INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
VOLUME-2, ISSUE-1, JAN-MAR-2012 ISSN 2231-4490
Accepted: Nov-2011 *Research Article*

ANTAGONISTIC EFFECTS OF *PSEUDOMONAS FLUORESCENS* AND *BACILLUS SUBTILIS* ON *MELOIDOGYNE INCOGNITA* INFECTING *VIGNA MUNGO* L.

Ambreen Akhtar; Hisamuddin; Abbasi and Rushda Sharf

Department of Botany, Aligarh Muslim University, Aligarh, 202002, U.P. India

ambreen.qazi08@gmail.com

ABSTRACT: Plant growth promoting rhizobacteria (PGPR) are known to enhance growth and vigor of various plant species specially leguminous pulse crop plants. Application of *Pseudomonas* sp. and *Bacillus* sp. significantly reduced infectivity rate of *Meloidogyne incognita on Vigna mungo*. Growth parameters in terms of shoot length, root length, shoot fresh and dry weights, root fresh and dry weights and number of nodules per plant were found significantly increased in the plants treated with the two bacteria as compared to control. Maximum inhibition of root knots (42.79 per plant) was observed in plants inoculated with *M. incognita* and simultaneously treated with 20 ml of *Pseudomonas fluorescens* and *Bacillus subtilis*.

Key words: *Bacillus subtilis, Meloidogyne incognita, Pseudomonas fluorescens,* PGPR and *Vigna mungo*

INTRODUCTION

Black gram, (*Vigna mungo* L.) is an important and widely cultivated pulse crop contributing substantially to the annual production of pulse grains. It is rich in phosphoric acid and it shows allelopathic effect on wheat, maize, gram and lentil (Sahi *et al*., 2002). Black gram prefers water retentive, stiff, loamy and heavy soil; it does well on both black cotton and brown alluvium soils. Black gram has the capability to draw atmospheric nitrogen for which it has to depend on the soil microbes of a particular group. They stimulate the plants to form nodules on the roots within which they grow, multiply and convert atmospheric nitrogen into a form available to the plants. During the last few decades the production and yield of black gram has declined due to attack by pathogenic agents. The root- knot nematode, *Meloidogyne incognita* Chitwood, an important nematode pest on black gram, is one of the major constraints in increasing the production.

The rhizosphere is a dynamic environment where mutual relationships among nematodes, plant and environment are often of chemical nature. The abundance of parasitic nematodes around roots may be due to attractants produced by the root microflora as well as the root exudates (Kafznelson and Henderson 1962, 1963). One of the biological factors affecting nodule formation or dysfunctioning of existing nodules is the occurrence of phytoparasitic nematodes in the rhizosphere. The nematodes are capable of producing above ground disease symptoms like stunting, wilting, chlorosis and ultimately reduced yield. They directly damage roots by producing galls, lesions, root-pruning, stubby roots etc. Available literature shows that *Meloidogyne* spp. adversely affect nodulation and nitrogen fixation in pulse crop plants (Haung 1987 and Taha 1993). PGPR rhizobacteria colonize plant roots and promote plant growth and/or reduce damage caused by the disease or the insects. Among PGPR, PSB (Phosphate Solubilizing bacteria) supply P to plants (Keneni *et al*., 2010). Bacteria of the groups *Bacillus* and *Pseudomonas* have proven to be the most powerful phosphate solubilizing bacteria. The objectives of this study were to investigate the effect of the simultaneous application of *M. incognita*, *Bacillus* sp. and *Pseudomonas* sp. on plant growth, nodulation and nitrogen fixation in black gram cv. Pant U-30.

In the soil, heterogeneously distributed microbial communities including symbiotic rhizobia and other plant growth promoting rhizobacteria perform a dynamic role in plant nutrition by transforming nutrients in soils that are beneficial to plant growth through a process called biogeochemical cycling, and directly transporting these nutrients to plants (Ahmed *et al*., 2009). These microbes determine the nutrient pool of soils and facilitate growth and development of the plants (Khan *et al*., 2009). An eco-friendly approach recently advocated to enhance the crop production is the use of PGPR as bio-inoculants.

The PGPR is known to facilitate the plant growth through nitrogen fixation; solubilization of insoluble phosphorus; production of compounds like siderophores, phytohormones, antibiotic and antifungal metabolites; and induced systemic resistance (Zaidi *et al.*, 2009). In this regard, numerous PSB have successfully been used as commercial biofertilizer in sustained agricultural production systems (Vassileva *et al*., 2010; Zaidi *et al.*, 2009). Soil bacteria resposible for nitrogen fixing symbiosis with some leguminous plants are generally named as rhizobia which include more than 50 species distributed in genera *Rhizobium*, *Ensifer, Mesorhizobium*, *Azorhizobium* and *Bradyrhizobium* (Valazquez *et al*., 2010). During rhizobium- legume interaction, rhizobia induce nodule formation in the root system of host plant by *nod* factors. The rhizobia, inside the nodules then convert nitrogen into ammonia for uptake by host plants while legumes provide nutrients to rhizobia (Spaink 2000). Application of PSB as inoculants in green gram has also been reported to increase the nodule number (Vikram and Hamzehzarghanim 2008).

Leghemoglobin (also leghaemoglobin or legoglobin) is an nitrogen or oxygen carrier, because naturally occurring oxygen and nitrogen interact similarly with this protein; and a hemoprotein found in the nitrogen-fixing root nodules of leguminous plants. But nitrogen is necessary for the cycle to occur. It is produced by legumes in response to the roots being infected by nitrogen-fixing bacteria, termed rhizobia, as part of the symbiotic interaction between plant and bacterium: roots uninfected with *Rhizobium* do not synthesise leghemoglobin. Leghemoglobin has close chemical and structural similarities to hemoglobin, and, like hemoglobin, is red in colour. The protein was believed to be a product of both plant and the bactreium in which the apoprotein is produced by the plant and the heme (an iron atom bound in a porphyrin ring) is produced by the bacterium (O'Brian *et al*., 1987). Newer findings however, indicate that the heme moiety is also produced by the plant (Santana *et al*., 1998).

MATERIALS AND METHODS

Seeds of black gram (*Vigna mungo*) var. Pant U-30, susceptible to nematode were sown in 15 cm diameter earthen (five seeds per pot) pots filled with autoclaved soil and mixed with compost (3:1). Prior to sowing the seeds were disinfected by 1% NaOCl for five minutes. After germination, the seedlings were thinned to one per pot.

Culturing of nematodes

In order to get a large number of second-stage juveniles of *Meloidogyne incognita* for the experiment, the population of the test nematode was developed from a single egg mass on tomato plants grown in cemented beds containing autoclaved soil well ahead of the experiment. The required numbers of freshly hatched second-stage juveniles were collected from this pure culture as per the requirement.

Extraction of nematodes

For isolation of juveniles (J2) of root-knot nematode, infected tomato plants were uprooted and washed properly in running tap water. Egg masses were carefully removed from the roots by forceps and transferred to a double layered tissue paper resting on the wire-gauge placed on petridishes containing sufficient water so that the tissue paper wire-gauge assembly slightly submerged in water. The petridishes were covered to avoid water loss due to evaporation.

The eggs were hatched and second stage juveniles wriggled out of the tissue paper into the petridishes. The volume of nematode suspension thus obtained, was reduced and kept separately for further use.

Culturing of bacteria

Cultures of *Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from IARI, New Delhi and maintained on nutrient broth and King's broth media, respectively. The bacterial population reached $>10^9$ cells per ml within 7-10 days.

Inoculation of plant

After 10 days, plants were inoculated by adding required amount of inocula through four soil depressions made around each plant with 1,000 J_2 of *M. incognita* and 10 or 20 ml *B. subtilis* and *P. fluorescens* suspension alone and in simultaneous combinations (Table-2). All the treatments were replicated thrice. The plants were watered after inoculation and thereafter, whenever required. The experiment was terminated 60 days after inoculation. Plant length, fresh and dry weights of the roots and the shoots were measured; nodule number, from all harvests, were determined; (Southey 1986 and Oostenbrink 1996) number of root- knot per plant was counted. Percent decrease in each parameter was calculated with respect to controls. The data obtained were analysed statistically for standered error (Panse and Sukhatme 1989).

RESULT AND DISCUSSION

The soil and the pots used were autoclaved and the plants were not inoculated with the nematodes therefore root-knots were not encountered on control plants. Root length and the shoot length was found increased in all the plants treated with *Pseudomonas fluorescens* and *Bacillus subtilis* as compared to control. Increase in length was gradual as the amount of the bacterial suspension increased. Simultaneous inoculation with bacteria caused higher increased their individual inoculations. Enhancement in length was highest with the higher dose of inocula applied simultaneously (Table 1). Fresh weights and dry weights of the roots and the shoots exhibited the same trend of increase. Highest values of the weights were encountered at higher dose when the two bacteria were simultaneously applied into the soil. Since the plants were not inoculated with nematodes therefore galls were not encountered on any plants in all the treatments (Table 1). Number of root nodules formed by the rhizobacteria were higher in the plants treated with *Pseudomonas fluorescens* than with *Bacillus subtilis*. Increased amount of rhizobacteria increased the number of nodules per plants. Highest number of nodules were found on the plants treated with both the rhizobacteria simultaneously. The presence of *Bacillus subtilis* and *Pseudomonas fluorescens* in roots of black gram cv. Pant U-30 was beneficial for plant growth. In the absence of nematode, growth of bacterized plant was improved than unbacterized plant. Leghaemoglobin content of Vigna mungo L. nodules was significantly differed at different inoculation levels (Table 2 and 4). F6 treatment showed a significantly maximum leghaemoglobin content compared to rest of the treatments and control (0.1). Among all treatment levels F6 showed the significantly maximum leghaemoglobin content (3.7 mg/g) of tissue followed by T7 and F5, where as T1 showed no leghaemoglobin content because of the absence of nodules due to *M. incognita* inoculation. Data regarding nitrogen content revealed that inoculation methods and interaction between bacteria were highly significant (Table 1). Rhizosphere inoculation of bacteria significantly increased the nitrogen content in leaves as compared to control. The lowest nitrogen content were recorded in T1 treatment (0.08) (Table 2).

Table 1: Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on different parameters of *Vigna mungo* L.

Treatment/Parameters	Shoot length (cm)	Root length (cm)	Shoot Fresh weight (g)	Root Fresh weight (g)	Shoot Dry weight(g)	Root Dry weight(g)	Galls per plant	Nodules per plant
Control	26.66	16.53	15.73	18.73	9.76	8.63	-	16.63
F1	36.60	23.86	23.73	20.90	12.80	10.96	-	25.56
F2	33.66	20.76	21.93	19.70	11.73	9.76	-	22.63
F3	40.86	29.76	27.73	22.20	14.94	12.70	-	35.66
F4	37.76	26.76	25.66	21.80	13.80	11.83	-	29.60
****F5**	43.66	32.73	29.60	24.70	15.73	13.93	-	45.76
****F6**	46.86	33.60	31.90	29.86	17.73	15.73	-	48.56
S.E.	1.37	1.31	1.11	0.78	0.55	0.51	-	2.46
LSD (0.05)	2.98	2.85	2.41	1.69	1.19	1.11	-	5.36

F1= P. fluorescens (10 ml)
F2= B. subtilis (10 ml)
F3= P. fluorescens (20 ml)
F4= B. subtilis (20 ml)
F5= P. fluorescens (10 ml) + B. subtilis (10 ml) (simultaneous)
F6= P. fluorescens (20 ml) + B. subtilis (20 ml) (simultaneous)
**Simultaneous
Mean of three replicates

Table 2: Effect of *P. fluorescens* and *B. subtilis* on Leghaemoglobin, nitrogen and chlorophyll content in *Vigna mungo* L.

Treatments/Parameters	Leghaemoglobin content mg/g nodule	Nitrogen content in leaves (mg/g)	Chlorophyll content (mg/g)
Control	0.1	0.2	1.21
F1	0.3	0.2	1.53
F2	0.2	0.1	1.30
F3	1.5	0.2	1.82
F4	1.1	0.2	1.61
****F5**	2.5	0.2	2.21
****F6**	3.7	0.2	2.45
S.E.	±0.28	±0.01	±0.09
LSD (0.05)	0.61	0.02	0.19

Chlorophyll content of crop plant was significantly differed at different inoculation levels (Table 2 and 4). F6 treatment showed significantly maximum chlorophyll content compared to rest of the treatments and control (1.21 mg/g).

Table 3: Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* antagonistic to *M. incognita* in different parameters of *Vigna mungo* L.

Treatments/Parameters	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root Fresh weight (g)	Shoot Dry weight (g)	Root Dry weight (g)	Galls per plant	Nodules per plant
Control	26.66	16.53	15.73	18.73	9.76	8.63	-	16.63
T1	15.93	9.63	10.70	10.83	4.73	4.03	65.33	-
T2	22.70	13.73	20.83	12.73	7.76	5.80	57.33	22.33
T3	18.66	10.80	18.70	11.73	6.83	4.86	55.33	20.33
T4	24.76	14.80	24.80	14.93	10.90	7.873	51.33	26.33
T5	19.70	12.73	22.13	14.00	8.70	5.76	53.33	25.33
T6	35.66	19.70	27.76	20.70	14.90	11.70	47.33	42.33
T7	40.63	21.80	30.93	22.06	17.00	13.70	42.33	47.33
S.E.	±1.67	±0.82	±1.27	±0.83	±0.80	±0.67	±3.90	±2.88
LSD (0.05)	3.58	1.75	2.72	1.78	1.71	1.43	8.36	6.17

Mean of three replicates
T1- M. incognita (1000 J2)
T2- M. incognita + P. fluorescens (10 ml)
T3- M. incognita + B. subtilis (10 ml)
T4- M. incognita + P. fluorescens (20 ml)
T5- M. incognita + B. subtilis (20ml)
T6- M. incognita + P. fluorescens (10ml) + B. subtilis (10ml)
T7- M. incognita+ P. fluorescens (20ml) + B. subtilis (20ml)

Table 4: Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* antagonistic to *M. incognita* on leghaemoglobin, nitrogen and chlorophyll content in *Vigna mungo* L.

Treatments/Parameters	Leghaemoglobin content mg/g nodule	Nitrogen content in leaves (mg/g)	Chlorophyll content (mg/g)
Control	0.1	0.20	1.21
T1	00	0.08	1.07
T2	0.1	0.10	1.12
T3	0.1	0.10	1.09
T4	0.2	0.10	1.19
T5	0.96	0.10	1.14
T6	2.1	0.15	2.11
T7	2.9	0.17	2.17
S.E.	±0.24	±0.009	±0.10
LSD (0.05)	0.52	0.01	0.21

When the plants were inoculated with the nematode, *Meloidogyne incognita*, significant reductions in lengths and weights were observed. Improvement in their parameters, however, was noticed when *Pseudomonas fluorescens* and *Bacillus subtilis* were added into the soil. The values of all the parameters were at per with control, in the plants inoculated with the nematode and treated with higher dose of *Pseudomonas fluorescens*.

Significant increase in all the parameters considered were noticed with the application of simultaneous dose of the PGPR; highest growth was found in T7 where the plants were inoculated with the nematode and were treated with higher dose of the bacteria (Table 2). *Meloidogyne incognita* subdued the lengths and the weights of roots of black gram, significantly. However, in the presence of *Pseudomonas* and *Bacillus*, the damage to plant growth was significantly less, except in treatment, where bacterial applications followed nematode inoculation at the same inoculum level. Nematode infestation diminished the number of nodules on root system in all the treatments and followed the trend of as that of plant growth. The gall number was higher on unbacterized plants than on bacterized ones. In the absence of bacteria growth of black gram was poor. Bacterial application resulted in increased plant growth and lesser damage to nematode inoculated plant thereby, indicates the presence of bacteria is beneficial for plant growth. The principal effect of *M. incognita* was suppression in plant growth and adverse effects of nematode was reduction in the presence of *Pseudomonas* (Joseph B., Patra RR, Lawrence R 2007) and *Bacillus* (Irina *et al*., 2002). Nematode infestation decreased the number of nodules on root system in all the treatments. Nodules were occasionally found on galls as reported by Hussey and Barker (1976) and Raut (1980). Suppressed nodulation was related to the size of the root system and thus confirmed the findings of Taha and Raski 1969 and Verdego *et al*., 1988. They also reported fewer nodules on plants infested with nematode, which was due to reduced root system. Development of nodules also depends on bacteria formation of infection threads, bacterial development and host mitotic activity, all of which are affected by phytochrome concentrations and translocations of nutrients. The dual inoculation of *Bacillus* and *Pseudomonas* to the black gram plants increased all the growth parameters with respect to the individual inoculation of either *Bacillus* or *Pseudomonas*. Lowest shoot length was recorded in the nematode alone treatment. In combination of *Pseudomonas, Bacillus* and *M. incognita* increased shoot length to the same degree as the plants inoculated with *Pseudomonas* (20 ml) and *Bacillus* (20 ml). The same trend occurred with root length, shoot and root weight also (Table-2). The plants inoculated with *M. incognita* exhibited a significant reduction in growth parameters when compared with control. The length of the roots and the shoots inoculated with the nematode and treated with rhizobacteria singly exhibited significant increases over the plants inoculated with the nematode but not treated with the rhizobacteria. In T6 and T7, where *M. incognita* inoculated plants were treated with lower or higher doses of rhizobacteria, significant reduction of T1 as well as over control were observed. *Pseudomonas* inoculation, either alone or in combination with *Bacillus* or with *M. incognita*, or together increased the nodule number significantly compared with the uninoculated control. The greater nodulation (48.33 per plant) was recorded in plants inoculated with 20 ml of *Pseudomonas* and *Bacillus* combination, which was significantly different from *Pseudomonas* alone treatment. Inoculation of nematode to the plants count no number of nodules while the combination of *Pseudomonas*, *Bacillus* and *M. incognita* was associated with the high nodule count (47.66 per plant) which was similar to the *Pseudomonas* and *Bacillus* (20ml) treatment. In general, combined application of *Pseudomonas* and *Bacillus* significantly increased nodulation and growth over *Pseudomonas* alone. There was reduction of growth and nodulation when nematodes were inoculated to the plants.

Different parameters such as shoot length, root length, dry and fresh shoot and root weight, number of knots per plant and number of nodules per plant increased with the (T7) 20 ml treatment of *Pseudomonas* and *Bacillus* in combination as compared with control. *Pseudomonas* and *Bacillus* in combination and alone increased the parameters significantly over the control (Table-1).

Among different nutrients essential to plants, deficiency of soil P is one of the most important aspects that limits the growth and development of the plants. Generally, the amount of P in soils is very low (Goldstein 1994). Rhizobacteria are known to enhance the plant productivity by solubilizing the mineral P (Khan *et al*., 2007).

In addition, phytohormones, like, IAA secreted by PGPR are reported to regulate many physiological activities of plants, such as cell enlargement, cell division, root initiation, growth rate, phototropism, geotropism, and apical dominance (Frankenberger and Arshad 1995). Siderophores directly stimulate the biosynthesis of other anti microbial compounds by increasing the availability of these minerals to the bacteria and may function in local and systemic host resistance in plants (Joseph *et al.,* 2007; Wani *et al*., 2008). The release of EPS (Exo-polysaccharide) by bacterial strain is an important activity as it provides protection to bacterial cells against dessication, phagocytosis, and phage attack and also help in nitrogen fixation by preventing high oxygen tension (Tank and Saraf 2003). EPS is also known to play an important role in concentrating nutrients and protecting bacteria from antibacterial agents (Costerton 1985). Furthermore, our data also showed that inoculated black gram plants had significantly increased number of nodules.

Leghaemoglobin content

Leghaemoglobin of nodules was adversely affected by nematode application. With the increase in nematode population there was gradual decrease in leghaemoglobin in all treatments. Leghaemoglobin was at its peak in the nodules of plants treated with PGPRs. *M. incognita* altered the function of nodules and adversely affected the leghaemoglobin in nodules as well as nitrogen uptake. Reduction in leghaemoglobin content of nodules due to nematode infestation may be the other reason for less fixation of dinitrogen, as leghaemoglobin play vital role in nitrogen fixation (Bergesen, 1961; Chahal & Rewari, 1977).

Chlorophyll content

In the present study there was significant increase in chlorophyll content which was observed in F6 treatment superior over control. The results have shown conformity with the observation made by Madhaiyan *et al.* (2004). Paulraj (2002) have also documented varied levels of chlorophyll content in Cardamom, rubber and coffee respectively due to bioinoculation with *Methylobacterium*.

Nitrogen uptake

The increase in N uptake due to combine inoculation of two or more organisms has been documented by several workers (Balamurugan and Ganasekaran, 1996; Patil *et al*. 1992; Devananda, 2000; Biswas *et al*. 2000; Senthikumar, 2003 and Holland, 1997) have well documented the increase in nutrients use efficacy when inoculation with *Rhizobium leguminosarum* by trifoli in wheat, corn, radish, mustard, rice and PPFMs in soybean respectively. *P. fluorescens* and *B. subtilis* provided increased nitrogen needed for healthy growth of plant. It can be justified by increased Nitrogen contents in leaves in bacterial treated plants. Smith (1949) reported that leghaemoglobin concentration was directly related to the amount of molecular nitrogen fixed during symbiotic process. Kundu and Gaur (1980) reported an increased wheat yield due to inoculation of *Azotobacter*, *B. polymyxa* and *P. striata* as combined inoculation over their single inoculants. The combined inoculation positively influenced the nitrogen uptake of plants and also noticed higher microbial populations of both nitrogen fixing and phosphate solubilizing bacteria in the mixed inoculations indicating possible synergistic interaction between the two groups. Aigawadi and Gaur (1988) reported that inoculation of *Rhizobium*, *P. straita* or *B. polymyxa* significantly increased the nitrogen and phosphorus uptake by chickpea.

REFERENCES

1) Ahemad M, Zaidi A, Khan MS and Oves M 2009 Factors affecting the variation of microbial communities in different agro-ecosystems. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, Heidelberg. Pp 301–324.
2) Costerton JW 1985 The role of bacterial exopolysaccharides in nature and disease. Dev Ind Microbiol 26 249–261.
3) Frankenberger WT Jr and Arshad M 1995 Phytohormones in soils: microbial production and function. Marcel Dekker Inc, New York.
4) Goldstein AH 1994 Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. In: Torriani-Gorini A, Yagil E, Silver S (eds) Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington DC. Pp 197–203.
5) Huang J S 1987. Interactions of nematodes with Rhizobia, In Vistas on Nematology (eds J.A. Veech and D.W. Dickson), Society of Nematologists, Hyaltsvitte. Pp 301-6.
6) Hussey RS and Barker KR 1976. Influence of nematodes and light sources on growth and nodulation of soybean. Journal of Nematology 8 48-52.
7) Irina NN, Boris AB, Nikolai FR, Nikoli DR and Dmitri GZ 2002. Nematicidal effects of the entomopathogenic bacteria Bacillus sp. in soil. Pedobiology 46 6 558-572.
8) Joseph B, Patra RR and Lawrence R 2007 Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicer arietinum L). Int J Plant Prod 2 141–152.
9) Katznelson H and Henderson VE 1962 Studies on the relashionships between nematodes and other soil microorganisms. In: Influence of Actinomycetes and fungi on Rhabditis (Cephaloboides) Oxycerca de Man. Canadian Journal of Microbiology 8 875-82.
10) Katznelson H and Henderson VE 1963 Ammonium as an 'Attractant' for a soil Nematode. Nature, 198, 907-908.
11) Keneni A, Assefa F and Prabu PC 2010 Isolation of phosphate solubilizing bacteria from the rhizosphere of faba bean of Ethiopia and their abilities on solubilizing insoluble phosphates. J Agric Sci Technol 12 79–89.
12) Khan MS, Zaidi A and Wani PA 2007 Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agron Sustain Dev 27 29–43.
13) Khan MS, Zaidi A, Wani PA and Oves M 2009 Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7 1–19.
14) Panse VG and Sukhatme PV 1989 Statistical methods for agricultural workers. Indian Council of Agricultural Research Publ. New Delhi. Pp 359.
15) Raut SP 1980 Effect of initial inoculum levels of M. incognita on plant growth and rhizobial nodulation of mungbean. Indian Journal of Phytopathology 33 351-353.
16) Sahi HN et al. 2002 Allelopathic influence of Vigna mungo (black gram) seeds on germination and radical growth of some crops plants. Plant Growth Regulation 38 69-74.
17) Southey JF 1986 Laboratory Methods for Work with Plant and Soil Nematodes. Ministry of Agriculture Fish and Food, London. Pp 202.
18) Spaink HP 2000 Root nodulation and infection factors produced by rhizobial bacteria. Annu Rev Microbiol 54 257–288.
19) Taha AHY 1993 Nematode interactions with root nodules Bacteria. In: Nematode Interactions, Khan MW (Ed) Chapman and Hall, London. Pp 202.
20) Taha AJ and Raski DI 1969 Interrelationship between root nodule bacteria, plant parasitic nematodes and their leguminous host. Journal of Nematology 1 201-211.
21) Tank N and Saraf 2003 Phosphate solubilization, exopolysaccharide production and indole acetic acid secretion by rhizobacteria isolated from Trigonella foenum-graecum. Ind J Microbiology 43 37-40.

22) Vassileva M, Serrano M, Bravo V, Jurado E, Nikolaeva I, Martos V and Vassilev N 2010 Multifunctional properties of phosphate solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Appl Microbiol Biotechnol 85 1287–1299.
23) Vela´zquez E, Garc0ıa-Fraile P, Ramırez-Bahena MH, Rivas R and Martınez-Molina E 2010 Bacteria involved in nitrogen-fixing legume symbiosis: current taxonomic perspective. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer- Verlag, New York.
24) Verdego S, Green CD and Podder AK 1988 Influence of M. incognita on nodulation and growth of pea and black gram. Nematologica 34 88-97.
25) Vikram A and Hamzehzarghani H 2008 Effect of phosphate solubilizing bacteria on nodulation and growth parameters of greengram (Vigna radiata L. Wilczek). Res J Microbiol 3 62–72.
26) Zaidi A, Khan MS, Ahemad M and Oves M 2009 Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56 263–284.
27) Bergersen FJ 1961 Leghaemoglobin content of legume nodules. Biochem Biophys Acta 50 576-578.
28) Chahal VPS and Rewari RB 1977 Leghaemoglobin and Bacteroid content in relation to nitrogen fixation. Journal of Research Punjab Agricultural University 14 386-388.
29) O'Brian MR, Kirshbom PM and Maier RJ 1987) Bacterial heme synthesis is required for expression of the leghemoglobin holoprotein but not the apoprotein in soybean root nodules. Proc Nat Acad Sci USA 84 23 8390–8393.
30) Santana MA, Pihakaski-Maunsbach K, Sandal N, Marcker KA and Smith AG 1998 Evidence that the plant host synthesizes the heme moiety of leghemoglobin in root nodules. Plant Physiol 116 4 1259–1269.
31) Kundu BS and Gaur AC 1980 Establishment of nitrogen fixing and Phosphate solubilizing bacteria in the rhizosphere and their effect on yield and nutrient uptake of wheat crop. Pl Soil 57 223-230.
32) Alagawadi AR and Gaur AC 1988 Associative effect of Rhizobium and Phosphate solubilizing bacteria on the yield and nutrient uptake of chickpea. Pl Soil 105 241-252.
33) Madhaiyan M, Poonguzhali S, Senthikumar H, Seshadari S, Chung H, Yang J, Sundaram SP and Tongmin SA 2004 Growth promotion and induction of systemic resistance in rice cultivar CO-47 (Oryza sativa L.) by Methylobacterium spp. Botanical Bulletin Academy Sin 45 315-325.
34) Paulraj A 2002 Effect of azophos on raising cardamon seedlings under in vitro condition. M. Sc. (Agri) Thesis, Tamilnadu Agric Univ Madurai.
35) Balamurugan S and Gunashekaran S 1996 Effect of combined inoculation of Rhizobium sp. and Phosphobacteria at different levels of phosphorus in groundnut. Madras Agric J 83 503-505.
36) Biswas JC, Ladha JK, Dazzo FB, Yanni YG and Rolf EBG 2002 Rhizobial inoculation influences seedling vigour and yield of Rice. Agron J 92 880-886.
37) Devanananda BJ 2000 Role of PGPR on growht and yield of Pigeon pea cultivars. M. Sc. (Agri) Thesis, Univ Agric Sci, Dhanwad.
38) Holfich G, Wiehe W and hecht-Buchholz C 1995 Rhizosphere colonization of different crops with growth promoting Pseudomonas and Rhizobim bacteria. Microbiol Res 150 139-147.
39) Holland MA 1997 Occams razor applied to hormonology are cytokinins produced by plants? Pl Physiol 115 865-868.
40) Patil AB 1992 Effect of combined inoculations of mineral nutrition and yield of leguminous crops. Ph.D. Thesis, State Agariarian Univ St Petersburg, Russia.
41) Senthikumar M 2003 Evaluating diazotrophic diversity and endophytic colonization ability Azorhizobium caulindodans and Methlobacterium sp. in bacterised biotized rice. Ph.D. Thesis, Tamilnadu Agric Univ, Coimbatore.