Seed coating treatments and foliar spray of micronutrients enhance quality of harvested seeds in garden pea (*Pisum sativum* L.)

Abstract

To study the effect of different seed coating treatments and foliar application of micronutrients on quality seed production in pea, analysis of harvested seed was done at laboratory of the Department of Seed Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP). Seeds from field experiment were harvested and were evaluated in the laboratory conditions based on the different parameters. In field experiment, there were seven different treatments comprising, seed coating with *Rhizobium*, Phosphate solubilizing bacteria, iron, zinc, carbendazim along with control and foliar application of micronutrients. From the investigation, it was observed that seed coating with *Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed and two foliar sprays of $ZnSO_4$ @ 0.3 % + $FeSO_4$ @ 0.3 % at 50 per cent flowering and 15 days later, significantly improved the germination (96.00 %), seedling length (19.29 cm), seedling dry weight (34.80 mg), SVI-I (1851.62), SVI-II (3341.38), germination in accelerated ageing test and in cold test (77.75 % and 80 %, respectively) and resulted in the lowest EC (16.75 µS cm^{-1}) of harvested seeds. Hence, it was concluded that seed coating with *Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed and two foliar sprays of $ZnSO_4$ @ 0.3 % + $FeSO_4$ @ 0.3 % performed best among all the treatments tested for quality seed production and it could be recommended for getting quality seeds in garden pea.

Key Words: Micronutrients, *Pisum sativum*, Phosphate solubilizing bacteria, Rhizobium, Seed coating

INTRODUCTION

The garden pea (*Pisum sativum var. hortense* L.) is a cool-season leguminous herbaceous plant that thrives in cool and moist climates. It is primarily cultivated for its tender, green seeds, which are highly valued as a food source. The garden pea (*Pisum sativum*), believed to have originated in the mediterranean region and first domesticated from its wild progenitor, *Pisum fulvum*, likely saw early cultivation in ancient civilizations such as those of Mesopotamia, Egypt and Greece, facilitated by trade routes and exploration, wherein Phoenician traders notably played a role in introducing peas to new territories Zohary and Hopf (2000). Pea, being a leguminous crop, plays a vital role in biological nitrogen fixation and contributes to soil fertility through introduction of a substantial amount of organic matter. Fresh pea pods, weighing 100 grams, have notable nutritional components. They provide a considerable amount of proteins (7.2 %) and carbohydrates (15.9 %), along with a small quantity of fat (0.1 %). Additionally, they contain carotene (83 µg), vitamin C (9 mg), thiamine (0.25 mg) and riboflavin (0.01 mg). Peas are also rich in essential amino acids and minerals like potassium, phosphorus, calcium, magnesium and iron (Choudhary, 2017).

Seed coating is a technique used to improve seed quality, where a special binder such as carboxy methyl cellulose (CMC) or gum arabic is utilized to apply microbial inoculants or chemicals onto the seed surface. This precise application of active ingredients has proven successful for seed inoculation in various crops (Jetiyanon *et al*., 2008; Oliveira *et al*., 2016; Rouphael *et al*., 2017; Accinelli *et al*., 2018; Rocha *et al*., 2018). CMC, being readily available, cost-effective and requiring a low application rate, is commonly used as a binder. According to Nyaga and Njeru (2020), rhizobium inoculation significantly increased nodulation, shoot dry weight, and yields by 22.7–28.6% compared to uninoculated controls. Phosphate solubilizing bacteria, another component of bio-fertilizers used in seed coating, help convert insoluble phosphates (such as tricalcium, iron and aluminum phosphates) into plant-available forms.

Iron serves as a constituent in numerous crucial enzymes, including cytochromes found in the electron transport chain, thereby playing a pivotal role in various biological processes. Within plants, iron participates in chlorophyll synthesis, crucial for sustaining chloroplast integrity and functionality (Rout and Sahoo, 2015). Jolli *et al*. (2020) in their study, concluded that foliar application of zinc, boron and iron resulted in significant increase in seed yield, fodder yield and shelling percentage in sweet corn. Zinc plays a crucial role in the reproductive development of plants. Zinc deficiency affects various stages of plant reproduction, including flowering, flower development, anthesis, gamete formation, fertilization and ultimately seed production. According the Choudhary *et al*. (2013), zinc is a crucial component for numerous enzymes, aiding in energy transfer, protein synthesis, and the synthesis of tryptophan, which serves as a precursor of indole acetic acid (IAA). It also plays a role in seed maturation and production. Based on these findings, the present study aimed to investigate the impact of seed coating and foliar sprays on quality seed production in garden pea.

MATERIAL AND METHODS

To investigate the effect of seed coating treatments and foliar spray of micronutrients on quality seed production of pea, analysis of harvested seeds was done at laboratory of the Department of Seed Science and Technology, Dr YS Parmar UHF, Nauni, Solan (HP) during the year 2019-20. Seeds of garden pea cv. ‘Pb-89’ from the field trial were harvested and evaluated in the laboratory conditions for different parameters. In field trial, there were seven different seed coating treatments namely, seed coating with *Rhizobium* @ 30 g kg^{-1} seed, seed coating with PSB @ 30 ml kg^{-1}, seed coating with *Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1}, seed coating with $FeSO_4$ @ 3 g kg^{-1}, seed coating with $ZnSO_4$ @ 3 g kg^{-1}, seed coating with carbendazim @ 2.5 g kg^{-1} along with control. Apart from seed coating, two foliar sprays of $ZnSO_4$ and $FeSO_4$ @ 0.3 % each were applied during 50 percent flowering and 15 days after the first spray in all treatments except control. Observations on various parameters viz., germination percentage, seedling length, seedling dry weight, seed vigour indices, electrical conductivity, germination percent after accelerated ageing test and cold test were observed as per the standard procedures. Experiments were conducted with four replications (ISTA, 2016) and results were statistically analyzed by using Completely Randomized Design (Panse and Sukhatme, 2000) following windows-based computer application OPSTAT developed by Sheoran (2006).

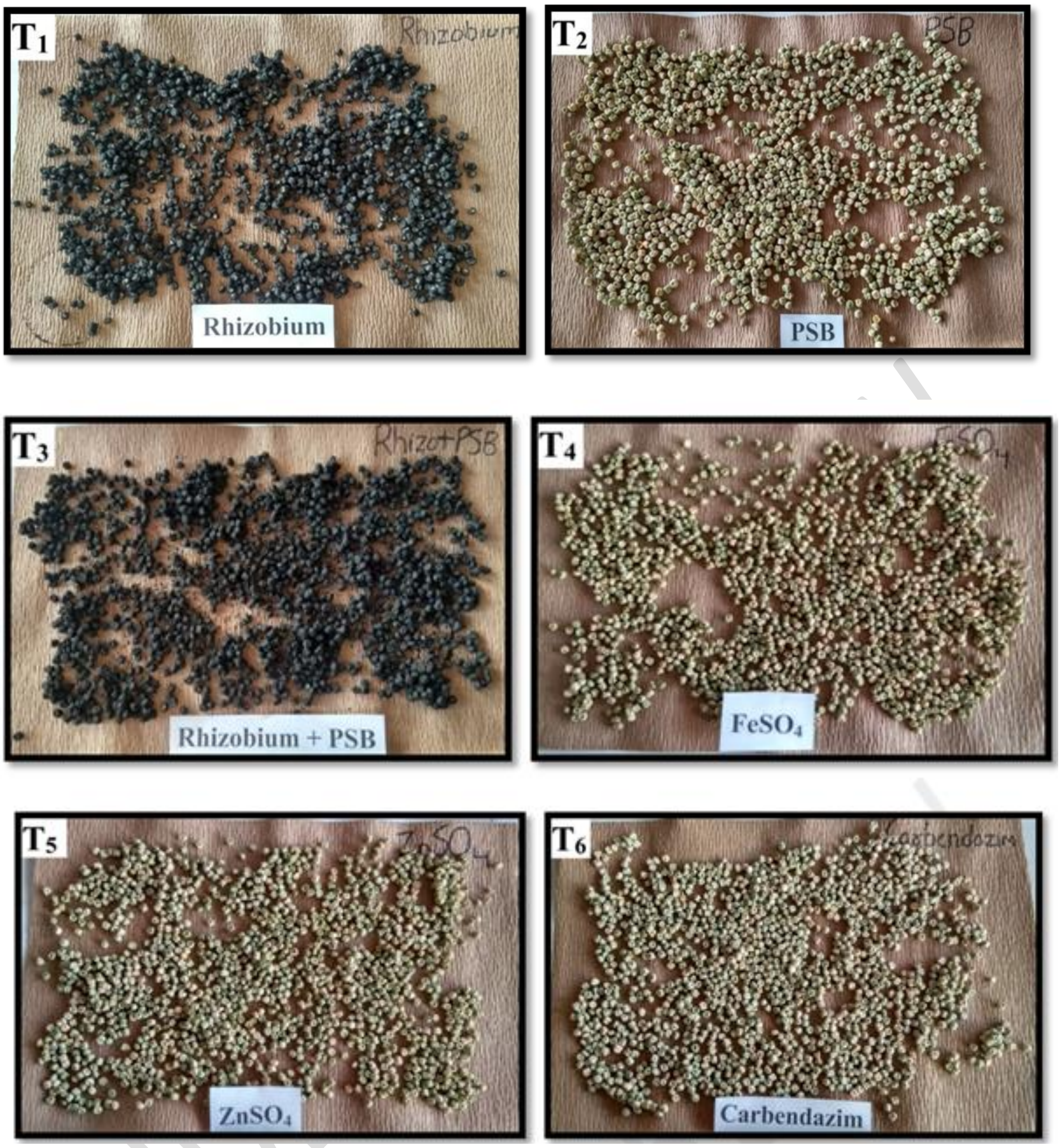

Plate 1: Seeds coated with different bio-fertilizers and chemicals

RESULTS AND DISCUSSION

The results of the investigation obtained w.r.t. various seed quality parameters after statistical analysis are presented and discussed hereunder:

Germination percentage

The analysis of the data (Table 1) revealed significant variations in germination percentage among different seed coating treatments. Treatment T_3, which involved seed coating with *Rhizobium* @ 30 g kg^{-1} and PSB @ 30 ml kg^{-1} seed along with two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %), had the highest germination (96.00 %). This result was statistically similar to treatment T_1, which included seed coating with *Rhizobium* @ 30 g kg^{-1} seed and two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %). Conversely, control treatment showed the lowest germination (88.25 %). The superiority in germination observed in treatment T_3 can be attributed to the positive effects of *Rhizobium* and phosphate solubilizing bacteria on the growth and seed weight of pea. These microorganisms likely contributed to the accumulation of greater food reserves, such as proteins and carbohydrates, within the seeds, thereby improving their quality and germination potential. This finding aligns with the results reported by Pandey (2018) in case of field pea. Additionally, the foliar sprays of zinc and iron may have further enhanced the germination process. Zinc is known to facilitate water uptake in plants, which could have positively influenced the water absorption by the seeds. Iron, on the other hand, aids in the absorption of other essential nutrients, potentially increasing their concentration within the seeds. This improved nutrient availability in the seed likely contributed to the higher germination observed. Furthermore, the enhanced rate of photosynthesis and production of photosynthates could have resulted in increased seed weight, ultimately leading to improved germination percentages of pea seeds. Yogeshwarappa (2005) discovered that the combination of *Rhizobium* and PSB, along with the application of micronutrients, resulted in a higher germination percentage in soybean. Similarly, Ranjithkumar (2017) reported that foliar application of iron led to an increased germination percentage in lentil.

Seedling length (cm)

There was a notable difference in seedling length among various seed coating treatments, as indicated in Table 1. Treatment T_3, which involved seed coating with *Rhizobium* at 30 g + PSB at 30 ml kg^{-1} seed along with two foliar sprays of $ZnSO_4$ (0.3 %) + $FeSO_4$ (0.3 %), exhibited the significantly highest seedling length of 19.29 cm. Contrarily, treatment T_7 (control) displayed the lowest seedling length (12.37 cm). The observed increase in seedling length in treatment T_3 might be due to the fact that application of biofertilizers resulted in increased seed weight, providing more nutrients for germination. This, in turn, promoted root and shoot growth, ultimately leading to enhanced seedling length. Similar results were reported by Pandey (2018) in field pea. Zinc plays a vital role in various processes related to auxin metabolism, such as tryptophan synthesis and tryptamine metabolism (Das, 1996).

Table 1: Effect of seed coating and foliar spray of micronutrients on germination (%), seedling length (cm), seedling dry weight (mg) and seed vigour indices in garden pea

	Parameters / Seed coating treatments**	*Germination (%)	Seedling length (cm)	Seedling dry weight (mg)	Seed vigour index-I	Seed vigour index-II
T_1	*Rhizobium* @ 30 g kg^{-1} seed	93.75 (9.68)a	16.90b	33.00b	1584.15b	3093.74b
T_2	PSB (Phosphate solubilizing bacteria) @ 30 ml kg^{-1} seed	92.25 (9.60)b	15.27c	32.68b	1408.07c	3015.05bc
T_3	*Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed	96.00 (9.80)a	19.29a	34.80a	1851.62a	3341.38a
T_4	$FeSO_4$ @ 3 g kg^{-1} seed	92.00 (9.59)b	16.44b	32.27b	1512.32b	2969.27c
T_5	$ZnSO_4$ @ 3 g kg^{-1} seed	91.00 (9.54)b	14.65c	28.74c	1333.99cd	2615.57d
T_6	Carbendazim @ 2.5 g kg^{-1} seed	90.25 (9.50)b	14.33d	28.50c	1292.84d	2571.79d
T_7	Control	88.25 (9.39)c	12.37e	27.32d	1091.43e	2411.32e

	Mean	91.93 (9.59)	15.61	31.05	1439.20	2859.73
	$CD_{0.05}$	0.14	0.91	0.86	92.78	122.85

***** Figures in the parenthesis represent square root transformed values

**Foliar application of $ZnSO_4$ + $FeSO_4$ each @ 0.3 % was given at two stages in all treatments except control

On the other hand, iron is essential for the formation of important structural components like cytochromes, hemes, hematin, ferrichrome and leghemoglobin, which are involved in oxidation-reduction reactions during respiration and photosynthesis. These factors may have contributed to increased seed weight, while the presence of auxin likely promoted the growth of both shoots and roots. Moreover, the foliar application of iron and zinc provided an ample supply of nutrients for optimal seed growth. Similar findings regarding root length have also been reported by Kiran (2006) in case of brinjal.

Plate 2: Germination (%) of harvested seed in treatments T_3 and T_7

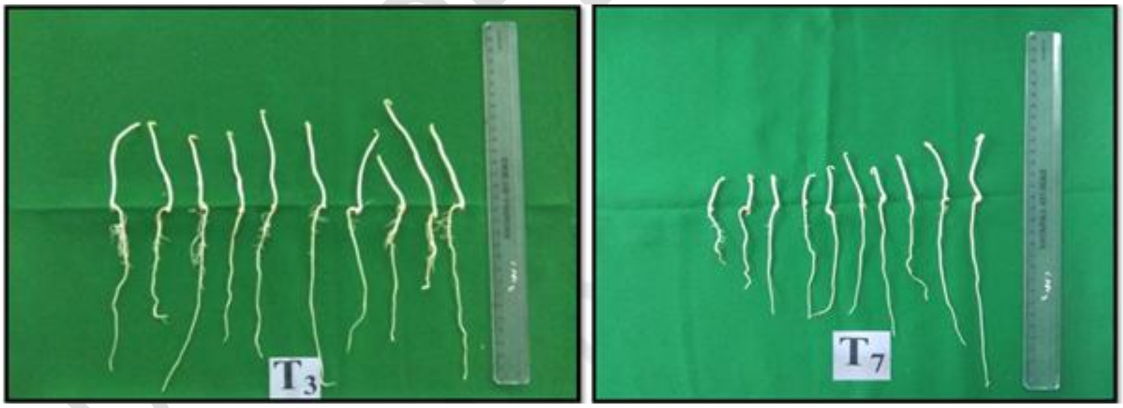

Plate 3: Seedling length (cm) of harvested seed in treatments T_3 and T_7

Seedling dry weight (mg)

An examination of the data w.r.t. seedling dry weight (Table 1), revealed that seed coating treatments significantly influenced the dry weight of seedlings as compared to control. Treatment T_3 [seed coating with *Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed and two foliar sprays of $ZnSO_4$ (0.3 %) + $FeSO_4$ (0.3 %)] showed the highest seedling dry weight (34.80 mg) differing significantly from the other treatments. In contrast, control treatment (T_7) resulted in the lowest seedling dry weight (27.32 mg). The application of biofertilizers through treatment T_3 led to the highest seedling dry weight, which attributable to enhanced germination, increased seedling length and higher seed weight. These factors resulted in seedlings with greater metabolic activity, ultimately leading to higher weight. These findings are in consonance with the research conducted by Pandey (2018) in field pea. Furthermore, the foliar spray of zinc and iron likely increased the concentration of nutrients in the seeds by promoting enzymatic activities that break down complex substances into simpler forms, facilitating easier absorption and utilization by the germinating seedlings. This enhanced nutrient availability further might have contributed to vigorous growth of the seedlings and ultimately resulted in increased seedling dry weight. These findings are consistent with those of Kiran (2006) in brinjal, supporting the positive impact of foliar sprays on seedling development.

Seed vigour indices

A careful examination of the data (Table 1) depicted significant variation in seed vigour index-I & II among different seed coating treatments. Seed vigour index directly reflects the seed capability to survive under adverse conditions. The highest seed vigour index-I (1851.62) & II (3341.38) were recorded in treatment in which dual inoculation of both the biofertilizers and two foliar sprays of $ZnSO_4$ (0.3 %) + $FeSO_4$ (0.3 %) were done i.e. in treatment T_3 while lowest seed vigour index-I (1091.43) & II (2411.32) were obtained in treatment where no inoculation was done (control). The high values of seed vigour indices in treatment T_3 was the resultant of more germination percentage and seedling length as well as dry weight in treatment T_3. The outcome indicated that seeds derived from treatment T_3 had high capacity to germinate and establish under unfavorable growing conditions. Moreover, the presence of bold seeds in treatment T_3 played a significant role in elevating the vigor indices within this treatment. Maruthi and Paramesh (2016) obtained similar findings in soybean, indicating that the seed vigour indices were enhanced. Similarly, Pandey (2018) also observed an increase in the seed vigour indices in field pea with the application of *Rhizobium*, phosphorus solubilizing bacteria and 100 % N.

Electrical conductivity (μS cm^{-1})

There was a significant variation in electrical conductivity among different seed coating treatments, as evident from the data presented in Table 2. The seeds treated with *Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed and two foliar sprays of $ZnSO_4$ (0.3 %) + $FeSO_4$ (0.3 %) in treatment T_3 exhibited the lowest electrical conductivity (16.75 μS cm^{-1}), which was statistically similar to the treatment T_1 consisting of seed coating with *Rhizobium* @ 30 g kg^{-1} and two foliar sprays of $ZnSO_4$ (0.3 %) + $FeSO_4$ (0.3 %). This indicated that the seeds harvested from these treatments had higher vigour compared to other treatments. On the contrary, the highest electrical conductivity (22.75 μS cm^{-1}) was observed in the control treatment (T_7). The measurement of electrical conductivity is a reliable indicator of seed membrane permeability, providing valuable insights into seed vigour. A higher electrical conductivity value signifies increased leaching of electrolytes, indicating weaker seed membranes and lower seed vigour. The lowest electrical conductivity recorded in treatment T_3 can be attributed to the presence of a robust membrane that restricted electrolyte leaching. The application of *Rhizobium* and phosphate solubilizing bacteria in T_3 might have enhanced nutrient availability for plant growth and development, thereby promoting cell membrane stability and reducing electrolyte leaching. Pandey (2018) also demonstrated similar results in field pea. Furthermore, foliar application of zinc and iron is known to enhance nutrient absorption and accumulation in seeds, contributing to improved cell membrane integrity. The increased stability of the membrane in treatment T_3 might have resulted in minimal electrolyte leaching, and yielding seeds with low electrical conductivity and high vigour. Yogeshwarappa (2005) also observed the lowest electrical conductivity in treatment consisting of 100 % NPK + *Rhizobium* + PSB + micronutrients, supporting the importance of nutrient supplementation in seed vigor. Similarly, Kiran (2006) reported reduced electrical conductivity in brinjal following foliar spray of iron and zinc.

Germination after accelerated ageing test (%)

There was a noticeable reduction in the percentage of germination across various treatments (Table 2). The treatments that exhibited higher germination percentages were associated with the presence of more vigorous seeds compared to other treatments. The seeds with the highest germination (77.75 %) were obtained in treatment T_3, which involved seed coating with *Rhizobium* @ 30 g and PSB @ 30 ml kg^{-1} seed, along with two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %). This treatment showed statistically similar germination performance to T_1, which included seed coating with *Rhizobium* @ 30 g kg^{-1} and two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %). However, the lowest germination (70.00 %) was recorded in control (T_7). The highest germination in treatment T_3 indicated the presence of vigorous seeds in this treatment. This could be attributed to the strong integrity of the cell membrane in the seeds and the higher phosphorus content in the plants, which might have been enhanced through the application of *Rhizobium* and phosphate solubilizing bacteria. Additionally, foliar application of zinc and iron could have contributed to stability of the cell membrane and provided essential nutrients to the developing seedlings. These factors led to the accumulation of more metabolites and photosynthates in the seeds, resulting in their enhanced vigour and increased ability to withstand ageing processes. During artificial ageing, DNA and mRNA can be damaged, and biochemical degradation of stored seed materials can occur, leading to a decline in seed vigor (Murthy *et al*., 2003). The higher germination observed after ageing in treatment T_3 may also be attributed to higher seed weight in this treatment. Maruthi and Paramesh (2016) also documented the highest germination percentage in soybean after artificial ageing of the seed obtained from treatment involving application of recommended dose of fertilizer along with *Rhizobium* and phosphate solubilizing bacteria.

Table 2: Effect of seed coating and foliar spray of micronutrients on electrical conductivity (µS cm^{-1}), germination percentage (%) after accelerated ageing test & cold test in garden pea

	Parameters / Seed coating treatments**	EC (µS cm^{-1})	*AAT (Germination %)	*Cold test (Germination %)
T_1	*Rhizobium* @ 30 g kg^{-1} seed	17.00a	76.75 (8.76)a	79.25 (8.90)a
T_2	PSB (Phosphate solubilizing bacteria) @ 30 ml kg^{-1} seed	19.25b	75.00 (8.66)b	78.75 (8.87)a
T_3	*Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed	16.75a	77.75 (8.82)a	80.00 (8.94)a
T_4	$FeSO_4$ @ 3 g kg^{-1} seed	20.00b	72.25 (8.50)c	75.00 (8.66)b
T_5	$ZnSO_4$ @ 3 g kg^{-1} seed	21.00c	73.00 (8.54)c	76.25 (8.73)b
T_6	Carbendazim @ 2.5 g kg^{-1} seed	19.25b	71.50 (8.46)d	74.75 (8.65)c
T_7	Control	22.75d	70.00 (8.37)d	72.75 (8.53)d
	Mean	19.43	73.75 (8.59)	76.68 (8.76)

	$CD_{0.05}$	1.32	0.10	0.07

***** Figures in the parenthesis represent square root transformed values
**Foliar application of $ZnSO_4$ + $FeSO_4$ each @ 0.3 % was given at two stages in all treatments except control

Germination after cold test (%)
The seeds that exhibited higher germination rates demonstrated their vigour and ability to thrive in adverse environmental conditions. The data collected from the cold test has been presented in Table 2. From the analysis of variance for the cold test data, it was observed that treatment T_3, which involved seed coating with *Rhizobium* @ 30 g kg^{-1} seed and PSB @ 30 ml kg^{-1} seed, along with two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %), recorded the highest germination (80.00 %). This result was statistically comparable to the germination per cent observed in treatment T_1 i.e. seed coating with *Rhizobium* @ 30 g kg^{-1} seed and two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %) and treatment T_2 [seed coating with PSB @ 30 ml kg^{-1} seed and two foliar sprays of $ZnSO_4$ (0.3 %) and $FeSO_4$ (0.3 %)]. On the other hand, the lowest germination percentage of 72.75 % was recorded in treatment T_7 (control). Treatment T_3 had vigorous seeds with robust characteristics, resulting in a higher germination percentage. This might be due to the synergistic effects of biofertilizers, which enhance seed quality by promoting photosynthetic efficiency, facilitating the translocation of nutrients and increasing the accumulation of essential food reserves such as proteins and carbohydrates. Additionally, foliar application of zinc and iron may have contributed to improved photosynthate transport, enhanced nutrient availability within the seeds and bolstered cell membrane integrity, ultimately leading to high seed vigour and germination.

CONCLUSION

From the present investigation, it can be concluded that treatment T_3 (seed coating with *Rhizobium* @ 30 g + PSB @ 30 ml kg^{-1} seed and two foliar sprays of $ZnSO_4$ @ 0.3 % + $FeSO_4$ @ 0.3 % at 50 percent flowering and 15 days later) has performed best among all the treatments tested for quality seed production and significantly enhanced all the seed quality parameters in garden pea.

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