Enhancing germination percentage and seed vigor in horticultural Crops through biopriming techniques

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Abstract

Seed biopriming induces a physiological condition that promotes germination and uniform seedling emergence. An investigation is carried out on different seeds such as carrot (*Daucus carota* subsp. *Sativus*), onion *(Allium cepa L.),* cauliflower *(Brassica oleracea),* radish, (*Raphanus sativus*), tomato *(Solanum lycopersicum*), spinach *(Spinacia oleracea),* fenugreek *(Trigonella foenum-graecum)*, and pea *(Pisum sativum)* to standardise bio-priming with *Bacillus siamensis* strain NKIT-9 to improve seed germination and seedling vigor index. To optimise the concentration of bio-priming seedlings were bioprimed overnight with *Bacillus siamensis* strain (NKIT-9) at varied concentrations (0.1mg/ml, 10µg/ml, 0.1µg/ml, and 10ng/ml). Bio-priming with *Bacillus siamensis* strain NKIT-9 gave highest values of germination percentage and seedling vigor index of carrot (at 0.1mg/ml), onion (at 0.1µg/ml), cauliflower at 0.1mg/ml, 10µg/ml, and 0.1µg/ml)*,* radish (at0.1mg/ml and 0.1µg/ml), tomato (at 0.1mg/ml), spinach (at 10ng/ml), fenugreek (at 10ng/ml) and pea (a10ng/ml) as compared with control. carrot, onion, cauliflower, radish, tomato, spinach, fenugreek, and pea exhibited higher germination percentages (60%, 100%, 100%, 100%, 80%, 90%, 100%, and 60% respectively) and seedling vigor indexes (148 ,720, 910, 880, 525, 580, 990, and 1018 respectively) when compared with control. Therefore, bio-priming with these concentrations is best suited for improving germination rate and seedling vigor of seeds.

Keywords: seed bio-priming, seedling vigor index, seed germination, hydro-priming, horticultural seeds.

1 Introduction

With the world population expected to reach 9.6 billion by 2050, the need to secure food security for all has never been more urgent. To fulfill this rising demand, food production must increase by a whopping 70% during the same time span. However, meeting such left goals requires novel ways that not only boost crop output but also ensure environmental sustainability.[1] Among these challenges is the need to protect crops from a variety of biotic stresses while accepting environmentally friendly and sustainable approaches, particularly the use of biocontrol agents (BCAs).

Seed germination is an important step in lifecycle of plants, marking the inception of dormant seeds' transformation into vigorous seedlings is a pivotal process for ensuring successful crop production. Maximizing germination rates and enhancing seedling vigor are imperative for achieving optimal yields and safeguarding food security, particularly in the face of climate change and escalating environmental pressures.[2] Within the ecosystem of soil, there are various interactions between organisms, soil bacteria occupying the rhizosphere have emerged as key players in both disease prevention and nutrient provision for plants. Consequently, the integration of microbial fertilizers containing Plant Growth Promoting Bacteria (PGPB) presents a compelling avenue for ensuring enduring soil fertility and sustained crop productivity. [3]
Traditional seed priming methods have long term solutions for improving germination and seedling performance. However, several limitations persist within these conventional approaches. After introducing biopriming which is promising alternative that involves pre-conditioning seeds through beneficial microorganisms, with the overarching goal of bolstering germination and seedling vigor. [4]

Microorganisms such as bacteria, fungi, and viruses have increasingly been harnessed for disease management in various crops. Among these, soil bacteria capable of colonizing the rhizosphere have garnered significant attention for their role in disease prevention and nutrient provision, underscoring the potential of microbial fertilizers containing PGPB as sustainable alternatives to conventional chemical methods.[5] In recent years, biopriming techniques have gained recognition as cost-effective and eco-friendly approaches to enhancing seed performance. These techniques not only stimulate growth but also confer stress tolerance, thereby contributing to the attainment of desired crop yields while minimizing environmental impact. [6]

This research paper aims into the efficacy of biopriming techniques in augmenting germination and seed vigor in carrot, onion, cauliflower, radish, tomato spinach, fenugreek and pea. This study seeks to elucidate the underlying mechanisms of biopriming and assess its applicability within contemporary horticultural contexts. Additionally, the paper will explore optimization strategies for biopriming, including the investigation of different concentrations of bio-priming agents, such as *Bacillus siamensis* strain NKIT-9, to determine their effects on seed germination and vigor. By advancing our understanding of biopriming and its potential benefits for horticultural crop production, this research endeavours to contribute to the development of sustainable and resilient agricultural systems, ultimately paving the way towards enhanced food security in the face of mounting global challenges.

2 Materials & method

2.1 Material

Seed, were procured from IARI, namely carrot (*Daucus carota subsp. Sativus)*, onion *(Allium cepa L.*), cauliflower (*Brassica oleracea*), radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), spinach (*Spinacia olecara*), fenugreek (*Trigonella foenum-graecum*), and pea (*Pisum sativum*). Alongside these seeds, chemical solutions consisting of 70% ethanol and 1% sodium hypochlorite were employed, for seed sterilization. Additionally, *Bacillus siamensis* strain NKIT-9 served as the microbial strain.

2.2 Biopriming of seed

Bacterial culture NKIT-9 (before 48 hours inoculated) was prepared by centrifuging at

15000 rpm for 15 minutes, collecting the supernatant, and pooling (combining) of pellet upto 1g. Serial dilutions of the bacterial culture were then made to obtain concentrations of 10^{-4} (0.1 mg/ml), 10^{-6} (10 μg/ml), 10^{-8} (0.1 μg/ml), and 10^{-10} (10 ng/ml). Spread plates were produced with 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10} dilutions. After that, seeds including carrot, onion, cauliflower, radish, tomato, spinach, fenugreek, and pea were surface sterilized with 70% ethanol followed by 1% sodium hypochlorite solution, washed with autoclaved milli-Q water, and dried on sterile filter paper. Ten surface-sterilized seeds were soaked in the bacterial culture concentrations of 10^{-4} (0.1 mg/ml), 10^{-6} (10 μg/ml), 10^{-8} (0.1 μg/ml), and 10^{-10} (10 ng/ml). while another ten seeds were soaked in autoclaved milli-Q water as a control. These seeds were then placed in an incubator shaker at 27°C overnight. Then, the soaked seeds were transferred to petri plates.

2.3 Germination percentage

The germination process of carrot (*Daucus carota subsp. Sativus*), onion (*Allium cepa L.*), cauliflower (*Brassica oleracea*), radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), spinach (*Spinacia olecara*), fenugreek (*Trigonella foenum-graecum*), and pea (*Pisum sativum*) seeds was observed daily over a period of seven days. On the 7th day, the number of seeds that had germinated was counted, and the germination percentage was calculated using the formula given in equation (i).

Germination percentage = (number of seeds germinated/total number of seeds) × 100. (i)

2.4 Seed vigor index

Root and Shoot lengths of individual seedlings were measured using a ruler in centi-meters. On the 7th day, seedling vigor index (SVI) was calculated for each seedling by multiplying its length by the germination percentage obtained earlier.

3 Result & discussion

3.1 Germination percentage

The biopriming of seeds with different concentrations of *Bacillus siamensis* (NKIT-9 strain) significantly influenced the germination percentage across various plant species. Growth of seedlings from seeds is shown in fig (2 and 3), and Data of Germination Percentage compared with control shown in fig (1). The result showed that carrot (*Daucus carota subsp. Sativus*), had highest germination percentage (60%) at bacterial concentration of 10^{-4}(0.1mg/ml), indicating its efficacy in enhancing germination compared to hydroprimed seeds (30%). Similarly, for onion (*Allium cepa L.*), the concentration of 10^{-8}(0.1μg/ml) showed the highest germination percentage (100%) compared to hydroprimed seeds (90%). In cauliflower (*Brassica oleracea*), all concentrations tested 10^{-4}(0.1mg/ml), 10^{-6}(10μg/ml), and 10^{-8}(0.1μg/ml) exhibited 100% germination, perform better than the control. radish (*Raphanus sativus)* showed enhanced germination across all concentrations tested, with 10^{-4} (0.1mg/ml) and 10^{-8}(0.1μg/ml) concentrations displaying the highest germination (100%) compared to the control (80%). Similarly, in fenugreek (*Trigonella foenum-graecum*) and pea (*Pisum sativum*), the concentration of 10^{-10}(10ng/ml) resulted in the highest germination percentage (60%) compared to the control (40%). However, in tomato (*Solanum lycopersicum*) and spinach (*Spinacia oleracea*), optimal concentrations varied, with 0.1mg/ml (10^{-4}) being the most effective for tomato and 10^{-10}(10ng/ml) for spinach.

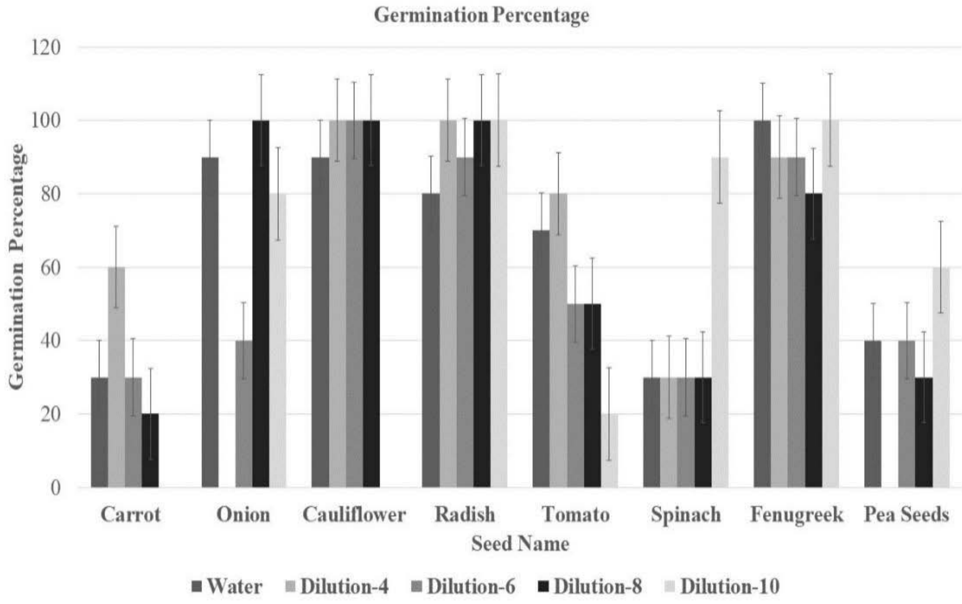


Fig. 1. Germination Percentage of selected horticultural crops i.e. carrot *(Daucus carota subsp. Sativus)* have 60% in bioprimed condition, onion *(Allium cepa L.)* have 100% in bioprimed condition, c*auliflower (Brassica oleracea)* have 100%in bioprimed condition, *r*adish, (*Raphanus sativus*) have 100%in bioprimed condition, tomato *(Solanum lycopersicum*) have 80% in bioprimed condition, spinach *(Spinacia ole*cara) have 90% in bioprimed condition, fenugreek *(Trigonella foenum-graecum)*, have 100% in bioprimed condition, and pea *(Pisum sativum)* have 60% in biopriming condition.

3.2 Seed vigor index

The seed vigor index, is an important parameter reflecting seed quality and initial seedling establishment, was significantly influenced by biopriming with *Bacillus siamensis* (NKIT-9) shown in fig (2). The maximum seed vigor index (274.3) was found in carrots at 0.1 mg/ml, which indicates that the seeds had more vigor than hydro primed seeds (135). Similarly, for onion, cauliflower, radish, tomato, and fenugreek, at concentration 10^{-8}(0.1µg/ml), 10^{-6}(10µg/ml), 10^{-8}(0.1µg/ml), 10^{-4}(0.1mg/ml), and 10^{-10}(10ng/ml) respectively of bacterial treatment resulted in higher seed vigor index (i.e 275, 745, 1071, 525, and 1140 respectively) compared to controls (have 225, 570, 208, 400, and 1040 respectively). However, in spinach, the seedling length was not measurable, indicating a potential limitation or lack of response to biopriming with *Bacillus siamensis* (NKIT-9). In pea, concentrations of 10^{-8}(0.1µg/ml) and 10^{-10}(10ng/ml) showed enhanced seed vigor index (205, and 240 respectively) compared to the control (120).

Table 1. Seed vigor index of various horticultural seeds

S.no.	Seeds name	Water	Dilutions Used			
			10^{-4} (0.1mg/ml)	10^{-6} (10µg/ml	10^{-8} (0.1µg/ml)	10^{-10} (10ng/ml)
1.	carrot	135.0	274.3	130.0	70.0	NM*
2.	onion	225.0	NM*	110.0	275.0	250.0
3.	cauliflower	570.0	530.0	745.0	650.0	NM
4.	radish	208.8	720.0	841.8	1071.9	720.0
5.	tomato	400.0	525.0	NM*	NM*	NM*
6.	spinach	300.0	NM*	NM*	NM*	NM*
7.	fenugreek	1040.0	745.6	381.8	498.7	1140.0
8.	pea seeds	120.0	NM*	90.0	205.0	240.0

*NM- Not Measurable

2a. water

2b. Bioprimed (10^{-4})

2c. water

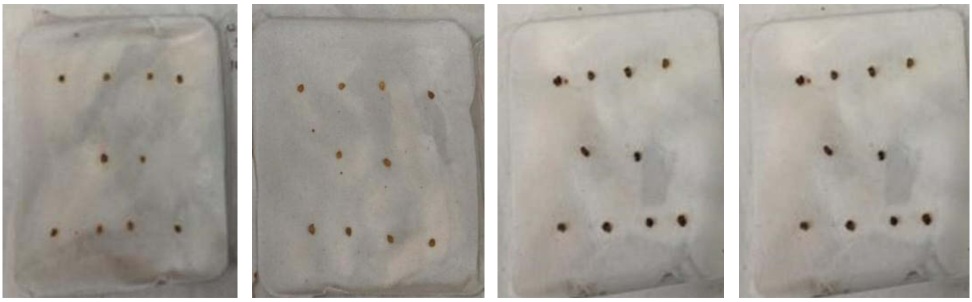
2d. Bioprimed (10^{-8})

2e. water

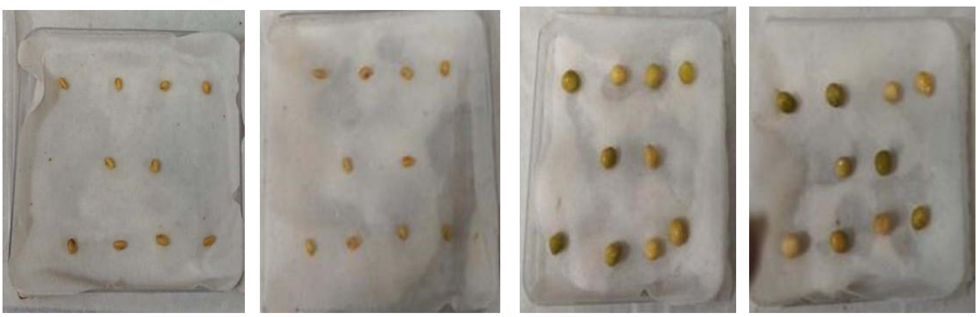
2f. Bioprimed (10^{-6})

2g. water

2h. Bioprimed (10^{-8})



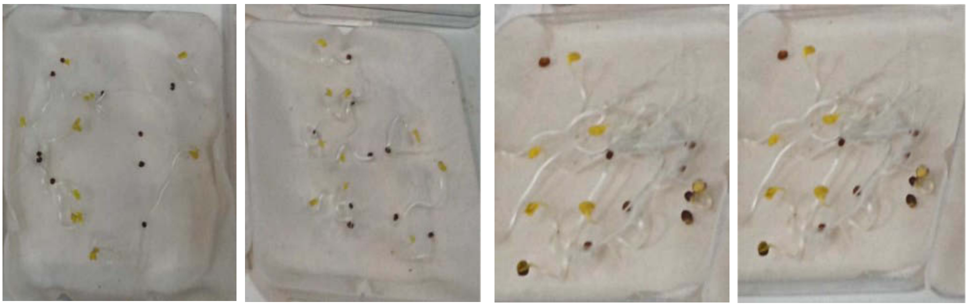
2i. water 2j. Bioprimed (10^{-4}) 2k. water 2l. Bioprimed (10^{-10})



2m. water 2n. Bioprimed (10^{-10}) 2o. water 2p. Bioprimed (10^{-10})

Figure 2. On 0 day Growth of seedlings from seeds (2a.) hydro primed carrot i.e. *Daucus carota subsp. Sativus* (2b.) biopriming with *Bacillus siamensis* (at concentration 10^{-4}) carrot i.e. *Daucus carota subsp. Sativus* (2c.) Hydro primed onion i.e. *Allium cepa L.* (2d.) biopriming with *Bacillus siamensis* (at concentration 10^{-8}) onion i.e. *Allium cepa L.* (2e.) hydro primed *cauliflower i.e. Brassica oleracea* (2f.) biopriming with *Bacillus siamensis* (at concentration 10^{-6}) *cauliflower i.e. Brassica oleracea* (2g.) hydro primed radish i.e. *Raphanus sativus* (2h.) biopriming with *Bacillus siamensis* (at concentration 10^{-8}) radish i.e. *Raphanus sativus* (2i.) Hydro primed Tomato i.e. *Solanum lycopersicum* (2j.) biopriming with *Bacillus siamensis* (at concentration 10^{-4}) tomato i.e. *Solanum lycopersicum* (2k.) hydro primed spinach i.e. *Spinacia ole*cara (2l.) biopriming with *Bacillus siamensis* (at concentration 10^{-10})spinach i.e. *Spinacia ole*cara (2m.) hydro primed fenugreek i.e *Trigonella foenum-graecum)* (2n.) biopriming with *Bacillus siamensis*(at concentration 10^{-10}) fenugreek i.e *Trigonella foenum-graecum* (2o.) hydro primed pea i.e. *Pisum sativum* (2p.) biopriming with *Bacillus siamensis* (at concentration 10^{-10}) pea i.e. *Pisum sativum.*

3a. water 3b. bioprimed (10^{-4}) 3c. water 3d. bioprimed (10^{-8})



3e. water 3f. bioprimed (10^{-6}) 3g. water 3h. bioprimed (10^{-8})

3i. water 3j. bioprimed (10^{-4}) 3k. water 3l. bioprimed (10^{-10})

3m. water 3n. bioprimed (10^{-10}) 3o. water 3p. bioprimed (10^{-10})

Figure 3. On 7th day Growth of seedlings from seeds (3a.) hydro primed carrot i.e. *Daucus carota subsp. Sativus* (3b.) biopriming with *Bacillus siamensis* (at concentration 10^{-4}) carrot i.e. *Daucus carota subsp. Sativus* (3c.) hydro primed onion i.e. *Allium cepa L.* (3d.) biopriming with *Bacillus siamensis* (at concentration 10^{-8}) onion i.e. *Allium cepa L.* (3e.) hydro primed *cauliflower i.e. Brassica oleracea* (3f.) biopriming with *Bacillus siamensis* (at concentration 10^{-6}) *cauliflower i.e. Brassica oleracea* (3g.) Hydro primed radish i.e. *Raphanus sativus* (3h.) Biopriming with *Bacillus siamensis* (at concentration 10^{-8}) radish i.e. *Raphanus sativus* (3i.) hydro primed tomato i.e. *Solanum lycopersicum* (3j.) biopriming with *Bacillus siamensis* (at concentration 10^{-4}) tomato i.e. *Solanum lycopersicum* (3k.) hydro primed spinach i.e. *Spinacia ole*cara (3l.) biopriming with *Bacillus siamensis* (at concentration 10^{-10})spinach i.e. *Spinacia ole*cara (3m.) hydro primed fenugreek i.e *Trigonella foenum-graecum)* (3n.) biopriming with *Bacillus siamensis (*at concentration 10^{-10}) fenugreek i.e *Trigonella foenum-graecum* (3o.) hydro primed pea i.e. *Pisum sativum* (3p.) biopriming with *Bacillus siamensis* (at concentration 10^{-10}) pea i.e. *Pisum sativum*

The findings highlight the potential of biopriming with *Bacillus siamensis* (NKIT-9) in enhancing germination percentage and seed vigor index across various plant species. The effectiveness of biopriming varied depending on the concentration of bacterial treatment and the plant species, suggesting the need for optimization based on specific crop requirements. The observed improvements in germination percentage and seed vigor index indicate the ability of *Bacillus siamensis* (NKIT-9) to promote seedling establishment and early growth, which are crucial for maximizing crop yield and quality.

4 Conclusion

In conclusion, the study demonstrates the significant potential of seed bio-priming with *Bacillus siamensis* strain NKIT-9 to enhance the germination percentage and seedling vigor index of various horticultural seeds. With the future issues of food security exacerbated by climate change, the development of sustainable strategies for boosting crop output is paramount. By creating a physiological condition that is favourable for enhanced seed germination and consistent seedling emergence, seed bio-priming presents an effective method. Through rigorous experimentation on a range of horticultural seeds including carrot, onion, cauliflower, radish, tomato, spinach, fenugreek, and pea, it was observed that specific concentrations of bacterial culture yielded optimal results. carrot seeds exhibited the highest germination percentage when bio-primed at 0.1mg/ml, while onion seeds performed best at 0.1µg/ml concentration. cauliflower seeds displayed enhanced outcomes across multiple concentrations, highlighting the versatility of bio-priming in different plant species.

Furthermore, the superior performance of bio-primed seeds in terms of germination percentage and seedling vigor index compared to hydro-primed seeds underscores the effectiveness of this technique. Notably, bio-priming with *Bacillus siamensis* strain NKIT-9 at specified concentrations resulted in substantial improvements across all tested horticultural seeds, indicating its broad applicability and efficacy. The findings of this research provide valuable insights for optimizing seed bio-priming protocols, thereby contributing to the development of sustainable agricultural practices. By leveraging bio-priming techniques, farmers can potentially enhance crop yields, mitigate risks associated with climate variability, and contribute to global efforts towards achieving food security. Further research is needed to explore the mechanisms underlying the observed enhancements and to assess the long-term effects of bio-priming on plant growth and yield under varying environmental conditions. Additionally, efforts should be directed towards scaling up bio-priming technologies and facilitating their adoption by farmers, thereby realizing their full potential in addressing the pressing challenges of food security in a changing climate scenario.

5 Future prospective

Molecular analysis could play a pivotal role in enhancing our understanding of the mechanisms underlying the positive effects of bio-priming with *Bacillus siamensis* strain NKIT-9 on seed germination and seedling vigor. One potential avenue for molecular analysis is to investigate the gene expression profiles of horticultural seeds treated with different concentrations of *Bacillus siamensis* strain NKIT-9 during bio-priming. This would involve techniques such as RNA sequencing (RNA-seq) to identify differentially expressed genes involved in seed germination and early seedling growth pathways. By elucidating the molecular pathways modulated by bio-priming, researchers can gain insights into the specific mechanisms through which *Bacillus siamensis* strain NKIT-9 enhances seed germination and seedling vigor. Furthermore, molecular markers associated with improved germination and vigor traits could be identified through techniques like quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS), facilitating marker-assisted breeding programs aimed at developing horticultural varieties with enhanced performance under adverse environmental conditions. Additionally, studying the microbial community dynamics in the rhizosphere of bio-primed seeds using metagenomic approaches could provide further insights into the interactions between beneficial microbes and plant hosts, contributing to the development of sustainable agricultural practices for boosting crop productivity and resilience in the face of climate change.

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