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Evaluating the Potential of Fluoride-Resistant Fungi as Plant Growth Promoters in Rhizosphere Soil from Madhya Pradesh and Rajasthan

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Abstract

This study aimed to assess the plant growth-promoting abilities of selected fluoride-resistant fungi isolated from rhizosphere soil samples in fluoride-impacted regions of Bhopal District Madhya Pradesh and Sikar District Rajasthan, India. The fungi evaluated included *Aspergillus niger*, *Trichoderma harzianum, Aspergillus flavus, Penicillium chrysogenum, Cladosporium cladosporioides,* and *Aspergillus tamarii*. We also examined IAA bioactivity, ammonia production, phosphate dissolving power, HCN production, and siderophore production. As positioning of the crops was found to vary with the addition of the fungi, it was also found that both *Aspergillus niger* and *Trichoderma harzianum* were the most effective in boosting plant growth metrics. This ability to synthesize the hormone IAA was also found to vary with the '*A. niger*' strain being the most consistent with elevated synthesis levels even in the presence of fluoride, indicating its potential to enhance plant stress tolerance. Likewise, the ammonia production assay confirmed the results obtained through the previous test, further establishing '*A. niger*' as an efficient donor of nitrogen, which was even further confirmed through the phosphate solubilizing assays. The assessments conducted for HCN production expanded the potential of the fungi as biocontrol agents, which was already attributed to their siderophores production as well as nutrient uptake mechanisms. This study underscores the important role of endophytic plant growth-promoting fungi (PGPF) that can thrive in fluoride-rich environments, helping to protect plants from

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fluoride-induced damage. By integrating these findings into sustainable agricultural practices such as bioaugmentation, organic amendments, and the cultivation of fluoride-resistant plant species the research lays the groundwork for biological strategies aimed at alleviating the negative effects of fluoride. The ultimate goal is to enhance agricultural productivity and improve soil health in areas impacted by fluoride contamination

Introduction

Fluoride contamination is a pressing environmental and public health challenge, particularly impacting arid and semi-arid regions by degrading water quality and posing significant health risks. Over the last decade, countries across Africa, Asia, and South America have reported fluoride levels surpassing the World Health Organization's safety standard of 1.5 mg/L,[1] with Rajasthan in India[2] experiencing elevated concentrations due to both geological and anthropogenic factors. Globally, more than 200 million people are affected by fluoride contamination,[3] notably in Africa, Asia, and parts of Europe. In countries like Iran, Pakistan, and Kenya, high fluoride levels in groundwater primarily result from natural weathering of fluorite-bearing minerals.[3] However, industrial emissions, phosphate fertilizers, and brick manufacturing also contribute significantly to anthropogenic fluoride discharges.[1]

India serves as a critical focus for studying fluoride pollution, affecting approximately 62 million people with conditions like dental and skeletal fluorosis, notably in regions like Rajasthan where fluoride levels range from 0.2 to 69.0 mg/L due to natural and human activities.[2] This widespread exposure underscores the urgent need for effective management strategies. Fluoride's impact stretches across environmental facets, including soil, water, and biological systems. Chronic exposure results in dental and skeletal fluorosis, with children being particularly susceptible due to developmental consequences. The agricultural footprint involves disrupted crop yield and quality, impacting processes like photosynthesis and nutrient uptake, and the systemic uptake of fluoride through irrigation exacerbates its presence in the human food chain.[4] Fluoride challenges agriculture by disrupting key physiological processes such as photosynthesis and respiration, causing stunted growth and reduced yields. While some plants like Camellia sinensis show tolerance, recent studies spotlight Plant Growth-Promoting Fungi (PGPF) as a natural means to enhance plant resilience to fluoride stress.[5] These fungi engage symbiotically with plants, boosting nutrient uptake and activating defense mechanisms to counter fluoride-induced oxidative stress. Rhizosphere fungi like Trichoderma and Penicillium alleviate fluoride toxicity by bioaccumulation, chelation, and strengthening antioxidative defenses, enhancing plant growth under abiotic stress conditions.[6] Additionally, these fungi detoxify fluoride by secreting organic acids that chelate fluoride ions, reducing their availability to plants.[7]

This research focuses on identifying and applying Plant Growth-Promoting Fungi (PGPF) as innovative solutions to mitigate the effects of fluoride contamination on soil and plant health. PGPF have demonstrated potential in enhancing plant growth and tolerance to various environmental stresses, including high fluoride levels, by optimizing nutrient absorption, activating plant defenses, and aiding in the detoxification of harmful ions through crucial biochemical pathways.[8] The goal is to establish sustainable and eco-friendly strategies using fluoride-tolerant fungi to alleviate fluoride toxicity in agriculture, improve nutrient uptake, and enhance plant resilience. This approach aims to boost crop yields and quality while promoting food security in fluoride-affected regions, aligning with sustainable agricultural practices to address this pervasive environmental challenge.

Materials and Methods

In the present study, we evaluated selected fluoride-resistant fungi for Plant Growth-Promoting Abilities including *Aspergillus niger, Aspergillus tamari, Trichoderma harzianum, Aspergillus flavus, Penicillium chrysogenum,* and*, Cladosporium cladosporioides*, all of which were isolated from the rhizosphere of plants in fluoride-impacted areas of Bhopal District Madhya Pradesh and Sikar District Rajasthan. The isolated fungal colonies were identified based on their morphological characteristics and further confirmed through molecular techniques, such as DNA sequencing, in our previous study.

Evaluation of Plant Growth-Promoting Abilities

Indole-3-Acetic Acid (IAA) Production

The ability of the isolated fungi to produce IAA was assessed using the Salkowski method.[9] Fungal cultures were grown in Luria-Bertani broth supplemented with 0.1% tryptophan. After 7 days of incubation at 28 ± 2°C, IAA was extracted using ethyl acetate and quantified at 530 nm using a spectrophotometer. Qualitatively analyzing IAA production, fungal cultures (e.g., *Aspergillus niger, Trichoderma harzianum*) were prepared with the same incubation and extraction procedures described. The Salkowski test was performed by mixing 1 ml of the ethyl acetate extract with 2 ml of Salkowski reagent and allowing it to stand for 30 minutes to develop color, indicating the presence of IAA.[10] The intensity of the color was compared against a control (LB broth with tryptophan) to assess qualitative differences in IAA production. Results were documented by photographing each sample, clearly labeling the fungal species and corresponding IAA levels, and compiling these into a single figure for visual representation. This approach enables clear visualization of the metabolites produced and highlights the plant growth-promoting potential of the analyzed fungi.

Ammonia Production

Ammonia production was determined using the Nessler's reagent method as described by Cappuccino and Sherman.[11] Fungal cultures were grown in peptone water, and after incubation, the presence of ammonia was confirmed with Nessler's reagent, allowing for colorimetric analysis. This method assists in evaluating the nitrogen availability provided by the fungi.[12]

Phosphate Solubilization

Phosphate solubilization capacity was evaluated on Pikovskaya's agar following the methodology of Pikovskaya[13] and Nautiyal.[14] The fungal isolates were inoculated into the medium, and after incubation, the solubilization halo around the colonies was measured to assess phosphorous solubilization efficiency.

Hydrogen Cyanide (HCN) Production

HCN production was measured using the alkaline method as described by Bakker and Schippers.[15] Fungal cultures were aseptically inoculated into a medium containing glycine, and after incubation, the production of HCN was assessed by the color change when reacting with NaOH and $FeCl_3$.

Siderophore Production

The production of siderophores was assessed using the Chrome Azurol S (CAS) assay, based on the method by Schwyn and Neilands[16] and Alexander and Zuberer.[17] Fungal isolates were grown in liquid medium containing CAS, and after incubation, the change in color indicated the presence of siderophores. Quantification was achieved by measuring the absorbance at 630 nm. For the assessment of siderophore production by fungal species through the observation of characteristic blue and orange zones, Potato Dextrose Agar (PDA) or Czapek's Yeast Extract Agar was prepared, and the plates were inoculated with selected fungal isolates using sterile techniques. After incubating the inoculated plates at 28 ± 2°C for 5-7 days, the plates were examined for clear zones around the fungal colonies. The presence of orange or blue zones indicated the type of siderophores produced; orange zones typically suggest hydroxamate-type while blue zones indicate catecholate-type siderophores. Control and Fluoride-Treated Conditions: Parallel experiments were conducted under control and fluoride-treated conditions (0.5%). Fluoride treatment involved adding specific concentrations of sodium fluoride to the growth media to simulate fluoride stress. The performance of fungal species under these conditions was compared to their respective controls to assess the impact of fluoride on their plant growth-promoting abilities.

Results

The study specifically examines IAA production, ammonia production, phosphate solubilization, and hydrogen cyanide (HCN) production in six key fungal species: *Aspergillus niger, A. tamari*, *A. flavus*, *Trichoderma harzianum*, *Penicillium chrysogenum*, and *Cladosporium cladosporioides*. Evaluating each species' ability to produce these metabolites under both control and fluoride-treated conditions indicates their potential to act as PGPFs, which can be crucial for environmentally friendly practices worldwide, particularly in regions affected by fluoride.

Table 1.1: provides absorbance readings at 530 nm, indicating IAA levels under control and fluoride-exposed (0.5%) conditions.

S. No.	Species	Control IAA (µg/ml) Mean	IAA under Toxicant (µg/ml) Mean	Control IAA - IAA under Toxicant (µg/ml) Mean
1	*Aspergillus niger*	27.71	24.88	2.83
2	*Trichoderma harzianum*	27.33	21.79	5.92
3	*Aspergillus flavus*	18.33	9.22	9.11
4	*Penicillium chrysogenum*	23.37	15.50	7.87
5	*Cladosporium cladosporioides*	14.26	8.51	5.75
6	*Aspergillus tamarii*	16.12	14.80	1.32

IAA Production

The analysis of indole-3-acetic acid (IAA) production demonstrated significant variability among the tested fungal species.

Data indicated that *Aspergillus niger* maintained the highest IAA production levels (27.71 µg/ml) despite a decrease to 24.88 µg/ml in the presence of fluoride, suggesting potential resilience to fluoride toxicity. In contrast, *Trichoderma harzianum* exhibited a decline from 27.33 µg/ml to 21.79 µg/ml, while *Aspergillus flavus* showed a substantial reduction from 18.33 µg/ml to 9.22 µg/ml, highlighting its sensitivity.

The findings presented in quantitative and qualitative tests (Table 1.1) highlight the variable resilience of different fungal species to fluoride stress, suggesting that species like *Aspergillus niger* could play an essential role in promoting plant growth in contaminated soils.[1,2]

Ammonia Production

Ammonia production serves as a crucial indicator of nitrogen availability for plants. *Aspergillus niger* displayed the highest ammonia production (178.94 µg/ml), though it decreased to 135.41 µg/ml under fluoride conditions, indicating strong but impaired ammonia production under stress (Table 1.2). *Trichoderma harzianum* maintained a reasonable level of ammonia production despite a modest decline. In contrast, *Penicillium chrysogenum*, *Cladosporium cladosporioides*, and *Aspergillus tamarii* showed almost complete inhibition under fluoride conditions, highlighting their vulnerability to fluoride toxicity. The findings suggest that while some species exhibit notable resilience, others are significantly affected by fluoride, potentially limiting their effectiveness as PGPFs.[18,4]

Table 1.2: summarizes the mean ammonia concentrations produced by the PGPF species both under control conditions and in the presence of fluoride.

S. No.	Species	Control Ammonia (µg/ml) Mean	Ammonia Under Toxicant (µg/ml) Mean
1	*Aspergillus niger*	178.94	135.41
2	*Trichoderma harzianum*	103.06	96.00
3	*Penicillium chrysogenum*	23.65	0.12
4	*Aspergillus flavus*	91.29	58.94
5	*Cladosporium cladosporioides*	10.71	ND
6	*Aspergillus tamarii*	8.35	ND

Phosphate Solubilization

Phosphate solubilization by fungi is crucial for enhancing soil fertility, particularly in environments characterized by nutrient limitations and chemical contaminants. The results for phosphate solubilization are summarized in Table 1.3.

Table 1.3: showcasing the effectiveness of these fungal species under control and fluoride-treated conditions.

S. No.	Species	Control Phosphate Solubilization (µg/ml) Mean	Phosphate Solubilization Under Toxicant (µg/ml) Mean
1	*Penicillium chrysogenum*	125.68	116.59
2	*Trichoderma harzianum*	137.05	105.23
3	*Aspergillus niger*	137.05	116.59
4	*Aspergillus flavus*	175.68	137.05
5	*Cladosporium cladosporioides*	50.68	46.14
6	*Aspergillus tamarii*	143.86	130.23

Aspergillus flavus exhibited the highest phosphate solubilization under control conditions at 175.68 µg/ml, but its ability decreased to 137.05 µg/ml when exposed to fluoride. This indicates a significant reduction yet suggests a capacity for maintaining functional solubilization. Similarly, *Trichoderma harzianum* showed a decline from 137.05 µg/ml to 105.23 µg/ml, reflecting a reduction due to fluoride stress.

In contrast, *Penicillium chrysogenum*, while initially effective, faced considerable reductions in their phosphate solubilization capabilities under fluoride conditions. The declines emphasize the importance of these functions in agricultural settings, where nutrient uptake is critical for plant health.[7,19]

The ability of these fungi to solubilize phosphate under fluoride stress is vital for improving soil nutrient profiles. The findings suggest that species such as *Aspergillus niger* and *Trichoderma harzianum*, despite some decline, can still play important roles in enhancing phosphorus availability in contaminated soils.

Hydrogen Cyanide Production

The production of hydrogen cyanide (HCN) by beneficial fungi serves as an important mechanism of biocontrol, as HCN can inhibit the growth of pathogens.

Table 1.4: The HCN production data from the selected fungal species under different experimental conditions.

S. No.	Species	Control Hydrogen Cyanide (HCN) (Absorbance at 625 nm) Mean	HCN Under Toxicant (Absorbance at 625 nm) Mean
1	*Aspergillus niger*	0.090	0.018
2	*Aspergillus flavus*	0.098	0.092
3	*Penicillium chrysogenum*	0.072	0.069
4	*Trichoderma harzianum*	0.0123	0.0115
5	*Aspergillus tamarii*	0.059	0.051
6	*Cladosporium cladosporioides*	0.0104	0.0080

In the present study (Table 1.4) *Aspergillus niger* showcased the highest HCN production under control conditions but experienced a drastic reduction in levels when exposed to fluoride, indicating high sensitivity and potential metabolic disruption. The ability to produce HCN, despite fluoride toxicity, points to its implications for biocontrol mechanisms. While the analyzed fungal species showed varying degrees of resilience to fluoride, those like *Aspergillus niger* and *Trichoderma harzianum* exhibit significant PGP potential through IAA, ammonia, and phosphate production, whereas their ability to synthesize HCN reveals their potential as biocontrol agents. The modest reductions in HCN levels suggest that such species may still be employed effectively in agricultural practices, even under fluoride-stressed conditions.

Siderophore Production

Siderophores are essential for iron acquisition in fungal species, particularly in environments where iron availability is restricted by various stressors. By quantifying the production of siderophores in the examined fungal species, we can gain important insights into their capacity to enhance plant growth through improved nutrient uptake. This study specifically investigates the siderophore production potential of four plant growth-promoting fungi: *Aspergillus niger, Trichoderma harzianum*, *Aspergillus flavus*, and *Penicillium chrysogenum*, all of which demonstrated strong plant growth-promoting potential in other assays conducted during this research.

Table 1.5: outlines the siderophore production levels measured under control and fluoride conditions.

S. No.	Species	Control Siderophore % (Mean ± SD)	Siderophore % Under Toxicant (Mean ± SD)
1	*Aspergillus niger*	82.28 ± 0.67	79.65 ± 0.74
2	*Trichoderma harzianum*	56.25 ± 0.84	54.87 ± 0.68
3	*Aspergillus flavus*	36.38 ± 0.83	31.21 ± 0.69
4	*Penicillium chrysogenum*	63.69 ± 0.65	58.00 ± 0.78

In the present study (Table 1.5) *Aspergillus niger* maintained the highest siderophore production levels, with only a slight decrease when exposed to fluoride. This resilience indicates its potential effectiveness in mobilizing iron, which is essential for plant growth, particularly in iron-deficient soils. Similarly, *Trichoderma harzianum* showed a minor reduction in its siderophore production, suggesting that it can also maintain iron acquisition capabilities under stress. Conversely, other species such as *Aspergillus flavus* and *Penicillium chrysogenum* displayed significant declines in siderophore production upon exposure to fluoride, indicating their sensitivity to environmental stressors. The reduction in iron-mobilizing abilities due to fluoride exposure could limit these fungi's effectiveness as PGPFs, particularly in contaminated environments.

Discussion

This study underscores the multifaceted roles and mechanisms by which select fungi function as plant growth-promoting agents in fluoride-contaminated agricultural systems. Notably, species like *Aspergillus niger* and *Trichoderma harzianum* significantly enhance plant health through the production of indole-3-acetic acid (IAA), ammonia, phosphate solubilization, and siderophore production.[20] Although fluoride exposure impairs metabolic functions, it also presents opportunities for utilizing these fungi in bioremediation to restore soil health.[21]

Maintaining essential nutrient cycling under fluoride stress is critical, highlighting the importance of these resilient fungi in enhancing soil fertility and plant growth in contaminated areas. This can play a vital role in supporting food security and ecosystem health. The observed sensitivity and resilience variations among fungal species facilitate the strategic selection of the most appropriate strains for specific environmental contexts, with adaptive species serving as effective bioamendments in fluoride-affected soils to boost agricultural productivity and soil vitality.[22]

The findings reveal significant variability in the resilience and efficacy of the examined fungi. *Aspergillus niger* exhibited robust IAA production despite fluoride exposure, indicating its resilience, while *Aspergillus flavus* showed reduced production, reflecting its sensitivity. Concerning ammonia production, *Aspergillus niger* maintained relatively high levels under fluoride stress, whereas *Penicillium chrysogenum* faced near-total inhibition, indicating its vulnerability. Although phosphate solubilization generally decreased due to fluoride, *Aspergillus flavus* retained some functionality, demonstrating adaptability in nutrient-poor environments. While *Aspergillus niger* showed elevated hydrogen cyanide (HCN) production under control conditions, its drastic reduction under fluoride suggests metabolic challenges, though it retains its biocontrol potential. Siderophore production by *Aspergillus niger* and *Trichoderma harzianum* remained stable, which is crucial for facilitating plant growth in iron-deficient soils, while other species displayed significant declines.[23,24] Overall, fungi like *Aspergillus niger* and *Trichoderma harzianum* emerge as promising candidates for promoting plant growth, particularly in fluoride-impacted regions. Their adaptability variability highlights the necessity of developing sustainable agricultural practices that are specifically tailored to address unique environmental challenges.

Conclusion

This research elucidates the role of soil as a medium in sustaining plant growth while displacing soil detrimental antibiotic activity or severe fluorine impact. Soils affected by fluorine were found to contain fungi that promote plant growth such as *Aspergillus niger* and *Trichoderma harzianum*, that performed well on axes like IAA production, ammonia synthesis, phosphate solubilization, and siderophore production. Due to their high level of tolerance with regard to fluoride, they could be utilized to promote plant growth and rehabilitate soil in polluted areas. It is important to point out that Aspergillus species are capable of enhancing the growth of plants, but depending on the condition, they can also be toxic. This dual nature emphasizes the need for careful consideration of their application in agricultural practices, particularly in fluoride-stressed environments.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Author Contributions

- **Ritu Kanthiya:** Data collection, Performing the experiments, and manuscript writing.
- **Rakesh Kumar Verma:** Conceptualization, Supervision and critical revision of the manuscript.
- **Deepak Bharti:** statistical analysis and help in manuscript writing.
- **Saloni Kanthiya:** Literature review and editing assistance.

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