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Effect of seed biopriming on cucumber seed quality and health parameters

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Abstract
Application of bioagents to seed is a practical choice for the safe and inexpensive use of beneficial microorganisms for sustainable agriculture as it focuses to benefit seed during seedling establishment which ultimately leads to improved plant growth throughout the growing season due to presence of advantageous microorganisms colonies established in rhizosphere and is a great alternative to harmful agrochemicals. Therefore considering its benefits to both the environment and plant, ten indigenous isolates of bioagents including five bacterial bioagents (*Bacillus licheniformis* strain B6, *B. pumilus* strain MK5, *B. subtilis* strain RDO10, *Pseudomonas aeruginosa* strain N2S6, *P. fluorescens* strain HB-13) and five fungal bioagents (*Trichoderma atroviride* ta001, *T. atroviride* ta002, *T. atroviride* ta003, *T. atroviride* ta004, *T. atroviride* ta005) were screened for their bio-efficacy as seed treatment of cucumber var. Solan Srijan under laboratory conditions following standard methods of seed quality and health testing as prescribed by International Seed Testing Association. Maximum germination percent, speed of germination, seed vigour parameter viz. seedling length, seedling dry weight, seed vigour index I, seed vigour index II as well as lowest seed microflora percent and seed infection percent were found in seeds bioprimed with *B. pumilus* strain MK5 and *T. atroviride* ta001 among their respective isolates.

Keywords: Bioagents, sustainable, seed, *Bacillus*, *Pseudomonas*, *Trichoderma*

Introduction
Cucumber (*Cucumis sativus* L.), family Cucurbitaceae, is a widely cultivated and most significant cucurbitaceous vegetable. It is an annual creeping vine that climbs trellises or other supports and surrounds them with thin, spiral tendrils and planted as a summer and rainy season crop. Lack of high yielding varieties, promising hybrids, and parthenocarpic types suitable for glass or poly house production are the reasons for the significant productivity gap between India and the rest of the globe. Farmers have become increasingly more dependent on agrochemicals as a relatively reliable way of crop protection that supports the economic stability of their operations. But due to environmental and health hazards, the utilization of agrochemicals in both agriculture and horticulture is becoming more restrictive and many of the chemicals are being prohibited. Since agriculture utilizes numerous resources (such as water, soil, energy, and air) and has a significant negative impact on the environment. As a result, advanced technologies and innovative cultivation techniques are almost entirely focused on minimizing this impact and protecting natural resources for future generations. The key to profitable agriculture is improved seed, which requires good germination in order to produce a healthy crop. Thus, the production of high-quality seed is a crucial component of food security. Seed treatment is a crucial tool to ensure healthy seed and seedling development and to help farmers in improving the crop's genetic and physiological potential. Bioagents may be used as suitable alternative to protect plants from indigenous pathogen populations and reduce the usage of synthetic chemicals and their potentially harmful side effects (Lugtenberg *et al*., 2001) [35]. Biological seed treatment is the most effective and efficient technique to protect seeds from soil-borne diseases at early stage of plant development (Singh *et al*., 2013) [8]. Microbial priming technique, generally known as 'Biopriming' intigrates the biological and physiological aspects of disease control and used as alternative way for managing numerous seed and soil transmitted diseases. Seed imbibition and bioagent inoculation are two components of the biopriming process (Callan *et*

al., 1990) [36]. As other priming method, this treatment improves germination rate and uniformity, but additionally, protects seeds against the soil and seed-borne diseases and microbial contamination. Muller and Berg (2008) [14] discovered that compared to other methods like pelleting and film coating, biopriming is a considerably more successful strategy for managing disease.

The aim of this paper is to highlight the bio-efficacy of different strains of biological control agents like *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp. on seed quality, health parameters in cucumber and therefore results in sustainable agriculture development. Second section of this paper shows the literature on the research work pertaining to the sustainable use of bioagents on plant species carried out in India and abroad by different researchers. The details of the materials used and methodologies adopted during the investigations are presented in third section of the paper. The results obtained from experiment during the study are presented in fourth section and further discussed. Finally, article is concluded at the end.

Theoretical background

Seed treatments with beneficial indigenous microbes have assisted in enhancing yield of many crops by offering protection from pests and diseases as well as a uniform crop stand across a wide range of soil types, cultural methods, and environmental conditions. The effect of fungal biocontrol agent, *Trichoderma* spp. on plant growth as well as yield promotion in various crops has been well documented in literature. The potential of the biocontrol agent *T. harzianum* was investigated by treating soybean seeds with this bioagent. The treated seeds had higher seed germination, seedling growth, yield, longer shoots and seedlings than untreated seeds (Anitha *et al*., 2013) [24]. Pill *et al*., (2009) [25] found faster seedling emergence and greater seedling shoot fresh weight in cucumber seeds treated with aqueous slurries of commercial preparations of *T. harzianum*, *T. virens* and their combination. Soil amendment with *T. harzianum* resulted in 30% increase in seedling emergence in cucumber and a significant increase in the concentration of Cu, P, Fe, Zn, Mn and Na in roots and shoots and 25 and 40% increase in the dry weight of roots and shoots of the inoculated plants was observed (Yedidia *et al*., 2001) [26]. Root colonization by *T. harzianum* provided long lasting resistance against stress and increased plant growth and nutrient uptake by increasing level of plant enzymes such as peroxidases, chitinases, β-1, 3 glucanases, lipoxygenase pathway hydro peroxide lyase and compounds like phytoalexins and phenols (Harman, 2006) [27]. According to Glick (1995) [28], PGPRs produce siderophores that chelate iron and make it available to the plant roots in addition to fixing atmospheric nitrogen that is transported to the plant and leads to improved plant growth. *Bacillus pumilus* and *B. licheniformis*, two PGPRs exhibited strong growth-promoting activities by producing high levels of bioactive C19 gibberellins and resulted in stem elongation in alder (*Alnus glutinosa*) plant (Gutierrez-Manero *et al*., 2001) [29]. The potential of *B. megaterium* to enhance growth in tea plant was investigated and it was found that the bacterium was capable of solubilizing phosphate, producing IAA, siderophore, and antifungal metabolites and hence promoted plant development and lower disease severity (Chakraborty *et al*., 2006) [30]. Similarly, Abeer *et al*., (2015) [31] found that the inoculation of *B. subtilis* significantly improved the root and shoot weight as compared to un-inoculated plants in Indian bassia (*Bassia indica*). Potentially, biopriming application can encourage faster and more even plant development and seed germination (Moeinzadeh *et al*., 2010) [32]. In comparison to the uninoculated control, plants with *Rhizobium* inoculation had more leaves, plant dry weight, and nodules per plant (Gendy, 2013) [33]. The combined application of rhizobial strains (*R. phaseoli*) and plant growth promoting rhizobacteria (*P. syringae* and *P. fluorescens*) under salt-stressed conditions resulted in increased shoot fresh weight, root fresh weight, number of pods per plant, pod fresh weight, total dry matter, relative water content, water use efficiency, potassium concentration in leaves, sodium concentration in leaves, nitrogen concentration in grains of mung bean and improved growth and productivity as compared with the uninoculated control (Ahmad *et al*., 2012) [34]. Based on the theoretical background presented in this section, more attention needs to be given in exploiting bio-control agents by testing them *in-vitro* as well as under heterogeneous field conditions.

Materials and Methods

The 6 months old seeds of cucumber cv. Solan Srijan were collected from the department of Seed Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, (Himachal Pradesh) India. The culture tubes of the bioagents were procured from their respective sources (Table 1) and were grown on the nutrient agar medium ((bacterial bioagents) and potato dextrose medium (*Trichoderma* spp.) for one week. These cultures were maintained at 4 °C in refrigerator and sub-culturing of cultures was done once at fortnight interval on respective medium at incubation temperature of 25±1 °C. Experimental research on seeds/plants, including the collection of plant/seed material, complied with relevant institutional, national and international guidelines and legislations. All methods were carried out in accordance with relevant guidelines and prior approval was undertaken from Director of Research, Dr Y.S. Parmar University of Horticulture and Forestry, Solan (HP), India.

Seed treatment: Apparently healthy seeds with no cracks or other visible deformities were selected and surface sterilized with 1.0% sodium hypochlorite (NaOCl) solution for 3-5 minutes. Seeds were than rinsed three times with sterilized distilled water and dried under laminar air flow chamber on sterilized blotting paper (Jain *et al*., 2012) [9]. The surface sterilized seeds were then soaked in the spore/cell suspensions of bacterial bioagents and *Trichoderma* spp. (10^8 and 10^6 cfu per ml, respectively) for 6-8 hours, separately. The treated seeds were shade dried to bring down their moisture content to original moisture content (Fig. 1).

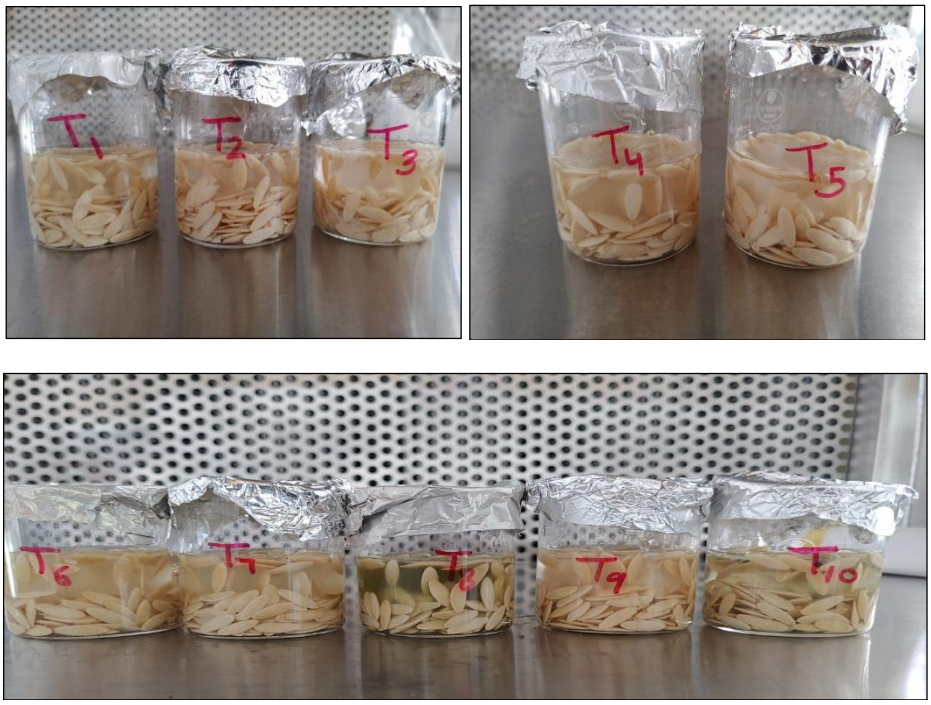

Fig 1: Seed treatment with different bioagents

Table 1: Details of biological control agents used under the study.

Sr. No.	Bioagent	Source
1	*Bacillus licheniformis* strain B6	Soil Microbiology Laboratory, Department of Soil Science and Water Management, Dr YSP UHF, Nauni, Solan (HP)
2	*Bacillus pumilus* strain MK5	-do-
3	*Bacillus subtilis* strain RDO10	-do-
4	*Pseudomonas aeruginosa* strain N2S6	-do-
5	*Pseudomonas fluorescens* strain HB-13	-do-
6	*Trichoderma atroviride* ta001	Biological Control Laboratory, Department of Plant Pathology, Dr YSP UHF, Nauni, Solan (HP)
7	*Trichoderma atroviride* ta002	-do-
8	*Trichoderma atroviride* ta003	-do-
9	*Trichoderma atroviride* ta004	-do-
10	*Trichoderma atroviride* ta005	-do-

Seed germination: Hundred seeds from all replications of each treatment were used for conducting the germination test as per ISTA (Anonymous, 1985) [2]. This was carried out by using rolled paper method in the seed germinator at 25±2 °C and 85 percent relative humidity. The first and final counts of cucumber were taken on 4th and 8th day, respectively. Germination percentage was calculated by using the formula:

$$\text{Germination\%} = \frac{\text{Number of normal seedlings germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Speed of germination: To find out speed of germination, the number of germinated seedlings was counted on each day from 1st day to 8th day and the speed of germination was calculated as described by Maguire (1962) [12].

$$\frac{\text{Number of seedlings emerged}}{1^{st}\text{ day of sowing}} + \ldots\ldots\ldots + \frac{\text{Number of seedlings emerged}}{\text{Day of last count } (8^{th})}$$

Seedling length (cm): The length of seedlings was measured on 8th day of germination test. Ten normal seedlings selected at random were used to work out seedling length. Total seedling length was worked out by taking the total length of seedlings from the tip of the primary leaf to the tip of primary root with the help of scale and the mean value was expressed in centimeters.

Seedling dry weight (mg): Ten seedlings selected for measuring seedling length were also used to work out seedling dry weight. Seedlings were kept in oven at 103 °C±2 for 24 hours and their weight using electronic balance was measured and the mean value was expressed in mg.

Seed vigour index-I (SVI-I): Seed vigour index-I was calculated as per the formula given by Abdul-Baki and Anderson (1973) [1]:

Seedling vigour index-I (SVI-I) = Germination (%) x Seedling length (cm)

Seedling vigour index-II (SVI-II): Seedling vigour index-II was calculated as per the formula given by Abdul-Baki and Anderson (1973) [1]:

Seedling vigour index-II (SVI-II) = Germination (%) x Seedling dry weight (mg)

Seed microflora (%): The microorganisms associated with the seed were observed following standard blotter method and the incidence of microflora was calculated as:

$$\text{Seed microflora (\%)} = \frac{\text{Number of seeds showing microbial growth}}{\text{Total number of seeds used}} X100$$

Percent infected seeds: Seed infection was observed by following standard blotter method as per the recommendations of ISTA (Anonymous, 1985) [2]. The surface sterilized seeds of cucumber were kept on blotter paper (25 seeds per plate, 4 plates per replication). These plates with seeds were incubated at 25 °C temperature for 8 days. The number of infected seeds was observed and percent incidence was calculated by following the method:

$$\text{Percent infected seeds} = \frac{\text{Number of seeds showing microbial growth}}{\text{Total number of seeds used}} X100$$

Statistical analysis: Completely randomized design (CRD) was used to analyze data with four replications in each treatment. The data recorded was analyzed using MS-Excel and OPSTAT at 5% level of significance and all the observations in respect of germination (%), seed microflora (%) and infected seeds (%) were transformed into angular or square-root values.

Results

Effect of biopriming on seed quality parameters

Application of various treatments has a significant impact on seed germination traits. The effects of several treatments on seed germination were significant (Table 2). Treatment T_2 (biopriming with *B. pumilus* strain MK5) had considerably significantly higher germination (87.33%) and was at par with treatments T_4 (biopriming with *P. aeruginosa strain* N2S6) and T_6 (biopriming with *T. atroviride* ta001). Highest speed of germination (19.94) was observed in biopriming with *Bacillus pumilus* strain MK5 (T_2) than other bacterial isolates and biopriming with *T. atroviride* ta001 (T_6) (18.10) among *T. atroviride* strains. As shown in Table 3, biopriming with *T. atroviride* ta001 showed the maximum seedling length (33.04 cm) as well as dry weight (15.66) followed by biopriming with *B. pumilus* strain MK5 (T_2) (Fig. 2). Similarly, Highest seed vigour index-I and seed vigour index-II was observed in treatment T_2 (biopriming with *B. pumilus* strain MK5) 2868.24 and 1360.97 over control.

Table 2: Effect of different strains of biological control agents on germination in cucumber.

Treatment	Germination (%)	Speed of germination
T_1: *Bacillus licheniformis* strain B6	82.67 (9.09)	18.83
T_2: *Bacillus pumilus* strain MK5	87.33 (9.34)	19.94
T_3: *Bacillus subtilis* strain RDO10	81.00 (8.99)	18.18
T_4: *Pseudomonas aeruginosa* strain N2S6	84.33 (9.18)	17.87
T_5: *Pseudomonas fluorescens* strain HB-13	82.00 (9.06)	17.88
T_6: *Trichoderma atroviride* ta001	86.00 (9.27)	18.10
T_7: *Trichoderma atroviride* ta002	81.00 (9.00)	16.28
T_8: *Trichoderma atroviride* ta003	81.33 (9.02)	16.98
T_9: *Trichoderma atroviride* ta004	82.00 (9.05)	16.99
T_{10}: *Trichoderma atroviride* ta005	79.33 (8.91)	16.46
T_{11}: Untreated Control	74.67 (8.64)	14.07
CD $_{(0.05)}$	0.25	2.06

* Figures in parentheses are square root transformed values

Effect of biopriming on seed microflora percent and seed infection.

Seed biopriming with different biological control agents showed significant difference on occurrence of seed microflora (%). Minimum seed microflora (%) was observed in T_2 (biopriming with *B. pumilus* strain MK5) (1.67%) and at par with treatment T_5 (3.00%) and T_6 (2.67%). However, the maximum seed microflora (6.67%) was observed in untreated control. Biopriming with *T. atroviride* ta001 (T_6) resulted in minimum seed infection (0.33%) and was statistically at par with T_2 (biopriming with *B. pumilus* strain MK5). Whereas, maximum seed infection (5.33%) was recorded in untreated control (Table 4).

Table 3: Effect of biopriming with different strains of biological control agents on seed vigour in cucumber.

Treatment	Seedling length (cm)	Seedling dry weight (mg)	Seed vigour index-I (SVI-I)	Seed vigour index-II (SVI-II)
T_1: Bacillus licheniformis strain B6	31.23	14.62	2577.47	1208.42
T_2: Bacillus pumilus strain MK5	32.83	15.59	2868.24	1360.97
T3: Bacillus subtilis strain RDO10	31.59	15.34	2559.96	1240.26
T_4: Pseudomonas aeruginosa strain N2S6	30.10	14.07	2535.08	1186.22
T_5: Pseudomonas fluorescens strain HB-13	32.16	15.48	2638.60	1270.32
T_6: *Trichoderma atroviride* ta001	33.04	15.66	2839.93	1344.10
T_7: *Trichoderma atroviride* ta002	32.56	14.89	2638.01	1206.36
T_8: *Trichoderma atroviride* ta003	31.35	14.66	2546.31	1193.44
T_9: *Trichoderma atroviride* ta004	31.37	14.20	2574.49	1165.08
T_{10}: *Trichoderma atroviride* ta005	30.27	14.16	2397.19	1123.04
T_{11}: Untreated Control	26.57	12.94	1983.54	966.36
CD $_{(0.05)}$	2.70	0.97	241.03	94.15

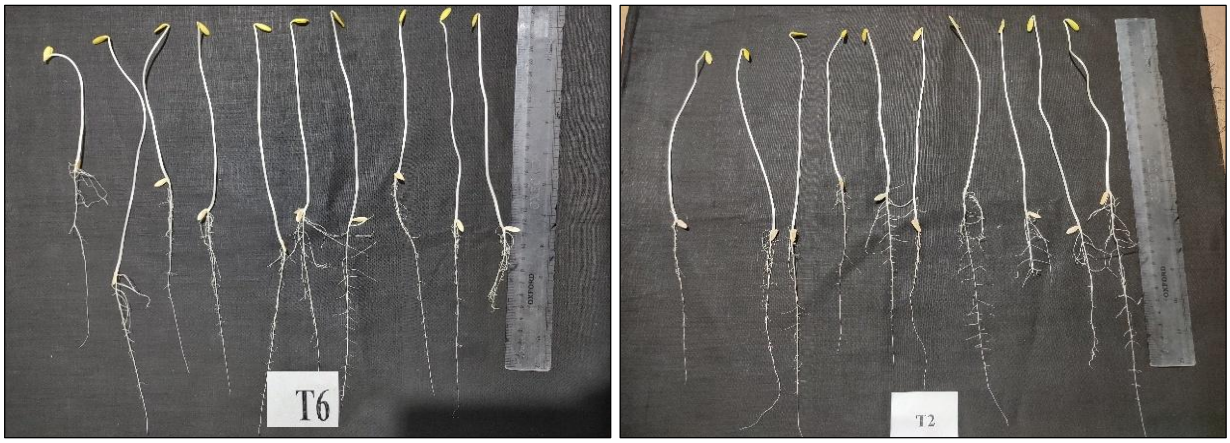

Fig 2: Seedling length after bioagent seed treatment

Table 4: Effect of biopriming with different strains of biological control agents on seed infection and microflora (%) in cucumber.

Treatment	Seed infection (%)	Microflora (%)
T_1: *Bacillus licheniformis* strain B6	1.00 (1.00)	3.33 (1.82)
T_2: *Bacillus pumilus* strain MK5	0.67 (0.67)	1.67 (1.28)
T_3: *Bacillus subtilis* strain RDO10	2.33 (1.47)	4.00 (1.99)
T_4: *Pseudomonas aeruginosa* strain N2S6	2.67 (1.61)	4.33 (2.06)
T_5: *Pseudomonas fluorescens* strain HB-13	1.33 (1.14)	3.00 (1.73)
T_6: *Trichoderma atroviride* ta001	0.33 (0.33)	2.67 (1.63)
T_7: *Trichoderma atroviride* ta002	2.00 (1.41)	5.33 (2.29)
T_8: *Trichoderma atroviride* ta003	1.67 (1.24)	3.67 (1.88)
T_9: *Trichoderma atroviride* ta004	2.00 (1.41)	5.00 (2.20)
T_{10}: *Trichoderma atroviride* ta005	3.00 (1.66)	3.67 (1.91)
T_{11}: Untreated Control	5.33 (2.31)	6.67 (2.54)
$CD_{(0.05)}$	0.56	0.47

* Figures in parentheses are square root transformed values

Discussion

In the present investigation, seed germination (%) was recorded higher with the seed treatment with *B. pumilus* strain MK5 and *T. atroviride* ta001. These results are in agreement with the findings of Sharma *et al*., (2023) [17], Sowmya *et al*., (2022) [19], Bharath *et al*., (2005) [3], Zheng and Shetty (2000) [23], Bharath *et al*., (2006) [4], Nezarat and Gholami (2009) [15] and Noumavo *et al*., (2013) in cucumber, watermelon, pea and maize; who reported the same results upon application of seeds with bioagents on seed germination (%) when compared to control. According to their findings, the increase in germination might be due to an increased synthesis of the phytohormones like gibberellins, which might have appreciably stimulated the activity of germination specific enzymes like α-amylase, proteases and nucleases involved in hydrolysis and assimilation of the starch in bioagents treated seeds. The increase in speed of germination might be due to similar reasons in present investigation. The findings for seedling length and dry weight are in agreement with the findings of Vishwas *et al*., (2017) [22] and Sujaya *et al*., (2019) [21] who noted that variations in seedling length may be associated to the stimulation of plant growth by bioagents that may produce growth-regulating substances like hormones upon seed imbibitions. The increase in seedling length might be due to establishment of endophytic population in rhizoplane and interior of roots which have adaptability to niche. This establishment is because of ability of bioagents to colonize the roots and it's interior and thus benefits the seedling and increase length. Increased seedling dry weight might be due to differences in germination seedling length correlated with the stimulatory effects of bioagents on plant growth.

Seed vigour index-I (SVI-I) and seed vigour index-II (SVI-II) was recorded maximum under seed treatment with *B. pumilus* strain MK5 and *T. atroviride* ta001. The vigour index values are closely correlated with an increase in seedling growth or dry matter production. Similar results were recorded by Indra *et al*., (2006) [8], Kanchana *et al*., (2014) [10], Subapriya and Geetha (2019) [20], Miljakovic *et al*., (2022) [13] and Sharma *et al*., (2023) [17]. This increase in seed vigour index might be due to the ability of bioagents to synthesize auxins, cytokinins, gibberellins, and salicylic acid accounts for the majority of the beneficial effects like cellular elongation and cellular division (Egamberdieva *et al*., 2017) [6].

Hadar *et al*., (2006) [7] also have recorded the antagonistic activities of *Trichoderma* against various storage pathogens. Occurrence of minimum seed microflora (%) and percent seed infection in bioagent treated seeds might be due to production of microbially active compounds, such as lytic enzymes, toxins, biocidal volatiles, and siderophores that chelate iron and antibiotics. Synthesis of various extracellular hydrolytic enzymes like chitinase and-1, 3-glucanase degrades the chitin and-1, 3-glucan constituent of cell wall plays an important role in antagonistic activities of bioagents against pathogens.

Conclusion

A safer substitute for the potentially dangerous agrochemicals is the application of different agriculturally significant microorganisms in agriculture. It can be concluded from the findings of present study that treatment comprised of seed priming with Bioagents viz. *Trichoderma atroviride* (ta001) and *Bacillus pumilus* was found superior

treatment for increasing seed quality seed germination, speed of germination, seedling length, seedling dry matter and seed vigour. This treatment also provided the control of seed infection as well as seed microflora.

Author contributions

NK Bharat[1] designed the investigation and contributed in the execution of the experiment as well as reviewing the whole script. Arshia[*1] and IshanT_2 contributed to conducting the study, data collection, analyze data and interpret the findings.

Research ethics

All relevant institutional, national, and international rules and regulations were followed when conducting experimental research on seeds or plants, including the collection of seed/plant material. Prior approval was undertaken from Director of Research, Dr Y.S. Parmar University of Horticulture and Forestry, Solan (HP), India.

Data availability

This manuscript does not have any supplementary data and the corresponding author will provide the original data if needed.

Competing Interests

The authors declare no competing interests.

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