EFFECT OF BIO-FORMULATION ON ENHANCEMENT OF SEED QUALITY AND YIELD IN GARDEN PEA

Abstract:

A research work was conducted at Assam Agricultural University, Jorhat during rabi season of 2021-2022 to evaluate the effect of different bio-formulations on enhancement of seed quality and yield in garden pea. The laboratory analysis were conducted in Complete Randomized Design for seed vigour characteristics whereas the field experiment was laid out in factorial Randomized Block Design for assessing plant growth and yield attributing characters. The experiment involvedtwo varieties of garden pea viz., Arkel and DS-10, each having seven treatments with three replications. The treatments comprised of T_1- untreated control, T_2- Hydropriming, T_3- seed priming with Trichoderma viride @ 5g/kg, T_4- PSB @ 10g/kg, T_5- Rhizobium @ 20 g/kg, T_6- PSB @ 10g/kg + Rhizobium @ 20 g/kg and T_7- Pseudomonas fluorescens @ 10 g/kg. Before sowing, the seeds of both the varieties were soaked in water for 4 hours and bio-primed with the above mentioned bio-formulations.The results revealed significantly difference amongst the treatments for most of the plant growth promoting and yield attributing characters.T_6 exhibited highest performance for most of the characters. Varietal performance of DS-10 was found better than Arkel.Laboratory observations also recorded the maximum germination (%) and seed vigour index I & II in the T_6 treatment. However, significant increasegerminationrelated characters and early seedling growth was exhibited by all the treatments over the control. The experiment thus revealed that although all bioformulations viz., PSB, Rhizobium, Trichoderma viride, Pseudomonas fluorescens alone and in combination could improveseed quality, seed yield & yield attributing characters but . the best performance could be achieved in T_6 i.e. by applying PSB@ 10g/kg + Rhizobium @ 20 g/kg. Therefore, it could be suggested that seed treatment with bio-formulations should be done for better seed production and seed yield in garden pea.

Key words: Garden pea, *Rhizobium*, PSB, *Trichoderma viride*, *Pseudomonas fluorescens*, seed vigour index (SVI)

INTRODUCTION:

Pea (*Pisum sativum* L.), one of the most popular annuallegume crop of India, belongs to family Leguminosae (Fabaceae) and sub-family Papilionoideae. It is a protein rich, self-pollinated, cool season vegetable crop grown throughout the world. There are two subspecies: *Pisum sativum* var. hortense, i.e. the garden pea with white flowers, and *Pisum sativum* var. arvense, i.e. the field pea with coloured blooms.Basically this crop is largely grown for its green tender pods and green seeds which are mostly used as vegetables and serve as excellent food source for human consumption. It is the third most important pulse crop in the world. India is ranked second, next to China both in terms of area and production (FAO, 2022). Major pea producing states in India are Uttar Pradesh, Bihar, Madhya Pradesh, Karnataka, Rajasthan, Punjab, Haryana, Himachal

Pradesh, West Bengal and Assam.In the northern plains, peas are grown during the *rabi* season from the beginning of October to the end of November. It is highly nutritive and contains high proportion of protein (25%), amino acids, sugars (12%), carbohydrate, vitamins A and C, calcium and phosphorus and a small quantity of iron. Straw of garden pea is used as fodder for livestocks. Its cultivation plays a vital role in promoting sustainable agriculture by maintaining soil fertility through biological nitrogen fixation in association with symbiotic *Rhizobium* prevailing in the root nodules.

Modern agriculture is becoming more and more reliant upon the supply of synthetic inputs such as chemical fertilizers and pesticides. However, long term application of chemicals reduces soil fertility and crop yield in the intensive cropping systems (Dadhich *et al*.,2011). Synthetic chemicals used in agriculture has a detrimental effect on agro-ecosystem and also result in health hazards. Hence, application of bio-formulations in crops is a sustainable approach from both ecological and economic viewpoint.The seed has consistently been a key factor in agriculture. The yield and quality of crop production are greatly influenced by seed quality. As food demand rises, it has become a challenge to produce high quality seeds in an efficient and effective manner. Therefore, some successful strategy must be used to ensure crop growth and increase seed yield. One of them is seed priming, which can be used as a seed invigoration treatment for rapid germination and early seedling establishment. Due to abiotic stress and various environmental conditions, the proportion of seed germination, field emergence and seedling vigour has been negatively impacted, which eventually leads to poor agricultural output. Therefore, to promote healthy and uniform germination as well as to maintain the vigour and viability of seeds, bio-priming offers one of the greatest alternatives to chemical fertilizers &pesticides. Keeping these facts in view, the present study on the "Effect of bio-formulations on enhancement of seed quality and yield in garden pea" was undertaken, which aimed at organic cultivation of garden pea by using seed priming treatments with different priming agents to hasten the rate of germination, improve seedling vigour, crop yield, soil fertility, resistance to biotic and abiotic stresses & ultimately enhance seed quality & yield.

MATERIAL AND METHODS:

The investigation was conducted in the Instructional Cum Research (ICR) Farm of Assam Agricultural University, Jorhat during rabi season of 2021-22. It belongs to upper Brahmaputra valley zone of Assam. Geographically, it is located at an elevation of 87m above mean sea level in 26° 45′ North latitude and 94° 12′ East longitude. The climate of Jorhat experiences a tropical monsoon rainforest with the temperature during winter ranges from 8°-15°C and during summer ranges from 35°-38°C.

The field experiment was laid out in Factorial Randomized Block Design with three replications and spacing of 30cm x 10cm. The plot size was 2 m x 0.9 m (1.8 m^2). The experiment consisted of seven treatment combinations involving three replications for two varieties (Arkel and DS-10). Details of the experimental treatments are shown in Table 1.

Table 1: Details of seed priming treatments

T_1	Control (un-treated)
T_2	Hydration (4 hours) + redrying
T_3	Hydration (4 hours) + redrying + *Trichoderma*@ 5 g/kg
T_4	Hydration (4 hours) + redrying + PSB @ 10 g/kg
T_5	Hydration (4 hours) + redrying + *Rhizobium* @ 20 g/kg
T_6	Hydration (4 hours) + redrying + PSB @ 10 g/kg + *Rhizobium* @ 20g/kg
T_7	Hydration (4 hours) + redrying + *Pseudomonas fluorescens* @ 10 g/kg

Healthy, uniform and dry seeds of both the varieties were used for priming treatments. For hydro priming, the garden pea seeds were soaked in water for 4 hours and then re-dried to initial moisture content. For bio-priming, the seeds were surface sterilized with 1.0% sodium hypochlorite (NaOCl) solution for 5 minutes and then dried. After that the seeds were washed three times with distilled water that had been sterilized and dried on sterilized blotter paper (Jain, *et al*., 2012) . The weight of the talc based bioagent formulations were measured out on a weighing balance in accordance with dose in order to cover the whole surface of seeds with the bioagents. The surface sterilized and dried seeds were bio-primed by soaking in the bioformulation of *Trichoderma viride*, *Pseudomonas fluorescens*, PSB and *Rhizobium* spp. After that seeds were shade dried to initial moisture content and sown in the field.

The laboratory analysis were conducted in Complete Randomized Design for seed vigour characteristics *viz*., germination (%), seedling length (cm), seedling dry weight (mg), Seed vigour index I (SVI-I) and Seed vigourinsex II (SVI-II). Plant growth promoting and yield attributing characters such as field emergence (%), final plant stand, days to 50% flowering, plant height (cm), branches per plant, leaves per plant, pods per plant, seeds per pod and seed yield per plant (g) were also recorded for observations.The data collected for each character was analyzed with the help of OPSTAT software.

RESULTS AND DISCUSSION:

Effect of bio-formulations on seed quality characters:

The findings pertaining to seed quality characters *viz.*, germination percentage, seedling length, seedling dry weight, seed vigour index I & II are presented in Table 2 and Table 3.

Table 2: Mean performance of germination (%), seedling length (cm) & seedling dry weight (mg) due to different priming treatments

Treat-ments	Germination (%)			Seedling length (cm)			Seedling dry weight (mg)		
	V1	**V2**	**Mean**	**V1**	**V2**	**Mean**	**V1**	**V2**	**Mean**
T_1	68.33	80.33	74.33	15.66	15.38	15.52	124.60	123.97	124.28
T_2	77.67	84.00	80.83	18.28	18.84	18.56	127.70	128.00	127.85
T_3	87.67	90.00	88.83	24.57	22.23	23.40	136.10	135.17	135.63
T_4	84.33	87.00	85.67	21.19	20.99	21.09	133.27	133.93	133.60
T_5	92.33	96.00	94.17	21.00	21.59	21.30	131.87	134.23	133.05
T_6	95.67	98.00	96.83	22.50	23.25	22.87	134.00	136.80	135.40
T_7	89.33	92.33	90.83	20.79	24.97	22.88	133.17	138.17	135.67
Mean	85.05	89.67	87.36	20.57	21.04	20.81	131.53	132.90	132.22
	Variety	Treatment	V x T	Variety	Treatment	V x T	Variety	Treatment	V x T
CD (p<0.05)	1.732	3.240	4.582	0.287	0.537	0.760	0.426	0.796	1.126
SE.m (±)	0.595	1.113	1.574	0.099	0.184	0.261	0.146	0.273	0.387

As evident from the data presented in Table 2, the maximum germination percentage (96.83%) was recorded in a combined seed priming treatment with PSB @ 10g/kg + *Rhizobium* @ 20g/kg (T_6) which was statistically at par with T_5 (94.17%)where the seeds were bioprimed with *Rhizobium* @ 20g/kg followed by T_7 *i.e.*, seed priming with *Pseudomonas fluorescens* @ 10g/kg. The untreated control exhibited lowest germination percentage. The mean germination (%) in different priming treatments ranged from 74.33% to 96.83%. All the treatments had significantly higher germination (%) as compared to untreated control. The priming treatments showed significant variations on germination (%).Significant variations were also observed in variety andinteraction of variety with treatment.Among the variety x treatment interaction V_2 (DS-10) recorded highest germination (98%) in T_6.

It was obvious from the study that seed priming with bioagents *viz. Trichoderma*, *Rhizobium*, *Pseudomonas* and PSB led to noticeably increased germination rate as compared to other treatments. Findings also exhibited that combined inoculation of bioagents result in higher seed germination (%). Similar results of seed germination due to biopriming have also been reported by Entesari*et al.*, 2013; Kumar, S. 2014; Naik, S. 2015; Sharma *et al.*, 2016; Vinay *et al.*, 2022 in crops like chickpea, soybean and garden pea.

Different seed priming treatments showed significant increase in seedling length and seedling dry weight as compared to untreated control. Maximum mean seedling length (23.40 cm) was recorded in T_3 (seed priming with *Trichoderma* @ 5g/kg) which was statistically at par with T_7 (seed treatment with *Pseudomonas fluorescens* @ 10g/kg) and $T_{6.}$ Among the variety x treatment interaction, highest seedling length was recorded in DS-10 variety when bio primed with *Trichoderma viride*. These results were in accordance with Naik, M., 2015.From the study, maximum seedling dry weight (135.67 mg) was recorded in T_7 which was statistically at par with T_3 and T_6.

Table 3: Mean performance of Seed Vigour Index-I& Seed Vigour Index-IIdue to different priming treatments

Treatments	Seed Vigour Index-I			Seed Vigour Index-II		
	V1	**V2**	**Mean**	**V1**	**V2**	**Mean**
T_1	1070.38	1,236.02	1,153.20	8,515.03	9,961.50	9,238.27
T_2	1,419.22	1,582.28	1,500.75	9,916.66	10,751.27	10,333.97
T_3	2,153.50	2,003.08	2,078.29	11,931.07	12,165.53	12,048.30
T_4	1,787.62	1,826.13	1,806.88	11,239.40	11,652.03	11,445.72
T_5	1,938.60	2,072.88	2,005.74	12,176.17	12,886.60	12,531.38
T_6	2,153.00	2,278.31	2,215.66	12,819.50	13,406.83	13,113.17
T_7	1,857.99	2,305.96	2,081.97	11,897.27	12,757.53	12,327.40
Mean	1768.61	1900.66	1834.64	11,213.59	11,940.19	11,576.89
	Variety	Treatment	V x T	Variety	Treatment	V x T
C.D (p <0.05)	48.191	90.156	127.500	239.820	448.663	NS
SE.m (±)	16.550	30.962	43.787	82.361	154.083	217.906

The studies carried out Negi *et al.*, 2020, Naik, M. 2015, Sharma *et al.*, 2016 also revealed similar results. The current results were in consistent with Monalisa *et al.*, 2017 who reported increased seedling dry weight due to seed bio-priming in common bean. Increased germination (%), root and shoot length of the seedlings might be the causes of rise in seedling dry weight.

Studies on seed vigour index revealed that T_6 showed the best results as compared to other priming treatments as shown in Table 4. Interaction of treatment with varietyin T_6showed highest values for both SVI-I and SVI-II. Highest seed vigour index in T_6 might be due to greater germination percentage, seedling length and seedling dry weight. The results were in agreement with Vinay *et al.*, 2022 and Pandey *et al.*, 2017. As per the results, bio-formulated seeds had higher seed vigour indices than the untreated control seeds. From the findings, although hydro priming caused improved seed germination, seedling length, seedling dry weight, and SVI-I and SVI-II but the effective bio priming treatments were found to be significantly better than hydro priming.

Table 4: Mean performance of Seed Vigour Index-I & Seed Vigour Index-II due to different priming treatments

Treatments	Seed Vigour Index-I			Seed Vigour Index-II		
	V1	V2	Mean	V1	V2	Mean
T_1	1070.38	1,236.02	1,153.20	8,515.03	9,961.50	9,238.27
T_2	1,419.22	1,582.28	1,500.75	9,916.66	10,751.27	10,333.97
T_3	2,153.50	2,003.08	2,078.29	11,931.07	12,165.53	12,048.30
T_4	1,787.62	1,826.13	1,806.88	11,239.40	11,652.03	11,445.72
T_5	1,938.60	2,072.88	2,005.74	12,176.17	12,886.60	12,531.38
T_6	2,153.00	2,278.31	2,215.66	12,819.50	13,406.83	13,113.17
T_7	1,857.99	2,305.96	2,081.97	11,897.27	12,757.53	12,327.40
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Effect of bio-formulations on plant growth and yield attributing characters observed under field condition:

The findings pertaining to growth parameters *viz.*, Field emergence (%), Final plant stand, days to 50% flowering, plant height (cm), branches per plant, leaves per plant are presented in Table 5 and Table 6 respectively.

Table 5: Mean performance of field emergence (%), final plant stand and days to 50% flowering due to different priming treatments

Treat-ments	Field emergence (%)			Final plant stand (no./plot)			Days to 50% flowering		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T_1	75.44	80.67	78.05	47.33	49.00	48.17	47.00	50.33	48.67

T_2	79.11	82.22	80.66	48.00	50.67	49.33	45.33	48.67	47.00
T_3	86.00	88.00	87.00	52.67	54.00	53.33	40.67	46.00	43.33
T_4	89.55	91.66	90.61	53.33	53.00	53.17	44.00	47.67	45.83
T_5	88.11	89.44	88.77	54.00	55.67	54.83	42.00	48.00	45.00
T_6	92.11	93.33	92.72	56.33	58.00	57.17	41.67	44.67	43.17
T_7	84.33	85.55	84.94	51.00	57.00	54.00	43.67	45.33	44.50
Mean	84.95	87.27	86.11	51.81	49.00	50.41	43.48	47.24	45.36
	Variety	Treatment	V x T	Variety	Treatment	V x T	Variety	Treatment	V x T
C.D ($p < 0.05$)	1.503	2.811	NS	0.800	1.496	2.116	0.975	1.824	NS
SE.m (±)	0.514	0.962	1.360	0.274	0.512	0.724	0.333	0.624	0.882

From the field study, data recorded for field emergence revealed that there were significant variations in emergence (%) due to different priming treatments, but the interaction of variety and treatment exhibited no significant difference among themselves. Highest value of field emergence (92.72%) was recorded in T_6which was statistically at par with T_4 whereas lowest value was recorded in untreated control. Highest field emergence (%)in $T_6$was due to application of *Rhizobium* and Phosphate solubilizing bacteria. Thisimproved the microbial activity which made vital biomolecules available to the plants during early stages of germination (Subba Rao, 1986). Final plant stand was found to be highest in T_6(57.17 no's) where the seeds were treated with combined application of *Rhizobium* + PSB. It was followed by T_5 and T_7.

In the study, data obtained for days to 50% flowering showed significant variations among the treatments but the interaction between variety and treatment was found non-significant. Days to 50% flowering was observed to be the earliest in T_6(43.17) which was statistically at par with T_3 (43.33). Late flowering was observed in Control T_1 (48.67) and in V_2 (DS-10) where no priming agents were applied. The early flowering could be attributed due to application of bio-formulations which in turn caused flower initiation.

Table 6: Mean performance of plant height, branches per plant and leaves per plant due to different priming treatments

Treat-ments	Plant height (cm)			Branches per plant			Leaves per plant		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T_1	35.89	39.34	37.62	11.89	13.18	12.53	41.78	44.89	43.33

T_2	40.85	43.38	42.12	13.44	15.22	14.33	45.78	47.78	46.78
T_3	48.08	51.54	49.81	15.89	17.44	16.67	54.11	57.22	55.66
T_4	52.22	52.73	52.48	17.33	18.80	18.07	55.33	52.88	54.11
T_5	54.15	59.11	56.63	18.95	19.11	19.03	58.11	55.11	56.61
T_6	58.43	63.91	61.17	18.63	21.53	20.08	62.56	68.33	65.44
T_7	50.37	56.42	53.40	14.22	16.43	15.33	46.55	63.44	54.99
Mean	48.57	52.35	50.46	15.76	17.39	16.58	52.03	55.66	53.85
	Variety	Treatment	V x T	Variety	Treatment	V x T	Variety	Treatment	V x T
CD ($p < 0.05$)	1.839	3.441	NS	1.091	2.040	NS	2.178	4.075	5.762
SE.m (±)	0.629	1.177	1.665	0.373	0.698	0.987	0.745	1.394	1.971

The highest plant height was observed in T_6*i.e.*, seed treatment with PSB @ 10g/kg + *Rhizobium* @ 20g/kg (61.17) followed by T_5(56.63). Treatment mean was found significant for plant height but interaction due to variety and treatment showed no significant variations. The highest plant height in T_6 was due to the application of *Rhizobium* which increased the populationof Rhizobia in the root zone. Thisresulted in high fixation of nitrogen from the atmosphere to the soil. *Rhizobium*couldaccess more phosphorus with the addition of PSB, which lead to an increase in root nodules. Phosphorus plays an important role in cell division and development which ultimately lead to an increase in plant height in garden pea (Das, 1996). The results were in accordance with Vinay *et al*., 2022, Bhat *et al.,* 2013 and Pandey *et al*.,2017 in pea. Mukherjee, D. (2016) also noted a considerable increase in plant height in combined application of *Rhizobium* and PSB along with Recommended Dose of Fertilizer in field pea.

From the experimental findings, it was observed that different priming agents had significant effect on branches per plant but interaction due to variety and treatment showed no significant difference. Highest branches per plant (20.08) was recorded in T_6,which was statistically at par with T_5(19.03) followed by T_4 and T_1. Negi *et al*., 2021 reported similar results where the branches per plant were increased when the seeds were bio primed with *Rhizobium* and PSB. Highest leaves per plant (65.44) was recorded in T_6 (PSB @ 10g/kg + *Rhizobium* @ 20g/kg) followed by T_5 and T_1. The mean values of leaves per plant in different priming treatments ranged from 43.33 to 65.44. All the priming treatments significantly enhanced the leaves per plant as compared to control.

Table 7: Mean performance of pods per plant, seeds per pod & seed yield per plant (g)

due to different priming treatments

Treat-ments	Pods/plant			Seeds/pod			Seed yield/plant (g)		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T_1	9.10	10.00	9.55	4.93	8.10	6.52	8.98	11.34	10.16
T_2	10.13	10.33	10.23	5.57	8.40	6.98	11.28	12.15	11.72
T_3	12.00	12.23	12.12	5.37	8.90	7.13	12.87	15.25	14.06
T_4	13.20	13.70	13.45	6.07	8.93	7.50	16.01	17.14	16.57
T_5	12.80	13.40	13.10	6.40	8.77	7.58	16.38	16.43	16.41
T_6	13.87	15.10	14.48	6.80	9.00	7.90	18.84	19.02	18.93
T_7	13.80	13.43	13.62	5.93	8.57	7.25	16.37	16.11	16.24
Mean	12.13	12.60	12.37	5.87	8.67	7.27	14.39	15.35	14.87
	Variety	Treatment	V x T	Variety	Treatment	V x T	Variety	Treatment	V x T
C.D ($p < 0.05$)	0.247	0.462	0.653	0.131	0.245	0.346	0.351	0.656	0.928
SE.m (±)	0.084	0.158	0.223	0.045	0.084	0.119	0.120	0.224	0.317

Statistical analysis of data for pods per plant revealed that there were significant difference among the priming treatments, varieties and the combination of treatment and variety (Table 7). Highest pods per plant (14.48) was recorded inT_6*i.e.*, Seed priming with *Rhizobium*+ PSB followed by T_7(13.62) which was statistically at par with T_4(13.45). The pods per plant was directly related to the yield of the plant because higher the number of pods, higher would be the yield. The results are in agreement with Sharma *et al.*,2018 in soybean and Bhat *etal.*, 2013 in field pea.

In the present study, significant difference in seeds per pod was observed due to application of different priming agents. Seed priming with *Rhizobium* + PSB recorded the maximum seeds per pod (7.90). It was found higher in DS-10 variety than Arkel, since the pod length was higher in DS-10 which contained more seeds per pod. Lower number of seeds per pod was recorded in untreated control. In addition to pods per plant, seeds per pod also have an impact on overall seed yield.The highest seeds per pod in T_6 may be attributed dueto the action of different bio-agents, which enhanced nutrient uptake, vegetative growth and better photosynthesis. This increased the plant’s biomass and raised the amount of proteins and carbohydrates resulting in higheraccumulation of seeds.This in turn produced more seeds per pod. Similar findings were also reported by Pandey *et al.*, 2017 in pea.

Seed yield is an important consideration in any study relating to seed production of a crop. Analysis of data on seed yield, revealed that all the treatments were found to enhance seed yield as compared to control. Seed priming with *Rhizobium* and PSB (T_6) recorded the maximum seed yield per plant (18.93) which was followed by treatment T_4 (16.57). There were significant variations among the treatments, varieties and combination of treatment and variety. The reason for higher seed yield recorded in T_6 might be due to beneficial effect of *Rhizobium* and PSB that enhanced field emergence resulting in more number of pods per plant and seeds per pod.This ultimately increased the seed yield in garden pea. Similar kind of studies were carried out by Lohitha *et al*., 2022 who reported higher seed yield in chickpea when the seeds were bio primed with *Rhizobium* and PSB. Higher seed yield was recorded by Pandey *et al*., 2017 also due to combined application of PSB and *Rhizobium* in field pea. Rani *et al*., 2017 also reported that grain yield in field pea was enhanced by combined inoculation of biofertilizers.

CONCLUSION:

In order to ensure a good crop production, modern agricultural technologies demand that every seed should readily germinate and produce vigorous seedlings. To produce good crop, the seeds should be properly invigorated by adopting various seed invigoration techniques like seed priming.From the presentstudy, it could be concluded that seed priming with combined application of PSB + *Rhizobium* for 4 hours exhibited spectacular performance in seed quality, yield and its attributing characters as compared to other treatments under field and laboratory conditions. From this investigation, combined application of PSB @10g/kg + *Rhizobium* @ 20g/kg may be promoted for future recommendation as a seed priming treatment in pea. However, multilocation trials in larger area should be done to confirm the benefits of biopriming before recommendation to farmers.

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