RESEARCH ARTICLE

EFFECT OF *PSEUDOMONASFLUORESCENS* AND ORGANIC MATTERAS A BIOFERTILIZER ON *SOLANUM MELONGENA* L. (BRINJAL)

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Abstract: The influence of biofertilizer inoculation,*Pseudomonasfluorescens* alone and recommended dose of organic solution on brinjal (*Solanum melongena L.*) crop was tested during the kharif season of the year 2019 at agricultural field Patanjali Bio-Research Centre, Haridwar, Uttarakhand. The results revealed significant improvement in growth characters such as height of plant, stem diameter, length of root, number of functional leaves, weight of fresh shoot and weight of dry shoot over the control. Similarly, number of fruits picked per plant and yield of fruits was more in inoculated crop.Itisoneofthemost popular and commercial crops grown in India and other parts of the world and rightly called as vegetable of masses. The common large-fruited forms are believed to have originated in Indo-Burma region. Fruits are moderate sources of vitamins and medicinal properties including de-collateralizing action.

Keywords: Biofertilizer, Eco-friendly, Organic matters, Plant growth, Soil health

INTRODUCTION

The use of organic solution (Biofertilizer) not only spoils the ground water, soil but also have deleterious effects by the emission of harmful gases. Forum for Nuclear Co-operation in Asia Bio-fertilizer Project, National Project on Organic Farming and All India Network Project on Bio-fertilizers aims to encourage use of bio fertilizers. Bio fertilizers improve the quantitative and qualitative features of many plants. Biofertilizers used in conjunction with chemical fertilizers improve crop productivity and nutrient use efficiency. Positive effect of *Pseudomonasfluorescens* on growth and yield of Brinjal also been reported by many workers. There is a positive influence on the growth and yield attributes of Brinjal.It is becoming difficult to meet the nutrient need of farming through chemical fertilizer alone and due to its higher costs; the concept of integrated plant nutrient supply system (IPNS) is gaining ground. Therefore, the investigation was planned and conducted to study the influence of liquid biofertilizers alone, dual and in different combinations with chemical fertilizers on the vegetative and reproductive growth, yield and quality attributes of *Solanum melongena* L.

China is the largest producer of Brinjal and contributes about 68.7% of the world's Brinjal production while India occupies second position in production with a share of 23.3%. However, the productivity of Brinjal is quite low in India. Thebrinjal,alsoknownas'eggplant'or'Guineasquash,'I n India, Brinjal occupies fourth position in area and sixth in production among the vegetable crops. Shoot-root borer, bacterial wilt, fusarin wilt, little leaf are the major threats to the Brinjal (Katiyar, 2000).

MATERIAL AND METHODS

There are following techniques are taken here

1. Isolation and identification of *Pseudomonasfluorescens*
2. Preparation of organic matters solution
3. Preparation of biofertilizer& apply

1. Isolation and identification of *Pseudomonasfluorescens* from rhizospheric soilof *Dulbergiasisso*.

i. Soil sample collection
ii. *Pseudomonasfluorescens* isolates from rhizosphere soil

- Preparation of soil dilution
- Preparation of spread plates
- Identification and characterization of bacterial isolates
- Morphologicalcharacterization
- Cultural Characterization
- Biochemical and Physiologicalcharacterization

i. Soil Sample collection:

Soil samples were collected from the location of Motherhood University,Roorkee, Bhagwanpur, (Uttarakhand) and 40gm soil samples were collected up to the depth of 10 to 15cm from the rhizosphere of *Dulbergia sissoo.* The soil intimately adhering to the roots was collected and mixed to provide a composite. Soil sample collected in polyethylene bags, stored at field moisture level and room temperature. The reference *Pseudomonas* strain was procured from Microbiology division, PBRI, Hardwar, Uttarakhand.

ii. *Pseudomonasfluorescens* isolates from rhizosphere soil:

Preparation of Soil Dilutions:

- Firstly, weigh out 20 g of soil sample in flask and add to 100ml of distilled water. Shake the suspension well and give label as "A".
- Prepare six 9ml water blank and give the label as

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B to G, before the soil settles, remove 1 ml of the suspension with a sterile pipette from suspension A and transfer it to a 9ml distilled water blank. Shake it well and give label as "B".

- Repeat this dilution step five times, each time with 1 ml of the previous suspension and 9ml distilled water blank. Label these sequentially as tubes C, D, E, F and G. This results in serial dilutions of 10^{-1} through 10^{-6} grams of soil per ml.

Preparation of Spread Plates for Bacterial Culture

- For the growth of bacterial colonies, take 6 pre-prepared Kings B' medium plates and label them as B, C, D, E, F and G. Vortex samples B, C, D, E, F and G and pipette 0.1 ml onto each plate.
- After this process, dip a glass spreader into ethanol. Place the spreader in a flame for a few seconds to ignite and burn off the ethanol. This will sterilize the spreader.
- Hold the spreader above the first plate until the flame is extinguished. Open the plate quickly, holding the lid close by. Touch the spreader to the agar away from the inoculum (Inoculum = cells used to begin a culture) to cool, and then spread the drop of inoculum around the surface of the agar until traces of free liquid disappear. Replace the plate lid.
- Re-flame the spreader and repeat the process with the next plate, working quickly so as not to contaminate the plates with airborne organisms.
- Incubate the bacteria plates at room temperature for some time. Make sure the plates are inverted during the incubation to prevent drops of moisture from condensation from falling onto the agar surface.

Enumeration

The plates incubated for a day at 30 ± 1°C were observed for the growth of *Pseudomonas* colonies on KB plates and the colonies are enumerated manually and recorded. Results are presented in the Table-1.

Table 1. Microbial population in the rhizosphere soil of *Dulbergiasisso*

S. No	Plates replicates	*Pseudomonasfluorescens* (x 10^{6}cfu / gm soil)
1	P.P-1	**4**
2	P.P-2	**3**
3	P.P-3	**3**
4	P.P-4	**2**
5	P.P-5	**2**
6	**P.P-6**	**2.65**

Identification of Bacterial Isolates

Morphological Characterization

All the 6 isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by

Cultural Characterization

Morphological characteristics of the colony of each isolate were examined on Nutrient agar and specialized medium and incubated for according to isolate. Cultural characterization of isolates observed by different characteristics of colonies such as shape, size, elevation, surface, margin, color, odor, pigmentation etc. were recorded as per Bergey's Manual of Determinative Bacteriology.

Biochemical and Physiological Characterization

Different biochemical tests performed and the protocols followed are briefly outlined below.

Hydrogen Sulfide Test

Sterilized hydrogen sulfide-Indole-Motility agar stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48h at 28°C. Visualization of black color along the line of inoculation indicated a positive reaction for the test.

Indole Production

Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28°C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red color were recorded as positive for indole production.

Catalase Test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of Pure isolates were taken on glass slides and one drop of H_2O_2(30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at 28°C. Then the tubes were kept in the refrigerator for 30 min at 4°C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test.

Starch Hydrolysis
Sterile starch agar plates were spotted with 10±l overnight broth cultures of the isolates and incubated at 28°C for 24-48 hour. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive reaction for the test.

Methyl Red Test
Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28±2°C for 48h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red color production was taken as positive and yellow color production was taken as negative for the test.

Voges Prausker's Test
To the PR sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37°C for 48h.After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink color in the broth was taken as positive for the test.

Citrate Utilization
Isolates were streaked on Simmon's citrate agar slants and incubated at 28±2°C for 24h. Change in color from green to blue indicates the positive reaction for citrate utilization.

Oxidase Test
The overnight cultures of the test isolates were spotted on plates poured with sterile trypicase soy agar and the plates were incubated for 24 h. at 28°C. After incubation, 2-3 drops of N, N, N', N'-tetramethyl- p-phenylene diamine dihydrochloride (Wurster's reagent) were added onto the surface of growth of each test organism. The isolates showing change of color to maroon were noted as oxidase positive.

Denitrification test
Sterilized nitrate broth tubes inserted with Durham's tube in inverted position were inoculated with overnight grown cultures of the test organisms and incubated at 25°C for 10-15 days. After incubation, the isolates which showed accumulation of gas in the Durham's tubes were scored as positive for denitrification.

Carbohydrate Utilization
All pure bacterial isolates were screened for the carbohydrate fermentation abilities using 4 different carbohydrates (lactose, sucrose, dextrose and mannitol) in Peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in color of Peptone broth was observed for utilization of particular carbohydrate present in broth.

Isolation of rhizobial*Pseudomonasfluorescens*
The microbial population in the rhizospheric soil of *Dulbergiasisso* was collected. Maximum population of *Pseudomonasfluorescens* was found in the university farm. The *Pseudomonas* population rangedbetween1-6.0x10^{6}cfu/soil.

Cultural and Morphological Characterization
Pseudomonasfluorescens based on their colony morphology on different media, cell morphology and Gram reaction. The bacterial isolates were named according to the crop and cultural characters presented in Table-2 *Pseudomonasfluorescens* (6 isolates), based on their colony morphology on different media, cell morphology and Gram reaction presented in the Table-2.

Table 2. Labelling of isolates according to crop and cultural characters.

	Pseudomonasfluorescens
Isolate name	**Soil sample/plant**
PP-1	***Dulbergia sissoo***
PP-2	***Dulbergia sissoo***
PP-3	***Dulbergia sissoo***
PP-4	***Dulbergia sissoo***
PP-5	***Dulbergia sissoo***
PP-6	***Dulbergia sissoo***

All theisolates developed small to medium, smooth, glistening colonies, out of the 8 isolates 5 isolates showed yellowish green color with light green pigmentation and the remaining isolates showed dull white colonies with no pigmentation. These isolates were Gram negative, small, single isolated rods without sporulation when observed.

Biochemical and Physiological Characterization
After the study of cultural and cell morphology, the isolates of the *Pseudomonasfluorescens* (6 isolates) were tested for different biochemical test viz., IMVIC test, oxidase test, catalase test, carbohydrate fermentation, denitrification, H2S production, starch hydrolysis, gelatin liquefaction etc. All the 6 isolates of *Pseudomonasfluorescens* showed positive results for catalase test and oxidase test whereas they were negative for Voges Prausker's test. For methyl red test PP-1, PP-2, PP-3, PP-6 isolates showed positive results. Out of 6 isolates 4 isolates showed positive results for starch hydrolysis, only 2 isolates showed positive results (Kumar, *et al.,* 2003).

For gelatin liquefaction, 6 isolates showed positive results for citrate utilization, only 5 isolates showed positive results for H2S test, denitrification, PP-1, PP-2, PP-3, PP-4 isolates respectively showed positive results.

2. PREPARATION OF ORGANIC MATTERS SOLUTION

Preparation of Organic Matters solution:

- 20 liters: Cow urine, Dried cow dung
- 03 Kg: Neem leaves extract
- 500 g: Green Chili extract
- 250 g: Garlic extract
- 500 g: Tobacco
- 250 g: Datura leaves extract
- 250 g: Calotropis (Aak) leaves extract
- 250 g: Ginger leaves extract
- Cow urine and dried cow dung

Nitrogen, phosphorus and potassium are the three major nutrients required for healthy plant growth. Cow urine contains significant amounts of both nitrogen and potassium. Research shows that only 20% of nitrogenous materials consumed by cattle are absorbed and 80% is excreted in urine and dung. 52%of Nitrogen returns in the form of urine while 28% return in form of dung 61-87% phosphorus and 82-92% potash was also obtained from urine.

- After analysis it is found that micronutrients increase in soil after application of cow urine.
- Color of leaves is greener than with the use of urea application.
- Residual effect of cow urine is present in next crop.
- Improves the Soil Texture
- Creates good environment in soil for earthworm growth.
- Cow urine sprayed after 14 days of storage in cool place works as insecticide against aphids and other insects.
- It serves as growth promoter of plants.

The spraying of urine not only provides nitrogen for plants but also protects the plants from aphid and other insects and provides resistance to diseases (Kumar, *et al.*,2003).

3. PREPARATION OF BIOFERTILIZER& APPLY

The experiments were laid down during kharif season of 2019. The Randomized Complete Block Design with four replications was adopted in field experiments. The sowing of experimental materials was done on 17thJune and transplanting on 22ndJuly 2019 at agricultural field. Patanjali Bio-Research Centre, Haridwar, Uttarakhand. The brinjal variety Syngenta Green-Crown was given a spacing of 80-85 cm between two plants and 90-100 cm between twolines.

Overall, the soils of experimental plots were medium, black, alkaline, with available N (158 kg/ha), P (7.38 kg/ha), K (443 kg/ha), organic C (0.42%), electrical conductivity (0.181 dSm^{-1}) and with 60.87% water holding capacity. Apply recommended dose of *Pseudomonasfluorescens* and organic solution. The other agronomic practices were followed uniformly during cropping season and need based protection measures were taken. First application (07thAug. 2019) constitutes half dose of *Pseudomonasfluorescens* and organic solution. Second application (05thSept. 2019) constitutes remaining half dose.

Observations were recorded on height of plant, stem diameter, length of root, number of functional leaves, weight of fresh plant, weight of dry plant, number of fruits picked per plant, yield of fruit. Observations were recorded by selecting randomly two plants from treatment for length of root, weight of fresh plant and weight of dry plant (Giri, *et al.*, 2003).

RESULT AND DISCUSSION

Height of the plant studied from 15 to 130 Days After Planting (DAP). The max. plant height at 150 DAP (98.64 cm) was found, whereas the min. was in control (71.44 cm). The treatments T-1, T-2, T- 3,T-4andT-5haveshown37.43%,37.54%,28.96%, 17.67% and 11.03% increased height per plant respectively over the control. Recommended dose of *Pseudomonasfluorescens* and organic solution is appeared to be compensated by the combined treatment of *Pseudomonasfluorescens* and organic solution. Mean stem diameter was under investigation from 15 to 150 DAP. The max. stem diameter at 150 DAP (3.33 cm) was found, whereas the min. was in control (2.69 cm). The treatments T-1, T-2, T-3, T-4 and T-5 have shown 23.79%, 15.69%, 16.04%, 11.89% and 6.38% increased mean stem diameter per plant over the control. A secretion of growth hormones and availability of nutrients and moisture influenced positively the stem diameter (Ram, *et al.*, 2002).

Average length of root per plant was studied from 30 to 165 DAP. The max. length of root at 165 DAP (57.14 cm) was found, whereas the min. was in control (41.73 cm). The treatments T-1, T-2, T-3, T-4 and T-5 have shown 36.93%, 26.05%, 13.04%, and 10.38% and 5.56% increased length of root per plant respectively over the control. Mean number of functional leaves per plant was observed from 30 to 130 DAP. The max. number of leaves at 120 DAP (552.38) was found. The treatments T-1, T-2, T-3, T-4 and T-5 have shown 51.51%, 27.06%, 19.55%, 13.10% and 05.67% more mean number of leaves respectively per plant over the control (Bhan, *et al.*, 1997).

Average weight of a fresh shoot was observed from 30 to 130 DAP. The max. weight of a fresh shoot at 130 DAP (791 g) was found in, whereas, the min. was recorded in the control (582 g). The treatments T-1, T- 2, T-3, T-4 and T-5 have shown 35.91%,35.22%, 16.32%, 12.89% and 7.90% increase of average weight of a fresh shoot over the control. The chemical fertilizers and combined biofertilizer treatments recorded significantly more weight due to the proper nutritional supply. The treatments T-1, T-2, T-3, T-4 and T-5 have shown

46.94%, 41.84%, 21.43%, 16.33% and 7.14% increase of average weight of a dry shoot respectively over the control, (Table 3) (Bhan, *et al.*,1997)

The maximum number of fruits picked per plant during the crop time was recorded, whereas the min. fruits picked per plant were in control (26.38). The treatments T-1, T-2, T-3, T-4 and T-5 have shown 44.92%, 52.81%, 33.09%, 24.64% and 11.30%more fruits picked per plant respectively over the control. The maximum yield of brinjal fruit per plant during the crop time was recorded;whereas the min. yield per plant was in control (1651.96 g). All the treatments were significantly superior in fruit yield over the control. The treatments T-1, T-2, T-3, T- 4 and T-5 have shown 54.61%, 52.33%, 36.09%, 23.48% and 11.89% more fruit yield per plant respectively over the control, (Table 4).Brinjal fruit yield in treatment of *Pseudomonasfluorescens*in combination with organic matter as a biofertilizer.

Table 1. Brinjal parameters according to days.

Parameter	Observation	T-1	T-2	T-3	T-4	T-5	T-6
Height, cm	90 DAT	59.23	86.12	80.45	73.88	69.24	**86.26**
	150 DAT	71.44	98.26	92.13	84.06	79.32	**98.18**
Stem diameter, cm	90 DAT	1.86	2.12	2.24	2.07	1.98	**2.72**
	150 DAT	2.69	3.08	3.06	2.98	2.78	**3.33**
Root length, cm	90 DAT	24.54	28.37	27.11	26.57	26.11	**30.33**
	165 DAT	41.73	52.60	47.17	46.06	44.05	**57.14**
Number of leaves	90 DAT	327.85	412.36	388.67	371.24	344.25	**502.48**
	120 DAT	364.58	463.25	435.86	412.35	385.24	**55.38**
Weight of fresh plant, g	90 DAT	441	598	548	521	493	**639**
	150 DAT	582	787	677	657	628	**791**
Weight of dry plant, g	90 DAT	73	112	97	91	86	**114**
	150 DAT	**98**	**139**	**119**	**114**	**105**	**144**

Table 2. Brinjal fruit yield as influenced by different treatments.

Parameter	T-1	T-2	T-3	T-4	T-5	T-6
No. of fruits pick^{-1} plant^{-1}	2.93±0.03	4.47±0.03	3.90±0.01	3.65±0.02	3.26±0.01	4.25±0.07
No. of fruits plant^{-1}	26.38±0.29	40.31±0.28	35.11±0.12	32.88±0.26	29.36±0.12	38.23±0.66
Fruits yield pick^{-1} plant^{-1}, g	183.55±0.20	279.60±0.11	249.79±0.36	226.65±0.17	205.38±0.20	283.85±0.05
Fruit yield plant^{-1}, g	1651.96±1.80	2516.46±1.03	2248.12±3.32	2039.91±1.57	1848.44±1.83	2554.7±0.49

Table 3. Brinjal disease or pest infestation (%)

Infestation	T1	T2	T3	T4	T5	T6
Shoot/root b	25.00	8.33	16.67	16.67	13.33	16.67
Fruit borer	24.81	22.64	19.40	20.44	20.25	21.30
Little leaf	33.33	8.33	25.00	16.67	25.00	20.25
Average over control	26.71	30.62	--	33.64	50.14	28.90

All the treatments appeared to be the significantly superior over the control as the average infestation was less by 26.71 to 50.14% as compared to the control population, (Table-5). It suggests that the biofertilizers secretes some antibiotic substances or growth hormones. Similarly, the treatments of fertilizers are more susceptible to infestation but due to availability of nutrients make them more resistant as compared to the control population. The treatments *Pseusomonasfluorescens* + organic solution has shown 66.68% and 46.68% less shoot-root borer infestation respectively over the control. The treatments *Pseusomonasfluorescens* + organic solution has shown 21.81% and 18.38% less of fruit borer infestation respectively over the control. The treatments *Pseusomonasfluorescens* + organic solution has shown 75.0% and 49.98% less of little leaf infestation respectively over the control. These findings are supported by Ghanbahadur, *et. al*., (2005) So, the proper dose of biofertilizer replaces chemical fertilizer.

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