

Exploring The Potential Fungal Metabolites as a Natural Bio-Stimulant for Soybean Seed Germination

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Abstract—Biostimulants are environment-friendly agricultural inputs that can improve plant health and yield potential under environmental stressors. Soybeans subjected to extreme temperatures during the growing seasons impacts plant health and performance. Seed germination and seedling vigor were assessed in the culture filtrate of seven different soybean seed mycoflora. *Rhizopus oryzae* culture filtrate showed the largest reduction in seed germination percentage (2.2%), followed by *Aspergillus flavus* and *Aspergillus niger*. *Rhizopus oryzae* culture filtrate was found to reduce the length of the plumule and radicle, followed by *Drechslera* spp. These mycoflora have a direct impact on seed germination, either by reducing the seed's viability by depriving it of nutrients or by damaging seeds that have been stored. To the best of our knowledge, this is the first study that shows the biostimulant effect of *Aspergillus flavus* and *Aspergillus niger*. *Rhizopus oryzae* culture filtrate was found to reduce the length of the plumule and radicle, followed by *Drechslera* spp. growth and metabolome, increasing the knowledge on fungal bioresources for the development of biostimulants.

Index Terms—Soybean, Biostimulants, metabolites, Seed germination.

I. INTRODUCTION

Globally, soybeans are a vital legume crop and a highly prized food source for both people and animals (Pagano & Miransari, 2016). The soybeans (*Glycine max* (L) Merr.) have the capacity to produce more edible oil, which can be mashed and consumed as vegetarian protein meat. Fodder is made from crushed soybean seeds (Sobko et al., 2020). Over the last two decades, soybeans have been popularized in Karnataka. Among the many factors influencing soybean yield and emergence, seed-borne microbes have a significant impact. Fungi are the

microorganisms that have the biggest impact on the longevity and quality of seeds. Numerous fungi degrade the seeds while they are being stored (Ahmed et al., 2016)

Therefore, as is stated in the UN 2030 Agenda Sustainable Development Goals (SDG 2.4), it is imperative to promote a more resilient and sustainable agriculture that is based on ecologically friendly techniques and solutions capable of maintaining and repairing ecosystems and natural resources (United Nations, 2015). In this regard, biostimulants offer an intriguing sustainable alternative that could be crucial in boosting crop yield and resilience in challenging environmental circumstances, reducing the use of agrochemicals, and addressing the consequences of climate change (Castiglione et al., 2021; Del Buono, 2021; Ganugi et al., 2021). Additionally, compared to agrochemicals, microbial biostimulants have the benefit of not accumulating over time, being less hazardous, and having a rare potential to select resistant strains of diseases and pests (Sangiorgio et al., 2020).

Seed germination is a major determinant of plant development and final yield establishment but strongly reliant on the plant's abiotic and biotic environment. In the context of global climate change, classical approaches to improve seed germination under challenging environments through selection and use of synthetic pesticides reached their limits (Rétif, F., et al. 2023).

The success of crop production depends on two distinct and subsequent stages: germination and emergence. Abiotic stress and changes in management can affect these stages. Water

imbibition by the seed, rupture of the seed coat, and the appearance of a radical are all part of germination. Utilizing the seed's stored resources to support early development until they transform into phototrophic organisms is necessary for this shift from seed to active growth (Nonogaki et al., 2014; Ali & Elozeiri, 2017; Szczerba et al., 2021). The proper emergence and establishment of uniform seedlings are crucial components linked to final yield and are frequently regarded as the most vulnerable stage of a crop's life cycle (Fenner & Thompson, 2005). Numerous studies have demonstrated the detrimental effects of metabolites generated by a range of seed-borne fungus on seed germination and seedling vigor. They are known to produce harmful metabolites that, if ingested, might be fatal (Amza, J. 2018; Park et al., 2023).

Plants are confronted with various and intricate interactions involving numerous environmental factors (Ghadirnezhad Shiade et al. 2023a,b). When exposing by biotic and abiotic stress, plants experience disruptions in their metabolic processes, leading to decreased overall growth and reduced productivity (González Guzmán et al. 2022; Ghadirnezhad Shiade et al. 2023c).

This study evaluated the efficacy of biostimulants in improving germination, emergence, and growth under varying growing temperature conditions using various phenotyping methods. In the present investigation attempt was to observe the effect of fungal metabolites on seed germination and seedling vigour of soybean, and the results are presented in this paper.

II. MATERIALS AND METHODS

The effect of culture filtrate on seed germination and seedling vigor was investigated using a total of seven different soybean seed mycoflora (Gowda et al., 2020). *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, *Drechslera* spp, *Fusarium* spp., *Cladosporium* spp., and *Rhizopus oryzae* were used for the study (Ahmed et al., 2016).

Composition of Richards Broth (1 litre)

- Potassium nitrate (KNO₃): 10.00g

- Monopotassium dihydrogen phosphate (KH₂PO₄): 5.00g
- Magnesium sulphate (MgSO₄): 2.50g
- Ferric chloride (FeCl₃): 0.02g
- Sucrose (C₁₂H₂₂O₁₁): 50.00g
- Distilled water: 1000ml

Preparation of Fungal Culture Filtrates and Seed Treatment

Preparation of culture media: In conical flasks (250 ml), 50 ml of Richard's broth medium was taken and sterilised.

Fungal culture inoculation & Filtrate: The discs (fungal colony) from days seven-day-old culture were inoculated into the flask and incubated for 21 days at a room temperature of 25±2 °c. Culture filtrates were obtained by filtering the contents through Whatman filter paper (Park et al., 2023).

Seed Soaking Treatment: One hundred healthy soybean seeds were separately soaked in these culture filtrates for 12h.

Seed germination test: Later, ten seeds were inoculated in sterilized plates containing moist blotter paper and allowed to germinate. Seeds soaked in uninoculated sterilized water served as a control. Petri plates were incubated at 25±2 °C for 7 days. After the incubation period, seed germination and seedling vigour were recorded.

III. RESULTS AND DISCUSSION

All of the fungi culture filtrate reduced seed germination and seedling vigor in comparison to the control. The *Rhizopus oryzae* culture filtrate showed the greatest decrease in seed germination (2.2%) compared to the control (8.71%), as indicated by the results (Table 1). Next in line were *Drechslera* spp (3.9%), *Aspergillus niger* (2.6%), and *Aspergillus flavus* (2.7%). *Rhizopus oryzae* is recognized to impact the germination of some oil seeds, despite its high effectiveness on soybeans. The *Fusarium* spp example showed the least amount of seed germination reduction (5.9%).

Table 1. The effects of culture filtrates on soybean seed germination and seedling vigor

Mycoflora	Germination of the seed	Seedling Vigour	
		Plumule length (cm)	Radicle length (cm)
Control	8.71	12.34	16.04
<i>Aspergillus niger</i>	2.6	2.82	4.17
<i>Aspergillus flavus</i>	2.7	2.84	4.97
<i>Alternaria alternata</i>	4.9	2.89	5.17
<i>Rhizopus oryzae</i>	2.2	1.63	2.26
<i>Cladosporium spp.</i>	4.5	2.3	3.4
<i>Drechslera spp.</i>	3.9	1.76	2.51
<i>Fusarium spp.</i>	5.9	4.69	5.61
Mean	4.42	3.90	5.51

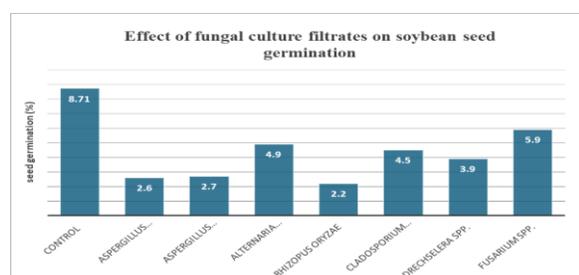


Fig.1 Effect of fungal culture filtrates on soybean seed germination.

The culture filtrate of *Rhizopus oryzae* (1.63 cm) and *Drechslera spp* (1.76 cm) showed the greatest reduction in plumule length in terms of seedling vigor (fig.1). However, the culture filtrate of *Fusarium spp* showed the least loss in plumule length (4.69 cm) when compared to the control (12.34 cm).

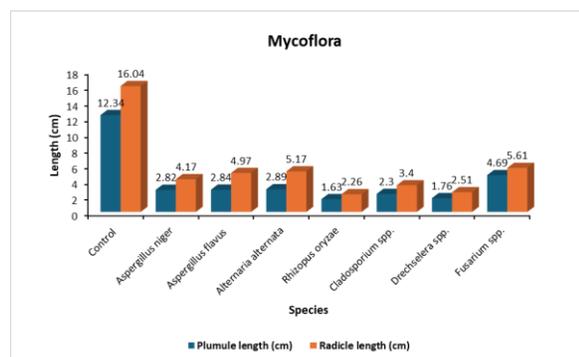


Fig. 2 The mycoflora

In comparison to the control (16.04 cm), the culture filtrate of *Rhizopus oryzae* also showed the largest reduction in radicle length (2.26 cm), followed by *Drechslera spp* (2.51 cm). The culture filtrate of

Alternaria alternata showed the smallest reduction in radicle length (5.17cm) (fig.2). Toxic metabolites can be absorbed, translocated, and interfere with the seed and seedling's normal metabolic processes during germination and seedling growth, which can reduce seed germination. The toxic culture filtrates of *Aspergillus flavus* and *Aspergillus niger* prevented plumule length decrease and germination. These findings align with those of Chandra et al. Additionally, Singh and Swami discovered that the *Aspergillus flavus* culture filtrate decreased pearl millet seed germination, plumule length, and radicle length. The culture filtrate of *Fusarium spp*, *Aspergillus* species, *Cladosporium* species, and *Curvularia lunata* has been shown by Subbaraja to limit root and shoot elongation as well as seed germination. As a result, it seems that *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium spp*, and *Rhizopus oryzae* have a direct impact on seed germination. They either reduce the viability of the seed by making it lack nutrients or by filtering out mycotoxic substances that are detrimental to the seed while it is being stored.

IV. CONCLUSION

Environmentally friendly agricultural inputs known as biostimulants can enhance plant health and yield potential in the face of environmental challenges. Extreme weather throughout the growing season affects the health and productivity of soybeans. In the culture filtrate of seven distinct soybean seed mycoflora, seed germination and seedling vigor were evaluated. These findings collectively support the conclusion that the fungi tested (specifically *Rhizopus oryzae*, *Drechslera spp*, *Alternaria alternata*, *Aspergillus flavus*, and *A. niger*) produce toxic metabolites (mycotoxic substances) in their culture filtrates. These substances, when absorbed, are capable of interfering with the normal metabolic processes during germination and seedling growth, resulting in a significant reduction in seed viability and seedling vigor, particularly the length of the radicle. This effect poses a serious challenge to seed health and crop establishment, particularly for seeds stored under conditions that permit fungal growth.

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