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Efficacy of Potassium Solubilizing Fungi isolated from the Rhizospheric Soil of Paddy

R. Ranjitha[1], Alagappan Gandhi[*2]

[1]*Department of Botany, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu- 608002, India*

[2]*Assistant professor, Department of Botany H.H. the Rajah's College, Pudukkottai, Tamilnadu, India*

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Abstract

Improving soil fertility is essential for enhancing agricultural productivity, with potassium (K) being a crucial nutrient for plant growth. In soil, potassium is predominantly found in insoluble forms, making it unavailable to plants. This study aims to identify and evaluate fungal strains capable of solubilizing insoluble potassium sources to improve soil fertility. Soil samples were collected from paddy fields in Pudukkottai, Tamil Nadu, and potassium-solubilizing fungi were isolated using modified Aleksandrov agar and Potato Dextrose Agar media. Ten fungal isolates were screened for their ability to solubilize potassium, with KSF1 demonstrating the highest solubilization index (SI) of 1.51. Quantitative analysis revealed that KSF1, identified as *Aspergillus niger*, released the highest amount of potassium from mica (42.16 mg/25 ml) on the 10th day of incubation. This isolate showed superior solubilization capabilities compared to others. The findings suggest that *Aspergillus niger* KSF1 is a highly effective potassium solubilizer, offering a sustainable approach to enhancing soil fertility and supporting agricultural productivity.

* **Corresponding Author:** Alagappan Gandhi ✉ drgbiofert@gmail.com

Introduction

Improving soil fertility is one of the most common methods of agricultural production. Potassium (K) is one of the most important plant nutrients for increasing plant production. In plants, K plays a vital role in numerous biochemical and physiological activities such as photosynthesis, respiration, energy generation, nucleic acid biosynthesis cell, osmotic regulation, and enzyme activation (Masood and Bano, 2016). Most soil potassium, around 98%, is in the form of insoluble potash and cannot be used by plants and just 1-2% is available for plants Meena *et al.*, (2016).To enhance potassium availability for plants, large amounts of fertilizer are regularly applied in the fields. However, many of these fertilizers are converted to insoluble forms after application and require continuous reapplication (Kang *et al.*, 2002). Microorganisms are essential to the natural potassium cycle, with certain fungal strains in the soil capable of dissolving minerals such as mica, feldspar, and potassium aluminium silicate. Filamentous fungi, especially those from *Aspergillus* species, produce significant quantities of organic acids, which are essential for breaking down insoluble substrates containing potassium (B. Bagyalakshmi, 2012; Basak *et al.*, 2022; Lian *et al.*, 2008; Samah M. *et al.*, 2023). The various microorganisms and minerals capable of releasing potassium from minerals have been recently reviewed (Etesami *et al.*, 2017; Sharma *et al.*, 2016). The fungus has been reported to have a higher solubility of insoluble potassium than bacteria.

A wide range of soil fungi are reported to dissolve insoluble phosphorus such as *Aspergillus niger*, which is the most common fungus having the ability to dissolve potassium-bearing minerals. The goal of this research is to find fungi that can bind potassium from various forms of potassium that are unavailable. If successful, this could provide a sustainable and environmentally friendly method of boosting agricultural yields and improving soil fertility.

Materials and methods

Collection of Soil Sample

Soil samples were collected from the rhizosphere of paddy in Pudukkottai district (10.419990°N and 78.781712°E) of Tamil Nadu mentioned in the Fig. 1. These samples were placed in polythene bags, transported to the laboratory, and stored in a refrigerator until they could be processed.

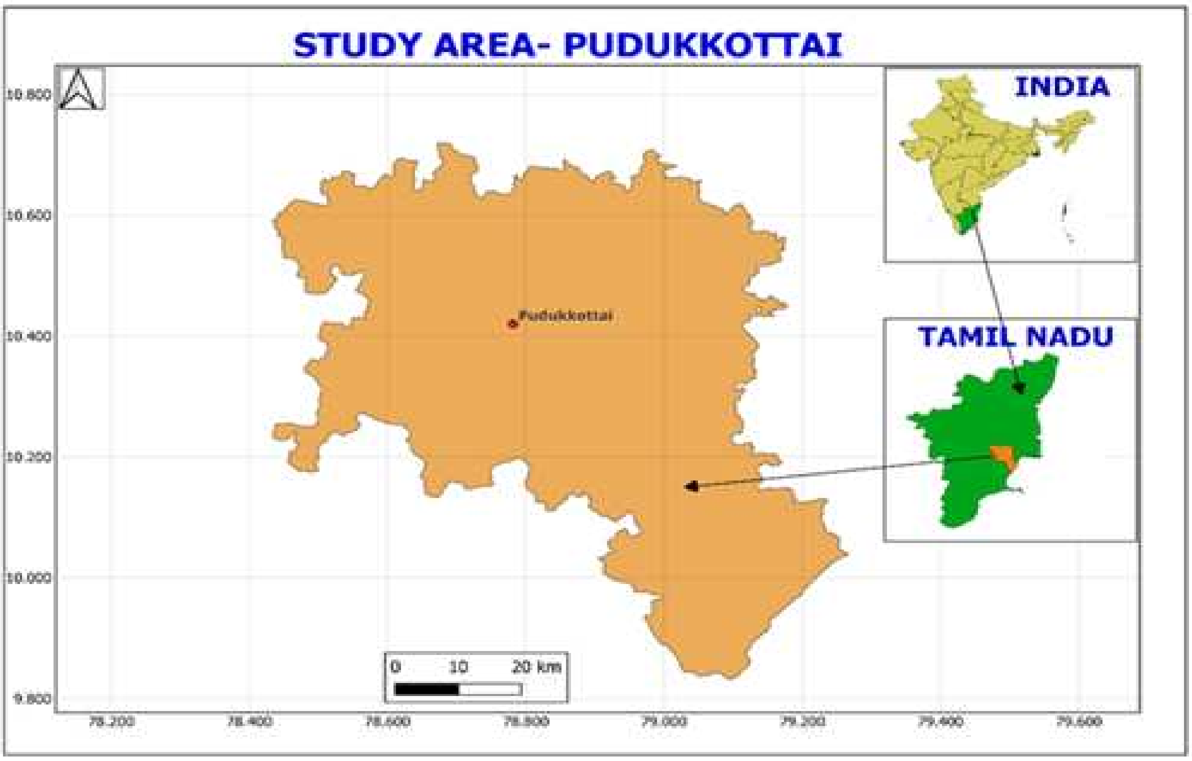

Fig. 1. Map of the study area Pudukkottai district of Tamil Nadu.

Culture media for isolation

To isolate and preserve potassium-solubilizing fungi (KSF), a modified Aleksandrov agar medium was utilized, composed of magnesium sulfate (0.5g), potassium aluminosilicate mica (0.1g), glucose (5g), ferric chloride (0.005g), calcium phosphate (2g), agar (20g), and distilled water (1 L), with a pH of 7.2 ± 0.2 (P. Sugumaran and B. Janarthanum, 2014). For fungal culture isolation and maintenance, Potato Dextrose Agar (PDA) was used, which included potato infusion (200g), dextrose (20g), and agar (15g), with a pH of 5.6 ± 0.2. The pH of both media was adjusted with 1N NaOH or 1N HCL, and the media were sterilized by autoclaving at 121°C for 15 minutes.

Isolation and screening of KSF

Soil samples were used to isolate potassium-solubilizing fungi by using Potato Dextrose Agar (PDA). Ten grams of soil were mixed with 90 ml of distilled water in a 250 ml of Erlenmeyer flask. From which one ml of the supernatant was diluted in 9 ml of distilled water, resulting in dilutions of 10^{-1} to 10^{-6}. One ml from each dilution was plated on PDA and incubated at 28 ± 2°C for 5-7 days. Colonies were repeatedly cultured on PDA plates until pure. These pure cultures were stored in the refrigerator for further use.

To screen for potassium solubilizers, isolates were inoculated onto Aleksandrov Agar with 0.5% mica and incubated at 28 ± 2°C for 5 days. Colonies forming clear halo zones were identified as potassium solubilizers.

Solubilization of index

Suspensions were prepared in distilled water for isolated fungal cultures. The optical density of each culture was adjusted at 0.3 using a colorimeter at wavelength 520nm. The 10µl suspension of each isolate was placed on an Aleksandrov agar plate and incubated at 28±2°C for 5 days in the incubator. The solubilization index (SI) was measured using the following formula (Khandeparkar's and Bhosle 2004; Ramesh *et al.*, 2014; Yaghoubi Khanghahi *et al.*, 2018).

Solubilizing index = Colony diameter + Halo zone diameter / Colony diameter

Identification

The fungal cultures were identified based on colony characteristics and microscopic examination (H. Barnett and B. Hunter, 1998; J. Gilman 2008).

Quantitative estimation of KSF

A volume of 25 ml of sterile Aleksandrov broth, containing potassium aluminosilicate (mica, feldspar) as the insoluble potassium source, fresh suspension (OD (600nm) = 0.16) of each isolate, which had been prepared in sterile saline. The inoculated cultures were incubated at 28±2°C for 5 days with constant shaking at 120 rpm. Following incubation, the cultures were centrifuged at 10,000 rpm for 10 minutes. Subsequently, 1 ml of the supernatant was extracted and the concentration of soluble potassium was determined via flame spectrophotometry, following the method described by (Zhang and Kong, 2014). The entire experimental procedure was performed in triplicate for each isolate.

Results and discussion

Ten fungal isolates were obtained from the rhizospheric soils of a paddy field based on their ability to form transparent halo zones on the Aleksandrov agar medium. These isolates were named as KSF1, KSF2, KSF3, KSF4, KSF5, KSF6, KSF7, KSF8, KSF9, and KSF10.

Out of these, one isolate KSF1 was selected for further analysis due to their larger zones of clearance on Aleksandrov agar medium (Fig. 2.), indicating their potential as potent potassium solubilizers. Fungi that produce halo zones around their colonies are considered effective potassium solubilizers and are predominantly from the *Aspergillus* genus, with *Aspergillus niger* being a prominent species, as supported by the findings of (Lopes-Assad *et al.*, 2010; Chandrashekar *et al.*, 2014).

The solubilization indexes (SI) of these isolates were calculated using the ratio of the diameter of the zone clearance (X) to the diameter of growth (Y). KSF1 demonstrated the highest SI of 1.51, indicating strong solubilization activity, followed by KSF7 (1.43), KSF2 (1.29), and KSF5 (1.24). These results suggest that KSF1 has the highest capability for solubilization, compared to KSF7, KSF2, and KSF5. The solubilization index was determined using the selection ratio method described by Khandeparkar's and Bhosle (2004), as listed in (Table 1).

Table 1. Potassium solubilization index values of selected bacterial isolates determined using Khandeparkar's selection ratio.

Isolates	X(mm)	Y(mm)	Solubilization index (SI)
KSF1	48.2	32	1.51
KSF2	40	31	1.29
KSF5	36	30.6	1.24
KSF7	42	29.3	1.43

X-(Halo zone +colony) diameter Y- Colony diameter.

Additionally, the morphological characterization of KSF1 on potato dextrose agar showed that the culture initially exhibited white mycelium for two to three days, which turned black as the conidia developed. The presence of guttulate hyphae and uniseriate conidiophores confirmed its classification within the *Aspergillus* genus, consistent with the findings of (Kang *et al.*, 2002; Gautam and K, G. A. 2013).

Table 2. Quantitative Estimation of K Solubilizing Activity of the Isolates from mica Sources.

Isolates	5th day	7th day	10th day
Control	2.06±0.078	2.15±0.09	2.17±0.15
KSF1	22.12±0.26	30.31±0.12	42.16±0.41
KSF2	9.42±0.48	11.01±0.48	12.93±0.23
KSF5	6.87±0.39	8.26±0.5	11.05±0.29
KSF7	12.36±0.44	15.74±0.30	19.96±0.47

All values are presented as mean ± SD based on triplicate measurements.

The findings underscore KSF1's superior efficiency as a potassium solubilizer, followed by KSF7, KSF2, and KSF5. KSF1, in particular, presents promising applications for enhancing potassium availability in soils, thereby improving crop growth and yield. Mechanisms driving these isolates' solubilization capabilities to fully leverage their potential in sustainable agriculture.

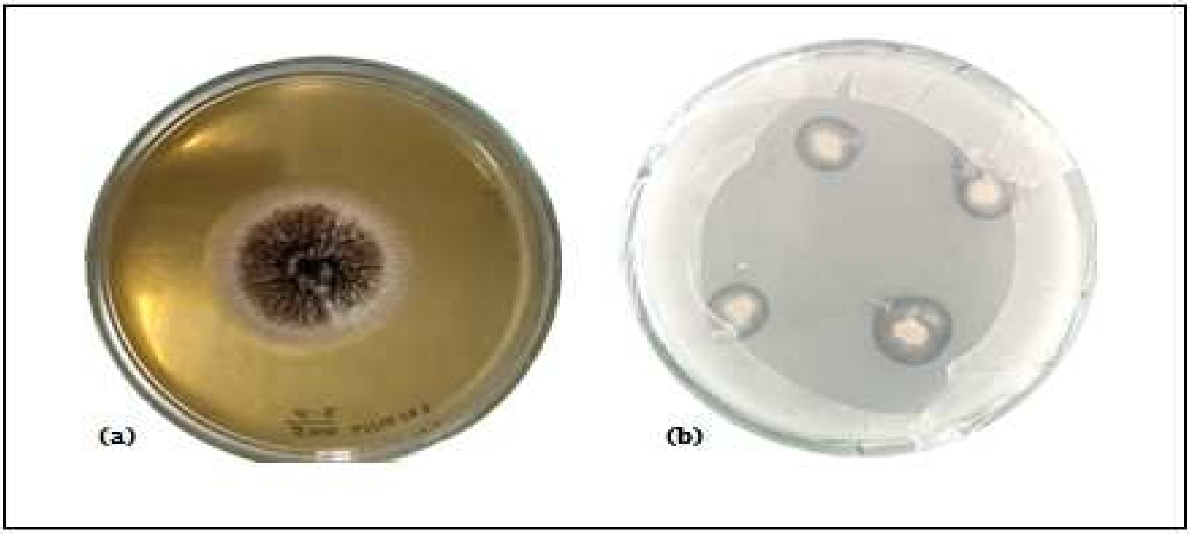

Fig. 2. (a) *Aspergillus niger* & (b) Halo zone formation.

The study measured the amount of potassium (K) released from mica in a broth by different isolates on the 5th, 7th, and 10th days after incubation. The results, as shown in (Table 2), indicate that the amount of K released from the mineral increased with longer incubation periods, reaching its highest levels at 10 days after incubation (DAI). On the 10th day, the K released by the strains ranged from 11.05 mg/25 ml to 42.16 mg/25 ml. Additionally, a decrease in pH was observed as the incubation time increased from 5 to 10 days. Among the strains, KSF1 released the most K, while KSF5 released the least on the 10th day.

Conclusion

In this study, ten fungal isolates from the rhizospheric soils of a paddy field were evaluated for their potential as potash solubilizers, based on the formation of transparent halo zones on Aleksandrov agar medium. Four isolates (KSF1, KSF2, KSF5, and KSF7) exhibited significant zone clearance and were identified as potent potassium solubilizers, primarily belonging to the *Aspergillus* sp., particularly *Aspergillus niger*. The solubilization indices (SI), calculated as the ratio of the diameter of the zone clearance (X) to the diameter of growth (Y), revealed that KSF1 had the highest SI (1.51), followed by KSF7 (1.43), KSF2 (1.29), and KSF5 (1.24). All isolates were found to be efficient in the solubilization of mica indicating that KSF1 is the most efficient solubilizer. Morphological characterization of KSF1 on potato dextrose agar showed initial white mycelium that turned black as conidia developed, with guttate hyphae and uniseriate conidiophores confirming its classification within the *Aspergillus* sp.

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