Original Research Article

Enhancing Maize Seed Vigour through Seed Biopriming using Bioagents

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 *Trichodermaharzianum* (96.75 %) and *Trichodermaafroharzianum* (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 shoot length(12.29 cm) followed by *T. harzianum* (11.12 cm). while both were at par with each other in dry weight.*Nigrosporasphaerica*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 and Vigour Index

UNDER PEER REVIEW

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 **(Yadav *et al*., 2016).** Among different fungal diseases of maize, banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* Kuhn f. sp. *sasakii*Exner **(Ogoshi, 1987)**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 **(Izhar and Chakraborty, 2013; Gao *et al*., 2014).**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 seedswith 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 **(Chapman and Harris, 1981).** Additionally, the careless application of chemicals disrupts the natural ecological balance by eliminating beneficial soil microbes **(Neergaard, 1988).** 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 **(Pitt, 1981).** 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 **(Baliukonieneet *al*., 2003).**

Plantmicrobiotacolonizesall plantorgansplayscrucial roles,providing nutrients to plants, stimulating seed germination, promoting plant growth anddefendingplantsagainstbiotic andabioticstresses. Plants host diverse microbes that colonize on or in their tissues include bacteria, fungi, oomycetes, nematodes, protozoa, algae,viruses,archaea,andarthropods(**Bueeet *al*. 2009; Raaijmakers *et al*. 2009**). Based on their habitats, plant-associatedmicrobialcommunitiesarereferredtoasrhizospheremicrobiome,rhizoplanemicrobiome,phyllospheremicrobiomeandendospheremicrobiome.Differentmicrobiome interacts with host plants and affect plants in various ways. Plant-associatedmicrobial organisms can potentially have positive and negative impacts on plant growth,developmentandhealth.Directeffectsonplantgrowthanddevelopmentbymicroorganisms include improved nutrient accessibility such as nitrogen fixation andphosphate solubilization; altered microenvironments such as changed acidity (pH) andhormonal stimulation (phytohormone production). Microorganisms are also involved inthesuppressionofplantdiseaseseitherdirectly(throghantibioticsproduction) or indirectly via induced systemic resistance and growth promotion (**Wu *et al*., 2020**).

However no literature is available on isolation of microbiome associatedwith BLSB of maize and its use in the maize production. Therefore,presentinvestigation aimsatenhancing maize seed vigour through seed priming with fungal and bacterial microbiomes isolated from banded leaf and sheath blight of maize.

MATERIALS AND METHODS

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, effectiveisolates *viz*., *T. harzianum*, *T. afroharzianum*, *N. sphaerica* 2D,*Nigrosporasphaerica*1 and *N.zimmermanii* 1C were evaluated for seed germination and seedling vigour test by using multipot tray method.

UNDER PEER REVIEW

Similarly, out of 17 bacterial isolates, the best performing bacterial isolates namely *Enterobacter cloacae*,*Pseudomonas stutzeri*,*Klebsiella pneumoniae*, *Brevibacilluslimnophilus*, *Bacillus aerophilus*and*Pseudomonas sihuinsis* were also tested for seed germination and seedling vigour test by using multipot tray method.

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× 10^6cfu) 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 × 10^8cfu) 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.

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.

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.

UNDER PEER REVIEW

Standard Germination Test (%)

The shoots rising above soil surface were counted as germinated. Germinationwas recorded on 6th day and the number of normal seedlings was counted and expressed as per cent germination**(Sinha and Kumar., 2019).**

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

Root and Shoot Length (cm)

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

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.

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.

Vigour Index

Seedling vigour indexwas calculated according to the formulae suggested by **Aosa (1983).**

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

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

UNDER PEER REVIEW

Completely Randomized Design (CRD). Different treatments were compared using critical difference (CD) value at 0.05 (1%) level of significance.

RESULT AND DISCUSSION

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*, *Brevibacilluslimnophilus*, *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 *Brevibacilluslimnophilus* (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), *Brevibacilluslimnophilus*(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), *Brevibacilluslimnophilus*(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:

UNDER PEER REVIEW

The highest vigour index was observed in *Pseudomonas sihuinsis* (1979.52) followed by *Bacillus aerophilus*(1717.31), *Brevibacilluslimnophilus*(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 *Pseudomonassihuinsis*, *Pseudomonas stutzeri*, *Enterobacter cloacae,Bacillus aerophilus*and *Brevibacilluslimnophilus* have beenfound superior in improving the growth parameters. Earlier works in maize (Revathi and Vanangamudi, 2014), pearl millet (Niranjan Raj *et al*., 2004) and sorghum (Raju *et al*.,1999) with *Pseudomonasfluorescens* have been reported with similar results. Maize seeds primed with *Bacillus subtilis* increased the seedling emergence (Junges, *et al.*, 2013). 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.

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
Brevibacilluslimnophilus	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

Influenceof different fungi on seed germination and seedling vigour of maize

UNDER PEER REVIEW

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

Germination :

Themaximum seed germination was recorded in *T. afroharzianum* (97.50%) which was statistically at par with *T. harzianum*(96.75 %) followed by *Nigrosporasphaerica*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 %).

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 *Nigrosporasphaerica*1 (0.72). lowest valves wasobserved in control (0.69 %).

Dry Weight:

The highestdry weight was recorded in *T.harzianum*(3.88) which was at par with *T. afroharzianum*(3.76) followed by *Nigrosporasphaerica*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), *Nigrosporasphaerica* 2D (1700.50), *Nigrosporasphaerica* 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.

UNDER PEER REVIEW

Table 2. Influenceof 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
Nigrosporasphaerica 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

Different species of fungal bio-agent on seed biopriming affected seed germination, root: shoot ratio and seedling growth parameters significantlyafter 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 beenfound superior in improving the growth parameters. In previous studies *Trichodermaharzianuma*and *T.asperellum*have been reported with similar results (**Sinha and Kumar, 2019 &Akladious and Abbas, 2014,)** No reports are available in literature with respect to seed biopriming with *Trichodermaafroharzianum.* It seems that this species of *Trichoderma* have been isolated and tested on maize for the first time. Similarly, *Nigrosporasphericaand Nigrosporazimmerrmani* have alsobeen evaluated first time though found less effective than *Trichoderma* spp.

Conclusion:

Fungal bio-agents *Trichodermaharzianuma*and *T. afroharzianum* and in bacterial bio-agent *Pseudomonas sihuinsis* significantly increased germination, root: shoot ratio and seedling vigour,which ultimatelyenhanced 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

UNDER PEER REVIEW

growing populations while minimizing the environmental impact of conventional farming practices.

References

Akladious, S.A. and Abbas, S.M., 2014. Application of *Trichodermaharzianum* T22 as a biofertilizer potential in maize growth. *Journal of Plant Nutrition*, 37(1): 30-49.

Aosa, I. (1983). Seed Vigour Testing Handbook. Contribution No. 32 to the handbook on seed testing. pp. 88.

Baliukoniene, V., Bakutis, B., and Stankevicius, H. 2003. Mycological and mycotoxicological evaluation of grain. *Annals of Agricultural and Environmental Medicine*, 10(2): 223- 227.

Buee, M., De Boer, W., Martin, F., Van Overbeek, L. and Jurkevitch, E., 2009. The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil*, 321: 189-212.

Chapman, R. A. and Harris, C. R., 1981. Persistence of four pyrethroid insecticides in a mineral and an organic soil. *Journal of Environmental Science and Health Part B*, 16(5): 605-615.

Gao, J., Chen, Z., Luo, M., Peng, H., Lin, H., Qin, C., Yuan, G., Shen, Y., Ding, H., Zhao, M., Pan, G. and Zhang, Z. 2014. Genome expression profile analysis of the maize sheath in response to inoculation to *Rhizoctonia solani*. *Mol. Biol. Rep*., 41: 2471-2483.

Izhar, T. and Chakraborty, M. 2013. Genetic analysis of banded leaf and sheath blight resistance (*Rhizoctonia solani*) in maize. *J. Pharmacogn. Phytochemical*., 1: 1-5.

Junges, E., Toebe, M., Santos, R.F.D., Finger, G. and Muniz, M.F.B., 2013. Effect of priming and seed-coating when associated with *Bacillus subtilis* in maize seeds. *RevistaCienciaAgronomica*, 44: 520-526.

Kumar, S., Arya, M.C. and Ranjit Singh. 2007. Efficiency of Pseudomonas fluorescens and Trichoderma harzianum as bio-enhancers in tomato at high altitude in central Himalayas. Indian J. Crop Sci., 2(1): 79-82.

UNDER PEER REVIEW

Neergaard, P., 1988. Seed Pathology, Vol. I. The Macmillan Press Ltd. London, U.K, pp. 840.

Niranjan Raj, S., Chaluvaraju, G., Amruthesh, K.N., Shetty, H.S., Reddy, M..S. and Kloepper, J.W. 2003a. Induction of growth promotion and resistance against downy mildew on pearl millet (Pennisetum glaucum) by rhizobacteria. Plant Dis., 87: 380-384.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and interspecific groups of *Rhizoctonia solani* Kuhn. *Annu. Rev. Phytopathol.*, 25: 125-143.

Pitt J. I. 1981. Food Spoilage and Biodeterioration In: Biology of Conidial Fungi Vol.-2 editor Garry T. Cole and Bryce Kendrick, Academic Press New York. pp. 111-137.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y., 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, 321: 341-361.

Raju, N.S., Niranjana, S.R., Janaradhana, G.R., Prakash, H.S., Reddy, H.S. and Mathur, S.B. 1999. Improvement of seed quality and filed emergence of Fusarium moniliforme infected sorghum seeds using biological agents. J. Sci. Food and Agric., 79: 206-212.

Revathi, R. and Vanangamudi, K. (2014). Biopriming of maize hybrid seeds with biocontrol agents for improving germination and vigour. *Madras Agricultural Journal*, *101*(1/3), 59-65.

Sinha, A. and Kumar, S., 2019. Effect of bio-control agents treatments on Maize seed vigour by Standard method. *International Archive of Applied Sciences and Technology*, 10(4): 61-70.

Wu, F.L., Li, Y., Tian, W., Sun, Y., Chen, F., Zhang, Y., Zhai, Y., Zhang, J., Su, H. and Wang, L., 2020. A novel dark septate fungal endophyte positively affected blueberry growth and changed the expression of plant genes involved in phytohormone and flavonoid biosynthesis. *Tree Physiology*, 40(8): 1080-1094.

Yadav, O.P., Prasanna, B.M., Yadava, P., Jat, S.L., Kumar, D., Dhillon, B.S., Solanki, I.S. and Sandhu, J.S., 2016. Doubling maize (*Zea mays*) production of India by 2025–Challenges and opportunities. *Indian Journal of Agricultural Sciences*, 86(4): 427-434.

UNDER PEER REVIEW

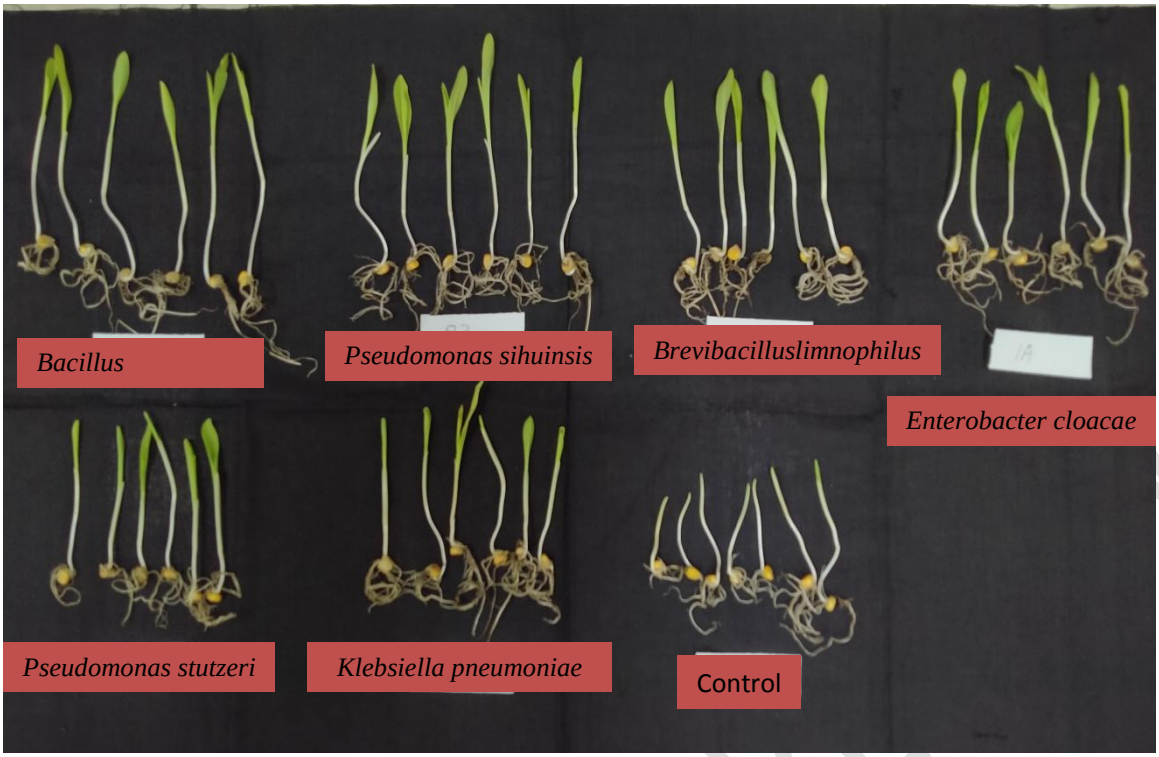

Plate: 1. Influence of different bacteria on seed germination and seedling vigour of maize

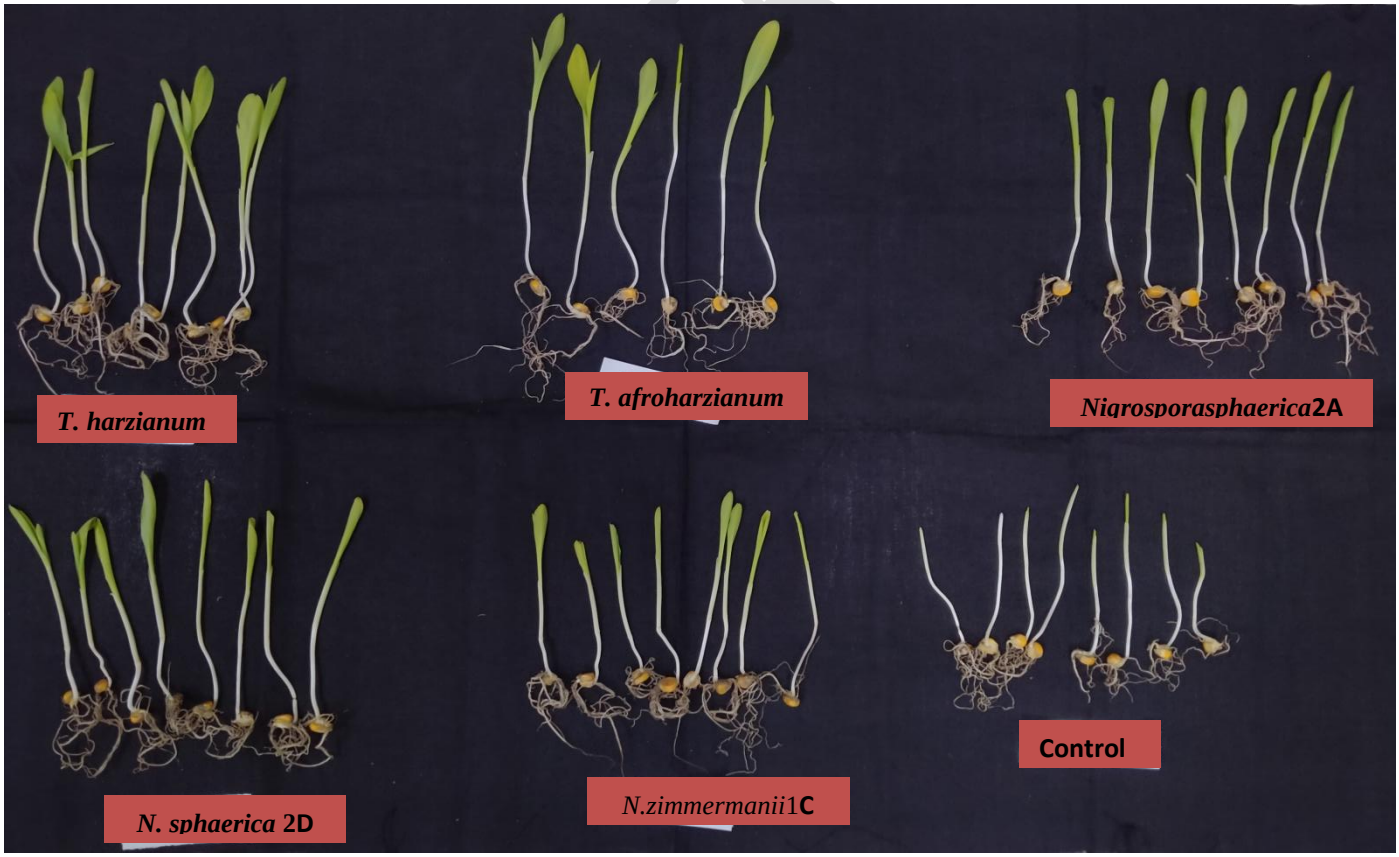

Plate: 2. Influence of different fungi on seed germination and seedling vigour of maize