

Original Research Article

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## Demonstration on Efficacy of *Pseudomonas fluorescens* and *Azotobacter* sp. against *Meloidogyne incognita* in Farmers Field of Assam

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### ABSTRACT

Field demonstration trials on efficacy of *Pseudomonas fluorescens* and *Azotobacter* sp. against *Meloidogyne incognita* were carried out in six different farmers' fields viz., Namtemera, Kakodunga, Naromari and Misimiati in Golaghat district, Kamalabari in Majuli district and Dichangmukh in Sivasagar district of Assam that were infested by root knot nematode, *Meloidogyne incognita*. The farmers of each location were educated with the demonstration technique of enrichment and soil application methods of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha) on the field. The demonstrated technique i.e. *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha) was compared with existing farmers technique as untreated control. Analyzed data reveal that, application of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost was found to be best in increasing the plant growth parameters viz., shoot and root length (cm), number of nodules per root system and yield (q/ha) of black gram and decreasing nematode infection (number of galls and egg masses) and multiplication (nematode population in soil) than untreated control. However, the yield of black gram in demonstration fields was ranged from 6.15-8.80q/ha whereas in untreated control it ranged from 4.50-5.70 q/ha. The percent increase in the yield with improved practice over control was recorded in the range of 36.67-66.00 %. The technology and extension gap ranged between 3.70-6.35 q/ha and 1.65-3.30 q/ha and it emphasized the need to educate the farmer's through various means for the adoption of improved / recommended production technology to decrease the gaps. Similarly, the technology and extension index were ranged between 29.60-50.80 % and 26.83-39.76% which are on lower side indicating greater scope for adoption of new techniques by the farmers. The benefit-cost (B :C) ratio in each location was found to be favorable which ranged from 1: 1.5 to 1: 2.03 and this higher benefit cost ratio indicates economic viability of the demonstrated technology among the farmers.

#### Keywords

*Pseudomonas fluorescens*,  
*Azotobacter* sp.,  
Black gram and  
*Meloidogyne incognita*

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### Introduction

The plant-parasitic nematodes are dominant species in the nematode world and it comprises of 4100 species of plant-parasitic nematode (PPN) (Jones *et al.*, 2013). Among

them, the root-knot nematode *Meloidogyne incognita* attack not only more than two thousands of plant species but they also caused five per cent of global crop loss (Hussey and Janssen, 2002). This nematode exhibit obligate parasitic relationship with the

host plant and they produce giant cell as feeding cell and act as a metabolic sink which diverts all the nutrient towards them (Davis *et al.*, 2004). They produced galls on the roots and very easy to recognize with naked eyes. In Assam, yield losses in black gram due to *M. incognita* were recorded to the tune of 13.19-23.50 percent (Anon, 2011). But during the last few decades, the production and yield of the black gram declined and expected target could not be achieved. The root-knot nematode, *M. incognita*, is one of the major constraints in the production of black gram.

The application of chemical can control the nematodes but the continuous application of chemicals can cause a harmful effect on the non-target species and increase their residual toxicity in the soil. Biological control is one of the possible safe alternatives to pesticides for the disease management and is likely to be free from the toxic residual effects (Siddiqui and Mahmood, 1999). However, agriculture production depends on availability and use of quality and quantity of farm inputs. The chemical fertilizer is believed to be a vital input for boosting up agricultural production. It had played a significant role in increasing agriculture production in the country. Long-term application of nitrogen-phosphorus-potassium (NPK) based fertilizers has a pronounced effect on the biochemical properties of soil which in turn leads to shift in the microbial populations. The changes in soil organic carbon (SOC), nitrogen (N) content, pH, moisture and thus the variation in nutrient availability to microbes have been observed due to long-term application of fertilizer in crops (Bunemann and McNeill, 2004 and Wu *et al.*, 2005). In general the biofertilizers are believed to be the potential alternative to chemical fertilizers in improvement of soil fertility for sustainable crop production. Biofertilizers are best defined as biologically active products or microbial inoculants viz., formulations

containing one or more beneficial bacteria or fungal strains in easy to use and contained carrier materials which add, conserve and mobilize the crop nutrients in the soil. In other words, biofertilizer is a substance which contains living microorganisms and when applied to seed, plant surfaces, or soil they can colonize the rhizosphere or show endophytic nature and promotes growth by increasing the availability of nutrients to the host plant (Timmusk *et al.*, 2011). Biofertilizers play a very vital role in enhancing soil fertility by fixing atmospheric N, both, symbiotically and non-symbiotically. However, apart from this they can also solubilize insoluble soil phosphates and produce plant growth substances in the soil (Illmer and Schinner, 1995 and Khan *et al.*, 2007). At the present time, a need has arisen for replacing the chemical fertilizers by using organic fertilizers such as biofertilizers. Therefore, it is very essential to demonstrate the impact of the biocontrol agent as well as biofertilizer. There are some negative issues regarding application of biocontrol agents as well as biofertilizers because it requires special care for long-term storage since they are alive.

They must be used before their expiry date. In case other microorganisms contaminate the carrier medium or if growers use wrong strain, they are not as effective. So there is need to create awareness and willingness for the use of biocontrol agent and bio-fertilizers in the farming community and also spreading of knowledge about the availability and usefulness of supplementary nutrients to enrich the soil. Therefore, Indian agricultural scientists recently working on the utilization of native beneficial microorganism so that they can be easily mass produced and easily applied in the field so that farmers can learn and/or adopt the technology. However, a wide gap exists between the available techniques and its actual application by the farmers

which is reflected through poor yield in the farmers' fields. There is a tremendous opportunity for increasing the production and productivity of black gram crop by adopting the improved technologies. The root knot nematode act as a hidden enemy for the farmers and still farmers thought that it is a rhizobial gall and kept the root inside the soil as result the infestation in soil is increased and caused huge losses to the next crop. Therefore, demonstration on efficacy of *Pseudomonas fluorescens* and *Azotobacter* sp. against *Meloidogyne incognita* in farmer's field of Assam may be helpful. The basic objective of this programme is to demonstrate the improved proven technology of field trial in the farmers' field to bring in enhanced application of modern technologies to generate yield data and collection of farmers' feedback.

The crop fields of Namtemera, Kakodunga, Naromari and Misimiati in Golaghat district, Kamalabari in Majuli district and Dichangmukh in Sivasagar district are having a great potential of black gram production twice in a year due to favourable soil and agro-climatic conditions but in the last few decades, the yield attribute could not be achieved because of many drawbacks including hidden enemy of farmers, root knot nematode which causes huge losses. It is also observed that majority of the farmers of this region still practice traditional farming as they are unaware about the use of biocontrol agents and biofertilizers. Therefore, for creating awareness amongst the farmers, efforts have been made to popularize the protection technologies through community approach.

The technology that is found the best in two years' field trial have been demonstrated with an objective to evaluate the efficacy of *Pseudomonas fluorescence* and *Azotobacter* sp. on plant growth parameters and nematode

multiplication along with yield performance of black gram in comparison with existing technology of farmers' in farmers field. .

## **Materials and Methods**

### **Source of biocontrol agent and biofertilizers**

The liquid formulation of biocontrol agent *viz.*, *Pseudomonas fluorescens*, were obtained from the Department of Plant Pathology, AAU, Jorhat-13 and solid formulations of biofertilizer *viz.*, *Azotobacter* sp. were obtained from the Department of Soil science, AAU, Jorhat.

### **Demonstration site**

The demonstration trials (Figure 1) were conducted in six different farmers' fields of three districts *viz.* Namtemera, Kakodunga, Naromari and Misimiati in Golaghat district, Kamalabari in Majuli district and Dichangmukh in Sivasagar district.

### **Source of seed**

Black gram seeds of the variety PU- 31 were obtained from the Krishi Vigyan Kendra, Kamrup, Guwahati and was used for the demonstration at farmers' field.

### **Demonstrated technologies under community approach**

#### **Source of enriched compost**

The enriched compost was obtained from the DBT center, AAU, Jorhat-13.

#### **Estimation of initial nematode population**

In every location, the farmers are well trained regarding collection of soil samples for the estimation of initial nematode population. For

that 20 soil samples consisting of 10 sub samples were collected by adopting random sampling method. After that, it is mixed thoroughly and 200 cc of soil was processed by modified Cobb's sieving and decanting technique (Christie and Perry, 1951) in the laboratory, Department of Nematology, AAU, Jorhat. The average population of the samples was taken as initial population.

### **Enrichment of bioagents and biofertilizers**

In every location the farmers are trained regarding enrichment of biocontrol agent and biofertilizer. For enrichment, *P. fluorescens* and *Azotobacter* sp. were mixed with enriched compost and covered with gunny sheet and kept as such for their growth for 15 days. Occasional sprinkling of water was made for moistening. Before application they were mixed thoroughly and applied as per the rates mentioned in the treatments.

### **Treatment details and layout**

In each location, a plot of land measuring 1000m<sup>2</sup> infested with *M. incognita* was selected and divided into two parts. One part *i.e.* T<sub>1</sub> applied with *P. fluorescens* and *Azotobacter* sp. enriched with enriched compost @ 1% (1ton enriched compost /ha) and second part *i.e.* T<sub>2</sub> by Farmer practice.

### **Sowing of seeds**

The treated seeds were sown in line @ 25 kg/ha by maintaining the spacing of 30cm x 10cm and at a depth of 5 cm. After sowing, seeds were covered with thin layer of soil.

### **Weeding and Harvesting**

Hand weeding was done at 15 days of interval. Harvesting was done at maturity of the crop.

### **Observations**

The data on shoot length (cm), fresh shoot weight (gm), number of nodules, galls, egg masses per root system, final nematode population in soil and yield per plot were recorded at harvest.

### **Final nematode population**

For recording the final nematode population in soil, five sub-samples from each plot were collected randomly to make a composite sample of about 1kg, mixed thoroughly in the laboratory and 200 cc of soil was processed by modified Cobb's sieving and decanting technique (Christie and Perry, 1951) for extraction of nematodes. The demonstrations on farmer's fields were regularly monitored right from sowing to harvesting. The grain yield of demonstration crop was recorded and analyzed. Different parameters like Technology gap, Extension gap, Technology index and Extension index were used for calculating gap analysis. The detail of different parameters is as follows:

1. Extension gap = Demonstration yield - Farmers practice yield
2. Technology gap = Potential yield - Demonstration yield
3. Technology index = Potential yield - Demonstration yield x 100/ Potential yield.
4. Extension index = Demonstration yield- Farmers practice yield x 100/Demonstration yield

### **Statistical analysis**

Statistical analysis was done by using web based agricultural statistics software package (WASP) version 2.0 at 5 percent of T value for that two sample t test is used for the analysis of data and each village represents single replication.

## Results and Discussion

The demonstration conducted during 2016-17 in six different farmers' fields of three districts viz. Namtemera, Kakodunga, Naromari and Misimiati in Golaghat district, Kamalabari in Majuli district and Dichangmukh in Sivasagar district of Assam indicated that T<sub>1</sub> i.e. application of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha) shows better result as compared to T<sub>2</sub> i.e. farmers' practice at each location. It is evident from the result that under demonstrated plots, performance of black gram was comparatively much better than the farmers practice. The analyzed data presented in Table 1, 2 and 3 reveal that, application of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost was found to exhibit significantly better results in increasing the plant growth parameters viz., shoot length (cm), fresh shoot weight (gm), number of nodules per root system of black gram and decreased the nematode infection (number of galls and egg masses per root system) and multiplication (nematode population in soil) than the farmers practice as untreated control. *P. fluorescens* and *Azotobacter* sp. are capable of surviving and colonizing the rhizosphere of all crop fields and is reported to promote plant growth by secreting auxins, gibberellins and cytokinins (Vidhyasekaran, 1998). Native isolates of *P. fluorescens* and *Azotobacter* sp. are reported to be effective in suppressing the population of root knot nematode *M. incognita* in banana (Jonathan *et al.*, 2006) and black gram (Bharali *et al.*, 2019a). Bharali *et al.*, (2019b) has proved the antagonistic effect of culture filtrates of *P. fluorescens* and *Azotobacter* sp. on the eggs and juveniles of *M. incognita*. Initial application of *P. fluorescens* prior to invasion protects the crop from the pathogens by strengthening the cell wall structure and causing biochemical and physiological

changes in the plant system (Chen *et al.*, 2000). However, the application of *P. fluorescens* (Khan *et al.*, 2012) and *Azotobacter* spp. (Martinez-Toledo *et al.*, 1988) increased IAA in plant and plays a major role in the development of rhizobial nodules on the legume plant. Plant growth promoting rhizobacteria viz., *P. fluorescens* reported to induce systemic resistance (ISR) in banana against plant parasitic nematodes (Hasky-Gunther *et al.*, 1998 and Shanthi and Rajendran, 2006). The yield performance of black gram under demonstrated plot was comparatively much higher than the farmers practice. The maximum yield was recorded in the demonstrated plot i.e soil application of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha) of Namtemera followed by Kamalabari, Dichangmukh, Misimiati, Naromari and Kakodunga than farmer's practice and the reason behind the varied yield performance may be due to the varied soil fertility and agro-climatic conditions. Similar yield improvement in the crops were given by Hiremath *et al.*, (2007) in Onion and Mishra *et al.*, (2009) in Potato, Katare *et al.*, (2010) and Meena *et al.*, (2012) in Rapeseed-mustard. The result clearly indicate the positive possessions of demonstration over the farmers practice towards increase in the yield of black gram in *M. incognita* infested soil in different district of Assam with its positive effect on yield trait. However, higher technology gap was recorded in the village Kakodunga followed by Naromari, Misimiati, Dichangmukh, Kamalabari and Namtemera which reveals the need to educate the farmer's through various means like village level training, on campus training etc. for adoption of improved / recommended production technologies to decrease the gaps. It can also be reduced by giving the more demonstrations in the districts and motivating the farmers for the implementation of improved technologies. Whereas, higher extension gap was recorded



in the village Dichangmukh followed by Kamalabari, Namtemera, Misimiati, Naromari and Kakodunga, respectively. However, this gap might be attributed to not only the different variables like bioagents and bio fertilizers, those were considered as critical inputs for the demonstration but also adoption of improved technology which resulted in higher grain yield than the traditional farmers practices. Anyway, if the farmers will adopt new technologies, it will lead to the farmers to discontinue the old technology. Now, it is revealed that the extension gap can be bridge through participatory approach (Mukhopadhyay, 2002). The extension agencies can effectively communicate the improve technologies to farming community for the better production. This finding is similar with the findings of Hiremath and Nagaraju, (2010), Raj *et al.*, and (2013). The technology index and extension index showed the feasibility of evolved technology at the farmers’ fields. The lower the value of

technology and extension index, the more is the feasibility of technology. Such variation in the indexes may be due to variation in soil fertility status, weather conditions, non-availability of water and insect pest attack on the crop. Pathogenicity of *M. incognita* that causes more damage to the crop in farmers practice since it is a control treatment and resulted in lower value of the indexes. However, to avoid such gap it is better to advice the farmers to implement the recommended technology and skills during the process of demonstration. Further, the benefit-cost (B: C) ratio in each location was found to be favorable and it ranged from 1: 1.50 in Kakodunga to 1: 2.03 in Naromari. The higher benefit cost ratio of demonstrations (Table 3) clearly signifies the economic viability of the demonstrated technique *i.e.* soil application of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha).

**Table.1** Average plant growth parameter of black gram in *M. incognita* infested soil under demonstration and existing farmers practice

Treatment	Shoot length (cm)	Shoot weight (gm)	Number of nodules per root system
T <sub>1</sub>	46.56 ± 2.094	21.37 ± 0.383	24.67 ± 1.37
T <sub>2</sub>	43.85 ± 1.078	17.52 ± 0.235	18.00 ± 1.41
T <sub>value</sub>	2.825	20.98	8.35
T <sub>value at 0.05</sub>	2.228		

T<sub>1</sub>- *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha) and T<sub>2</sub>- Farmers practice (Untreated control)

**Table.2** Average nematode multiplication under demonstration and existing farmers practice

Treatment	Number of galls per root system	Number of egg masses per root system	FNP (200cc)
T <sub>1</sub>	17.33 ± 1.63	16.33 ± 1.033	240.00 (15.48 ± 0.99)
T <sub>2</sub>	30.33 ± 1.033	26.17 ± 1.72	371.67 (19.23 ± 1.73)
T <sub>value</sub>	16.48	11.99	4.58
T <sub>value at 0.05</sub>	2.228		

T<sub>1</sub>- *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost @ 1% (1ton enriched compost /ha) and T<sub>2</sub>- Farmers practice (Untreated control)

**Table.3** Productivity, technology gap, extension gap, technology index, extension index and B:C ratio of black gram in *M. incognita* infested soil under demonstration and existing farmers practice

Name of Village	Yield (q/ha)			Gap between potential Yield and Demonstration Yield (q/ha)	% yield increase over control	Technology gap (q/ha)	Extension gap (q/ha)	Technology index (%)	Extension index (%)	B:C Ratio
	Potential	Demonstration	Control (Farmers practice)							
Namtemera	12.50	8.80	5.70	3.70	54.39	3.70	3.10	29.60	35.23	1: 1.76
Kakodunga		6.15	4.50	6.35	36.67	6.35	1.65	50.80	26.83	1: 1.50
Naromari		6.40	4.60	6.10	39.13	6.10	1.80	48.80	28.13	1: 2.03
Misimiati		7.40	4.80	5.10	54.17	5.10	2.60	40.80	35.14	1: 1.57
Kamalabari		8.50	5.40	4.00	57.41	4.00	3.10	32.00	36.47	1: 2.01
Dichangmukh		8.30	5.00	4.20	66.00	4.20	3.30	33.60	39.76	1: 1.87

**Figure.1** Demonstration at A. Misimiati, B. Kakodunga, C. Naromari, D. Namtemera, E. Kamalabari and F. Dichagmukh



**A. Misimiati**



**B. Kakodunga**



**C. Naromari**



**D. Namtemera**



**E. Kamalabari**



**F. Dichagmukh**



It is evident from the results of the present investigation that the biofertilizers which are generally used for better growth and yield of the crop, may also serve as a component in integrated management of root-knot nematode, *M. incognita* infecting black gram. Thus combined application of *P. fluorescens* and *Azotobacter* sp. enriched in enriched compost might be an eco-friendly and cost effective nematode management package for the farmers who are facing the problems of root-knot nematode, *M. incognita* infecting black gram.

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