen under micro aerophilic conditions and are frequently associated with root and rhizosphere of a large number of agriculturally important crops and cereals. Due to their frequent occurrence in the rhizosphere these known as associative diazotrophs.

Sen (1929) made one of the earliest suggestions that the nitrogen nutrition of cereal crops could be met by the activity of associated nitrogen fixing bacteria such as *Azospirillum*.

After establishing in the rhizosphere, *Azospirilla* usually but not always, Promote the growth of plants (Okon, 1985; Tilak and Subba Raw, 1987; Bashan and Holgwin, 1997) Despite their N₂ – fixing capability (1 10kg N/ha), the increase in yield is mainly attributed to improved

root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake (Dewan and Subba Raw, 1979; Okon and Kapulnik, 1986; Flik *et al.*, 1994).

Tripathi *et al.* (1998) reported accumulation of compatible solutes such as glutamate, Proline, glycine betaine and trehalose in response to salinity/ Osmolarity in *Azospirillum* sp. Usually, Proline plays a major role in osmoadaptation through increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline in *A. brasilense*.

Saleena *et al.* (2002) have studied the diversity of indigenous *Azospirillum* sp. Associated with rice cultivated along the coastline of Tamilnadu on the basis of mutational studies of *Azospirillum*. Kadouri *et al.* (2003) suggested a role of PHB synthesis and accumulation in enduring various stresses, *viz.* UV radiation, heat osmotic pressure, osmotic shock and desiccation.

3.5.3. Phosphobacteria

In a study on green gram, the highest grain yield and Relative Agronomic Efficiency (RAE) were obtained under MRP application at 25kg P₂O₅/ha along with seed treatment of phosphobacteria at 400g/ha seeds as reported by Ramamoorthy and Arokia Raj (1997).

In another study at NPRC, Vamban (Ramamoorthy *et al.*, 1997) on rainfed red gram, the results revealed that the highest grain yield was obtained (449 kg/ha under 125kg N + 37.5 Kg P₂O₅/ha as MRP with seed inoculation of Phosphobacteria. This treatment was significantly superior to other treatments but at per with higher dose of P (50 kg/ha as MRP).

3.6. Nitrogen fixation in pulse crops

Solaiappan *et al.* (1994) reported that the basal application of 6.25 kg N and 12.5kg P₂O₅/ha followed by foliar spray of 3% DAP (70 DAS) recorded higher seed yield. In the same study seed treatment with *Rhizobium* recorded higher yield of 10.26q/ha with a BC ration of 1.86 and the treatment was at par with the treatment where in seeds were treated with super PO₄.

Pulses are the major sources of dietary protein in the vegetation diet in our country. Besides being a rich source of protein, they

maintain soil fertility through biological N₂ fixation in soil and thus play a vital role in furthering sustainable agriculture (Kanniyan, 1999).

Pulse seeds treated with specific strains of *Rhizobium* increase the yield through for better nodulation and maintenance of organic matter in the soil (Saxena and Tilak, 1999).

Pulses are the major sources of dietary protein in the vegetarian diet in our country. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture (Kanniyan, 1999).

Pulse seeds treated with trains of *Rhizobium* increase (favourable CN ration is 10:1) the yield through for better nodulation and maintenance of organic matter in the soil (Saxena and Tilak, 1999)

Combined inoculation of *Rhizobium* with phosphobacteria (*Bacillus megaterium* and *Pseudomonas striata*) for red gram, black gram, green gram and bengalgram increased has grain yield for maximum grain yield was recorded by the combination of rhizobial strain with phosphobacteria with full dose of N & P in red gram (986 kg/ha). It increased 34.70 per cent higher yield than the uninoculated control. Dual inoculation with half dose of fertilizer gave and yield of 880 kg/ha which is 20.22 per cent higher that control.

3.7. Use of biofertilizers for increasing pulse production

Pulse crops have been an important component of agriculture since ancient times. These leguminous plants of symbiotic association with soil bacterium, the *Rhizobium* forms nitrogen fixing root nodules which are agronomically significant as they provide an alternative to the use of energy expensive nitrogenous chemical fertilizer. Despite the large and increasing use of nitrogenous fertilizers in agriculture, estimates suggest that biological nitrogen fixation contributes at least four times more nitrogen to the soil throughout the world. On a global basis these symbiotic association between legume and *Rhizobium* may reduce about 70 million tons of atmospheric nitrogen to ammonia per annum which amounts to about 40% of all biologically fixed nitrogen per year (Burns and Hardy, 1975.)

. REFERENCES

- Arunachalam, V., G.D. Pungle, M.Dutta P.T.C. Nambiar and P.J.Dart. 1984. Efficiency of Nitrogenase activity and nodule mass in producing the relative performance of genotypes assessed by a number of characters in groundnet (*Arachies hypogaea*). *Exp. Agric.* 20: 303 – 309.
- Bashan, Y. and Holguin, G. 1997. *Azospirillum* – plant relations: environmental and physiological advances (1990- 1996). *Can. J. Microbiol.* 43:103-121.
- Brockwell, J. 1962. Studies on seed pelleting as an aid to legume seed inoculation. I. Coating Materials, adhesives, and methods of inoculation. *Aust.J.Agric. Res.* 13: 638 – 649.
- Brockwell, J., A.Diatloff, R.J.Roughly and R.A. Date. 1982. Selection of rhizobia for inoculants. In: Nitrogen fixation in legumes (ed.J.M Vincent) Academic Press, Sydney. pp.173 – 191.
- Burns, R. C and R.W.F. Hardy 1975. Nitrogen fixation in bacteria and higher plants. Springer Verlag, New York p.189.
- Carroll, B.J., D.L. Mc Neil and P.M. Cresshoff. 1985. A super nodulation and nitrate – tolerant symbiotic (nts) soybean mutant. *Plant Physiol.* 78: 34 – 40.
- Click, B.R. (1995). *Can. J. Microbiol.* 41: 109 – 117.
- Cooper. (1959). *Soil fertilizers*, 22: 227 – 233
- Countinho, H.L.C., Oliveria, V.M., Lovato, A., Maia, A.H.N. and Manfio, G.P. 1999. Evaluation of the diversity of rhizobia in Brazilian agricultural soils cultivated with soybeans. *Appl. Soil. Ecol.*, 13:159-167.
- Cruz., G.N. Stamford, N.P Silva, J.A.A. and Chamber – Perez, C. 1997. Effects of inoculation with Bradyrhizobium and urea application on N-fixation and growth of yam bean. *Tropical Grassland*, 33:23-27.
- Subba Rao, N.S. (1982). Biofertilizers, In: Advances in Agricultural Microbiology (Ed. Subba Rao. N.S.). Oxford and IBH Publ. Co., New Delhi. Pp 219 – 242.
- Subba Rao, N.S. (2002). Soil Microbiology. Oxford and IBH publ co. Ltd. New Delhi.
- Subba Rao, N.S. and Singh, C.S. (1985). *Zent. Microbial.* 140: 97 – 102.
- Subba Rao, N.S. and Tilak, K.B.R. (1977). Souvenir Bull. Directorate of Pulse Development, Govt. of India.
- Sundara Rao, W.V.B., Sen, A.N, and Gaur, Y.D. 1969. Survey and isolation of root nodule bacteria in Indian soils, Final Rep, Rep – PL- 480 Scheme, Division of Microbiology, IARI, New Delhi, India.
- Tang, W.H. (1994). In: Improving Plant Productivity with Rhizosphere Bacteria (Eds Reder, M.H. Stephens, P.M. and Bowen, G.D.). CSIRO, Adelaide. Pp. 267 – 278.
- Thompson, J.A. 1980. Production and quality control of legume inoculants. In: Methods for evaluating biological nitrogen fixation, ed. F.J. Bergerson) John Wiley and Sons Ltd. Chichester. pp. 489 – 533.
- Tien, T.M. Guskin, M.H. and Hubbel, D.H. (1979). *Appl. Environ. Boil.* 37: 1012 – 1024.
- Tilak, K.V.B.R. (1991). Bacterial biofertilizers, ICAR, New Delhi. Pp. 66.
- Tilak, K.V.B.R. 1993. Bacterial Biofertilizers, ICARI, New Delhi, India. Pp 4-33.
- Tilak, K.V.B.R. and Subba Rao N.S. 1987). *Biol. Fertile. Soil*, 4: 97 – 102.
- Tilak, K.V.B.R. and Subba Rao, N.S. 1987. Association of *Azospirillum brasilense* with pearl millet (*Pennisetum amarciumum*) (L.) Leeke). *Boil. Fertil. Soils*, 4:97 – 102.
- Tinker, P.B. (1984). *Plant Soil*, 76: 77 – 91.
- Tripathi, A.K., Mishra, B.M. and Tripathi, P. 1998. Salinity stress responses in plant growth promoting hizobacteria. *J. Biosci.* 23:463- 471.
- Van Peer, R, and Schippers, B., (1989) *Can. J. Microbial.*, 35: 456 – 463.
- Venkataraman, G.S. (1972). Algal Biofertilizers and Rice cultivation, today and Tomorrow Printers and Publ. New Delhi. Pp. 75.
- Young, J.P.W. 1992. Phylogenetic classification of nitrogen – fixing organisms. In : Biological nitrogen Fixation (eds Stacey, G., Burris, R. and Evans, H.J) Chapman and Hall, New York, pp .43-86.
- Postgate, J. 1998. Nitrogen fixation, Cambridge University press, Cambridge, 3rd edn, pp.112.
- Preeti Vasudevan, Reedy, M.S Kavitha. S, Vellusamy.P., David Paulraj. R.S. Purosothaman. S.M. Brinda Priyadarisini. V, Bharathkumar. S, Kloepper.J.W, and Gnanamanickam. S.S. (2002). Role of biological preparations in enhancement of rice seedling growth and grain yield. *Curr. Sci.*, **83 (9)** : 1140 – 1143.
- Ramamoorthi, K., and Arokia Raj. 1997. Agronomic effectiveness of organic sources and MRO to phosphorus economy in rainfed green gram. *Madras Agri. J.*, **84(10)**:593-595.
- Ramamoorthi, K., Balasubramanian, A. and Arokia Raj. 1997. Response of rainfed black gram (*Phaseolus mungo*) to phosphorus and sulphur nutrition in red lateritic soils. *J. Agron*, 42(1): 191-193.

- Rewari, R.B. (1984 & 1985). Summarized result of Microbiology Trials. All India coordinated Research project on Improvement of pulses, ICAR, New Delhi.
- Rewari, R.B., and Tilak, K.V.B.R.1988. Microbiology of pulses. In: Pulse Crops(Eds. Baldev,B., Ramnujam, S. and Jain,H.K.), Oxford & IBH, New Delhi, India. pp. 4-33.
- Rupela O.P. 1994. Screening for intracultivaral variability for nodulation of chickpea and pigeonpea. In: Linking Biological Nitrogen Fixation Research in Asia. Report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes. (eds. O.P. Rupela, j.V.D.K. Kumar Rao, S.P. Wani and C. Johansen) pp 75-83. international crop Research Institute for the Semi – Arid Tropics, Patancheru, Andhra Pradesh, India.
- Saleena,L.M., Rangarajan,S. and Nair,S.2002. Diversity of *Azospirillum* strains isolated from rice plants grown in saline and nonsaline coastal agricultural ecosystems. *Microbiol.Ecol*,44(3): 271 – 277.
- Salema, M.P. C.A. Parker, D.K.Kidby and D.L. Chatel 1982. Death of rhizobia on inoculated seed. *Soil Biol. Biochem.* 14: 13 – 4.