International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 15, 2018, pp.-6784-6787.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

EFFECT OF *Pseudomonas* sp. BASED MICROBIAL FORMULATION ON GREEN POD YIELD AND SHELLING PERCENTAGE OF *Pisum sativum*

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Received: July 23, 2018; Revised: August 10, 2018; Accepted: August 11, 2018; Published: August 15, 2018

Abstract: The effect of solid carrier based microbial formulation containing *Pseudomonas* UN7 and NPK fertilizers on green pod yield and shelling percentage on pea *cv*. PB-89 was investigated by seed treatment method. Plant growth promoting activity of *Pseudomonas* UN7 was also investigated in the present work. *Pseudomonas* UN7 showed phosphate solubilizing activity, Indole acetic acid production and iron chelating compound *i.e*., siderophore producing activity. A field experiment was conducted to study the effect of biofertilizers on green pod yield and shelling percentage of pea, which resulted in increased with the inoculation of *Pseudomonas* UN7 integrated with different NPK combinations compared to either sole application of Talc, FYM or combinations of both (Talc + FYM). The experiment was conducted in randomized block design with three replications in sandy loam soil. The experiment comprised 24 treatment combinations of four levels of fertility (Control, FYM, 50,75, and 100 NPK). The treatment FYM +NPK_{50} + *Pseudomonas* UN7 recorded the highest green pod yield, plant height and shelling percentage of (102.16 q/ha), (39.33 q/ha) and (39.50 q/ha) respectively, which was statistically at par with FYM +NPK_{100}. The lowest green pod yield (30.36 q/ha) was recorded when the plots were supplemented with talc (control). The above treatments thus resulted in saving of 50 % NPK fertilizers.

Keywords: Solid carrier, NPK, *Pseudomonas*, FYM, Rhizobacteria, Organic farming, Phosphate solubilization, Indole Acetic Acid, Siderophore

Citation: Negi Urvashi, *et al*., (2018) Effect of *Pseudomonas* sp. Based Microbial Formulation on Green Pod Yield and Shelling Percentage of *Pisum sativum*. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 15, pp.- 6784-6787.

Introduction

For improving productivity and sustainability in agriculture New and novel solutions are sought. Soil fertility and plant health is affect by plant growth promoting microbes. In last few years, for developing plant growth promoting formulations, there has been an upsurge in the selection of beneficial microbes. The region near the roots commonly Known as 'rhizosphere' presents intense microbial activity, in the presence of existing microbial community [1,2]. Plant growth promoting microorganisms are a multiple group of microbes like bacteria, fungi, actinomyces and other flora that colonize roots, may be present on surface of the plants and also can exist in the intracellular parts of plant which impart to promote the growth of plants. PGPR term was initially for the first time used by [3] for micro-organisms closely associated with rhizosphere region. It is well understood that, in the rhizosphere only 1-2% of bacteria promote plant growth. These inoculants are known to result in improvement of crop growth and yield or provide protection to the crop from pests and diseases. The genera commonly used as biofertilizers are *Rhizobium, Bacillus*, *Pseudomonas etc*. The genera commonly used as biocontrol agents are *Pseudomonas*, *Bacillus, Burkholderia, Agrobacterium etc*. These organisms suppress plant disease by production of antibiotics, production of indole acetic acid, phosphate solubilization, siderophores, or by induction of systemic resistance or any other mechanism [4].

The plant growth promoting microbes were commonly characterized: (i) proficient to colonize the root surface (ii) they must compete with other microflora, survive and multiply, to express their plant growth promotion/protection activities, and (iii) they must help to promote plant growth [5]. Plant growth promoting microorganisms (PGPM) mediated growth and yield promotion occurs by the alteration of the whole microbial community in environment niche through the production of various intermediate and by products [6]. Generally, PGPM promote plant growth directly by either facility by acquisition of required nutrients (nitrogen, phosphorus and essential minerals) or plant hormone levels modulation, or indirectly by decreasing the inhibitory effects of various soil borne or plant pathogens on plant growth and development in the forms of biocontrol agents [7]

To achieve the maximum growth promoting interaction between PGPM and crop seed or seedlings it is important to explore how the microbe exerting their effects on plant and whether the effects are affected or altered by various environmental factors, including the presence of other micro- organisms, therefore, it is necessary to develop efficient strains in field condition. PGPM can mediate plant growth by different direct and indirect mechanisms [8, 9]. Some of the mechanisms commonly observed are; (1) due to solubilization/mobilization increased availability of nutrients; (2) biological nitrogen fixation; (3) plant protection from diseases and pests by producing hormones, antibiotics, siderophores, hydrogen cyanide, *etc.* (Medeiros *et al*. 2005; Keel and Mayrhofer 2009); (4) production of plant hormones like IAA, cytokinin's, gibberellic acid, *etc*.; (5) improving the tolerance to salt stress, drought stress, *etc*.; (6) by production of the enzyme 1-aminocyclopropane- 1-caroxylate (ACC) deaminase lowering of ethylene levels in plants [9]. Often-ally among the plant rhizosphere bacteria *Pseudomonas* predominate and have received tremendous attention due to their wide spread distribution in soil, ability to colonize rhizosphere and capacity to produce an array of enzymes and metabolites [10]. Legume-rhizobia symbiosis is also stimulated by PGPR through various modes of action, the most commonly action being the IAA-induced increase in root growth providing more sites for nodulation and through the supply of phosphorus required for nodule formation and nitrogen fixation by rhizobia [11,12]. Several microbial inoculants are used as biofertilizers, which improve the uptake of nutrients like nitrogen, phosphorus, potassium, sulphur, iron, *etc*. Biofertilizers are carrier-based formulations containing viable cells of efficient strains of N-fixing, P-solubilizing or cellulolytic

microorganisms used for application to seed or soil, intended to improve soil fertility. Mode of application of biofertilizers depend on the type of crop. Application of biofertilizer to the field under cultivation is essential. Mixing of microorganisms in carrier materials enables sustainability issues like easy-handling, long term storage and high effectiveness of biofertilizer [13]. The carrier is any material which can be used as a delivery vehicle of viable micro-organisms from the laboratory to the field. The type and the quality of carrier is an important factor to determine the microbial population density and shelf life of biofertilizer. Properties of a good carrier material are; good moisture holding capacity, available in adequate quantity, inexpensive, easy to process and sterilize, nontoxic to inoculated bacterial strains and plants [14]. The aim of formulating viable cells in carriers is to facilitate the delivery and handling processes, and to ensure the adequate cell viability to maintain the efficacy of the cells [15]. Hence present study was carried out to validate the effect of plant growth promoting rhizobacteria on Pea.

Materials and methods

Revival of Bacterial Isolate

Bacterial *Pseudomonas* UN7 (previously isolated from Cold desert of Lahaul & Spiti) was procured from repository of Basic science department, DR YS Parmar UHF, Nauni (HP). Which was stored and maintained as glycerol stocks (25% v/v) at – 80°C for long term storage. Culture isolate *Pseudomonas* UN7 was revived from glycerol stock on Trypticase Soy Agar (Pancreatic digest of casein 17.0, Papaic digest of soybean meal 3.0, NaCl 5.0, K_2HPO_4 2.5, Dextrose 2.5, Agar 15.0, pH 7.3 ± 0.2) plates and incubated at 28±0.1 °C for 24 h.

Colony Morphology

Characteristics such as shape, colour, margin, opacity were observed on TSA.

Gram Staining

A thin homogenous bacterial smear was prepared on a clean glass slide from the bacterial culture grown on TSA for 48 h, air-dried and heat-fixed. The smear was covered with crystal violet stain for 1 min, washed with de-ionized water and flooded with Gram's iodine solution for 1 min. The slide was washed with de-ionized water and decolorized with absolute alcohol until no violet colour came off. The smear was counter stained with 0.5% safranin for 30 s, washed with de-ionized water, blot-dried and observed under Microscope using oil immersion objective.

Screening of *Pseudomonas* UN7 for Plant Growth-Promoting Attributes

Phosphate solubilisation

Initial screening to evaluate phosphate solubilization by the bacterial isolates was done on modified Pikovskaya agar (Glucose:10.0, $(Ca_3PO4)_2$:5.0, $(NH_4)_2SO_4$:0.5, KCl: 0.2, $MgSO_4$:0.1, $FeSO_4$: 0.01, $MnSO_4$: 0.01, Yeast extract: 0.5, Bromo phenol blue (0.4%): 3.0 ml, Agar: 18.0, Ph 7.0± 0.2) with tricalcium phosphate as the inorganic form of phosphate [16]. A loop full of each culture was placed on the agar plates and incubated at 28°C for 5 days. A clear zone around the colonies was scored as phosphate solubilization zone. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone.

Production of IAA-Like Auxins

Fifty millilitre NB containing 0.1% DL-tryptophan (Nutrient broth (NB) Peptone:5 g, NaCl: 5g, Beef extract:1.5 g, Yeast extract:1.5 g, pH was adjusted to 7.4 and the final volume was made to 1000 ml using distilled water. was inoculated with 500 µl of 24 h old bacterial cultures and incubated in a refrigerated incubator shaker) at 180 rpm for 48 h in dark. The bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4°C. Estimation of indole-3-acetic acid (IAA)-like auxins in the supernatants was done using colorimetric assay [17].

Production of Siderophores

Glassware

The glassware used for siderophore detection, quantification and antagonistic studies were cleaned in 20% HCl to remove iron and rinsed with deionized water.

Screening

Siderophore production by the bacterial isolates was detected on Chrome azurol sulphonate (CAS) plates (CAS:60.5 mg, Iron (III) solution: 10 ml (1mM FeCl3 .6H2 O, in 10 mM HCl), HDTMA: 72.9 mg, PIPES: 30.24 g, NaOH: 12 g, Agar: 15g) as described [18]. The CAS assay is based on the high affinity of siderophores for ferric iron, whereby ferric iron bound to the dye, is complexed and released from the dye:

dye Fe^{3+} (blue) + siderophore = siderophore Fe^{3+} + Orange to pale yellow coloured dye

24 hours old bacterial cultures were spot inoculated on CAS agar plates then incubated at 28°C for 5 days. The yellow-orange halos around the bacterial colonies indicating the production of siderophores were measured. Zone of siderophore production was determined by subtracting the diameter of bacterial colony from the diameter of total zone inclusive of the colony.

Field Experiment

The present investigation was done with the pea var. PB-89 at vegetable farm. The experiment was conducted on sandy loam soil with the pH 5.1, organic compound (1.65%), available N, P and K was 238, 14.8, 395 kg/ha, respectively. The experiment consisting of eight treatments was laid in randomized block design with three replications in plot size of 7.0m2. The treatments included control (Talc), FYM (20 tons/ ha), NPK 50%, NPK 75%, NPK 100% (16:32:16 kg/ha), NPK + FYM, Biofertilizers (*Pseudomonas* UN7) and NPK 50%+FYM+ *Pseudomonas* UN7. The experiment was laid out in a randomized complete block design with three replications. Method of application was treatment of seed for 30 minutes before sowing. Experimental crop was grown under irrigated condition as per the recommended agronomic practice. The effect of treatment was evaluated on pooled basis on pea yield, plant height and shelling percentage.

Growth Parameters

Plant Height (CM)

The height was measured in five-tagged plants in each treatment from ground level to the tip of fully opened leaves of main stem with the help of meter scale at 30, 45 and 60 days after sowing.

Green Pod Yield (q ha^{-1})

Green pod yield per plot (g) was recorded with help of digital weighing balance and converted into green pod yield per ha (q) from each treatment.

Shelling Percentage

Shelling percentage was calculated with taking total weight of pods in a plot and total weight of seed in a plot by following formula:

$$Weight\ of\ seed\ = \frac{Shelling\ percentage}{Total\ weight\ of\ pod} \times 100$$

Statistical Analyses

All experiments were performed in triplicate, and the results were expressed as the mean.

Fig-1 Purification of *Pseudomonas* UN7 on Tryptone soy agar (TSA).

Fig-2 Gram Staining

Results and Discussion

Morphological Characterization

Plant growth promoting cold desert isolate *Pseudomonas* UN7 was characterized morphologically. Colony morphology such as shape, color, margin and opacity were observed on TSA plates. Isolate was round in shape, yellow in color, regular in margin, smooth in surface and opaque colony [Fig-1]. Microscopic characterization through Gram staining showed that the isolate *Pseudomonas* UN7 was gram negative rod (pink colour) shaped [Fig-2].

Screening of Isolate *Pseudomonas* UN7 for Multiple Plant Growth Promoting Attributes

Phosphate Solubilisation

The results showed development of white halo around colonies by *Pseudomonas* UN7, hence showing the phosphate solubilisation attribute. The strain showed considerable phosphate solubilisation with halo size 25mm [Fig-3]. Major constraints of crop production due to rapid binding of applied phosphorous into fixed forms not available to the plants leading to phosphorous deficiency. Phosphate solubilization have been reported for many bacterial cultures *Pseudomonas* species [19].

Fig-3 Zone of tricalcium phosphate solubilization on modified Pikovskaya agar at 28±0.1°C after 5 days Incubation.

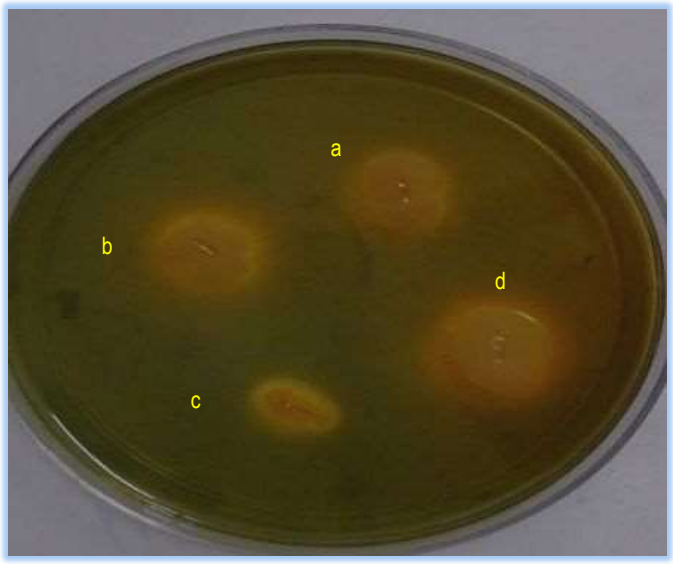

Fig-4 Siderophore production on Chrome Azurol Sulphonate media after 5 days incubation at 28 ± 0.2°C. (a-c) other rhizobacteria (d) *Pseudomonas* UN7 Author original work

Siderophore Production

The result showed the development of orange halo around colonies showed Siderophore production with halo size 15mm [Fig-4]. Plant growth is also influenced by an important trait of microorganisms that is the production of iron chelating compound siderophore which is reported as suppressor of fungal pathogen by rendering the unavailability of iron in the surrounding rhizospheric area [3]. The type and biological activity of siderophores produced by plant growth-promoting *Pseudomonas* has been considered important for their ability in controlling the plant pathogens [20]. Many *Pseudomonas* species including *P. amygdale, P. asplenii, P. aeruginosa, P. chlororaphis, P. cichorii, P. fluorescens, P. fucovaginae, P. meliae, P. poae, P. pseudomallei, P. putida, P. stutzeri, P. syringae,* and *Pseudomonas* spp. have been reported to produce siderophores.

Production of IAA-Like Auxins

Phosphate-solubilizing *Pseudomonas* UN7 showed the production of IAA-like auxins 16.38 µg ml^{-1} in tryptophan supplemented medium [Table-1]. For screening of effective PGPR strains potential for auxin biosynthesis by rhizobacteria can be used as a tool. The strains which produce the highest number of auxins *i.e.*, indole acetic acid and indole acetamide (IAM) in non-sterilized soil, causes maximum increase in growth and yield of the wheat crop [22]. Several species of fluorescent *Pseudomonas* such as *P. fluorescens* NJ101 [23], were reported as good auxin producers.

Table-1 *Production of IAA-like auxins by Pseudomonas UN7 in tryptophan supplemented medium after 48 h of incubation at 28±0.1°C*

Auxin production (µg ml^{-1})	
Strain	Colorimetric
Pseudomonas UN7	16.38
Values are the mean of three replicates	

Table-2 *Effect of microbial inoculants and NPK fertilizers on different traits of pea*

SN	Treatments	Green pod yield (q/ha)	Plant height (cm)	Shelling percentage (%)
1	Talc	30.36	31.71	26.53
2	FYM	36.16	34.00	28.10
3	FYM + Talc	40.20	35.00	28.03
4	FYM + NPK (50%)	53.00	36.33	30.36
5	FYM + NPK (75%)	62.56	39.67	40.40
6	FYM + NPK (100%)	85.10	39.33	39.50
7	*Pseudomonas* UN7	70.5	32.5	26.70
8	FYM + NPK (50%) + *Pseudomonas* UN7	102.46	46.66	47.36
	CD at 5%	14.03	6.83	1.39
	C.V. (%)	12.15	10.31	2.12

Effect on Green Pod Yield, Plant Height and Shelling Percentage

There was significant difference in all the treatments. Pod yield was significantly maximum than the control and other NPK dose treatments. Treatment combination of FYM+NPK50% + *Pseudomonas* UN7 showed the maximum green pod yield of 102.16q/ha following application on NPK 100% showed the green pod yield of 85.10 q/ha. Sole application of Talc (control) showed the minimum green pod yield of 30.36q/ha. The results obtained here are comparable with the results obtained [24]. Increase in green pod yield may be attributed to better availability of nutrients. This high performance of treatment combination of FYM, NPK 50% and bio-fertilizer may probably be due to augmented nutrient supply to crop by increasing availability through exploitation of natural processes like biological nitrogen fixation, solubilization of insoluble P and decomposition of organic matter. Therefore, artificial seed treatment is often needed to restore the population of effective strains of *Pseudomonas* in rhizosphere [25]. Effect of treatment combination on plant height was significantly greater for all the treatments including control. Treatment combination of FYM+NPK50% + *Pseudomonas* UN7 showed the highest plant height of 46.66 q/ha. However, treatment combination of FYM + NPK (100%) showed the plant height of 39.99q/ha following FYM + NPK (50%) 36.33 q/ha. Combined application of biofertilizer showed the better results as compared to the other treatments.

Sole application of Talc showed the lowest plant height. [24] also reported the better plant height attribute in organic management of crops. Shelling percentage of pea var. PB-89 increased significantly with the inoculation of microbial inoculants integrated with different NPK combinations compared to either sole application of talc, FYM or combinations of both (talc + FYM). Treatment combination of FYM+NPK50% + *Pseudomonas* UN7 showed the maximum shelling percentage of 47.26 q/ha following application of FYM + NPK (100%) showed shelling percentage of 39.50 q/ha. Sole application of Talc showed the lowest shelling percentage of 26.53 q/ha [Table-2].

Application of research: Research is applicable to reduce the use of chemical fertilizers by using these microbial based fertilizers. Farmers can reduce the application of hazardous fertilizers, biofertilizer is selective, safe and eco-friendly to food and environment.

Research Category: Use of biofertilizer to promote organic farming.

Abbreviations: PGPR- Plant growth promoting rhizobacteria, IAA- Indole Acetic Acid, IBA-Indole Butyric Acid, FYM- Farm Yard Manure, NPK- Nitrogen Phosphorous and Potassium, CD- Critical Difference, CV-Critical Variation.

Acknowledgement / Funding: Author thankful to Regional Horticultural Research & Training Station and KVK, Sharbo, Kinnaur, 172 107, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh 173230.

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Research project name or number: Research station trials

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Walker T.S., Bais H.P., Halligan K.M., Stermitz F.R. and Vivanco J.R. (2003) *Journal of Agricultural and Food Chemistry*,51, 2548-2554.
[2] Hawkes C.V., DeAngelis K.M. and Firestone M.K. (2007) *In the Rhizosphere Cardon Z. and Whitbeck J. (eds). New York, NY, USA, Elsevier*,1–31.
[3] Kloepper J.W., Zablotowick R.M., Tipping E.M. and Lifshitz R. (1991) *The Rhizosphere and Plant Growth. Kluwer Academic Publishers, Dordrecht, Netherlands*, 315-326.
[4] Tenuta M. (2003) *www.umanitoba.ca/afs/agronomists_conf/2003/pdf/tenuta_rhizoba cteria.pdf*.
[5] Kloepper J.W. and Schroth M.N. (1981) *Phytopathology*, 71, 590–592.
[6] Glick B.R., Cheng Z., Czarny J., Cheng Z. and Duan J., (2007) *European Journal of Plant Pathology,* 119, 329–39.
[7] Glick B.R. (1995) *Canadian journal of Microbiology*, 41, 109–117.
[8] Hayat R., Ali S., Amara U., Khalid R. and Ahmed I. (2010) *Annals of Microbiology*, 60, 579– 598.
[9] Glick B.R., Patten C.L., Holgin G. and Penrose D.M. (1999) *Imperial College Press, London*, pp 267.
[10] Jha B.K., Pragash M.G., Cletus J., Raman G. and Sakthivel N. (2009) *World Journal of Microbiology and Biotechnology*, 25, 573-581.
[11] Rosas S.B., Andrez J.A., Rovera M. and Correa N.S. (2006) *Soil Biology and Biochemistry*, 38, 3502-3505.
[12] Miao S.J., Qiao Y.F., Han XZ. and An M. (2007) *Pedosphere*, 17, 36-43.
[13] Trevors J.T., Van Elsas J.D., Lee H. and Van Overbeek LS. (1992) *Soil Microbial Releases* 1, 61- 69.
[14] Bhatia P., Harsh N.S.K., Dubey R.C. and Kaushik P.K. (2017) *Indian Forester*, 143 (4), 350-354.
[15] Bazilah A.B.I., Sariah M., Zainal Abidin M.A. and Yasmeen S. (2011) *International Journal of Agriculture & Biology*,13, 198– 202.
[16] Babu S.V., Triveni s., Subhash R. and Satyanarayana J. (2017) *Int.J.Curr.Microbiol.App.Sci*., 6(10), 2090-2101.
[17] Khan A.L., Halo. B.A., Elyassi A., Ali S., Al-Hosni K., Hussain J., Al-Harrasi A., and Jung L.I. (2016) *Electronic Journal of Biotechnology*,21,58-64.
[18] Christina Jenifer A., Aruna Sharmili S., Anbumalarmathi J., Umamaheswari K. and Shyamala K. (2015) *Pelagia Research Library*, 5(10),41-45.
[19] Jha B.K., Pragash M.G., Cletus J., Raman G. and Sakthivel N. (2009) *World Journal of Microbiology and Biotechnology*, 25, 573-581.
[20] Chaiharn M., Chunhaleuchanon S. and Lumyong S. (2009) *World Journal of Microbiology and Biotechnology*, 25, 1919-1928.
[21] Sayyed R.Z., Naphade B.S. and Chincholkar S.B. (2005) *Recent Trends in Biotechnology*, 1-16.
[22] Khalid A., Arshad M. and Zahir ZA. (2004) *Journal of Applied Microbiology*,96, 473-480.
[23] Bano N. and Musarrat J. (2004) *FEMS Microbiology Letters*, 231, 13-17.
[24] Pandey A.; Trivedi P.; Kumar B.; Palni L.M.S. (2006) *Current Microbiology*,53(2),102–7.
[25] Srivastava S.N.L. and Verma S.C. (1984) *Legume Research*, 7(1), 37-42.