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Effect of Seed Bio-priming with Cold Tolerant *Pseudomonas fluorescens* and Soil Application of Cow Urine on Germination, growth and Nutrient Uptake in Pea

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Abstract: Two pot experiments were carried out at College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand (India) during 2018-2019 to investigate the effect of seed biopriming with cold tolerant *Pseudomonas fluorescens* and soil application of cow urine on germination and growth of pea. Experiments consisted of eight treatments viz; control (T_1), lime + RDF @ 20:30:30 kg/ha (T_2), soil application of 10% cow urine + RDF (T_3), seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF (T_4), FYM @ 10 t/ha + RDF (T_5), FYM @ 10 t/ha + seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF (T_6), 10 % cow urine + seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF (T_7) and 10 % cow urine + FYM @ 10 t/ha + RDF (T_8) replicated thrice. The results revealed that seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF recorded significantly higher germination percentage and seedling growth at 25 DAS in pot experiment I. Also the application of 10 % cow urine + FYM @ 10 t/ha + RDF had maximum value for growth, yield attributes and seed yield in pot experiment II. These findings suggested the role of cold tolerant *Pseudomonas fluorescens* in improving the germination of pea. Also the use of cow urine along with FYM was found more efficient in increasing the growth and yield of pea.

Introduction

Pea (Pisum sativum L.) is one of the most important annual leguminous crops in many countries; including India which belongs to the family fabaceae (leguminousae). This is an important Rabi pulse crop with an area of 530000 hectare and production of 5345000 tonnes in the country (FAOSTAT 2017). It is utilized mainly as a vegetable and also

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consumed as pulses. Being a cool season pulse crop, it is mostly grown in temperate region throughout the world. In India, it is cultivated mainly as a summer vegetable in the hilly areas of north India and as a winter vegetable in the plains of north India. Pea is considered as money spinner to the farmers of hill as they fetch more remuneration due to it's off - season cultivation. Chemical fertilizers have played a prominent role in increasing the quantity of food produced in the country but decreased its 'nutritional quality' and also 'soil fertility' over the years if used in imbalanced form (Sinha *et al*. 2010, Rawat *et al.* 2016a). The inorganic source of nutrients alone cannot meet the requirement of cropping systems and crops because of their high cost and residual effects of chemical (Rawat *et al.* 2016b). So, the alternate focusing has been on the integrated nutrient management system aimed at decreasing the use of mineral fertilizers by integrating organics. A judicious use of beneficial microorganisms and organic manures may be effective not only in supplementing the essential plant nutrients, sustaining the crop productivity, quality and soil health, but also in supplementing inorganic fertilizers for the crops (Jaipaul *et al*. 2011, Debnath *et al.* 2019, Srivastava *et al.* 2015). The beneficial microorganisms applied on soil or as seed treatment has evidently increased the growth, yield and uptake of primary, secondary and micronutrients in various crops (Vaid *et al.* 2019, Vaid *et al.* 2020, Bargaz 2018) The use of *Pseudomonas fluorescens* was effective in improving the seed quality such as seed germination, seedling vigour index and nutritional quality such as protein and carbohydrates content of sorghum (Prathibha and Siddalingeshwara 2013). So seed bio-priming with this beneficial microorganism can be used to improve the quality of seed. The importance of FYM in increasing the yield and quality of crops on sustainable basis along with its residual effect on succeeding crops by improving the soil physical conditions and soil fertility is well recognized (Sangma *et al*. 2018, Pachauri *et al.* 2021).

Apart from FYM and bio-fertilizer, use of cow urine is another alternative among organic supplements as it is easily available in the rural areas in India. It has been considered that cattle urine is very useful in agricultural operations as a bio-pesticide and bio-fertilizer (Khanal *et al*. 2011). It is rich source of essential plant nutrients and has disinfectant and prophylactic properties thus improve soil fertility and purify the atmosphere (Pathak and Ram 2013). This nutrient source is available to farmers free of cost in their own house; being organic in nature it is eco friendly and if used in crops has no adverse effect on ecosystem and human health. Therefore, it seems that cow urine under livestock based integrated farming system has a great potential for use as soil application in crop production.

Despite the beneficial effect of various organic sources on the productivity of crops, the reports available on the effect of organic sources especially like cow urine and cold tolerant *Pseudomonas fluorescens* on the germination and growth of pea are meagre. Therefore, keeping in view the importance of pea in the cropping pattern of Uttarakhand as it is sown twice in a year in the hills of the state and is a significant source of income for the farmers in the area, the present investigation has been undertaken to assess the effect of seed bio-priming with cold tolerant *Pseudomonas fluorescens* and soil application of cow urine on germination and growth and nutrient uptake in pea.

Materials and Methods

The pot experiments were conducted during Kharif season of 2018 at Green house of Department of Basic Sciences, College of Forestry, V.C.S.G Uttarakhand University of Horticulture and Forestry, Ranichauri, Tehri Garhwal, Uttarakhand to investigate the effect of seed bio-priming with cold tolerant *Pseudomonas fluorescens* and soil application of cow urine on germination and growth of pea**.** The variety used was 'Arkel' which was procured from Gaja, Research and Extension Centre, V.C.S.G. UUHF, College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand, India. This variety of pea have plants dwarf in height, white flowers, wrinkled seeds, dark green coloured pods, 7 to 10 seeds per pod and length of pod is about 8.5 cm. Its shelling percentage is 40% and green pod yield is about 8 to 10 t/ha. The pot experiments was performed with eight treatment combinations viz., control (T_1), lime + RDF@ 20:30:30 kg/ha (T_2), soil application of 10 % cow urine + RDF@ 20:30:30 kg/ha (T_3), seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF@ 20:30:30 kg/ha (T_4), FYM @ 10 t/ha + RDF@ 20:30:30 kg/ha (T_5), FYM @ 10 t/ha + seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF@ 20:30:30 kg/ha (T_6), 10 % cow urine + seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF@ 20:30:30 kg/ha (T_7) and 10 % cow urine + FYM @ 10 t/ha + RDF @ 20:30:30 kg/ha (T_8) replicated thrice. For the experiments, plastic pots of diameter (26 cm) were filled by 8 kg soil collected from the Horticulture Block of College of Forestry, Ranichauri. In pot experiment I, twenty seeds were sown in each pot. The germination was scored every day after first seed germinated in any of the treatments and continued till all seeds germinated in any of the treatments using the following formula:

$$Germination\ percent = \frac{Seed\ germinated}{Total\ seed\ sown} \times 100$$

After 25 days of sowing, five plants were up rooted and washed to remove the adhering soil and observations of seedling length, fresh weight, dry weight, plant vigour index were recorded and its nutrient concentration (N, P, S) was also analysed. The seedling/plant vigour index was calculated by using two different methods given by Abduli baki and Anderson (1973).

Vigour index I = Standard germination (%) × Seedling length (cm)

Vigour index II = Standard germination (%) × Seedling dry weight (g)

For pot experiment II**,** ten seeds were sown in each pot. After a week of germination, five healthy seedlings were maintained in the pots. At harvesting, five seedlings were uprooted to record the growth attributes, yield and yield attributes of pea was also recorded. The concentration of N, P and S was determined in seed and straw samples of pea. For

Table 1: Effect of seed bio-priming and soil application of cow urine on germination and seedling growth of pea at 25 DAS under pot experiment I

Treatment	*Germination (%)*	*Seedling length (cm)*	*Seedling fresh weight (g)*	*Seedling dry weight (g)*	*PVI I*	*PVI II*
T_1	40.00	21.40	1.97	0.79	856.00	31.47
T_2	50.00	17.33	1.21	0.62	866.68	31.00
T_3	50.00	21.97	1.85	0.82	1098.33	41.00
T_4	80.00	28.93	2.59	0.97	2314.73	77.86
T_5	60.00	22.13	2.26	0.83	1328.00	49.60
T_6	70.00	23.87	2.14	0.80	1670.45	56.24
T_7	75.00	23.93	1.96	0.81	1795.00	61.00
T_8	75.00	26.23	2.21	0.86	1967.50	64.75
SEM	0.25	0.26	0.36	0.17	16.32	1.24
CD at 5%	0.75	0.77	0.11	0.05	48.93	3.73

*PVI = Plant value index

Table 2: Effect of seed bio-priming and soil application of cow urine on nutrient concentration in seedling at 25 DAS under pot experiment I

Treatment	*Nitrogen concentration (%)*	*Phosphorus concentration (%)*	*Sulphur concentration (%)*
T_1	1.45	0.19	0.44
T_2	1.54	0.17	0.47
T_3	1.53	0.24	0.49
T_4	1.72	0.32	0.45
T_5	1.64	0.38	0.52
T_6	1.53	0.23	0.47
T_7	1.58	0.34	0.51
T_8	1.63	0.32	0.48
SEM	0.009	0.007	0.006
CD at 5%	0.03	0.02	0.02

Table 3: Effect of seed bio-priming and soil application of cow urine on the growth attributes of pea at harvesting stage under pot experiment II.

Treatment	*Number of nodules*	*Plant height (cm)*	*Root length (cm)*	*Fresh shoot weight (g)*	*Fresh root weight (g)*	*Dry shoot weight (g)*	*Dry root weight(g)*
T_1	15.00	21.53	14.50	3.43	0.68	0.94	0.14
T_2	17.00	22.88	13.53	3.60	0.70	0.97	0.12
T_3	14.00	25.27	14.93	3.97	0.66	1.03	0.16
T_4	17.00	26.85	16.19	4.16	0.67	1.30	0.18
T_5	19.00	25.47	15.96	4.08	0.76	1.16	0.16
T_6	14.00	23.66	13.63	3.27	0.70	1.31	0.20
T_7	16.00	23.71	14.43	3.08	0.64	0.99	0.12
T_8	21.00	28.76	16.63	4.47	0.77	1.39	0.19
SEM	0.51	0.32	0.12	0.04	0.009	0.01	0.004
CD at 5%	1.52	0.95	0.37	0.13	0.03	0.03	0.01

Table 4: Effect of seed bio-priming and soil application of cow urine on yield attributes and yield of pea under pot experiment II.

Treatment	*No. of branches/ plant*	*No. of pods/ plant*	*No. of seeds / pod*	*Pod length (cm)*	*Pod width (cm)*	*Pod yield/ plant (g)*	*Seed yield/ plant (g)*
T_1	6.80	2.77	2.70	4.35	0.81	2.80	2.13
T_2	6.90	2.40	2.83	4.47	0.85	4.20	3.47
T_3	7.03	3.27	2.50	4.21	0.88	3.40	2.80
T_4	6.90	3.97	3.60	4.75	0.93	5.13	4.30
T_5	7.43	3.93	3.07	5.29	0.89	4.27	3.37
T_6	6.97	3.60	2.80	5.18	0.88	5.00	4.10
T_7	7.00	3.30	3.10	4.54	0.83	3.10	2.20
T_8	8.13	4.17	3.80	5.45	0.95	5.47	4.40
SEM	0.15	0.07	0.06	0.05	0.02	0.98	0.65
CD at 5%	0.44	0.21	0.19	0.16	0.07	0.29	0.19

*Values of No. of seeds per pod, No. of branches and pods per plant depicted in the table are average of five plants in the pot.

Table 5: Effect of seed bio-priming and soil application of cow urine on nutrient content in shoot and seed at harvesting under pot experiment II.

Treatment	*Nitrogen concentration (%)*		*Phosphorus concentration (%)*		*Sulphur concentration (%)*	
	Shoot	*Seed*	*Shoot*	*Seed*	*Shoot*	*Seed*
T_1	1.57	2.26	0.23	0.35	0.36	0.53
T_2	1.60	2.35	0.32	0.39	0.33	0.58
T_3	1.63	2.30	0.27	0.37	0.37	0.60
T_4	1.68	2.74	0.30	0.41	0.37	0.68
T_5	1.82	2.48	0.43	0.39	0.45	0.59
T_6	1.65	2.58	0.27	0.37	0.38	0.59
T_7	1.77	2.66	0.31	0.45	0.36	0.65
T_8	1.76	2.51	0.42	0.39	0.42	0.61
SEM	0.01	0.02	0.006	0.006	0.006	0.005
CD at 5%	0.03	0.05	0.02	0.02	0.02	0.01

determination of nutrient concentration in plant parts, total nitrogen content was determined in percentage by modified micro Kjeldahl's method (Jackson 1967). Total phosphorous content was determined by using Vanadomolybdate phosphoric acid yellow colour method using Spectrophotometer at 470 nm **(**Jackson 1967**)**. Total sulphur content was determined by turbidimetrically using colorimeter at 440 nm (Chesnin and Yien, 1950). The statistical analysis of the data was done by the method recommended by Panse and Sukhatme (1987) using the Completely Randomized Design with the help of STPR-3 software developed by College of Basic Science and Humanities, G.B.P.U.A&T, Pantnagar, Uttarakhand, India.

Result and Discussion

Among the different treatments, seed bio-priming with cold tolerant *Pseudomonas fluorescens* + RDF@ 20:30:30 Kg/ha (T_4) showed the highest germination percentage, seedling length, seedling fresh weight, seedling dry weight and seedling vigour index (I & II) at 25 days after sowing in pot experiment I. These results are in agreement with the findings of Mishra *et al.* (2010) and Kumar (2011). Enhancement of seed germination percentage may be attributed to the role of beneficial micro organism *Pseudomonas fluorescens* in increasing the availability of nitrogen and phosphorus in the soil and making them available to the germinating seed with the consequent enhancement in the metabolic activity (Ram *et al*. 2011). In case of nutrient content, the use of seed bio-priming + RDF recorded maximum nitrogen concentration in shoot at 25 DAS while maximum phosphorus and sulphur concentration in shoot at 25 DAS was observed under the treatment which received FYM along with RDF in pot experiment I. The synergistic effect of organic and inorganic fertilizers which resulted in more release of macro and micro nutrients enhanced the root growth thereby increasing nutrient content in the plant. Similar results were also reported by Sutaria *et al.* (2010), Awomi *et al.* (2012) and Geletu and Mekonnen (2018).

In pot experiment II, the highest growth attributing characters in pea viz. number of nodules, plant height, root length, shoot fresh and dry weight, root fresh, dry weight, number of branches, number of pods, pod length, pod width, number of seeds per pod, pod yield per plant and seed yield per plant were recorded under the treatment containing 10 % cow urine + FYM + RDF. The integration of organic manures with inorganic fertilizers enhanced the availability of essential nutrients and these nutrients being important constituents of enzymes, nucleotides, protein and chlorophyll are involved in various metabolic processes which have direct impact on vegetative and reproductive phases of plant (Baghdadi et al. 2018). Application of nutrient rich cow urine along with FYM and RDF might also be the possible reason for better vegetative growth i.e. plant height, root length and fresh and dry weight of plants because cow urine is known for the presence of growth promoting auxin like *indole*-3-*acetic acid* (IAA) in it (Zhang, 2000) and it is rich in essential nutrients (Ramachandrudu and Thangam, 2007). Cow urine contains many of those elements that are needed by plants, such as N, P, K, Na, Ca (Phrimantoro, 1995). The results have got close conformity with the findings of Meena and Bheemavat (2009), Devakumar *et al*. (2014) and Veeresha *et al.* (2014). Significantly higher seed yield was recorded under the application of cow urine + FYM + RDF (T_8) which was at par with the treatment with seed bio-priming + RDF (T_4) might be due to higher plant growth and increased number of branches in T_8 and the *Pseudomonas fluorescens* which is a PGPR, was able to increase the vegetative growth of plant due to its ability to produce growth promoting hormones thereby leading to more photosynthetic area resulting in higher seed yield levels. The results of present study were in agreement with the finding of Veeresha *et al*. (2014).

In case of nutrient concentration the increased concentration of nutrients (N, P and S) in shoot at the stage of harvesting was recorded under the application of FYM + RDF. This might be due to increased solubilisation of nutrients by the organic acids released due to formation of humus and increased biological activity in the presence of carbon source like FYM. Similar results were also reported by Chaudhary *et al.* (2016). The maximum nitrogen and sulphur concentration in seeds was recorded in treatment applied with seed biopriming + RDF**.** While, highest phosphorus content in seeds was recorded in cow urine + seed bio-priming + RDF. The role of *Pseudomonas fluorescens* as PGPR in the present investigation can also be associated with its role in improving the nutrient use efficiency thereby increasing the uptake of these nutrients by the crop. Similar results were reported by Dekhane *et al.* (2011) and Geletu and Mekonnen (2018).

The findings of this study suggested the role of cold tolerant *Pseudomonas fluorescens* in improving the germination of pea. Also the use of cow urine along with FYM was found more efficient in increasing the growth and yield of pea in the pot experiment. Considering the positive effect of seed bio-priming with cold tolerant *Pseudomonas fluorescens* and soil application of cow urine in the present investigation, long term effect of these organic nutrient sources can be explored for improving the crop yield and sustainability of the soil health.

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