RESEARCH ARTICLE Legume Research- An International Journal, Volume 49 Issue 5: 869-875 (May 2026)

Influence of Seed Bio-priming with Microbial Inoculants on Germination, Seedling Growth, Vigour and Enzyme Activity in Chickpea

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10.18805/LR-5403

ABSTRACT

Background: Chickpea (*Cicer arietinum* L.), a staple pulse crop with significant nutritional, economic and agricultural importance, is known for its diverse uses and contributions to both human diet and livestock feed. To enhance its productivity and resilience, particularly in the face of abiotic and biotic stresses, innovative seed enhancement techniques such as bio-priming with plant endophytes have emerged as a promising approach to improve seed quality and overall crop performance.

Methods: In this laboratory investigation during 2022, chickpea seeds were surface sterilized with sodium hypochlorite (1%) for five minutes and then washed thrice with sterile water. Later the seeds were bio-primed with different bioagents with the seed to solution ratio of 1:3 for different duration hours. Blotter method was used to observe seed germination. Primed seeds were dried back to original moisture content (24 hrs) and then used to assess the seed quality parameters.

Result: The results showed that, priming chickpea seeds with *Paenibacillus polymyxa* for 6 hours resulted in significantly enhanced seedling growth and quality compared to all other treatments and control indicating its effectiveness in improving seed quality and growth.

Key words: Bio-priming, Chickpea, Enzyme activity, *Paenibacillus polymyxa,* Seed germination, Seedling vigour.

INTRODUCTION

Chickpea, known scientifically as *Cicer arietinum* L. and commonly referred to as gram, Bengal gram, or Spanish pea, is one of the oldest and most significant pulses cultivated both in Asia and Europe. As a major contributor to pulse production, chickpea holds a crucial position in terms of area, consumption and its role in sustaining subsistence farming systems. In India, chickpea is an important protein source in the human diet and is used in various forms including split chickpea (dal), 'Besan', fresh green leaves (sag) and as a vegetable (chhole). The plant's by-products, such as husks and dal bits, are valuable cattle feed, while its straw serves as excellent fodder. Chickpea is also noted for its medicinal value, particularly in blood purification.

Seeds are the fundamental and most essential component for achieving sustainable agriculture. Poor and slower seed germination limits seedling growth, ultimately reducing crop yield. The successful establishment of early seedlings requires rapid and uniform emergence and root growth. Developing techniques for fast and homogeneous seed growth could be a sustainable approach to improving agricultural productivity (Osburn and Schroth, 1988). In this regard, "seed priming" is a sustainable method to enhance seed quality, germination and establishment, leading to improved yields and plant performance. Primed seeds have been shown to withstand various abiotic and biotic stresses, resulting in better seed emergence and crop productivity (Roy *et al*., 2024). Priming improves various biochemical processes including synthesis of various antioxidant enzymes, soluble sugar, regulates hormonal functions and thus improve plant growth. The seedlings from primed seeds exhibit enhanced activities of catalase (CAT), peroxidase (POD), glutathione reductase (GR). They also regulate proteins like aquaporins, tonoplastic intrinsic proteins, dehydrins, lateembryogenic proteins and initiate various metabolic processes including protein synthesis, DNA repair (Diya *et al*., 2024). Over the years, various plant

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How to cite this article: Koradhanyamath, P., Basave Gowda, Shakuntala, N.M., Doddagoudar, S.R., Arunkumar, H., Savitha, A.S. and Satyanarayan, R. (2026). Influence of Seed Bio-priming with Microbial Inoculants on Germination, Seedling Growth, Vigour and Enzyme Activity in Chickpea. *Legume Research*. **49(5):** 869-875. doi: 10.18805/LR-5403.

Submitted: 15-08-2024 **Accepted:** 31-03-2025 **Online:** 16-08-2025

growth-promoting microbes have been used to biologically enhance seeds. Among these, plant endophytes are increasingly gaining attention in agricultural research. Seed bio-priming is being focused as it ensures the entrance of endophytes into the sides along with avoiding the effect of high temperature (Pramod *et al*., 2022).

Endophytes are microorganisms that reside in plant tissues for all or part of their life cycle without causing visible harm (Wilson, 1993). Due to their symbiotic nature, these endophytes can colonize a diverse array of plant hosts and are increasingly recognized for their potential in seed bio-priming. This technique combines biological and physiological approaches to enhance seed protection and growth (Afzal *et al*., 2016). Endophytes can influence interactions with pests and pathogens, offering potential as biocontrol agents. The use of endophytes in seed bio-priming can significantly improve various aspects of seed and plant development. These benefits include enhanced germination rates, increased inorganic phosphate uptake, better overall plant growth and ultimately, higher seed yield and quality. The primary aim of this study was to evaluate how seed bio-priming with endophytes affects key seed quality parameters such as germination and vigour.

MATERIALS AND METHODS

Seeds and culture collection

The present investigation was carried out at Seed Unit, UAS, Raichur during the year 2022. Chickpea variety JG-11 seeds were obtained from the Seed Unit at UAS, Raichur. *Rhizobium* bioagents were sourced from the Bio-input Entrepreneurship Centre at the College of Agriculture, Raichur. Additionally, six endophytes were sourced from the ICAR-National Bureau of Agriculturally Important Microorganisms in Kushmaur, Mau, Uttar Pradesh, while two endophyte cultures were acquired from the Department of Plant Pathology at the University of Agricultural Sciences, Raichur, Karnataka.

Preparation of endophyte inoculums

Potato dextrose broth (PDB) was prepared for fungal cultures, while nutrient broth (NB) was used for bacterial cultures. Fungal cultures were added to Potato Dextrose Broth (PDB), whereas bacterial cultures were added to Nutrient Broth (NB). These cultures were incubated at 25± 2°C for 14 days for fungi and 2 days for bacteria. Following incubation, the culture filtrates were obtained by filtering through pre-sterilized conical flasks using Whatman No. 1 filter paper. The filtrates were then stored at 4°C in a refrigerator until used for seed bio-priming. The concentration of the bio-priming agents was determined using a hemocytometer under a light microscope. For fungal cultures, the concentration was calibrated to 1×10^3 conidia/ml and for bacterial cultures it was calibrated to 1×10^8 cfu/ml.

Seed bio-priming protocol

Chickpea seeds were subjected to surface sterilization (1%) for five minutes and then rinsed three times with sterile water. Later the seeds were primed with the seed to solution ratio of 1:3 for chickpea using different bio priming agents as per factor-II for different durations hours as per factor-I and then the primed seeds were dried back to original moisture content (24 hrs) and then used to assess the seed quality parameters by following standard procedure of International Seed Testing Association (Anonymous, 2016). Each treatment was allocated with 4 replications, each replication with 50 seedlings. Seed quality parameters were assessed at the end of the eighth day. Statistical analysis was conducted using a Two-Factorial Completely Randomized Design and DMRT analysis was done using R-software.

List of bioagents and soaking duration used in the study.

Factor-I : Soaking duration	Factor II: Bioagents
H_1: 2 hours	T_1: *Bacillus amyloliquefaciens* (Bacterial culture 1×10^8 cfu ml^{-1})
H_2: 4 hours	T_2: *Bacillus subtilis* (Bacterial culture 1×10^8 cfu ml^{-1})
H_3: 6 hours	T_3: *Fusarium nygamai* (Fungal culture 1×10^3 conidia ml^{-1})
	T_4: *Bacillus cereus* (Bacterial culture 1×10^8 cfu ml^{-1})
	T_5: *Paenibacillus polymyxa* (Bacterial culture 1×10^8 cfu ml^{-1})
	T_6: *Piriformospora indica* (Fungal culture 1×10^3 conidia ml^{-1})
	T_7: *Trichoderma hamatum* (Fungal culture 1×10^3 conidia ml^{-1})
	T_8: *Stenotrophomonas maltophilia* (Bacterial culture 1×10^8 cfu ml^{-1})
	T_9: *Rhizobium* (Bacterial culture 1×10^8 cfu ml^{-1})
	T_{10}: Control

RESULTS AND DISCUSSION

This experiment was performed to evaluate the role of bioagents on seed quality in chickpea. A total of 10 bioagents were studied. Seed priming with these bioagents had shown a notable effect on chickpea.

Influence of bio-priming on seed quality parameters of chickpea

Significant influence on germination percentage, seedling length, dry weight, seedling vigour index-I and II, speed of germination and electrical conductivity of chickpea seeds were noted for various different bio agents and duration of priming (Table 1). With respect to priming duration, priming of chickpea seeds with different bio agents for seed germination percentage was determined to be non-significant but significant for seedling length, dry weight, seedling vigour index-I and II, speed of germination and electrical conductivity. However, seed priming for 4 and 6 hours had recorded maximum seed germination (95.1%)

Table 1: Influence of bio-priming on seed germination (%), seedling length (cm), seedling dry weight (g), seedling vigour index-I and seedling vigour index-II of chickpea.

Treatments (T)	Priming duration (H)																			
	Seed germination (%)				Seedling length (cm)				Seedling dry weight (g)				Seedling vigour index-I				Seedling vigour index-II			
	2	4	6	Mean	2	4	6	Mean	2	4	6	Mean	2	4	6	Mean	2	4	6	Mean
T_1: *Bacillus amyloliquefaciens* (1×10^8 cfu ml^{-1})	96.0^{ab}	96.0^{a}	98.5^{c}	96.8	36.5^{d}	35.8^{a}	40.8^{a}	37.7	2.2^{f}	2.1^{c}	2.5^{ef}	2.3	3504de	3437^{cd}	4019^{ef}	3653	211^{fg}	202^{c}	246^{d}	220
T_2: *Bacillus subtilis* (1×10^8 cfu ml^{-1})	94.5^{ab}	97.0^{a}	97.5^{bc}	96.3	33.6^{c}	36.9^{de}	38.4^{e}	36.3	1.9^{cd}	2.2^{cd}	2.4^{e}	2.2	3175^{c}	3579de	3744^{d}	3500	180^{d}	213^{cd}	234^{d}	209
T_3: *Fusarium nygamai* (1×10^3 conidia ml^{-1})	94.0^{ab}	94.5^{a}	91.5^{ab}	93.3	33.0^{bc}	33.1^{ab}	29.6^{ab}	31.9	1.8^{c}	1.8^{a}	1.5^{a}	1.7	3102^{bc}	3128^{ab}	2708^{a}	2979	169^{c}	170^{ab}	137^{ab}	159
T_4: *Bacillus cereus* (1×10^8 cfu ml^{-1})	92.5^{ab}	93.5^{a}	94.5^{abc}	93.5	31.1^{ab}	32.5^{ab}	33.5^{cd}	32.4	1.6^{ab}	1.7^{a}	1.8^{c}	1.7	2877^{ab}	3039^{ab}	3166^{c}	3027	148^{b}	159^{a}	170^{c}	159
T_5: *Paenibacillus polymyxa* (1×10^8 cfu ml^{-1})	97.5^{b}	97.5^{a}	99.0^{c}	98.0	37.4^{d}	38.1^{e}	41.5^{f}	39.0	2.2^{f}	2.3^{d}	2.6^{f}	2.4	3647^{e}	3715^{e}	4109^{f}	3823	215^{g}	224^{d}	248^{d}	229
T_6: *Piriformospora indica* (1×10^3 conidia ml^{-1})	96.0^{ab}	96.0^{a}	98.0^{bc}	96.7	36.7^{d}	35.6^{cd}	39.3^{ef}	37.2	2.2^{f}	2.1^{c}	2.4^{e}	2.2	3523de	3418^{cd}	3851de	3597	211^{fg}	202^{c}	235^{d}	216
T_7: *Trichoderma hamatum* (1×10^3 conidia ml^{-1})	95.5^{ab}	94.0^{a}	95.5^{abc}	95.0	35.2^{cd}	33.0^{ab}	34.7^{d}	34.3	2.1^{ef}	1.8^{ab}	1.9^{d}	1.9	3362^{cd}	3102^{ab}	3314^{c}	3259	201^{f}	169^{ab}	181^{c}	184
T_8: *Stenotrophomonas maltophilia* (1×10^8 cfu ml^{-1})	92.5^{ab}	94.0^{a}	92.50^{abc}	93.0	31.0^{ab}	32.7^{ab}	31.7^{bc}	31.8	1.6^{b}	1.8^{ab}	1.6^{b}	1.7	2868^{ab}	3074^{ab}	2932^{b}	2958	148^{b}	169^{ab}	148^{b}	155
T_9: *Rhizobium* (1×10^8 cfu ml^{-1})	95.5^{ab}	95.0^{a}	95.0^{abc}	95.2	35.2^{cd}	34.3^{bc}	34.0^{d}	34.5	2.0^{de}	1.9^{b}	1.9^{d}	1.9	3362^{cd}	3259^{bc}	3230^{c}	3283	191^{e}	181^{b}	181^{c}	184
T_{10}: Control	91.5^{a}	93.0^{a}	89.0^{a}	91.2	29.4^{a}	31.3^{a}	28.9^{a}	29.9	1.5^{a}	1.7^{a}	1.4^{a}	1.6	2690^{a}	2911^{a}	2572^{a}	2724	137^{a}	158^{a}	134^{a}	143
Mean	94.6	95.1	95.1		33.9	34.3	35.2		1.9	1.9	2.0		3211	3266	336^{5}		181	185	191	
Factors	H	T	H×T		H	T	H×T		H	T	H×T		H	T	H×T		H	T	H×T	
S.Em±	0.4	0.8	1.4		0.1	0.3	0.5		0.01	0.01	0.03		14	26	45		1	1	2	
CD @ 1%	NS	2.4	4.2		0.5	0.9	1.5		0.02	0.05	0.08		40	73	127		2	3	6	

Legend NS: Non significant.

and (95.1%) compared to 2 (94.6%) hours. Seed priming for 6 hours had recorded maximum seedling length (35.2 cm), seedling dry weight (2.0 g), seedling vigour index-I (3365), seedling vigour index II (191), speed of germination (21.0) and minimum electrical conductivity (0.60 dsm^{-1}) compared to 2 (33.9 cm), (1.9 g), (3211), (181), (20.4) (0.65 dsm^{-1}) and 4 (34.3 cm), (1.9 g), (3266), (185), (20.5), (0.62 dsm^{-1}) hours respectively.

While comparing the different bio agents, significantly higher seed germination (98.0%), seedling length (39.0 cm), seedling dry weight (2.4 g), seedling vigour index-I (3823), seedling vigour index II (229), speed of germination (22.1) and lower electrical conductivity (0.37 dsm^{-1}) was noticed in chickpea due to *Paenibacillus polymyxa* relative to other treatments and control (91.2%) (29.9 cm), (1.6 g), (2724), (143), (19.6), (0.93 dsm^{-1}) respectively. Among the interactions priming chickpea seeds with *Paenibacillus polymyxa* for 6 hrs had showed the maximum seed germination rates significantly (99.0%), seedling length (41.5 cm), seedling dry weight (2.6 g), seedling vigour index-I (4109), seedling vigour index II (248), speed of germination (22.8) and lower electrical conductivity (0.30 dsm^{-1}) as relative to all other treatments. Control had showed the minimum seed germination (89.0%), seedling length (28.9 cm), seedling dry weight (1.4 g), seedling vigour index-I (2572), seedling vigour index II (134), speed of germination (19.4) and highest electrical conductivity (1.01 dsm^{-1}) primed for 6 hrs.

From the results it was found that *Paenibacillus polymyxa* significantly improved the seed quality parameters. This could be attributed to production of plant growth hormones like GA and IAA and also production of secondary metabolites. Increased GA and IAA might trigger the enzyme activity of α-amylase which is responsible for early germination through maximizing the availability of starch assimilation, GA and IAA might have played an important role on seed germination and radical length (Maiyappan *et al*., 2010). Endophytes are known to produce a wide variety of phytohormones including gibberellins (GAs), auxin (IAA) and abscisic acid (ABA) and IAA (You *et al*., 2012). Indole-3-acetic acid (IAA) and auxin identified as the most abundant and wide spread that mediates an enormous range of developmental and growth responses including embryo symmetry establishment, initiation of cell division, promote vascular differentiation, root initiation and apical dominance as well as environmental responses such as gravitropism and phototropism. Besides its hormonal functions, indole-3-acetic acid (IAA) is involved in the stimulation of the ethylene synthesis. Reports of earlier work showed that the ability of endophytic fungi to produce gibberellins (GAs) in pure culture and their role in promotion of germination has widely been reported (Hamayun *et al*., 2009) in soybean.

The seeds of pulse crops prone to imbibition injury below or above soaking periods hence a safe period of soaking in priming medium is necessary. Reports of earlier workers have indicated four to eight hours soaking suitable for the bean and related crops. (Ghassemi-Golezani *et al.,* 2010 in pintobean; Shah *et al*., 2012 in mungbean) found maximum priming hours was four hour for one variety and six hour for another under early moisture stress condition and concluded that varieties of same crop might require varied priming hours. So, duration of priming is very important and it differs for each crop and variety.

Speed of germination was attributed to higher per cent germination of seeds over time. From the results it was evident that *Paenibacillus polymyxa* found having higher speed of germination, this could be attributed to secretion of certain harmones such as cytokinin and auxin, which stimulates for better absorption of water which in turn helps in germination of seeds (Zahir *et al*., 2004) thus the rate of germination, might have improved. Similar outcomes were also noted by Shukla *et al*. (2015) in wheat; Piri *et al*. (2019) in cumin. The electrical conductivity in seeds bio primed with *Paenibacillus polymyxa* was less over control this may be due to protection of the seeds by the endophyte from infecting pathogens, thus reduces the seed infection, cracks and aberrations of the seed coat and reduce the leaching of the electrolytes. Comparable results were also observed by Umadi *et al*. (2018) in soybean; Estevez-Geffriaud *et al.* (2020) in maize.

Influence of bio-priming on enzyme activities of chickpea

The data regarding dehydrogenase activity (OD value), catalase activity and peroxidase enzyme activity shown in Table 2 revealed significant differences among various bio agents and duration of priming. Significant differences for dehydrogenase activity (OD value), catalase activity and peroxidase enzyme activity were noted with respect to priming duration. Higher dehydrogenase activity (0.73), catalase activity (0.47 µmol min^{-1} mg^{-1} protein) and peroxidase enzyme activity (2.16 µmol min^{-1} mg^{-1} protein) was recorded in seed priming for 6 hours compared to 2 (0.70 µmol min^{-1} mg^{-1} protein), (0.45 µmol min^{-1} mg^{-1} protein), (2.09 µmol min^{-1} mg^{-1} protein) and 4 (0.71 µmol min^{-1} mg^{-1} protein), (0.46 µmol min^{-1} mg^{-1} protein), (2.12 µmol min^{-1} mg^{-1} protein) hours respectively.

The experimental results of dehydrogenase activity (OD value), catalase activity and peroxidase enzyme activity with respect to different bio agents in chickpea was revealed statistically significant. Significantly higher dehydrogenase activity (0.87), catalase activity (0.65 µmol min^{-1} mg^{-1} protein) and peroxidase enzyme activity (2.73 µmol min^{-1} mg^{-1} protein) was noticed in chickpea due to *Paenibacillus polymyxa* followed by *Bacillus amyloliquefaciens* (0.84) (0.60 µmol min^{-1} mg^{-1} protein) (2.62 µmol min^{-1} mg^{-1} protein) *Piriformospora indica* (0.83) (0.58 µmol min^{-1} mg^{-1} protein) (2.58 µmol min^{-1} mg^{-1} protein) and *Bacillus subtilis* (0.80) (0.55 µmol min^{-1} mg^{-1} protein) (2.38 µmol min^{-1} mg^{-1} protein) and differed significantly over uninoculated control (0.55) (0.29 µmol min^{-1} mg^{-1} protein) (1.57 µmol min^{-1} mg^{-1} protein) respectively. Perusal of Table 2 revealed that interactions

Table 2: Influence of bio-priming on speed of germination, electrical conductivity (dsm^{-1}), dehydrogenase activity (OD value), catalase enzyme activity (µmol min^{-1} mg^{-1} protein) and peroxidase enzyme activity (µmol min^{-1} mg^{-1} protein) of chickpea.

Treatments (T)	Priming duration (H)																			
	Speed of germination				Electrical conductivity (dsm^{-1})				Dehydrogenase activity (OD value)				Catalase enzyme activity (µmol min^{-1} mg^{-1} protein)				Peroxidase enzyme (µmol activity min^{-1} mg^{-1} protein)			
	2	4	6	Mean	2	4	6	Mean	2	4	6	Mean	2	4	6	Mean				
T_1: *Bacillus amyloliquefaciens* (1×10^8 cfu ml^{-1})	21.1^{bc}	20.6^{abc}	22.4^{b}	21.4	0.43^{a}	0.54^{b}	0.33^{ab}	0.43	0.83^{c}	0.80^{cd}	0.88^{cd}	0.84	0.59^{e}	0.56^{e}	0.65^{e}	0.60	2.56^{de}	2.45^{c}	2.85^{g}	2.62
T_2: *Bacillus subtilis* (1×10^8 cfu ml^{-1})	20.4^{abc}	21.3^{bc}	22.1^{b}	21.3	0.65^{d}	0.42^{a}	0.37^{c}	0.48	0.69^{c}	0.84^{cd}	0.87^{cd}	0.80	0.43^{c}	0.60^{f}	0.61^{d}	0.55	1.95^{b}	2.55^{cd}	2.63^{f}	2.38
T_3: *Fusarium nygamai* (1×10^3 conidia ml^{-1})	20.1^{ab}	20.3^{ab}	19.5^{a}	20.0	0.72^{e}	0.69^{d}	0.92^{g}	0.78	0.63^{ab}	0.66^{ab}	0.54^{ab}	0.61	0.37^{b}	0.42^{c}	0.27^{a}	0.35	1.84^{b}	1.93^{b}	1.48^{ab}	1.75
T_4: *Bacillus cereus* (1×10^8 cfu ml^{-1})	19.6^{a}	19.9^{ab}	20.3^{a}	19.9	0.85^{f}	0.80^{f}	0.65^{e}	0.77	0.55^{ab}	0.56^{ab}	0.68^{a}	0.60	0.29^{a}	0.34^{ab}	0.39^{b}	0.34	1.62^{a}	1.75^{a}	1.86^{c}	1.74
T_5: *Paenibacillus polymyxa* (1×10^8 cfu ml^{-1})	21.6^{c}	21.8^{c}	22.8^{b}	22.1	0.41^{a}	0.39^{a}	0.30^{a}	0.37	0.85^{c}	0.86^{d}	0.89^{d}	0.87	0.64^{f}	0.63^{f}	0.67^{e}	0.65	2.67^{e}	2.65^{d}	2.86^{g}	2.73
T_6: *Piriformospora indica* (1×10^3 conidia ml^{-1})	20.7a^{bc}	20.6^{abc}	22.7^{b}	21.3	0.49^{b}	0.50^{b}	0.34^{b}c	0.44	0.83^{c}	0.80^{cd}	0.87^{cd}	0.83	0.58^{e}	0.54^{e}	0.61^{d}	0.58	2.52^{d}	2.47^{c}	2.74^{fg}	2.58
T_7: *Trichoderma hamatum* (1×10^3 conidia ml^{-1})	20.5^{abc}	20.2^{ab}	20.5^{a}	20.4	0.54^{c}	0.70^{d}	0.57^{d}	0.60	0.78^{bc}	0.65^{bc}	0.73^{bc}	0.72	0.52^{d}	0.36^{b}	0.48^{c}	0.45	2.36^{c}	1.82^{a}	2.12^{e}	2.10
T_8: *Stenotrophomonas maltophilia* (1×10^8 cfu ml^{-1})	19.6^{a}	20.0^{ab}	19.8^{a}	19.8	0.89^{f}	0.76^{e}	0.83^{f}	0.83	0.54^{a}	0.61^{a}	0.56^{a}	0.57	0.30^{a}	0.35^{ab}	0.28^{a}	0.31	1.64^{a}	1.79^{a}	1.59^{b}	1.67
T_9: *Rhizobium* (1×10^8 cfu ml^{-1})	20.5^{abc}	20.5^{ab}	20.4^{a}	20.5	0.55^{c}	0.60^{c}	0.63^{e}	0.59	0.75^{bc}	0.71^{bc}	0.70^{bc}	0.72	0.51^{d}	0.46^{d}	0.45^{c}	0.47	2.24^{c}	2.01^{b}	1.99^{d}	2.08
T_{10}: Control	19.5^{a}	19.8^{a}	19.4^{a}	19.6	0.95^{g}	0.83^{f}	1.01^{h}	0.93	0.54^{a}	0.56^{a}	0.54^{a}	0.55	0.28^{a}	0.32^{a}	0.26^{a}	0.29	1.53^{a}	1.74^{a}	1.45^{a}	1.57
Mean	20.4	20.5	21.0		0.65	0.62	0.60		0.70	0.71	0.73		0.45	0.46	0.47		2.09	2.12	2.16	
Factors	H	T	H×T		H	T	H×T		H	T	H×T		H	T	H×T		H	T	H×T	
S.Em±	0.1	0.1	0.2		0.004	0.006	0.011		0.003	0.006	0.011		0.003	0.005	0.009		0.01	0.02	0.03	
CD @ 1%	0.2	0.4	0.7		0.010	0.018	0.032		0.010	0.018	0.031		0.008	0.015	0.026		0.03	0.05	0.09	

effect between duration of priming and bio control agents for dehydrogenase activity (OD value), catalase activity and peroxidase enzyme activity was assessed to be statistically significant. Among the interactions priming chickpea seeds with *Paenibacillus polymyxa* for 6 hrs had achieved significantly the higher level dehydrogenase activity (0.89), catalase activity (0.67 µmol min^{-1} mg^{-1} protein) and peroxidase activity (2.86 µmol min^{-1} mg^{-1} protein) as comparision to all other treatments and control (0.54) (0.26 µmol min^{-1} mg^{-1} protein) (1.45 µmol min^{-1} mg^{-1} protein) primed for 6 hrs respectively.

All living seeds undergo respiration, which leads to the production of enzymes such as dehydrogenase. Therefore, dehydrogenase activity is commonly used as a biomarker to assess seed *via*bility. An enhancement in dehydrogenase activity following seed priming can be attributed to the activation of enzyme activity during the priming process and heightened cell cycle activity, potentially stimulated by endophytes. Similar enhancements in dehydrogenase activity due to seed priming have been observed in previous studies. For example, Sharma *et al*. (2017) observed increased dehydrogenase activity in Aloe vera, while Devi *et al*. (2021) reported similar findings in pigeon pea. These studies imply that priming can effectively enhance enzymatic activity, reflecting improved seed *via*bility and potential for successful germination.

Paenibacillus polymyxa was found to be superior and effective in increasing catalase and peroxidase activity this might be because of growth hormones produced by these endophytes increses the antioxidant enzymes like catalase and peroxidase. The growth harmones like GA3 counteracts the free radicals produced due to oxidative damage by increasing the production of osmolytes like proline, protein and sugar contents and antioxidant enzymes like peroxidase, polyphenol oxidase and catalase also overcome the limitations created by environmental stress such as osmotic effects, ion toxicity and nutritional imbalance which promote better seedling growth and ultimately improve the plant vigour (Jamil and Rha, 2007) in sugar beet. Lalngaihawmi *et al*. (2018) opined that with respect to time and concentration, the rice seeds dipped in 100 per cent concentration of *Pencillium citrinum* for 30 minutes recorded the highest dehydrogenase activity, catalase activity and peroxidase activity compared to 15 minutes.

CONCLUSION

Based on the laboratory experiments, *Paenibacillus polymyxa* was identified as the most effective bio-priming agent for improving seed quality parameters in chickpea, outperforming other tested bio-agents. Among various priming durations, a six-hour treatment was determined to be the most effective. The combination of *Paenibacillus polymyxa* with a six-hour priming period yielded the best results concerning seed quality parameters evaluated. The investigation indicates that low-quality seeds benefit from bio-priming treatments to enhance their quality before sowing. This study illustrates that seed bio-priming can significantly improve seed quality in chickpea. Therefore, it is recommended to treat chickpea seeds with suitable bio-agents prior to sowing to achieve better seed quality.

ACKNOWLEDGEMENT

I would like to acknowledge my guide, my advisory committee members and the Head of the Seed Unit for providing the necessary facilities and encouragement that greatly supported the completion of this research.

Conflict of interest

All authors declared that there is no conflict of interest.

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