Biological Forum – An International Journal **15(10): 885-891(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In vivo evaluation of seed biopriming with *Bacillus, Pseudomonas* sp. and actinobacterial isolates on seed germination and seedling vigour of chilli (*Capsicum annuum* L.)

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(Received: 12 August 2023; Revised: 02 September 2023; Accepted: 26 September 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: This research was conducted at the Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, over the period of 2019-2021. Seed priming, a technique involving the hydration of seeds to activate metabolic processes without actual germination, followed by subsequent drying, has been recognized for its ability to enhance germination rates, seedling establishment, and stress tolerance across various crops. In this particular study, our objective was to assess the efficacy and potential of three distinct bacterial strains in promoting seedling vigour and improving germination percentages in the Byadgi dabbi, chilli variety. To achieve this, we employed a bio-priming method in which the chilli seeds were soaked in solutions containing eight different isolates of *Bacillus, Pseudomonas* sp. and actinobacteria strains for a standardized duration of 12 hours. Among the eight isolates from these three bacterial groups, *B. subtilis*, AUDP209, and AUDT636 exhibited significant and positive responses regarding seed germination rates, with values of 71.00%, 73.00%, and 75.00%, respectively. Similarly, these three isolates displayed enhanced seedling vigour, with corresponding values of 1168, 1104, and 1231, surpassing the performance of other isolates and the control group. Moreover, these specific isolates also led to greater root and shoot lengths. When comparing the three bacterial groups, it was observed that actinobacteria, which includes AUDP209 and AUDT636, proved to be the most effective in terms of enhancing both germination percentages and seedling vigour. This research sheds light on the potential benefits of employing specific bacterial strains, such as B. subtilis, AUDP209, and AUDT636, for bio-priming in chili cultivation, which can significantly contribute to improved seed germination and seedling development, ultimately enhancing crop productivity.

Keywords: Actinobacteria, *Bacillus* sp., *Pseudomonas* sp., Seed biopriming, Seedling vigour.

INTRODUCTION

Chilli, scientifically known as *Capsicum annuum* L., holds a significant status as a global vegetable crop, owing to its immense importance and economic value. Indian chilli, in particular, enjoys worldwide recognition for two key commercial attributes: its vibrant colour and its pungency levels. This member of the solanaceous family is primarily cultivated for its green, edible fruits and for dried chilli, a highly sought-after spice in the global market. Moreover, it serves as a rich source of essential vitamins such as C, A, and B.

In the Indian context, chilli is a vital cash crop, cultivated to meet both domestic consumption and international export demands. India stands as the foremost producer and exporter of chilli globally, with an impressive production exceeding one million metric tons in the year 2020. Leading the pack in chilli production within India are states like Telangana, Karnataka, Madhya Pradesh, Orissa, Gujarat, Assam, Punjab, Rajasthan, Uttar Pradesh, and Mizoram. Over the past three decades, the cultivation area dedicated to ripe, red, and dry chilli fruits in India has fluctuated, ranging from 634 to 921 thousand hectares. This variable landscape has yielded a total production of dry chilli ranging from 364 to 895 thousand tons, with an average yield per hectare fluctuating between 574 and 957 kilograms. Notable among the regions contributing to this production are the districts of Dharwad, Nagpur, Prakasam, Khammam, Guntur, and Warangal. In Dharwad district, specifically, the chilli cultivation area has shown variability over the past two decades, spanning from 31 to 82 thousand hectares. This fluctuation is primarily influenced by the previous year's chilli prices and prevailing weather conditions. As per IMSCS chilli has only 60 per cent minimum

germination, so it's of great importance to increase the minimum germination over and above 60 per cent which will be an incremental achievement in seed quality attributes, as poor, delayed and erratic germination of chilli seeds is one of the reasons for low yield of chilli.

Seed priming represents a pre-sowing seed treatment designed to induce a physiological state that enhances seed germination efficiency. Most seed priming methods are based on seed imbibition, allowing seeds to enter the initial, reversible stage of germination without allowing the radical to protrude through the seed coat. Subsequently, primed seeds exhibit accelerated and synchronized germination, resulting in more robust young seedlings that are better equipped to withstand abiotic stresses when compared to seedlings from unprimed seeds.

Biopriming, a form of seed priming, involves the use of living bacterial inoculum, particularly plant growth-promoting rhizobacteria (PGPR). Interest in PGPR research has surged due to its potential to enhance crop growth and yield. Numerous studies have reported significant yield improvements in various crops when inoculated with PGPR (Iswandi *et al*., 1987; Javed *et al*., 1996; Khalid *et al*., 1997; Zahir *et al*., 1997, 1998a, 1998b). To maximize the benefits of inoculation, the selection of the most effective PGPR is crucial. However, a standardized approach for selecting effective PGPR has been lacking.

Directly using rhizobacterial strains in the field without prior screening is a labor-intensive process that relies on effective rhizobacteria screening before field application. Inoculating seeds with *biological* agents in conjunction with priming has, in several instances, been shown to enhance and stabilize the efficacy of these biological agents (Callan *et al*., 1990, 1991; Harman *et al*., 1989; Warren and Bennett, 1999). Additionally, bio-osmopriming treatment has demonstrated the ability to promote uniform germination and enhance plant growth when combined with bacterial coatings (Bennett, 1998).

Specifically for chilli cultivation, there is currently no established methodology for selecting effective biological agents or for applying them to seeds. This study's objectives are two fold: (1) to screen and compare the effectiveness of eight strains of *Bacillus, Pseudomonas* sp. and actinobacteria in enhancing seed germination and seedling growth, and (2) to identify the most efficient strain among the top three isolates of *Bacillus, Pseudomonas* sp. and actinobacteria.

MATERIAL AND METHODS

The investigation on *In-vivo* evaluation of seed biopriming with *Bacillus, Pseudomonas* sp. and actinobacterial isolates was carried out at the Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad during 2019-2021.

A. Collection of culture

The *Bacillus, Pseudomonas* sp. and actinobacteria isolates were collected from culture collection at Department of Biotechnology, College of Agriculture, UAS, Dharwad and *Bacillus subtilis* was collected from Institute of Organic Farming, UAS, Dharwad. The collected isolates were spotted and sub cultured in Nutrient agar, King's B and Starch casein agar media (pH 7) and incubated for 3, 2 and 7 days, respectively, in incubator at 30 ºC.

***Bacillus* isolates**: AUUB109, AUUB112, AUUB98, AUUB57, AUUB119, AUUB54, AUUB117 and *Bacillus subtilis*.

***Pseudomonas* isolates**: AUDP204, AUDP207, AUDP209, AUDP218, AUDP219, AUDP223, AUDP264 and AUDP265.

Actinobacteria isolates: AUDT535, AUDT574, AUDT592, AUDT608, AUDT620 and AUDT636, AUDT504 and AUDT606.

B. Seed Bio Priming

The chilli seeds were surface sterilized using a 0.5% solution of sodium hypochlorite for a duration of five minutes, followed by three rinses with sterile water. Subsequently, *Bacillus, Pseudomonas* sp. and actinobacteria cultures, cultivated in Nutrient broth, King's B, and Starch casein broth for 3, 2, and 7 days respectively, were employed for the biopriming procedure. To perform biopriming, the seeds were soaked in the *Bacillus, Pseudomonas* sp. and actinobacteria cultures for a period of 12 hours, after which they were air-dried in the shade. These bioprimed seeds, alongside a control group treated with broth alone, were then subjected to the germination process, and various seed quality parameters were subsequently recorded.

C. Observations recorded

Seed Germination. The germination test was conducted by placing four sets of 100 seeds each onto rolled paper towels. These seed sets were then incubated within a controlled walk-in seed germination chamber, maintaining a temperature of 25 ± 2°C and relative humidity at 90 ± 5 percent. Seedling assessment occurred once the seedlings had reached a stage where all essential structures were fully developed and visible. Ample time was allowed for the seeds to germinate and exhibit all the necessary structures indicative of their potential to grow into normal plants under favourable conditions.

Seedlings meeting these criteria were categorized as "normal seedlings" and were tallied to determine the germination percentage. On the 14th day, the number of normal seedlings in each of the four replicates was counted, and the germination percentage was calculated in accordance with the guidelines outlined by ISTA (International Seed Testing Association), as detailed in the reference provided (Anon, 2013).

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Root Length. Following the germination test, ten randomly chosen normal seedlings from each treatment within each replication were selected on the 14th day. The measurement of root length was carried out from the tip of the primary root to the hypocotyl, and the average root length was expressed in centimetres.

Shoot Length. In the germination test, ten randomly chosen normal seedlings from each treatment within each replication were selected on the 14th day. The measurement of shoot length was conducted from the base of the primary leaf to the hypocotyl, and the average shoot length was expressed in centimetres.

Seedling Vigour Index (SVI). The vigour index was calculated using the formula provided and expressed as a numerical value, as outlined by Abdul-Baki and Anderson in 1973.

Seedling vigour index = Germination % × [Shoot length + Root length].

Seedling Dry Weight. In the germination test, the random ten seedlings previously utilized for root and shoot length measurements were placed within a paper packet and subjected to drying in a hot air oven, maintained at a temperature of 70° ± 2°C, for a duration of 24 hours. Subsequently, the seedlings were allowed to cool within a desiccator for 30 minutes, and the weight of the dried seedlings was meticulously recorded using a precision balance. This weight was expressed in milligrams (mg) for every set of 10 seedlings, following the methodology detailed by Evans and Bhatt in 1977.

RESULTS AND DISCUSSION

Microorganisms play a vital role in maintaining ecosystem balance through their involvement in various processes such as biodegradation, sewage treatment, enhancing soil fertility, and boosting agricultural productivity. Notably, microbes possess the capacity to stimulate seed germination and promote plant growth. Microbial products that enhance seed health and germination have also been commercialized in the form of biofertilizers. These specific bacteria are referred to as plant-growth-promoting rhizobacteria (PGPR), as described by Hashem *et al*. (2019).

Interestingly, bioprimed seeds have been shown to exhibit increased resilience against both abiotic and biotic stresses, ultimately leading to improved seed emergence and enhanced crop productivity, as observed in the study by Pralhad and Krishnaraj in 2020. In this experiment, we endeavoured to identify one strain each of *Bacillus*, *Pseudomonas* sp. and actinobacteria from a pool of eight strains, with the objective of pinpointing the most effective strains for enhancing seed germination and seedling vigour. Such studies are recognized as an efficient method for screening rhizobacteria and selecting PGPR strains with potential agricultural benefits.

A. In vivo evaluation of Bacillus strains for seed quality enhancing properties of chilli

Among eight strains of *Bacillus* spp. four of them showed significant difference in terms of seed germination *i.e.*, AUUB109, AUUB112, AUUB119 and *Bacillus subtilis* (69.63, 69.19, 68.00 and 71.00 %, respectively) compared to control, whereas, *Bacillus subtilis* showed significantly highest seed germination per cent (71.00 %) (Plate 1&2), on par with AUUB 112 (69.19 %), when compared to control (62.79 %) data represented in Table 1.

Similarly seeds bio primed with *Bacillus* spp. showed significant difference on seedling growth, dry weight and vigour index where significantly highest root length, shoot length, dry weight and seedling vigour index was observed in seeds primed with *B. subtilis* (10.24 cm, 6.22 cm, 39.97 mg and 1168, respectively), compared to control (6.19 cm, 4.34 cm, 35.14 mg and 661, respectively).

Seeds of chilli bioprimed with the inoculant of eight different *Bacillus* strains (AUUB109, AUUB112, AUUB98, AUUB57, AUUB119, AUUB54, AUUB117 and *Bacillus subtilis*) showed independent and significant response on seed germination and seedling vigour index, whereas, significantly higher seed germination, root length, shoot length, seedling dry weight and seedling vigour index (71.00%, 10.24 cm, 6.22 cm, 39.97 mg and 1168, respectively), was observed in seeds bio primed with *Bacillus subtilis* when compared to other strains and control (62.79 %, 6.19 cm, 4.34 cm, 35.14 mg and 661, respectively).

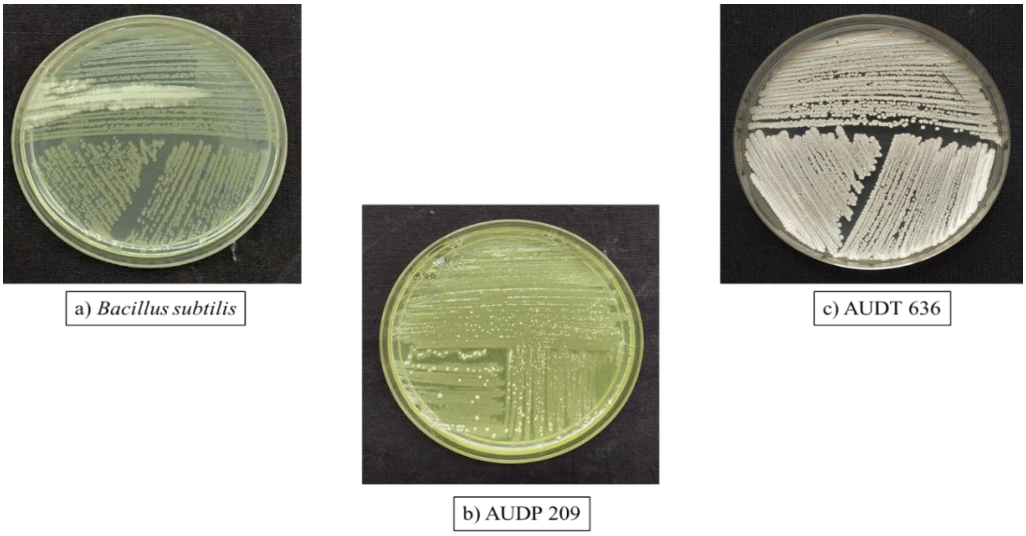

Plate 1. Screened isolates of *Bacillus subtilis*, AUDP209 and AUDT636.

These results are in conformity with Agrawal and Agrawal, 2013 who reported five bacterial isolates of *Bacillus* that showed potential in promoting plant growth activity by markedly improving seed germination, seedling vigour index, shoot and root length of tomato compared to the non-inoculated. Sundaramoorthy and Balabaskar, 2012 also observed increase germination rate in tomato seeds treated with *B. licheniformis* SV4 where early germination, by 40%, after two days of incubation was recorded. Such results were possible due to production of certain enzymes, solubilisation of nutrients or production of phytohormones by *Bacillus* (Olivia Devi, *et al.*, 2020), also *Bacillus* is involved in direct and indirect mechanisms that include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of 1 - amino cyclopropane -1-carbocylate (ACC) deaminase, antibiotics and volatile compounds and the difference in seed germination and SVI among the eight isolates might be due to the type and amount of secondary metabolites produced independently by each of the strains.

Table 1: Effect of seed bio-priming with *Bacillus* strains on seed quality of chilli.

Treatments	Seed germination %	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Seedling vigour index - I
Control	62.79 (52.45) *	6.19	4.34	35.14	661
AUUB109	69.63 (56.59)	7.63	5.61	38.83	922
AUUB112	69.19 (56.33)	8.06	5.50	35.43	938
AUUB98	65.00 (53.76)	7.35	5.65	38.74	845
AUUB57	64.33 (53.36)	8.43	5.72	39.32	910
AUUB119	68.00 (55.58)	8.88	5.92	39.57	1006
AUUB54	65.33 (53.96)	9.06	6.10	39.39	990
AUUB117	63.57 (52.90)	6.26	5.41	36.72	742
B. subtilis	71.00 (57.45)	10.24	6.22	39.97	1168
SE(m) ±	1.023	0.058	0.039	1.389	12.196
C. D. (1%)	4.164	0.237	0.158	NS	49.65
C. V.	2.636	3.558	2.480	NS	2.319

* values in the parenthesis are arcsin transformation

B. In vivo evaluation of Pseudomonas sp. strains for seed quality enhancing properties of chilli

Seeds bio primed with eight *Pseudomonas* sp. strains showed significant difference on seed quality of chilli, where significantly highest seed germination per cent was recorded in seeds primed with AUDP209 (73.00 %) (Plate 1&2) on par with AUDP265 (69.33 %) and significant compared to control (62.67 %) data as shown in Table 2.

Correspondingly significant difference was observed on root length, shoot length, dry weight and seedling vigour index in seeds primed with AUDP209 (9.06 cm, 6.07 cm, 39.30 mg and 1104, respectively) when compared to control (6.14 cm, 4.27 cm, 33.56 mg and 652, respectively).

Various strains of *Pseudomonas* sp. have been found to be effective in plant growth promotion by improving seed germination rate (Kloepper *et al*., 1991; Miller *et al*., 1990; Raj *et al*., 2004). Bio priming of chilli seeds with the inoculant of eight *Pseudomonas* sp. strains showed varied results among the strains, where significantly higher seed germination, root length, shoot length, seedling dry weight and seedling vigour index (73.00 %, 9.06 cm, 6.07 cm, 39.30 mg and 1104, respectively) was observed in AUDP209 compared to other strains and control (62.67 %, 6.14 cm, 4.27 cm, 33.56 mg and 652, respectively). So, the most consistently performing strain in seed germination and improving seedling growth was AUDP209 which was selected for the next experiment. Similar results were recorded by Moeinzadeh *et al*. (2010) when sunflower seeds were bio primed with 30 different strains of *Pseudomonas* sp. The significant effect observed in bioprimed seeds might be related to the production of secondary metabolites, enzymes and antibiotics which helps in improving seed health by protecting the seed from infection.

C. In vivo evaluation of actinobacterial strains for seed quality enhancing properties of chilli

Seed germination in chilli seeds showed significant difference among all the eight bio primed strains whereas, numerically highest seed germination was recorded in seeds primed with AUDT636 (75.00 %) (Plate 1&2) on par with AUDT504 (72.00 %) and significant when compared to control (61.68 %) data shown in Table 3.

Likewise, significant difference was recorded in root length, shoot length, dry weight and seedling vigour index in seeds primed with AUDT636 (9.77 cm, 6.64 cm, 37.80 mg and 1231, respectively) compared to control (5.67 cm, 4.38 cm, 33.30 mg and 620, respectively).

Table 2: Effect of seed bio-priming by *Pseudomonas* sp. strains on seed quality of chilli.

Treatments	Seed germination %	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Seedling vigour index - I
Control	62.67 (52.37) *	6.14	4.27	33.56	652
AUDP204	63.67 (52.96)	6.59	5.53	38.11	772
AUDP207	65.00 (53.76)	6.50	5.45	36.12	777
AUDP209	73.00 (58.73)	9.06	6.07	39.30	1104
AUDP218	66.67 (54.77)	7.21	5.59	38.20	853
AUDP219	68.67 (55.99)	8.43	5.66	38.74	967
AUDP223	65.33 (53.96)	8.85	5.84	38.93	960
AUDP264	67.67 (55.37)	7.17	5.57	38.71	862
AUDP265	69.33 (56.42)	6.21	5.38	35.37	803
SE(m) ±	0.949	0.024	0.029	0.783	11.80
C. D. (1%)	3.864	0.098	0.116	3.186	48.033
C. V.	2.458	1.535	2.105	3.619	2.376

* values in the parenthesis are arcsin transformation

Several Actinobacteria are previously known to enhance the seed germination and seedling vigour (Crawford *et al*., 1993). Actinobacteria collected were used for bio priming of chilli seeds where all the eight strains improved seed quality over control. AUDT636 showed significantly higher seed germination, root length, shoot length, seedling dry weight and seedling vigour index (75.00 %, 9.77 cm, 6.64 cm, 37.80 mg and 1231, respectively) than compared to other strains and control (5.67 cm, 4.38 cm, 33.30 mg and 620, respectively) as same reported by Supritha, 2021. Prahlad and Krishnaraj, (2020) reported that the seed biopriming with AUDT559 and AUDT632 strains of actinobacteria increased root length, shoot length, dry biomass and seedling vigour in peanut. Similar findings were reported by Karthika and Vanangamudi, (2013) in maize where priming enhanced seedling vigour, seedling length and dry weight. IAA is a common auxin, naturally occurring, and a product of L-tryptophan metabolism in microorganisms (Johri *et al*., 2003) which have the potential to promote seed germination (Bailey *et al*., 2002).

Table 3: Effect of seed bio-priming by actinobacterial strains on seed quality of chilli.

Treatments	Seed germination %	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Seedling vigour index - I
Control	61.68 (51.78) *	5.67	4.38	33.30	620
AUDT535	66.33 (54.56)	5.69	4.70	35.71	689
AUDT574	68.67 (55.99)	6.83	4.67	33.77	790
AUDT592	64.75 (53.61)	7.43	5.23	33.67	820
AUDT608	66.33 (54.57)	6.12	6.33	32.02	826
AUDT620	69.00 (56.20)	9.65	6.32	32.12	1102
AUDT636	75.00 (60.03)	9.77	6.64	37.80	1231
AUDT504	72.00 (58.09)	5.61	5.22	32.56	779
AUDT606	65.67 (54.16)	8.23	6.25	35.99	951
SE(m) ±	0.908	0.039	0.045	0.631	11.614
C. D. (1%)	3.699	0.162	0.018	2.569	47.279
C. V.	2.309	2.562	3.284	3.205	2.311

* values in the parenthesis are arcs in transformation

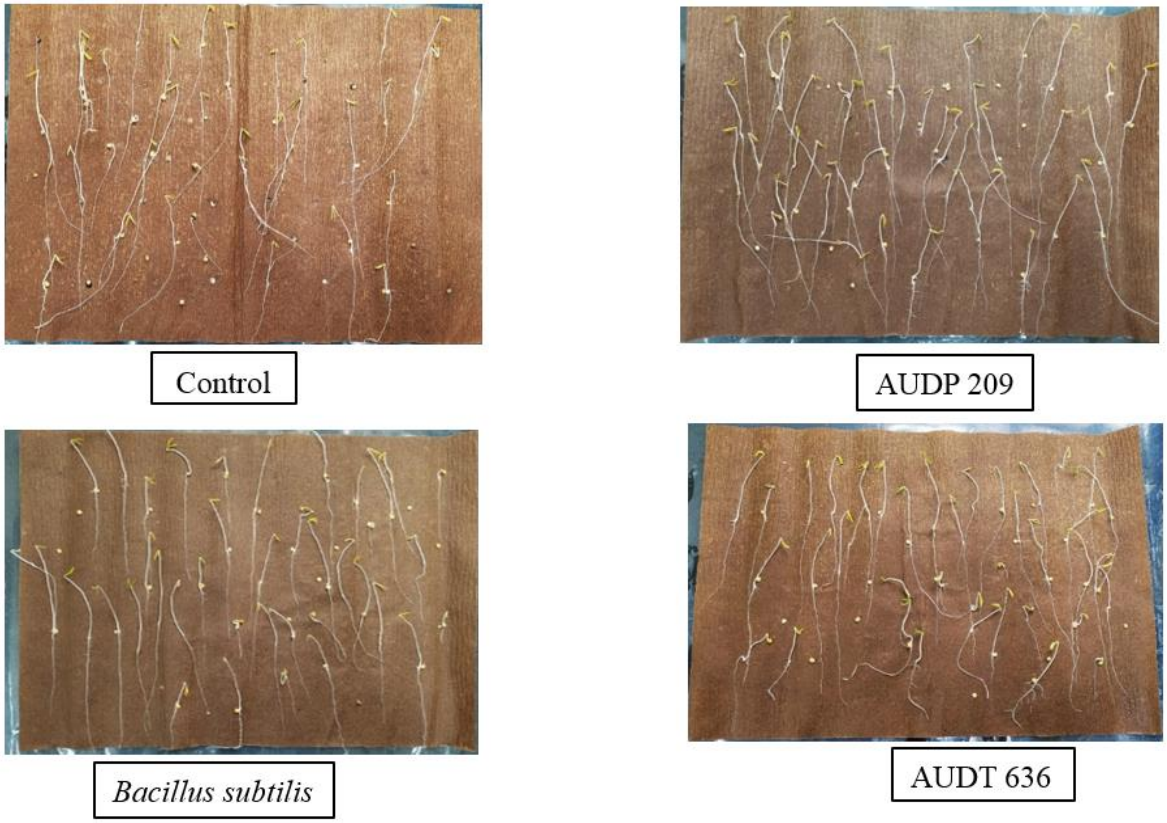

Plate 2: Effect of *B. subtilis*, AUDP 209 (*Pseudomonas* sp.) and AUDT 636 (Actinobacteria) on seed germination of chilli.

The improvement of seed germination and seedling vigour due to seed bio priming with actinobacteria particularly might be due to the in-situ production of secondary metabolites including auxins, gibberellins, cytokinins *etc*. Previously it was also reported that the isolates of actinobacteria synthesizing IAA enhanced growth and yield of wheat plants (Aldesuquy *et al*., 1998).

SUMMARY AND CONCLUSIONS

In vivo evaluation of *Bacillus, Pseudomonas* sp. and Actinobacteria was carried out with eight different strains for seed quality enhancing properties, among eight strains *B. subtilis*, AUDP209 and AUDT636 proved to be the prominent strains for enhancing seed germination and seedling vigour.

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How to cite this article: A. K. Kulsumbi, S. S. Chandrashekhar, J. S. Hilli, P. U. Krishnaraj and H. Ravikumar (2023). *In vivo* evaluation of seed biopriming with *Bacillus, Pseudomonas* sp. and actinobacterial isolates on seed germination and seedling vigour of chilli (*Capsicum annuum* L.). *Biological Forum – An International Journal, 15*(10): 885-891.