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Enhancing Maize Seed Vigour through Seed Biopriming Using Bioagents

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Maize stands as a crucial cereal crop on a global scale, encountering up to 112 diseases, with over 70 being seed-borne. Use of bioagents not only safeguards the environment but also enhances the cost-effectiveness of production initiatives and addresses concerns related to pesticide residue. Maize plants are vulnerable to a range of diseases that significantly diminish both crop yield and quality. One prominent disease is banded leaf and sheath blight, caused by the highly prevalent and destructive pathogen *Rhizoctonia solani* f. sp. *sasakii*. In present study, the seed treatments with bacterial and fungal bio- agents on maize seed (variety DOP-339). The seed quality parameters were recorded by multi-pots tray method. Seed priming with bacterial bio-agent *ie*. *Pseudomonas sihuinsis* (96 %), *Bacillus aerophilus* (94.67 %), *Pseudomonas stutzeri* (94.33 %) and *Enterobacter cloacae* (94 %) significantly increased seed germination over control (92.67 %). *Pseudomonas sihuinsis* showed the highest shoot length (11.84 cm), root length (8.78 cm) and vigour index (854.72). Similarly, seed biopriming with fungal bio-agents with *Trichoderma harzianum* (96.75 %) and *Trichoderma afroharzianum* (97.50 %) were at par with each other in seed germination over control (93.25 %). *T. harzianum* showed highest root length (9.02 cm), fresh weight (15.77 g) and seedling vigour index (883.81) followed by *T. afroharzianum*. *T. afroharzianum* showed highest

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shoot length (12.29 cm) followed by *T. harzianum* (11.12 cm). while both were at par with each other in dry weight. *Nigrospora sphaerica* 2A, *N. sphaerica* 7D and *N. zimmermanii* 8C also displayed positive effects on various parameters as compared to control.

Keywords: Maize; microbiome; biopriming; seed; germination; vigour Index.

1. INTRODUCTION

Maize (*Zea mays* ssp*. mays* L.) holds global significance as a staple in human diets, animal feed, and a key raw material for numerous industrial products. In India, maize currently finds primary usage in feed (63%), food (23%), starch industries (12%) and seed/miscellaneous purposes (2%). Projections suggest that by 2025, India will require 50 million metric tons (MT) of maize grains, of which 32 MT designated for the feed sector, 15 MT for industrial purposes, 2 MT for food, and 1 MT for seed/miscellaneous uses [1]. Among different fungal diseases of maize, banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* Kuhn f. sp. *sasakii* Exner [2] causes significant grain yield loss from 11 per cent to 40 per cent, even upto 100 per cent on some cultivars in warm and humid regions, where the conditions *viz.,* high relative humidity (90 per cent), an optimum temperature of 28 °C and rainfall during first week of infection were favorable for the pathogen [3,4]. The increasing global population presents significant challenges in meeting the demand for food. To address these challenges, efforts made include breeding high yielding disease resistant varieties, the application of chemicals to mitigate plant pathogens, insect pests, and the utilization of biological control agents to minimize plant pathogens. Chemical approaches, such as treating seeds with fungicides, have been implemented to enhance germination, vigour, crop establishment and overall yield. Nevertheless, the unregulated use of chemicals for plant disease control has led to environmental pollution and health risks [5]. Additionally, the careless application of chemicals disrupts the natural ecological balance by eliminating beneficial soil microbes [6]. In certain instances, farmers in developing nations find it challenging to afford the high expenses associated with chemical pesticides. Consequently, there is a growing focus on the exploration of safer approaches to manage seed and plant health. This shift has led to the creation of biopesticides specifically designed for managing seed-borne pathogens in food crops. Various factors contribute to the growth of mold, including unfavorable weather conditions, the high expenses associated with mechanical drying, damage from insects and rodents and temperature fluctuations leading to moisture movement during storage and transportation [7]. The subsequent mold growth can result in diminished germinability, discoloration, loss of milling properties, the development of rancidity through the production of free fatty acids, spoilage and the generation of mycotoxins. The deterioration of stored grains by fungi is a persistent issue within the Indian storage system, often leading to a reduction in both the quality and yield of grains [8].

Plant microbiota colonizes all plant organs plays crucial roles, providing nutrients to plants, stimulating seed germination, promoting plant growth and defending plants against biotic and abiotic stresses. Plants host diverse microbes that colonize on or in their tissues include bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, and arthropods [9,10]. Based on their habitats, plant-associated microbial communities are referred to as rhizosphere microbiome, rhizoplane microbiome, phyllosphere microbiome and endosphere microbiome. Different microbiome interacts with host plants and affect plants in various ways. Plant-associated microbial organisms can potentially have positive and negative impacts on plant growth, development and health. Direct effects on plant growth and development by microorganisms include improved nutrient accessibility such as nitrogen fixation and phosphate solubilization; altered microenvironments such as changed acidity (pH) and hormonal stimulation (phytohormone production). Microorganisms are also involved in the suppression of plant diseases either directly (throgh antibiotics production) or indirectly via induced systemic resistance and growth promotion [11].

However no literature is available on isolation of microbiome associated with BLSB of maize and its use in the maize production. Therefore, present investigation aims at enhancing maize seed vigour through seed priming with fungal and

bacterial microbiomes isolated from banded leaf and sheath blight of maize.

2. MATERIALS AND METHODS

2.1 Selection of Microbiome

Culturable micro-organisms were isolated from maize phyllobiome from different maize growing environments of Uttarakhand state. After screening of 45 fungal isolates against *R. solani* by dual culture, volatile and non-volatile assay for their antagonistic activity, effective isolates *viz*., *T. harzianum*, *T. afroharzianum*, *N. sphaerica* 2D, *Nigrospora sphaerica* 1 and *N.zimmermanii* 1C were evaluated for seed germination and seedling vigour test by using multipot tray method. Similarly, out of 17 bacterial isolates, the best performing bacterial isolates namely *Enterobacter cloacae*, *Pseudomonas stutzeri*, *Klebsiella pneumoniae*, *Brevibacillus limnophilus*, *Bacillus aerophilus* and *Pseudomonas sihuinsis* were also tested for seed germination and seedling vigour test by using multipot tray method.

2.2 Mass Multiplication

Pure culture were maintained by sub culturing on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) and stored in refrigerator for further use at 5 ± 1ºC temperature. For mass multiplication of fungal bio-agents potato broth and for bacterial bio-agents nutrient broth were used after autoclaving at 15 psi for 20 minutes. Cultures of fungal BCA were prepared in potato broth in 250 ml conical flasks by inoculating 10 mm disc of 7 to 10 days fungal BCA culture under aseptic conditions in laminar air flow chamber and incubated at 25 ± 1^0C in BOD incubator. After 10 days of incubation, the potato broth was thoroughly shaken and the resulting suspension (1× 10^6 cfu) was maintained. Similarly, for the preparation of bacterial biological control agents, the cultures were established in 250 ml conical flasks containing nutrient broth. This process involves inoculating a loopful of bacteria from a 48-hour-old bacterial culture under aseptic conditions within a laminar airflow chamber. The flasks are then incubated at a temperature of 25 ± 1°C in incubated shaker at 150 rpm. After 48 hours of incubation, the suspension (1 × 10^8 cfu) was used for seed priming. Seed bio-priming was done by treating 100 g maize seeds in 100 ml freshly prepared suspension of fungal or bacterial bio-agents separately for 15 minutes prior to seed sowing.

2.3 Soil Preparation

Field soil was mixed uniformly with vermicompost @ 200 g/kg of soil. Soil sterilization was done by autoclaving at 15 psi for 90 minutes before filling in pots.

2.4 Multi-pot Tray Method

The seeds of maize variety DOP-339 were used for pot experiment. Hundred seeds treated with individual bio-agents were sown per tray filled with the sterilized soil at 1-2 cm depth. Untreated seeds sown in trays served as a control. Four replications were maintained per treatment.

2.5 Standard Germination Test (%)

The shoots rising above soil surface were counted as germinated. Germination was recorded on 6^{th} day and the number of normal seedlings was counted and expressed as per cent germination [12].

$$G\ (\%) = [N_T \times 100]/N$$

Where,
N_T: Proportion of germinated seeds in each treatment for the final measurement
N: Number of seeds used

2.6 Root and Shoot Length (cm)

Root and shoot length was measured in centi-meter on 6^{th} day and average seedling length was calculated by using formula. Seedling length =Shoot length + Root length

2.7 Seedling Fresh Weight (g)

Normal germinating seedlings were collected in separate paper bags and the seedling fresh weight was measured in gram and average seedling fresh weight was calculated.

2.8 Seedling Dry Weight (g)

For dry weight determination, all replication seedlings were dried for 2-3 days in shade. These seedlings were placed in separate paper bags and then transferred into oven at 50ºC for 2 h four times. The average weight of all replications of germination seedlings was taken and seedling dry weight was expressed in grams.

2.9 Vigour Index

Seedling vigour index was calculated according to the formulae suggested by Aosa [13].

Vigour index = Germination (%) X (Root length + Shoot length)

2.10 Statistical Analysis

The statistical analysis of the experimental data was carried out using computer software OPSTAT. The data obtained from the laboratory experiments were analyzed statistically with Completely Randomized Design (CRD). Different treatments were compared using critical difference (CD) value at 0.05 (1%) level of significance.

3. RESULTS AND DISCUSSION

3.1 Influence of Different Bacteria on Seed Germination and Seedling Vigour of Maize

Over all mean of all seed quality parameter of DOP-339 variety revealed that, the seed treatment with bacterial microbiomes like, *Pseudomonas sihuinsis*, *Bacillus aerophilus*, *Brevibacillus limnophilus*, *Pseudomonas stutzeri*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were significantly superior over control (Table 1 & Plate 1).

Germination: Highest seed germination was recorded in *Pseudomonas sihuinsis* (96.00 %) %) followed by *Bacillus aerophilus* (94.67 %), *Pseudomonas stutzeri* (94.33 %), *Enterobacter cloacae* (94 %) which were statistically at par each other and least per cent germination was observed *Brevibacillus limnophilus* (93.67 %) compared to control (92.67 %) and *Klebsiella pneumoniae* (92.00 %).

R:S ratio: *Pseudomonas stutzeri* provided highest (0.77) root-shoot (R:S) ratio followed by *Pseudomonas sihuinsis* (0.74), *Brevibacillus limnophilus* (0.74), which were at par with each other and *Klebsiella pneumoniae* (0.72) and *Bacillus aerophilus* (0.72) were at par with each other and least root-shoot ratio was observed in control (0.62).

Dry Weight: The maximum dry weight was recorded in *Pseudomonas sihuinsis* (2.86 g) followed by *Bacillus aerophilus* (2.83 g), *Brevibacillus limnophilus* (2.72 g), *Pseudomonas stutzeri* (2.63 g), *Enterobacter cloacae* (2.36 g) and *Klebsiella pneumoniae* (2.28 g) compared to control (1.98 g).

Vigour Index: The highest vigour index was observed in *Pseudomonas sihuinsis* (1979.52) followed by *Bacillus aerophilus* (1717.31), *Brevibacillus limnophilus* (1589.58), *Pseudomonas stutzeri* (1550.78), *Enterobacter cloacae* (1387.44) and *Klebsiella pneumoniae* (1333.08) compared to control (974.88).

Highest shoot length (11.84 cm), root length (8.78 cm) and seedling length (20.62 cm) was observed in *Pseudomonas sihuinsis* and remaining were showing positive effects on all parameters except control. All the growth parameters like shoot-root length, fresh weight as well as dry weight of the plant, vigour index and root-shoot ratio, were significantly enhanced by the treatment.

In present study, seed bio-priming with *Pseudomonas sihuinsis*, *Pseudomonas stutzeri, Enterobacter cloacae, Bacillus aerophilus* and *Brevibacillus limnophilus* have been found superior in improving the growth parameters. Earlier works in maize [14], pearl millet [15] and sorghum [16] with *Pseudomonas fluorescens* have been reported with similar results. Maize seeds primed with *Bacillus subtilis* increased the seedling emergence [17]. In literature no reports are available on seed bio priming either with *Pseudomonas sihuinsis* or *Pseudomonas stutzeri*. It seems that these species of *Pseudomonas* have been isolated and tested on maize for the first time.

3.2 Influence of Different Fungi on Seed Germination and Seedling Vigour of Maize

Seed treatment with fungal bioagents isolated from maize *viz*., *Trichoderma harzianum*, *Trichoderma afroharzianum*, *Nigrospora sphaerica*, *Nigrospora zimmermanii* were significantly superior over control (Table 2 & Plate 2).

Germination: The maximum seed germination was recorded in *T. afroharzianum* (97.50 %) which was statistically at par with *T. harzianum* (96.75 %) followed by *Nigrospora sphaerica* 1(95.50 %), *N. sphaerica* 2D (95.00 %) and *N.zimmermanii* 1C (94.75 %), which were on par with each other and least per cent germination was observed in control (93.25 %).

Table 1. Influence of different bacteria on seed germination and seedling vigour of maize

Bacteria	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	R:S ratio	Fresh weight (g)	Dry weight (g)	Vigour Index
Enterobacter cloacae	94.00^{b}	8.64^{d}	6.12^{c}	14.76	0.71	12.86^{d}	2.36	1387.44
Pseudomonas stutzeri	94.33^{b}	9.28^{cd}	7.16^{b}	16.44	0.77	14.39^{c}	2.63	1550.79
Klebsiella pneumoniae	92.00^{d}	8.44^{d}	6.05^{c}	14.49	0.72	12.48^{d}	2.28	1333.08
Brevibacillus limnophilus	93.67^{bc}	9.78^{bc}	7.19^{b}	16.98	0.74	14.88^{bc}	2.72	1589.58
Bacillus aerophilus	94.67^{b}	10.53^{b}	7.61^{b}	18.15	0.72	15.59^{a}	2.83	1717.31
Pseudomonas sihuinsis	96.00^{a}	11.84^{a}	8.78^{a}	20.62	0.74	15.45^{ab}	2.86	1979.52
Control	92.67^{cd}	6.48^{e}	4.04^{d}	10.52	0.62	10.84^{e}	1.98	974.89
S. Em. ±	0.36	0.30	0.18	0.21	0.009	0.12	0.09	22.50
CD @ 1 %	1.10	0.92	0.56	0.66	0.028	0.37	0.29	68.91
CV	1.82	1.51	2.10	2.37	2.194	1.12	1.76	2.59

Table 2. Influence of different fungi on seed germination and seedling vigour of maize

Fungi	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	R:S ratio	Fresh weight (g)	Dry weight (g)	Vigour Index
T. harzianum	96.75^{a}	11.12^{b}	9.02^{a}	20.14	0.81	15.77^{a}	3.88	1948.55
T. afroharzianum	97.50^{a}	12.29^{a}	8.11^{b}	20.40	0.76	15.2^{b}	3.76	1989.00
Nigrospora sphaerica 1	95.50^{b}	9.41^{d}	6.62^{c}	16.03	0.70	13.18^{c}	3.38	1530.87
N. sphaerica 2D	95.00^{b}	10.21^{c}	7.69^{b}	17.90	0.75	14.71^{b}	3.45	1700.50
N.zimmermanii 1C	94.75^{b}	8.87^{e}	6.54^{c}	15.42	0.74	12.8^{c}	3.28	1460.10
Control	93.25^{c}	6.55^{f}	4.54^{d}	11.09	0.69	11.16^{d}	2.87	1034.14
S. Em. ±	0.58	0.05	0.15	0.38	0.009	0.12	0.09	18.28
CD @ 1 %	0.80	0.14	0.46	1.19	0.029	0.38	0.58	56.96
CV	1.50	1.79	1.99	3.97	2.224	1.15	2.34	1.96

Plate 1. Influence of different bacteria on seed germination and seedling vigour of maize

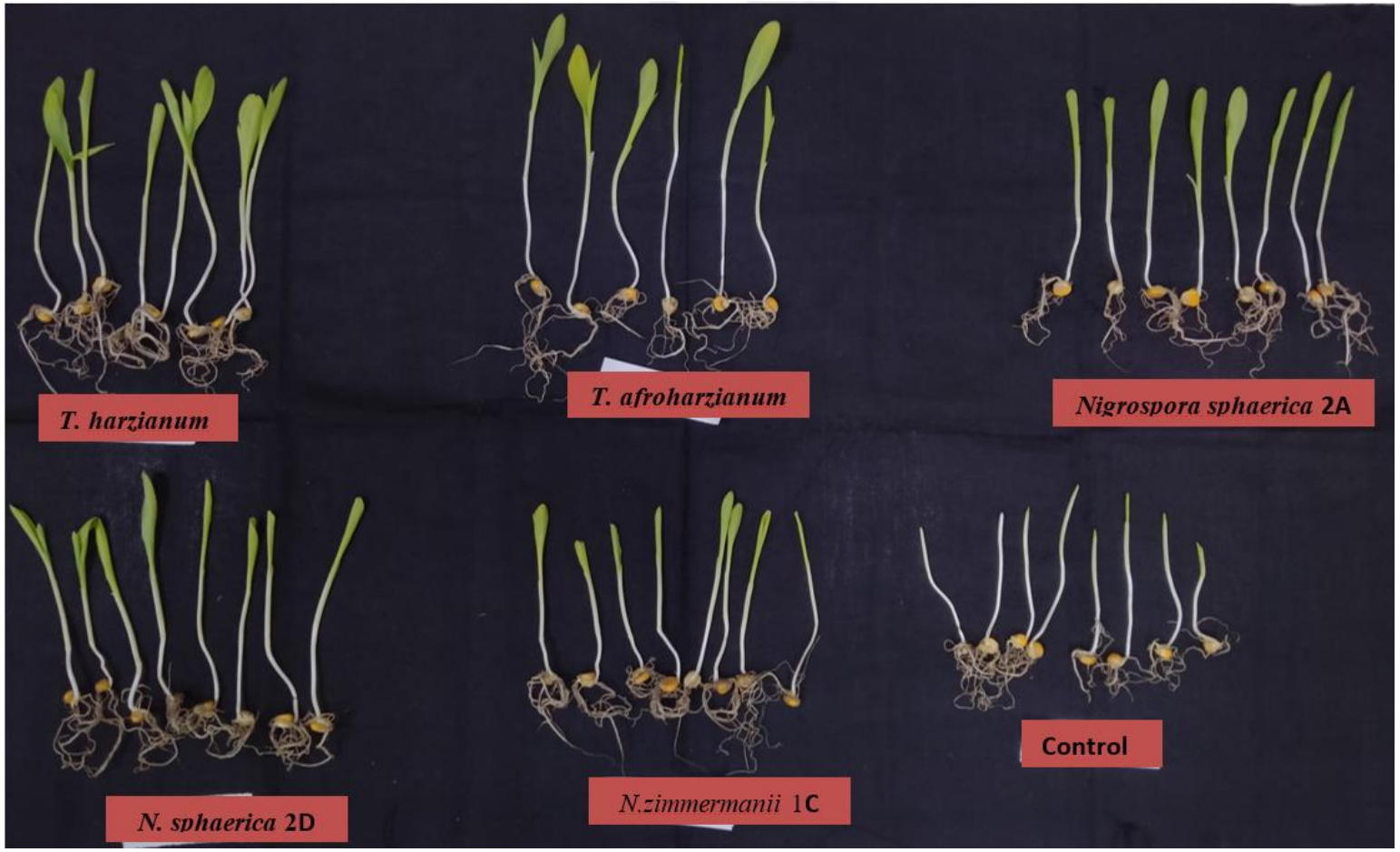

Plate 2. Influence of different fungi on seed germination and seedling vigour of maize

R:S ratio: *T. harzianum* provided highest (0.81) root-shoot (R:S) ratio followed by *T. afroharzianum* (0.76), *N. sphaerica* 2D (0.75), *N.zimmermanii* 1C (0.74) and *Nigrospora sphaerica* 1 (0.72). lowest valves was observed in control (0.69 %).

Dry Weight: The highest dry weight was recorded in *T. harzianum* (3.88) which was at par with *T. afroharzianum* (3.76) followed by *Nigrospora sphaerica* 1(3.38), *N. sphaerica* 2D (3.45) and *N.zimmermanii* 1C (3.28), which were on par with each other and least dry weight was observed in control (2.87).

Vigour Index: The highest vigour index was observed in *T. afroharzianum* (1989) followed by *T. harzianum* (1948.55), *Nigrospora sphaerica* 2D (1700.50), *Nigrospora sphaerica* 1 (1530.87) and *N.zimmermanii* 1C (1460.10) compared to control (1034.14).

Highest root length (9.02 cm) and fresh weight (15.77 g) was observed in *T. harzianum*. In contrast, highest shoot length (12.29 cm) and seedling length (20.40 cm) was observed in *T. afroharzianum* and remaining were showing positive effects on all parameters except control. All the growth parameters like shoot-root length, fresh weight as well as dry weight of the plant, vigour index and root-shoot ratio, were significantly enhanced by the treatment.

Different species of fungal bio-agent on seed biopriming affected seed germination, root: shoot ratio and seedling growth parameters significantly after seed bio-priming. The key parameters are the lengths of the roots and shoots, as the roots come into direct contact with the soil, absorbing water and supplying it to the entire plant. For this reason root and shoot length provides an important clue plant response to seed mycoflora. In present study, *Trichoderma harzianuma* and *T. afroharzianum* have been found superior in improving the growth parameters. In previous studies *Trichoderma harzianuma* and *T. asperellum* have been reported with similar results [12,18] No reports are available in literature with respect to seed biopriming with *Trichoderma afroharzianum.* It seems that this species of *Trichoderma* have been isolated and tested on maize for the first time. Similarly, *Nigrospora spherica* and *Nigrospora zimmerrmani* have alsobeen evaluated first time though found less effective than *Trichoderma* spp [19].

4. CONCLUSION

Fungal bio-agents *Trichoderma harzianuma* and *T. afroharzianum* and in bacterial bio-agent *Pseudomonas sihuinsis* significantly increased germination, root: shoot ratio and seedling vigour, which ultimately enhanced the plant growth and development which can be beneficial to overcome biotic and abiotic stresses. Integrating bio-priming into standard agricultural practices provides a practical and cost-effective solution, enhancing resilience and productivity for maize crops. This innovative approach not only improves economic viability but also promotes ecological balance by reducing reliance on chemical inputs and ensuring food security for growing populations while minimizing the environmental impact of conventional farming practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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