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Effect of Seed Pelleting with *Rhizobium* and Nitrogen Application on Yield and Quality of Cowpea Seeds

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ABSTRACT: Seed treatment with biofertilizers reduces demand of artificial fertilizers and reduces environmental pollution and cost of cultivation. By keeping this aspect in mind, an experiment was laid out to check the effect of seed pelleting with *Rhizobium* and different dose of nitrogen application on yield and quality of cowpea seeds. Experiment was laid out in a Randomized Block Design (Factorial) with different pelleting material i.e. no pelleting (P_1), *Rhizobium* liquid (P_2), *Rhizobium* powder (P_3) and *Rhizobium* liquid with jaggery (P_4) and 5 levels of nitrogen application i.e. 0% N of Recommended dose of fertilizer (F_1), 40% N of RDF (F_2), 60% N of RDF (F_3), 80% N of RDF (F_4) and 100% N of RDF (F_5). Results of experiment showed that maximum seed yield per plant and per hectare was found when seeds were pelleted with *Rhizobium* (liquid form) and yield was increased by 15.74% and 23.21% over control, respectively. Nitrogen application also significantly affected seed yield over control and maximum was found when 80% N of recommended dose of fertilizer (RDF) was applied. In case of nitrogen application, seed yield per plant and per hectare was increased by 10.34% and 13.71% over control, respectively. Seed quality of harvested seeds from field was also analyzed and found that seed pelleting with *Rhizobium* (liquid form) and 80% N of RDF produced high vigour seeds in comparison to control and other treatments.

Keywords: Cowpea, Nitrogen, Pelleting, *Rhizobium*, Seed yield, Seed quality.

INTRODUCTION

According to estimation by FAO, global population will be around 9.1 billion by 2050 (Cohen, 2005) and around (Barrett, 2010). To feed all the population, challenges in current agriculture like climate change, shrinking available land for agriculture, higher use of chemical fertilizers, depleting soil properties, yield stagnation of crops etc. have to be reduced by adopting sustainable agriculture (Gomiero *et al.,* 2011). A sustainable agriculture is one which depletes neither the people nor the land. Sustainable agriculture is known for environment protection, to maintain soil properties and to expand Earth's natural resource base (Gold, 2016). The increasing demand of crop yield and food can be achieved by use of biofertilizers like *Rhizobium*, *Azotobacter*, *Azospirillum* and blue green algae (BGA) for the betterment of crops (Mishra *et al.*,2013) and even for the health of soil (Singh *et al.,* 2014). *Rhizobium* fixes nitrogen symbiotically and stimulates plant growth by production of plant growth hormones (Tilak *et al.,* 2006), vitamins, by solubilization of insoluble phosphates, induction of systemic disease resistance and enhancement in stress resistance (Lebrazi and Benbrahim 2014). Interaction between *Rhizobium* and legume crops has potential to increase agricultural productivity with less dependence on non-renewable inputs like artificial fertilizers (Peoples *et al*., 2009).

Cowpea [*Vigna unguiculata* (L.) Walp], a member of Leguminosae family (Ng and Padulosi, 1991, Kumar *et al.,* 2022) is a crop of great economic importance for low-income population in developing countries (Verdier *et al.,* 1998; Singh, 1997) because it is good source of protein (Fatokun, 2002), carbohydrate, vitamins and minerals (Iqbal *et al.,* 2006). Being a leguminous crop, it also fixes nitrogen symbiotically with biofertilizers (Eke-Okoro *et al.,* 1999); although it requires nitrogen as a small basal dose for quick and better start because it is essential constituent of many enzymes, protein and chlorophyll. To ensure proper germination and growth of seedlings, there are different ways to apply biofertilizers to seeds of cowpea and seed pelleting with *Rhizobium*is one of them. Seed pelleting is a mechanism of applying needed material in such a

way that they affect the seed or soil at the seed-soil interface to ensure better performance of seed. Seed pelleting with *Rhizobium* can also reduce the negative effect on seed germination, seedling growth and plant growth in soil due to excessive use of urea (Bremner and Krogmeier, 1989). Pawar *et al.* (2014) reported that seed inoculation with *Rhizobium* show higher growth and yield in legumes when it is compared to application of *Rhizobium* in soil as biofertilizers. Besides, cowpea has inherent problems like flower shedding, shrinkable seeds, poor pod and seed setting due to deficiency of micronutrients, macronutrients, growth promoting substances and biotic stress and *Rhizobium* is known for providing plant growth promoting substances like auxin and to increase nutrient uptake. Keeping in view the above prospective, this study had been planned to determine the effect of seed pelleting with *Rhizobium* and nitrogen application on yield and quality parameters of cowpea crop.

MATERIALS AND METHODS

This experiment was conducted during *Kharif* season of 2015-16 at experimental farm of Dr. Y S Parmar UHF which is located at an altitude of 1250 meters above mean sea level with latitude of 35.5^{o}N and longitude of 77.8^{o}E in the mid- hill zone of Himachal Pradesh, India. Genetically pure seed of cowpea cv. Him Lobia 1 was used for experiment. Liquid and powder form of *Rhizobium* culture was obtained from Soil microbiology laboratory of Department of Soil Science and water management. Seeds were pelleted at CSIR-IHBT (Institute of Himalayan Bioresource Technology) Palampur, H.P., India. For seed pelleting clay and adhesive (45% Gum Arabica) was used. There were total 20 treatment combinations (4 level of seed pelleting i.e. P_1-Control, P_2-*Rhizobium* liquid + clay + adhesive, P_3- *Rhizobium* powder + clay + adhesive, P_4-*Rhizobium* liquid formulation + jaggary and 5 level of nitrogen application i.e. F_1-0 % Nitrogen of Recommended dose of fertilizer, F_2 -40% N of RDF, F_3 -60% N of RDF, F_4 -80% N of RDF, F_5 -100% N of RDF) and each treatment was replicated three times so there were total 60 plots of 1.8m × 1.5m (i.e. 2.7 m^2) size. There were total 40 plants in every plot and spacing was 45cm × 15cm. Urea is applied to these plots and recommended dose for cowpea is 45kg/hectare. Seed yield per plant and per hectare as yield parameters and germination %, speed of germination, seedling length, seedling dry weight, seed vigour index-I and seed vigour index-II as seed quality parameters were observed.

Table 1: Meteorological data on rainfall, temperature and relative humidity during the course of investigation.

Month	Mean Temperature (°C)	Mean Rainfall (mm)	Relative Humidity (%)
June	38.05	91.1	60
July	38.20	294.40	79
August	38.05	102.20	80
September	36.75	41.60	68
October	19.65	34.60	58

Source: Meteorological Observatory, Department of Environmental Sciences, Dr. YS Parmar University of Horticulture and Forestry, Nauni-173 230, Solan (HP)

RESULTS AND DISCUSSIONS

Seed pelleting with *Rhizobium* (liquid and powder form) and jaggary affected significantly seed yield of crop. Seed yield per plant was increased by 15.74% over control (no pelleting) when seed were pelleted with *Rhizobium* liquid form. Maximum average seed yield per plant (22.49 g) was found when seed were pelleted with liquid form (P_2) and minimum average yield per plant (19.43 g) was found in control (no pelleting) treatment. It might be due to that *Rhizobium* ensures proper nodulation in root zone which leads adequate nitrogen uptake (Vessey, 2003). *Rhizobium* also exhibits antibacterial and antifungal activities (Pawar *et al.*, 2014) which allow seeds for healthy seedling rise. Similar findings were obtained by Bohra *et al.* (1990); (Patel and Jadav, 2010); (Mishra and Solanki, 1996) and Rajput (1994) in cowpea. (Khanam *et al.*, 1994); (Albayrak *et al.*, 2006); (Khalil *et al.*, 1989) also found increased yield per plant in chick pea, Common Vetch (*Vicia sativa* L.) and mung bean respectively when seeds of these crops were inoculated with *Rhizobium*. Different dose of nitrogen application as application of urea also affected seed yield per plant significantly. Maximum average seed yield per plant (21.86 g) was found when 80% N of RDF was applied to plots and minimum (19.81 g) was found in control in which urea was not applied. Application of nitrogen in form of urea increased seed yield per plant because nitrogen helps in vegetative growth of plant and nitrogen also play a vital role to reduce nutritional deficiency especially at reproductive stage which leads to high number of pods per plant, seeds per pod and finally higher seed yield (Umeh *et al.*, 2011); (Thies *et al.*, 1991); (Pikul *et al.*,1997) also found increased yield in different legumes when seeds were treated with *Rhizobium*. (Upadhyay and Singh, 2016) also reported that recommended nitrogen dose increased seed yield in cowpea. The interaction effects due to seed pelleting and nitrogen application were found to be non-significant at 5% level of significance. Maximum average seed yield was found when 80% N of RDF (F_4)

applied and seeds pelleted with liquid form of *Rhizobium* (P_2). Minimum average seed yield per plant was found in P_1F_1 (control + 0% N of RDF). Around 28% yield per plant was increased in P_2F_4 over control (P_1F_1). Kishan *et al*., (2002) also reported that seed pelleting with *Rhizobium* and nitrogen application increase seed yield in cowpea cv. Pusa Phalguni. Same findings were also obtained by Mishra and Solanki, (1996) in cowpea and Soni *et al.,* (2021) in pigeonpea. Seed yield per hectare was significantly affected by different seed pelleting and nitrogen application treatments. Interaction effect of seed pelleting and nitrogen application was non-significant on seed yield per hectare though. When seeds were inoculated with liquid form of *Rhizobium* pelleting than total yield per hectare was increased by 20.1% over control. Among different doses of nitrogen application, 80% N of RDF gave maximum yield (23.96 q) which was 12% higher in comparison to control (0% N of RDF).Treatment combination of liquid *Rhizobium* pelleting (P_2) and 80% N of RDF (F_4) gave maximum (26.73 q) yield per hectare and minimum (19.39 q) was found in P_1F_1 (non-pellet seeds + 0% N of RDF). There was around 37% hike in seed yield per hectare when seeds were pelleted with *Rhizobium* liquid and 80% N of RDF were supplied to plots over control (non-pellet seeds + 0% N of RDF). Seed pelleting with Powder form of *Rhizobium* also increased the seed yield. The enhancement in seed yield per hectare due to seed pelleting with *Rhizobium* might be due to the carryover beneficial effects of pre sowing treatments with nutrients to the seeds which led to better plant growth during vegetative and reproductive phase and ultimately led to increased seed yield. Seed yield per hectare was increased linearly with the increasing levels of nitrogen fertilization with *Rhizobium* seed pelleting due to increase in plant height, number of branches per plant, number of pods per plant and number of seeds per pods. Harvested seeds from crop were analyzed for seed quality parameters like germination percentage, seedling length, seedling weight and vigour index and found that all the parameters were increased in comparison to seeds which were sown.

Table 2: Effect of seed pelleting with *Rhizobium* and nutrient management on seed yield per plant of cowpea.

Treatments	Seed yield per plant (g)					
	F_1	**F_2**	**F_3**	**F_4**	**F_5**	**Mean**
P_1	18.52	19.16	19.38	20.20	19.88	19.43
P_2	21.11	21.89	22.44	23.76	23.26	22.49
P_3	19.90	20.47	20.64	22.25	21.24	20.90
P_4	19.70	20.21	20.40	21.22	20.62	20.43
Mean	19.81	20.43	20.71	21.86	21.25	
Factor	P	F	P×F			
$CD_{0.05}$	0.39	0.43	NS			

Table 3: Effect of seed pelleting with *Rhizobium* and nutrient management on seed yield per ha of cowpea.

Treatments	Seed yield per ha (q)					
	F_1	**F_2**	**F_3**	**F_4**	**F_5**	**Mean**
P_1	19.39	20.23	20.46	21.93	21.17	**20.64**
P_2	22.70	23.76	24.80	26.73	25.94	**24.79**
P_3	21.40	22.01	22.20	24.15	23.05	**22.57**
P_4	20.80	21.73	22.14	23.03	21.98	**21.94**
Mean	**21.07**	**21.93**	**22.40**	**23.96**	**23.04**	
Factor	P	F	P×F			
$CD_{0.05}$	0.54	0.61	NS			

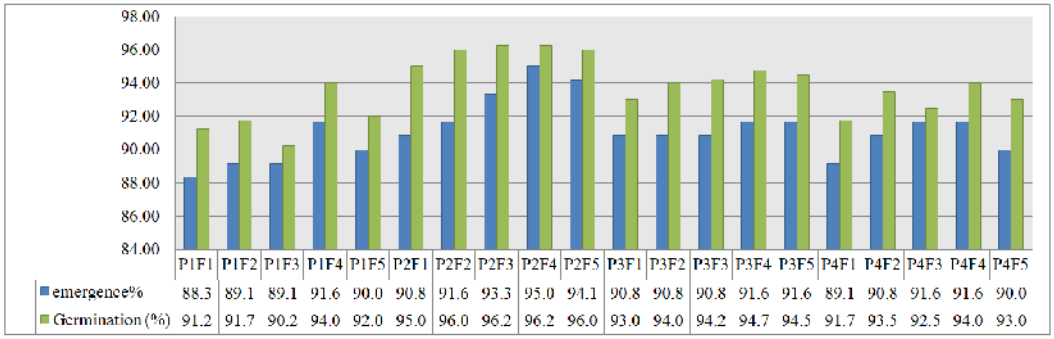

Fig. 1. Comparison of germination % of sown seeds and emergence of harvested seeds.

Among different types of pelleting treatments, maximum germination (95.90%) was found with *Rhizobium* liquid pelleted seeds (P_2) and minimum (91.85%) was found in non-pelleted seeds (P_1). The application of different nitrogen doses had also exhibited significant effect on germination. Maximum germination (94.75%) was found with application of 80% N of RDF (F_4) and found to be statistically superior to all other doses of nitrogen. However, minimum germination (92.75%) was found with the application of 0% N of RDF (F_1).Interaction effect of seed pelleting and nitrogen application on germination percentage was non-significant buy germination percentage increased in every treatment and maximum (96.25%) was found in P_2F_4 and minimum (91.25%) was in control (P_1F_1). It might be due to freshly harvested seeds from plants which had uptake proper quantity of nutrients during their growth. Due to proper supply of nitrogen during mother plant growth, mother plants might be produced high content protein seeds and high content of protein in seeds in known for higher germination. Similar results of increased germination due to seed inoculation with *Rhizobium* and nitrogen application was reported by Yadav and Malik (2005) in cowpea.

Table 4: Effect of seed pelleting and nitrogen application on seed quality parameters of cowpea.

Treatment	Germination %	Speed of germination	Seedling length (cm)	Seedling dry weight (mg)	Seed Vigour Index-I	Seed Vigour Index-II
Seed pelleting						
P_1	91.85 (9.64)	56.67	29.02	67.02	2665.95	6156.50
P_2	95.90 (9.84)	58.66	28.93	76.61	2774.08	7346.90
P_3	94.10 (9.75)	58.30	28.72	70.07	2702.31	6593.90
P_4	92.95 (9.69)	58.40	28.56	69.26	2654.43	6438.14
$CD_{0.05}$	0.03	0.71	NS	0.51	64.94	62.12
Nitrogen application						
F_1	92.75 (9.68)	57.10	28.65	69.46	2658.79	6447.40
F_2	93.81 (9.74)	57.46	27.94	70.12	2620.74	6582.93
F_3	93.31 (9.71)	57.95	28.32	70.80	2641.41	6613.39
F_4	94.75 (9.79)	58.97	29.92	71.98	2835.38	6823.56
F_5	93.88 (9.74)	58.55	29.19	71.34	2739.64	6702.04
$CD_{0.05}$	0.03	0.80	0.74	0.57	72.61	69.45
Interaction						
P × F $CD_{0.05}$	0.06	NS	1.48	NS	145.22	NS

P_1-Control, P_2-*Rhizobium* liquid + clay + adhesive, P_3- *Rhizobium* powder + clay + adhesive, P_4- *Rhizobium* liquid formulation + jaggary
F_1-0 % N of RDF, F_2 -40 % N of RDF, F_3 -60 % N of RDF, F_4 -80 % N of RDF, F_5 -100 % N of RDF

Both treatment; seed pelleting and nitrogen application had significant effect on speed of germination separately but interaction effect of both treatments was non-significant. In case of seed pelleting, maximum (58.66) speed of germination was found in P_2 and minimum (56.67) was found in control. Among treatment of nitrogen application, maximum (58.97) speed of germination was found in F_4 and minimum (57.10) was found in control (F_1). The biofertilizers and nitrogen doses provide different micronutrients to the mother plant at early stages of growth that play vital role in producing good quality seed and good quality seeds led to higher speed of germination. Increased speed of germination due to seed pelleting was also recorded by Shashibhaskar *et al*. (2009); Masuthi *et al*. (2009).

Different pelleting treatments and nitrogen application had significant effect on seed vigour index-I. Among different pelleting treatments, maximum (2774.08) SVI-I was found in P_2 and minimum (2665.95) was found in non-pelleted seeds (P_1). Among different nitrogen application, maximum (2835.38) SVI-I was found in F_4 and minimum (2658.79) was found in F_1. Interaction of seed pelleting and nitrogen application had also significant effect on SVI-I and maximum (2952.33) SVI-I was found in P_2F_4 and minimum (2556.70) was found in P_1F_2. Micronutrients provided to the mother plant during the early stages of growth helps to produce uniform, bold and vigorous seed that results in good germination and seedling length that led to enhanced SVI-I. Increased seed vigour index-I might be due to increased germination and seedling length. Similar findings of increased seed vigour index-I due to seed pelleting with *Rhizobium* and nitrogen application was reported by Kishan *et al*. (2001) in cowpea cv. Pusa Phalguni.

Different types of pelleting and different doses of nitrogen had significant effect on seed vigour index-II in cowpea. Among different types of pelleting, maximum seed vigour index-II (7346.90) was found with *Rhizobium* (liquid form) pelleted seeds and minimum (6156.50) was found in the non-pelleted seeds (P_1). The application of different nitrogen doses also exhibited significant effect on seed vigour index-II. Maximum seed vigour index-II (6823.56) was found with application of 80% N of RDF (F_4) and found to be

statistically superior to all other doses of nitrogen. However, minimum seed vigour index-II (6447.40) was found with the application of 0% N of RDF (F_1). The interaction effects due to seed pelleting and nutrition management were found to be non-significant at 5% level of significance. Masuthi *et al*. (2009) reported that the seed pelleting provides the nutrient to the mother plant during early stages of growth which led to formation of bold and vigours seeds. The enhanced germination and seedling dry weight due to vigorous seeds led to enhanced SVI-II.

The biofertilizers and nitrogen doses provide different micronutrients to the mother plant at early stages of growth that play vital role in seed set, seed size and quality. Higher seed quality might be due to well-developed seeds with higher thousand seed weight and additional supply of micronutrients due to seed pelleting to the mother plant which led to the production of bold and vigorous quality seeds. The good quality seeds led to higher speed of germination and seedling vigour (length and dry weight).The increased seedling length of seeds harvested from the crop raised from pelleted seeds has also been reported by (Masuthi *et al*., 2009) in cowpea. Seedling dry weight. Similar findings of increased seed vigour index-I due to seed pelleting with Rhizobium and nitrogen application was reported by Swaroop *et al*., (2001) in cowpea; Khatana *et al.* (2021) and in chickpea by Patil *et al*. (2002).

Table 5: Interaction effect of seed pelleting with *Rhizobium* and nutrient management of seed quality parameters of harvested seeds.

Interaction treatment	Germination %	Speed of germination	Seedling length (cm)	Seedling dry weight (mg)	Seed Vigour Index-I	Seed Vigour Index-II
P_1F_1	91.25 (9.61)	55.94	28.25	66.32	2578.15	6052.36
P_2F_1	95.00 (9.80)	57.73	30.45	75.23	2892.90	7146.96
P_3F_1	93.00 (9.70)	57.09	28.00	68.10	2604.43	6332.94
P_4F_1	91.75 (9.63)	57.67	27.90	68.20	2559.68	6257.34
P_1F_2	91.75 (9.63)	56.07	27.88	66.36	2556.70	6088.25
P_2F_2	96.00 (9.85)	57.89	27.25	75.78	2615.68	7274.36
P_3F_2	94.00 (9.75)	57.79	28.68	69.49	2695.40	6532.24
P_4F_2	93.50 (9.72)	58.09	27.98	68.85	2615.18	6436.86
P_1F_3	90.25 (9.55)	56.54	28.90	67.27	2608.83	6071.08
P_2F_3	96.25 (9.86)	58.62	27.00	76.84	2599.23	7395.45
P_3F_3	94.25 (9.76)	58.40	28.95	69.69	2727.83	6567.88
P_4F_3	92.50 (9.67)	58.25	28.43	69.40	2629.75	6419.15
P_1F_4	94.00 (9.75)	57.57	30.55	67.80	2872.08	6373.16
P_2F_4	96.25 (9.86)	59.68	30.68	77.96	2952.33	7503.84
P_3F_4	94.75 (9.79)	59.53	29.15	72.13	2762.73	6833.21
P_4F_4	94.00 (9.75)	59.11	29.30	70.04	2754.38	6584.03
P_1F_5	92.00 (9.64)	57.22	29.50	67.37	2713.98	6197.67
P_2F_5	96.00 (9.85)	59.40	29.28	77.23	2810.25	7413.91
P_3F_5	94.50 (9.77)	58.68	28.80	70.93	2721.18	6703.22
P_4F_5	93.00 (9.70)	58.89	29.18	69.83	2713.18	6493.34
$CD_{0.05}$	0.06	NS	1.48	NS	145.22	NS

P_1-Control, P_2-*Rhizobium* liquid + clay + adhesive, P_3- *Rhizobium* powder + clay + adhesive, P_4- *Rhizobium* liquid formulation + jaggary

F_1-0 % N of RDF, F_2 -40 % N of RDF, F_3 -60 % N of RDF, F_4 -80 % N of RDF, F_5 -100 % N of RDF

CONCLUSION

Seed pelleting with *Rhizobium* was found to be a recognized technique to increase seed yield in cowpea. Seed pelleting with *Rhizobium* and proper nitrogen application are known to increased seed quality parameters. Still there is always room for improvement and research in every area but cowpea seed treatments with *Rhizobium* could be recommended for sowing in Himachal Pradesh conditions.

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Conflicts of Interest. None.

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