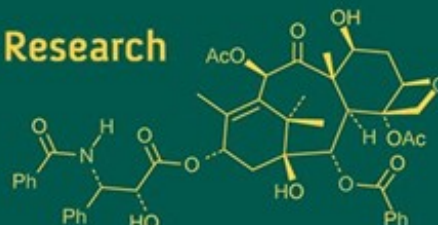
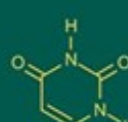


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Evaluation of potassium solubilization by rhizospheric *Aspergillus* isolates on potassium aluminosilicate medium

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ABSTRACT

Potassium is an essential nutrient for plants but most of the potassium in soil is stuck inside minerals and difficult for plants to absorb. Microorganisms can release potassium by making acids that lower the pH. Fungi *Aspergillus* is one among ubiquitous fungi present in diverse rhizospheric soil. In the study 21 isolates of fungus *Aspergillus* were collected from the rhizospheric soil of different crops and characterized phenotypically based on morphological and cultural features on PDA media. They were further screened on Aleksandrov agar medium for K solubilization ability. A total of 16/21 *Aspergillus* isolates showed K solubilization zones in range of (14.63 mm- 45.50 mm) after 7 days of incubation. Among these 08/16 isolates namely S8, S7A2, S12*, S2, S7A3, E5, Isol.1 and S5 showed higher K-solubilization ability. They showed efficient K solubilization zone (D) value in the range of 38.25 mm (S7A3) to 45.50 mm (S5), Solubilization efficiency (SE) value in range of 0.00 (S8, S12*, S2, S7A3, E5 and S5) to 1.63 (E5) and Solubilization index (SI) value in range of 1.00 (S8, S12*, S2, S7A3, S7(A), and S5) to 1.14 (E5). Results show that some rhizospheric *Aspergillus* isolates have K solubilization ability although with different K solubilization potential. The solubilization potential estimation depends on the parameter used viz. solubilization zone (D), SE and SI values.

Keywords: Potassium solubilization, *Aspergillus*, Rhizosphere, Potassium aluminosilicate, K-solubilizing fungi, Aleksandrov medium, Solubilization zone

Introduction

Potassium is one of the most important plant nutrients for increasing plant production. In plants, potassium 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 (Ranjitha *et al.*, 2024) [1].

Soil-plant-microbe interaction has got much importance in recent decades. Many types of microorganisms are known to inhabit soil, especially crop rhizosphere and play an important role in plant growth and development (Mirsam *et al.*, 2021) [2]. It is well known that a considerable number of microorganisms (bacterial and fungal) species possess a functional relationship and constitute a holistic system with plants. They are able to easily multiply in a rhizosphere to promote plant growth and yield (Jabin *et al.*, 2017) [3]. Although K is one of the most important macronutrients and the eighth most abundant element on earth comprising about 2.1% of the earth's crust, plant uptake of K is limited due to its lesser availability in the soluble form (Bhattacharya *et al.*, 2016) [4]. Soil solution potassium plays a vital role in providing the pathway for potassium uptake from the soil by plant roots (Öborn *et al.*, 2005) [5].

It is generally accepted that the major mechanism of mineral K-solubilization is the action of organic acids synthesized by rhizospheric microorganism. Productions of organic acids results in acidification of the microbial cells and its surrounding environment which promote the solubilization of mineral K (Jain *et al.*, 2022) [6]. The potassium-solubilizing ability of microorganisms is commonly screened using Aleksandrov media containing an insoluble K-bearing mineral potassium alumino silicate. The appearance of a clear or altered zone surrounding the microbial colony provides a preliminary indication of mineral dissolution (Aleksandrov *et al.*, 1967; Etesami *et al.*, 2017) [7]. Recent studies have shown that filamentous fungi like *Aspergillus*, *Penicillium*, and *Fusarium* can also be solubilize K through insoluble sources such as potassium alumino silicate under in vitro conditions (Etesami *et al.*, 2017, Saha *et al.*, 2016) [7]. The aim of research is to identify different fungi isolates of *Aspergillus* from various local rhizospheric soil samples and evaluate their ability to solubilize potassium.

Material and Methods

Fungal isolates used for K solubilization studies

The present study was carried out at the laboratory of Department of Recombination Techniques, College of Biotechnology Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P., India during 2023-24. A total of 21 rhizospheric fungal isolates were included in the initial experimental set: S2, S3C, S4, S5, SS6A, SS6B, S7(A), S7A2, S7A3, S7A4, S7A*, S7Bx, S8, S10, S12*, E1, E5, U1, U2, U3 and Isol.1. The isolates had been obtained from different rhizospheric soil samples (Sisauli, Muzzafarnagar, U.P., Daurala, Meerut U.P. and College of Biotechnology, SVPUAT, Meerut U.P.) and maintained as pure fungal cultures on PDA plates at 4°C. Composition of PDA medium- Potato- 200g, Dextrose- 20g, Agar- 20g, Distilled Water- 1000 ml. The Fungal cultures were grown on PDA plates, incubated at $30 \pm 2^\circ\text{C}$ for 04 days. The cultures were observed for colony morphology and absence of any contaminations. These fresh and uniform subcultures of different fungal isolates were used for subsequent K solubilization studies.

Culturing fungal isolates on Aleksandrov medium

Aspergillus isolates 21 no. were screened using Aleksandrov K-Solubilization medium. Aleksandrov-type media conventionally contain glucose as the carbon source together with $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CaCO_3 , $\text{Ca}_3(\text{PO}_4)_2$, FeCl_3 and an insoluble K-bearing mineral Potassium aluminosilicate $\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$. Actively growing pure cultures of different fungal isolates were initiated on PDA plates, for 48 hours. Freshly growing fungal culture bits of 5-8 mm size was cut using Cork borer and transferred onto Aleksandrov medium plates containing 0.005 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g FeCl_3 , 2.0 g CaCO_3 , 5 g glucose, 2.0 g, calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) 0.100g, Potassium aluminosilicate $\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$ 2.00 g and 20.00 g of agar (pH 7.2) (Aleksandrov *et al.*, 1967, Hu *et al.*, 2006) [8] and incubated at $30 \pm 2^\circ\text{C}$. After incubation, the clear zones formed around the colonies were observed at different time intervals of 1 Day to 7 days.

Screening and evaluation of K solubilization potential of *Aspergillus* isolates

A total of 16/21 *Aspergillus* isolates that were preliminary screened, were further cultured on Aleksandrov Agar medium and incubated under the $30 \pm 2^\circ\text{C}$, for 7 days. Colonies of *Aspergillus* isolates formed a clear halo zones, which was measured at different days. The analysis of each isolate was done in three replications. Fungal growth was recorded as colony diameter (d), while the corresponding potassium-solubilization or halo zone diameter (D) was measured around the growing colony. The solubilization index (SI) was calculated as D/d , and the solubilization efficiency (SE) was calculated as $(D - d)/2$. (Gayal and khandeparkar's., 1979) [9]

a. Solubilization Index (SI) = D/d

b. Solubilizing efficiency (SE) = $(D-d)/2$

d = Fungal colony diameter.

D = Total diameter (Fungal colony zone diameter + Clear zone of K solubilization)

Screening was carried out on the basis of the study of formation of halo zone and colony growth of different isolates. The colony diameter (d) and total diameter (D) were calculated for each replication. All the reading was recorded with average value and its standard deviation.

Results

This research highlights ecological variations, K-solubilization and adaptability of *Aspergillus* in different soil types, offering insights into their ecological roles, soil nutrient dynamics and their interactions with plants in the major crop systems. The solubilization ability of 16 no. isolates after 07 days are enlisted in the Table 1 and the solubilization zones formed after 5 days of incubation is depicted in Figure 1.

Table 1. Analysis of comparative Potassium solubilization potential of different *Aspergillus* fungal isolates on Aleksandrov after (7th day)

S.no	Isolate Code	d* (mm)		D* (mm)		SE (D-d)/2	SI D/d
		Mean	SD	Mean	SD		
1.	S2	34.00	±3.27	34.00	±3.27	0.00	1.00
2.	S3C	18.00	±1.41	18.00	±1.41	0.00	1.00
3.	S5	45.50	±1.73	45.50	±1.73	0.00	1.00
4.	SS6A	13.63	±0.75	16.75	±2.22	1.56	1.23
5.	SS6B	30.00	±5.35	30.00	±5.35	0.00	1.00
6.	S7(A)	34.75	±4.99	34.75	±4.99	0.00	1.00
7.	S7A*	35.00	±1.63	35.00	±1.63	0.00	1.00
8.	S7A2	35.00	±1.83	35.00	±1.83	0.00	1.00
9.	S7A3	38.25	±4.19	38.25	±4.19	0.00	1.00
10.	S7A4	35.50	±3.32	35.50	±3.32	0.00	1.00
11.	S8	34.25	±2.50	34.25	±2.50	0.00	1.00
12.	S10	21.75	±2.07	25.50	±1.91	1.88	1.17
13.	S12*	30.25	±3.77	30.25	±3.77	0.00	1.00
14.	ISOL.1	10.00	±2.45	27.75	±3.77	8.88	2.78
15.	U3	10.00	±2.45	29.75	±3.86	9.88	2.98
16.	E5	25.50	±0.58	34.50	±1.00	4.50	1.35

d- Colony diameter, D- Halo zone diameter. SI- Solubilization Index, SE- Solubilization Efficiency

Initially the d value (colony diameter) represents the actual growth of the *Aspergillus* colony on the medium. A higher d value indicates greater radial fungal growth, whereas a lower d value indicates slower or limited colony growth. According to the (Table 1), the d values showed considerable variation among the *Aspergillus* isolates. The highest colony diameter was recorded was for S5 (45.50 mm), followed by S7A3 (38.25 mm), S7A4 (35.50 mm), S7A* (35.00 mm),

S7 (A) (34.75 mm), S8 (34.25) and S2 showed d values of 34.00 mm, respectively. Moderate colony growth was observed in SS6B (30.00 mm), S12* (30.25 mm), and E5 (25.50 mm). The lowest d value was recorded for U3 (10.00 mm) followed by SS6A (13.63 mm) and S3C (18.00 mm). This indicates differential growth ability of *Aspergillus* isolates on Aleksandrov medium, thereby indicating their diversity and culture characteristics.

Subsequently the D value (total diameter) represents the combined diameter of the fungal colony and the surrounding solubilization zone. Therefore, a higher D value can indicate a larger overall area affected by the isolates solubilizing activity. The highest D value was also recorded for S5 (45.50 mm), followed by S7A3 (38.25 mm), S7A4 (35.50 mm), S7A* (35.00 mm). S7 (A), S8 and S2 showed D values of 34.75, 34.25 and 34.00 mm, respectively. The lowest D value was observed for SS6A (16.75 mm). This indicates differential solubilization ability of *Aspergillus* isolates on Aleksandrov medium, where some habitats have isolates with superior K-solubilization ability.

The SE (Solubilization Efficiency) values showed considerable differences among the isolates, viz. S2, S3C, S5, SS6B, S7 (A), S7A*, S7A2, S7A3, S7A4, S8 and S12* showed SE value of 0.00 mm, indicating that no additional measurable zone was observed beyond the fungal colony, which means the growth of fungal hyphae and amount of acid production for solubilization remains equal. The positive SE

value was observed for few isolates viz. five isolates showed the SE value above zero and the order is as follows, SS6A (1.56), S10 (1.88), E5 (4.50), Isol.1 (8.88), U3 (9.88). This shows that 05 isolates produced solubilization zones beyond their hyphae/ mycelium growth, indicating these isolates produced higher amount of organic acid that diffuse and solubilized potassium much beyond their d value or mycelium growth.

A similar pattern was observed for SI (Solubilization Index) There was 05 isolates observed SI value above one and the order is as follows, S10 (1.17), SS6A (1.23), E5 (1.35), Isol.1 (2.78), U3 (2.98). All other isolates recorded an SI of 1.00, because their D and d values were identical hence SI which is ratio of D/d becomes 1.00.

The Figure 1, shows solubilization zones by different *Aspergillus* isolates viz. S2, S5, S12*, S7A3, S7 (A) and E5 are shown on day 5th of incubation in Aleksandrov medium.

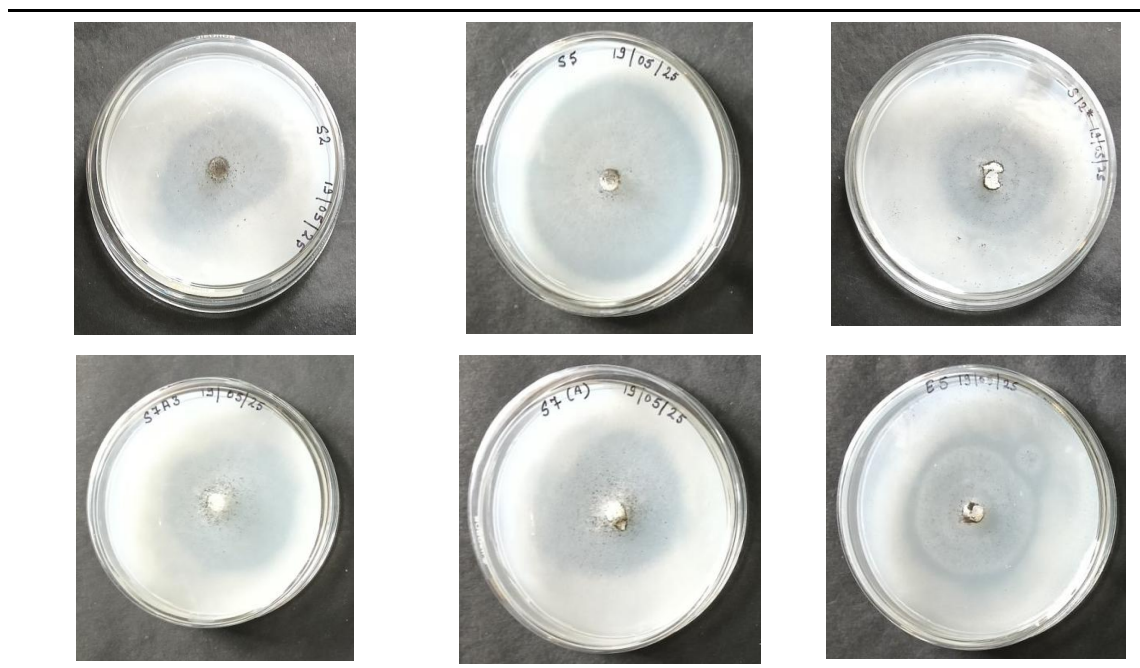


Figure. 1: Potassium solubilization by *Aspergillus* isolates from different rhizospheric soil sample on Aleksandrov medium at 5th day of incubation.

Discussion

The *Aspergillus* isolates from different native rhizospheres varied in size of K-solubilization zones formed on the Aleksandrov medium plate. Measuring both colony diameter (d) in the range of 10.00 to 45.50 and total diameter including the solubilization zone (D) in the range of 16.75 to 45.50, tells us two different things. A large d value means the fungus grew fast and spread widely on Aleksandrov medium. When D value is bigger it indicates higher solubilization zones and if D value is more than d value, the fungus has solubilized K beyond its colony growth. Thus for fast growing fungi where d and D values are close together the SI value will be 1.00 and SE values will be 0.00, then the D values may be used for estimation of solubilization ability. Other screening studies on Aleksandrov medium have used the same measurements to calculate D/d or a solubilization index (SI) value. The current study showed SI value range from 1.00 to 2.98 in fungal cultures tested. For example, Sugumaran and Janarthanam (2012) reported SI values i.e. D/d values between 1.04 and 1.87 across K-solubilizing fungi, including *Aspergillus terreus* and *A. niger*. Comparing isolates through colony and halo zone measurements is also common in work on K-solubilizing *Aspergillus* (Muthuraja *et al.*, 2021) [10]. In present study the SE value was in range of 0.00 to 9.88. The SE values on Aleksandrov medium using

following khandeparkar's selection measurement formula (SE) = $(D-d)/2$ was used to calculate solubilization efficiency of different micro-organisms as reported by (Jabin *et al.* (2017) [3].

The results do have limits as the mineral dissolves on solid medium depends on growth rate of different isolates, the metabolites diffusion through the agar, medium composition and the incubation conditions. The high SE and SI of isolate U3 therefore shows strong organic acid production and indicates mobilization of K beyond mycelium diameter. Previous work on K-solubilizing *Aspergillus* used this method while selecting isolates for K solubilization from halo-zone screening and then further testing K release in Aleksandrov broth by flame photometry method (Ashrafi-Saiedlou *et al.*, 2024) [11]. Although, screening on Aleksandrov agar medium plates provide better demonstration of solubilization ability and growth patterns of different microbial isolates.

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