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Assessment of soil respiration in response to incorporation of different crop residues

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ABSTRACT

C:N ratio of crop residues is the key factor dictating the decomposition of crop residues and soil respiration. Crop residue incorporation is one of the best residue management options, which not only enhances soil health, but also reduces environmental pollution. To investigate the effects of various crop residues *viz.*, paddy, sunflower, cotton, and red gram on soil respiration, an incubation study was conducted for 120 days at soil science laboratory, School of Agriculture, SR University, Warangal. Soil respiration was measured by alkali trap method, at different days after incubation. The results indicated that incorporating crop residues significantly increased soil respiration rates, with the highest CO_2 emissions observed in treatments with both residues and nitrogen. Among the treatments, soil with paddy residue and nitrogen showed the highest respiration rate, demonstrating the synergistic effect of residue incorporation and nitrogen addition in enhancing organic matter decomposition. The lowest soil respiration was recorded in soil alone (control) treatment throughout the incubation period. The study concludes that the incorporation of crop residues, especially when combined with nitrogen, significantly enhances soil respiration. The research provides critical insights for developing strategies that promote sustainable agriculture, emphasizing the need for residue retention and appropriate nutrient management to maximize soil productivity while minimizing environmental impacts.

KEY WORDS: *Crop residues*; *C:N ratio*; *Soil respiration*; *CO_2 emissions*; *Crop residue incorporation*

1. Introduction

Crop residue incorporation in soils is a key practice in agricultural management. Residues provide organic C and N inputs to soils for maintaining or improving the soil stocks of these elements and, ultimately soil health and crop productivity (Janz *et al*., 2022). Crop residue decomposition may result in the formation of the hotspots of microbial N turnover processes (Janz *et al*., 2022). Rapid decomposition of soil organic matter, and consequent N loss, is considered as a major limitation for maintaining soil fertility and a threat to the environment. Cox *et al.* (2004)

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reported that 80% of the crop residues burning took place during the post-harvest period. There as on behind this is attributed to the crop patterns used to ensure a higher economic return which have limited time between two consecutive crop cultivations. Some farmers even resort to a cycle of three crops a year with a short gap between harvesting and sowing of the next crop. Burning of residues emits a significant amount of Green House Gases (GHGs). Heat from burning of residues elevates soil temperature causing death of beneficial soil organisms also reduces level of C and potentially mineralizable N in the upper (0-15 cm) soil layer. However frequent residue burning leads to complete loss of soil microbial population, though the effect is temporary, as the microbes regenerate. Moreover, residue decomposition in soils potentially releases ammonia gas which is a precursor of secondary aerosol, a harmful pollutant for the environment and human health (Ruijter and Huijsmans, 2012). The major environmental problem today is the global warming due to accumulation of gases like CO_2, CH_4, N_2O and Chlorofluoro Carbons along with water vapour in the atmosphere causing greenhouse effect through trapping outgoing thermal radiation and depletion of ozone layer in stratosphere, affecting several aspects of humanity on planet earth, due to increased temperature (0.3-0.6 °C) at the earth's surface.

Crop residues with a low C/N ratio (≤15) lead to significant N_2O emissions meanwhile, the incorporation of residues with a high C/N ratio (>40) produced insignificant changes or even reduced soil N_2O emissions (Akiyama *et al*., 2020). Specifically, the composition of organic N and C compounds in the soluble, cellulose, and lignin-like fractions determine the N mineralization potential of residues, and this affects the fate of the incorporated residue N, as it may be immobilized in soil fractions or lost to the environment through the gaseous and hydrological pathways (Lashermes *et al*., 2010). Synchronizing soil N availability with plant requirements can improve the soil–plant system N use efficiency and reduce N losses through leaching below the crop rooting depth and/or from gaseous emissions (Vanlauwe *et al*., 2001). Previous studies confirm that C and N dynamics are mainly controlled by the C/N ratio (Nicolardot *et al*., 2001), chemical components (e.g., lignin, total phenol, soluble sugar and nutrients) (Bonanomi *et al*., 2010) and heterogeneity of plant residues applied to soil. Paul *et al*. (2014) reported that plant residues with different physical or chemical properties cause differences in soil microorganism activity levels, metabolic pathways and even community structures, thus contributing to different soil C and N dynamics. Addition of organic materials to agricultural soil (with or without chemical fertilizers) is important for replenishing the annual C losses and for improving both the biological and chemical properties of the soils (Goyal *et al*. 1999). Soil microbial communities are the primary regulators of soil carbon and nutrient cycling processes. Differences in microbial community composition have the potential to affect the fate of carbon and nutrients during decomposition and may therefore influence the retention of C and provisioning of crop nutrients in agro ecosystems.

When these residues are incorporated into soil, they provide a source of carbon and nutrients for soil microbes. This can stimulate microbial activity, leading to increased decomposition of organic matter and nutrient cycling in the soil. Different microbes specialize in breaking down different types of organic residues, so the composition of microbial communities can shift

based on the type of organic matter added. Many studies have shown that residues with low C/N ratio are decomposed rapidly and lead to net N mineralization as they satisfy the N demands of microbes (Hadas *et al*., 2004).

Soil respiration is considered a good estimator of overall biological activity and has been proposed as a descriptor of soil quality (Doran and Parkin, 1994). The soil microorganisms break down complex molecules such as cellulose, hemi-cellulose, proteins and lignin into low-molecular-weight substances, which are then oxidized to CO_2 to produce energy or used to provide C for cell growth. The rate of decomposition is determined by the quantity and quality of organic substrates, the efficiency and population dynamics of various decomposer groups, and the soil's physico-chemical environment including moisture, temperature, oxygen, acidity, and redox potential (Kilham, 1994; Coleman and Crossley, 1996). Soil respiration measurements are increasingly used in studies of soil C cycling to detect early changes in decomposition rate of soil organic matter in response to various soil or crop management practices (Jensen *et al*., 1996; Rochette and Angers, 1999). The composition of easily and slowly mineralizable organic matter significantly differs among various residues and fresh green materials are generally decomposed faster than straw (Schmatz *et al*., 2017). Due to a decline in soil fertility and adverse physical conditions, application of organic material and N fertilizer is important for sustainable soil fertility and environment.

2. Material and Methods

The investigation was carried out in Soil Science Laboratory, School of Agriculture, SRU, Warangal, Telangana, which is located in Warangal district of Telangana state at 79°55' °E longitude and 18°029' N latitude. According to Troll's climatic classification, it falls under Semi Arid Tropical region (SAT). The experimental site is in Southern Telangana Agro-Climatic Zone. The soil sample for the study was collected from D block (field number 21D), at the SR University college farm. The surface soil (0–15 cm) is collected, air–dried and sieved for chemical analysis. The soil is sandy clay loam, with a pH of 7.48, EC of 0.4 dSm^{-1} and the organic carbon content was 0.78%.

Four predominant crop residues (Rice, Cotton, Sunflower and Red gram) available on-farm were selected (Table 1). Samples of crop residues were oven dried at 65°C in a hot air oven, and crushed with a willey mill before sieving through a 2 mm sieve. The treatment details were provided in the Table 2.

2.1 Experiment details

Location	: Soil Science Laboratory, School of Agriculture, SRU, Warangal, Telangana
Design	: Completely Randomized design (CRD)
Treatments	: 10
Replications	: 3
No. of residues employed	: 4 (Paddy, sunflower, cotton, red gram).
Rate of paddy straw employed	: Based on top residue available from respective crops in Telangana, that is possible to deploy succeeding crops
Frequency of sampling	: 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120 days after incubation
Duration of lab experiment	: 120 days

Table 1. Amount of residue added per 100 g soil

Sl. No.	Crop residue	Amount (g)
1.	Paddy	0.18
2.	Cotton	0.50
3.	Red gram	0.26
4.	Sun flower	0.16

Table 2. Treatment details

T_1	Control (Soil with no N and no residue)
T_2	Soil + N
T_3	Soil + Paddy residue
T_4	Soil + Sunflower residue
T_5	Soil + Cotton residue
T_6	Soil + Red gram residue
T_7	Soil + Paddy residue + N
T_8	Soil + Sunflower residue + N
T_9	Soil + Cotton residue + N
T_{10}	Soil + Red gram residue + N

*Nitrogen (N) @ 80 kg ha^{-1} will be applied as urea, to T_2, T_7, T_8, T_9 and T_{10}; 80 kg ha^{-1} is chosen because it is the amount employed for rabi maize at the time of sowing as 1/3rd of RDN)

*Recommended dose of Phosphorus (P) is applied uniformly to all treatments.

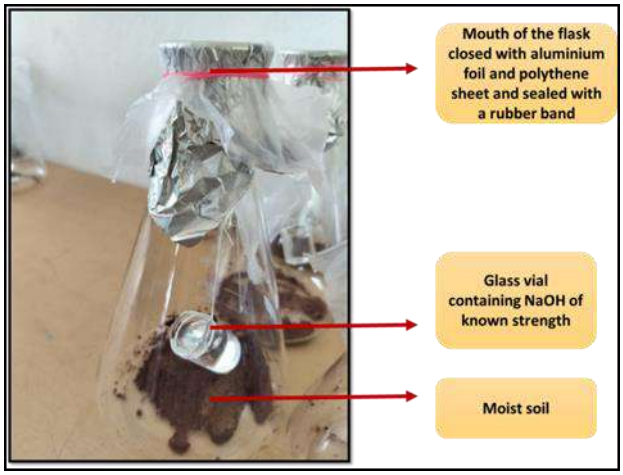
2.2 Pre-incubation

In 500 ml beaker, 100 g weighed soil was taken and the soil was kept at field capacity by adding 13 ml of distilled water and kept in dark for 10 days for pre-incubation. Pre-incubation was done prior to the start of incubation experiment to initiate microbial activity in the soil.

2.3 Laboratory incubation

Fresh soil, equivalent to 100 grams of dry soil samples, was pre-incubated, weighed, and transferred into polythene bags. After pre-incubation, residues were thoroughly mixed with the soil as per the treatment requirements and incubated for 120 days. Distilled water was frequently added to maintain a 60% water-filled pore space. The entire experiment followed a completely randomized design and was conducted under controlled room temperature conditions. Soil moisture was monitored and adjusted every five days by weighing the Zip lock bags and adding the necessary amount of distilled water.

The method measures the respiration activity of soil microorganisms as CO_2 production per time unit. When soil samples are incubated in a gas tight closed vessel at 30 ºC for 24 hours, the CO_2 produced is absorbed in sodium hydroxide (NaOH). Thus after adding barium chloride the sodium carbonate is precipitated as the hardly solute barium carbonate and the unused sodium hydroxide is titrated by hydrochloride acid (Ferreira *et al*., 2018).



C or CO_2 = (B-V) ×N×E

Fig. 1: Set-up for measurement of soil respiration by Alkali Trap Method

3. Results and Discussion

The result of the soil respiration rate of day two is shown in Fig. 1a. Soil respiration on day 2 was influenced by different residues with or without nitrogen addition. There was significant difference ($P < 0.05$) among the treatments. The Soil + Red gram residue (T_6) without nitrogen have shown the highest respiration rate 12.27 mg g^{-1}, which was on par with soil + cotton residue + N (T_9) 11.83 mg g^{-1}, followed by soil + paddy residue + N (T_7), soil + sunflower residue + N (T_8) and soil + N (T_2), which were statistically similar. The result indicated that, among all the treatments, those with addition of nitrogen have recorded higher respiration rate, compared to rest of the treatments. This might be due to the addition of N fertilizer accelerated C mineralization during early stages reported by Wang *et al*. (2004). The higher soil respiration in soil + red gram residue might be due to red gram residues have a relatively low C: N ratio and microbial community might efficiently utilize the nitrogen available from the red gram residues. Also according to Hadas *et al*. (2004), the C mineralization of residues in the early stage of decomposition is influenced by the amount of soluble C present in the added plant materials. Use of inorganic nitrogen application when organic residue of high C/N ratio was used might have either minimized the N immobilization or speed up the microbial decay (Jenkinson *et al*., 1985). In contrast, treatments without nitrogen soil alone

Fig. 1: Impact of different residues decomposition with or without in organic N on Soil Respiration at various days of incubation (2, 4, 6, 8 DAI).

(T_1), soil + paddy residue (T_3) and Soil + cotton (T_5) showed statistically similar but lower respiration rates, likely due to limited nutrient availability. The 34.77% overall increase observed with nitrogen, compared to treatments without nitrogen on day two, which highlights significant role N in enhancing nutrient availability. Additionally, nitrogen contributes to a decrease in the carbon-to-nitrogen (C) ratio of the residue. The result is in line with findings of Jenkinson *et al*. (1985) reported that Use of inorganic nitrogen application when organic residue of high C/N ratio was used might have either minimized the N immobilization or speed up the microbial decay (Jenkinson *et al*., 1985).

The soil respiration rates on day 4, is shown in Figure 1b, there was significant differences among treatments, particularly highlighting the influence of residues and nitrogen addition. Soil + Sunflower residue + N (T_8) shown significantly higher respiration rate compared to remaining treatments, followed by Soil + Red gram residue + N (T_{10}) 25.63 mg g^{-1}, Soil + Cotton residue + N (T_9) 25.50 mg g^{-1} and soil + N (T_2) 24.20 mg g^{-1}, which were statistically similar. The higher soil respiration rate in the Soil + Sunflower residue + N (T_8) treatment might be primarily due to sunflower residues having a lower C ratio, which leads to faster decomposition and increased microbial activity when combined with added nitrogen. This combination maximizes microbial growth and CO_2 release, whereas other residues with higher C ratios decompose more slowly, resulting in lower respiration rates. Kriauciuniene *et al*. (2012) and Munthali *et al*. (2015) reported that initial C/N ratios were the most critical variables in influencing decomposition of plant residues. Use of inorganic nitrogen application when organic residue of high C/N ratio was used might have either minimized the N immobilization or speed up the microbial decay (Jenkinson *et al*., 1985). The lowest respiration rate on day 4 was observed in soil alone (T_1), which was due to the absence of added organic residues and nitrogen. Without these inputs, there are fewer nutrients available for microbial activity, resulting in lower decomposition rates and reduced soil respiration. The 45.51% increase with nitrogen is primarily due to its impact on soil respiration. Nitrogen enhances microbial activity in the soil, which increases respiration rates and overall soil metabolic activity, leading to improved nutrient breakdown and availability.

The soil respiration rates on day 6, is presented in Fig. 1c. It is observed that, there was significant differences among treatments, particularly the influence of differences crop residues and nitrogen addition. Soil + N (T_2) recorded the highest respiration rate of 40.83 mg g^{-1}, which was significantly higher than all other treatments, followed by Soil +Sunflower +N treatment (T_8), which was 33.23 mg g^{-1}. This might be due to acceleration of mineralization. Also Singh (1991) reported that the nitrogen addition, is known to accelerate the mineralization of easily degradable organic carbon. The overall percentage increase in soil respiration rates due to the addition of nitrogen across all residue amended treatments is approximately 47.69%. This indicates a substantial enhancement in microbial activity and decomposition processes when nitrogen is added to the residues. Soil alone (T_1) had the lowest respiration rate of 5.27 (mg g^{-1}), which was significantly lower than all other treatments. This rate reflects minimal microbial activity in the absence of added nutrients or organic matter, serving as a baseline for comparison with the residue and nitrogen-amended treatments.

The soil respiration rates on day 8 is shown in Fig. 1d, reveal significant differences among treatments. Soil + sunflower residue + N (T_8) recorded the higher respiration rate of 40.63 mg g^{-1}, significantly higher than all other treatments, followed by Soil + red gram residue + N (T_{10}) showed a respiration rate of 35.07 mg g^{-1}, which was significantly higher than rest of the treatments. On day 8, the high soil respiration rates with sunflower residue + N (T_8) and redgram residue + N (T_{10}) are due to enhanced microbial activity. Nitrogen boosts microbial growth by alleviating nutrient limitations, leading to more efficient decomposition of organic matter. The increased decomposition results in higher carbon dioxide release, which lead to higher respiration rates. The percentage increase in soil respiration rates due to the addition of nitrogen across all residue treatments on day 8 is approximately 51.67% which reveals its critical role in enhancing microbial activity. Nitrogen boosts microbial growth and decomposition efficiency by providing essential nutrients, which accelerates organic matter breakdown and CO_2 release. In contrast, the soil alone (T_1) had the lowest respiration rate due to the absence of added nutrients and organic matter, limiting microbial activity and decomposition. Previous studies support these findings, including research by Zeng *et al*. (2010), which highlighted how nitrogen accelerates the breakdown of crop residues, particularly those rich in cellulose and hemicellulose. Similarly, Craine *et al*. (2007) found that nitrogen availability enhances microbial respiration by reducing the C ratio, allowing microbes to process organic carbon more efficiently. Fierer and Jackson (2006) further demonstrated that nitrogen amendments boost microbial biomass and enzymatic activity, leading to faster organic matter turnover and higher CO_2 emissions. Moreover, Cotrufo *et al*. (2013) reported that nitrogen additions increase the decomposition rate of recalcitrant plant residues, further contributing to higher soil respiration.

The soil respiration rates on day 10, were presented in Fig. 2a, which indicates significant differences among the treatments. Soil + red gram residue + N (T_{10}) exhibited the highest respiration rate of 46.17 mg g^{-1}, significantly higher than all other treatments. This may be because on day 10 there is active microbial population to process the red gram residues and also red gram residues (legume) have created more favorable environment compared to other residues for the microbes to break down the complex substances, which accelerated the decomposition rate and finally soil respiration. Muhammad *et al.* (2011) studied the total C mineralized from residues and suggested that decomposition of organic material in soil initially proceeded at faster rate. The decomposition attained a slower rate after about 15 days. It was mineralized within 14 days and remaining plant materials decomposed at a slower rate. The overall percentage increase in soil respiration rates due to the addition of nitrogen across all residue treatments on day 10 is approximately 53.65%. This indicates a significant improvement in soil respiration with the addition of nitrogen, underscoring its substantial impact on enhancing microbial activity and decomposition processes in the soil. Soil alone (T_1) recorded the lowest respiration rate of 6.60 mg g^{-1}, significantly lower than all other treatments. This low rate reflects minimal microbial activity in the absence of both added nutrients and organic matter, serving as a baseline for comparison with residue and nitrogen-amended treatments.

The soil respiration rates on day 15, as presented in Fig. 2b. There was significant differences among treatments, particularly the influence of crop residues and nitrogen addition. Soil + paddy residue + N (T_7) exhibited significantly higher respiration rate at 48.57 mg g^{-1}, this might be due to combine effect of paddy residue and nitrogen which result to significant increase in mineralization which result in lowering the C: N ratio of paddy residue. The result indicated that paddy residue start mineralization were others residues are almost in the mid of mineralization processes. The overall percentage increase in soil respiration rates due to the addition of nitrogen across all residue treatments on Day 15 is approximately 51.09%. This indicates that nitrogen addition leads to a significant enhancement in soil respiration, emphasizing its critical role in boosting microbial activity and decomposition processes in the soil. The decomposition attained a slower rate after about 15 days. Soil + N (T_2) recorded significantly lower respiration rate of 8.57 mg g^{-1}, compared to other treatments. This might be because the microorganisms have utilized the both added nitrogen and mineralized carbon present in the

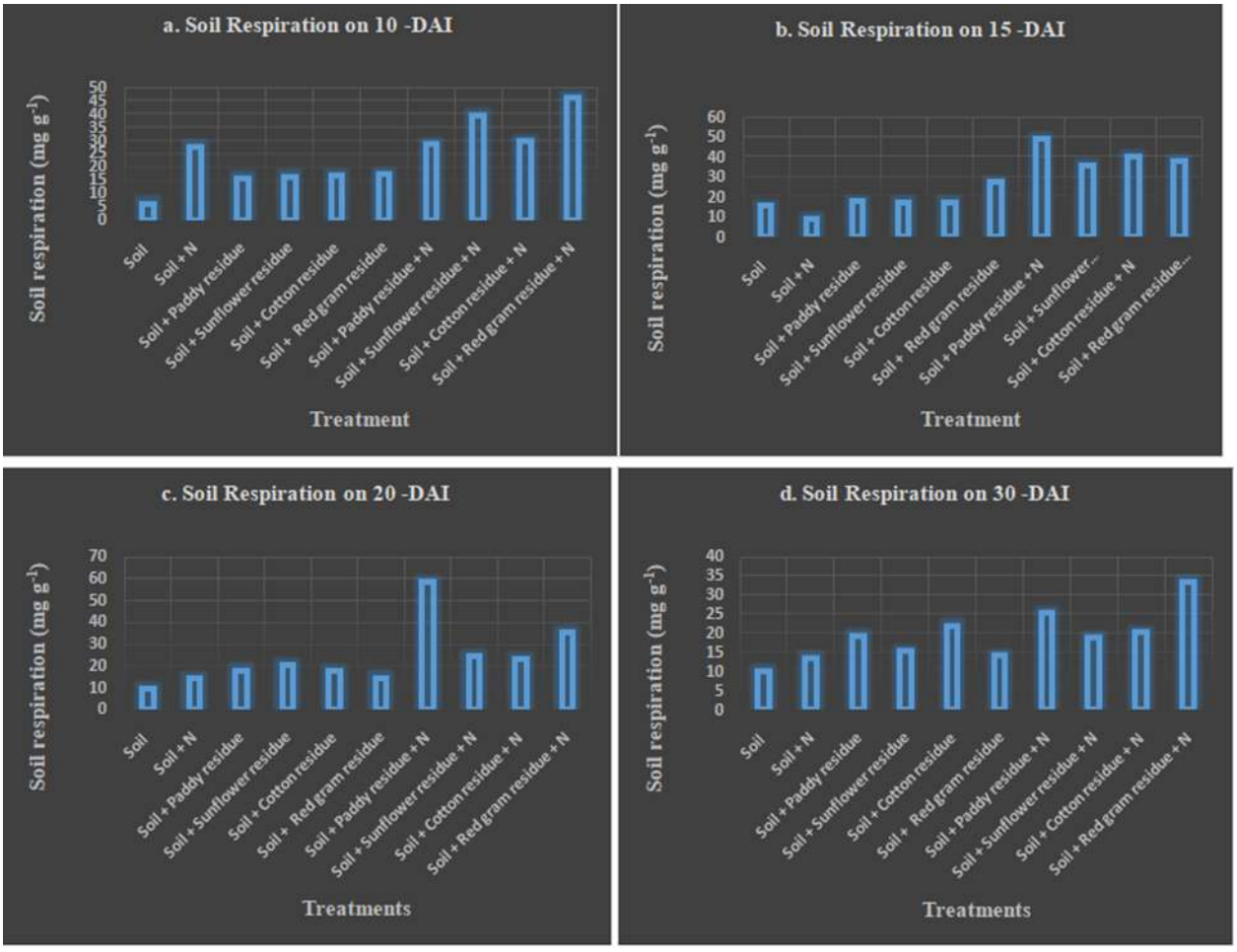


Fig. 2: Impact of different residues decomposition with or without in organic N on Soil Respiration at various days of incubation (10, 15, 20, 30 DAI).

soil. This suggests that while soil microbial activity is ongoing, the addition of organic residues plays a crucial role in further enhancing respiration rates.

The soil respiration rates on day 20, is presented in Fig. 2c. There was significant difference among treatments. The soil respiration rates observed on day 20 reflect the influence of various crop residues and nitrogen addition on microbial activity. Soil + paddy residue + N (T_7) recorded the highest respiration rate of 58.33 mg g^{-1}, significantly higher all other treatments. This high rate can be attributed to the combined effect of paddy residue and nitrogen. Despite paddy residue having a high C: N ratio, the addition of nitrogen helps overcome nitrogen immobilization by providing an immediate nitrogen source for microbes, thus accelerating the decomposition of organic matter and increasing microbial activity. This aligns with findings by Jenkinson *et al*. (1985), which state that nitrogen addition can enhance decomposition rates, even with residues of high C ratios. Soil alone (T_1) had a respiration rate of 9.80 mg g^{-1}, which was significantly lower among all treatments. This is expected, as the absence of added organic material or nitrogen limits microbial activity. The overall percentage increase in soil respiration rates due to the addition of nitrogen across all residue treatments on day 20 is approximately 49.90%. Balasubramanium *et al*. (1974) observed that the release of CO_2 by the soil amended with organic material was significantly more as compared control.

The soil respiration rates on day 30, is presented in Fig. 2d. There was significant difference among treatments. Significantly higher respiration rate was observed in the treatment with soil + red gram residue + nitrogen (T_{10}) 33.37 mg g^{-1}, followed by soil + paddy residue + nitrogen (T_7) 25.40 mg g^{-1} and soil + cotton residues (T_5) 22.07 mg g^{-1}, which were on par. These values indicate a strong enhancement of microbial activity due to the combined effects of nitrogen and crop residues. Red gram residues generally have a relatively low C ratio, which supports faster microbial decomposition and higher CO_2 emissions. When nitrogen is added, it further stimulates microbial growth and enzyme activity, enhancing the decomposition process and leading to increased soil respiration. Kriauciuniene *et al*. (2012) and Munthali *et al*. (2015) reported that initial C/N ratios were the most critical variables in influencing decomposition of plant residues. The overall percentage increase in soil respiration rates due to the addition of nitrogen across all residue treatments on day 30 is approximately 27.57%. This indicates that while nitrogen addition continues to enhance soil respiration, the degree of increase is less pronounced compared to earlier days. This may reflect the varying impacts of nitrogen over time and the dynamic nature of microbial activity and decomposition processes. The lowest respiration rates were recorded in treatments such as soil alone (T_1) 10.10 mg g^{-1}, indicating that the absence of crop residues or the sole addition of nitrogen results in significantly lower microbial activity.

The soil respiration rates on day 45, is presented in Fig. 3a, there was significant differences among the treatments involving various crop residues, nitrogen and their combinations. Soil with paddy residue (T_3) showed a significantly higher respiration rate of 26.47 mg g^{-1}. The elevated respiration rate in the paddy residue treatment indicates that microbial activity has been strongly stimulated by the paddy residue, which provides a

continual source of organic matter for decomposition. Over the 45-day period, the paddy residue has likely undergone substantial decomposition, enhancing microbial activity as nutrients are gradually released and utilized. Saidi *et al*. (2008) reported that a stable C: N ratio could be achieved after 95 days of decomposition. The soil respiration rate for soil alone (T_1) was 10.37 mg g^{-1} establishing the baseline, which was significantly lower among all treatments. On Day 45, the overall percentage increase in soil respiration rates due to nitrogen addition across all residue treatments is approximately 19.13 %. This indicates a modest enhancement in soil respiration with nitrogen, suggesting that while nitrogen still influences soil microbial activity and decomposition, its effect has diminished compared to earlier observations. This decrease in the rate of increase might be due to various factors such as changes in microbial activity, the decomposition stage of residues, or nutrient dynamics over time.

The soil respiration rates on day 60, is presented in Fig. 3b, which showed significant differences among the treatments involving various crop residues, nitrogen and their combinations. The higher respiration rate was observed in soil with red gram residue + nitrogen (T_{10}) at 23.57 mg g^{-1},

Fig. 3: Impact of different residues decomposition with or without in organic N on Soil Respiration at various days of incubation (45, 60, 75, 90 DAI).

followed by soil with paddy residue alone (T_3) with a respiration rate of 22.53 mg g^{-1}. On day 60, the higher soil respiration rate in the (T_{10}) treatment is due to advanced decomposition and optimal nutrient conditions. By this time, the red gram residues have fully decomposed, and the added nitrogen has maximized microbial activity and organic matter breakdown, leading to elevated CO_2 emissions. On day 60, the 16.50% increase in soil respiration rates due to nitrogen addition reflects advanced decomposition and stable microbial activity. As decomposition progresses, the impact of nitrogen stabilizes, leading to sustained but moderated increases in microbial respiration. This suggests that while nitrogen continues to enhance microbial activity, the effects have become more consistent over time. The soil alone treatment T_1 (10.067 mg g^{-1}) recorded lower respiration rate which was on par with T_2 (10.83 mg g^{-1}), T_6 (10.98 mg g^{-1}) and T_8 (10.93 mg g^{-1}). Reddy *et al*. (2018) reported that the addition of crop residues significantly enhances soil microbial activity and respiration by providing readily available carbon and nutrients. Similarly, Khan *et al*. (2012) demonstrated that nitrogen application leads to increased microbial biomass and respiration rates, particularly in soils enriched with organic residues.

The result of the soil respiration rate of day 75 is presented in Fig. 3c. Soil respiration on day 75 was influenced by different residues with or without nitrogen. On Day 75, the soil respiration rates was higher in soil with paddy residue alone (T_3) with a respiration rate of 18.8 mg g^{-1} and it was on par with soil + red gram +N (T_{10}) demonstrating a notable stimulation of microbial activity by paddy residue, though slightly lower than observed on earlier days. On Day 75, the higher soil respiration rate in (T_3) is due to the delayed mineralization associated with the high carbon-to-nitrogen (C) ratio of paddy residues. Over time, as nitrogen is gradually released from the residues, microbial activity increases, leading to sustained high respiration rates. This effect is comparable to Soil + red gram residue + Nitrogen (T_{10}), reflecting effective microbial stimulation from both types of residues. Soil with cotton residue (T_9) had the lowest respiration rate of 8.67 mg g^{-1}, which was on par with soil alone 9.67 mg g^{-1}. This might due to low C: N ratio of cotton. Use of inorganic nitrogen application when organic residue of high C/N ratio was used might have either minimized the N immobilization or speed up the microbial decay (Jenkinson *et al*., 1985). The reduction in the percentage increase of soil respiration rates due to nitrogen addition from day 75 might be because nitrogen becomes depleted, its stimulating effect on microbial activity wanes, and advanced decomposition stages lead to a stabilization of microbial processes. Additionally, residues without nitrogen start to mineralize and decompose more steadily, contributing to a natural increase in soil respiration rates independent of added nitrogen.

The result of the soil respiration rate of day 90 is presented in Fig. 3d. Soil respiration on day 90 was influenced by different residues with or without nitrogen. Soil with paddy residue recorded a respiration rate of 16.47 mg g^{-1}, demonstrating a strong stimulation of microbial activity by paddy residue, though this rate is lower than observed on earlier days and it was on par with soil + redgram + N (T_{10}). Soil alone (T_1) had a respiration rate of 7.87 mg g^{-1}, the lowest among all treatments, which was on par with T_2 (8.55 mg g^{-1}) and T_5 (8.40 mg g^{-1}). On Day 90, Soil + paddy residue (T_3) has a respiration rate of 16.47 (mg g^{-1}), showing continued microbial activity despite a

decrease from earlier peaks due to advanced decomposition and reduced nutrient availability. Soil + redgram residue + nitrogen (T_{10}) maintains a similar respiration rate, supported by the ongoing nutrient benefits of red gram residues and added nitrogen. Saidi *et al.* (2008) reported that a stable C: N ratio could be achieved after 95 days of decomposition. This indicates that both red gram residue + nitrogen and paddy residue alone have strong impacts on microbial activity, with red gram residue + nitrogen showing a comparable effect to paddy residue alone. On Day 90, the 3.45% increase in soil respiration rates due to nitrogen addition indicates a reduced effect over time. This is likely because of advanced decomposition stages, stabilization of microbial communities, and potential nutrient immobilization, which diminish the impact of additional nitrogen.

The result of the soil respiration rate of day 105 is presented in Fig. 4a. The results of soil respiration measured on day 105 reveal significant differences in microbial activity influenced by various residue treatments with or without nitrogen. The highest respiration rate was observed in the treatment with soil + paddy residue (T_3) 14.37 mg g^{-1} had the highest rates, followed by soil + red gram residue + N 14.40 mg g^{-1}. These two treatments showed statistically difference high respiration levels. In contrast, soil alone exhibited the lowest respiration rate of 4.40 mg g^{-1}, reflecting minimal microbial activity. The addition of nitrogen to soil alone increased this rate to 5.50 mg g^{-1}, a statistically significant improvement, and emphasizing nitrogen's role in enhancing microbial activity by improving nutrient availability. Saidi *et al*. (2008) reported that a stable C: N ratio could be achieved after 95 days of decomposition. The observed 5.60 % increase in respiration rates without nitrogen addition across all treatments indicates that microbial communities continue to actively decompose residues even in the absence of added nitrogen. N initially boosts microbial activity, its effect diminishes over time as it is used up, whereas the residual microbial activity continues to drive decomposition and soil respiration.

The results presented in Fig. 4b illustrate significant differences in microbial activity influenced by various residue treatments, particularly with or without nitrogen, on day 120. The highest respiration rate was recorded in the soil + paddy residue treatment (T_3) at 12.10 mg g^{-1}, highlighting the effectiveness of paddy residue in promoting microbial activity and stable mineralization. This elevated respiration rate indicates a continuous nutrient release from the paddy residue, providing a rich carbon source for microbes, which sustains activity even as carbon sources in other treatments become depleted. The soil + red gram residue + N treatment (T_8) achieved the second-highest respiration rate at 7.80 mg g^{-1}, attributed to the synergistic effect of red gram residue and added nitrogen, enhancing microbial activity. However, the benefits of nitrogen, while significant, do not match the long-term advantages provided by paddy residue. In contrast, the lowest respiration rates were seen in the soil alone (T_1) and soil + N (T_2), both at 3.67 mg g^{-1}. These low rates reflect limited microbial activity due to the lack of organic residues and the exhaustion of carbon sources in the nitrogen-only treatments. Overall, the increase in respiration linked to organic residues alone is approximately 14.57%, underscoring the crucial role of organic matter in stimulating microbial activity. This percentage reinforces that while nitrogen can enhance microbial processes, the sustained high

respiration rates and long-term stability are more effectively supported by high-quality organic residues like paddy. The persistent high respiration associated with paddy residue underscores its superior ability to maintain microbial activity and nutrient cycling over time.

The respiration rate of the residue added treatments was high compared to control, throughout the experiment (Fig. 5). Over time, soil respiration steadily decreases after rising gradually and peaking on day 15. This pattern most likely represents a rise in microbial activity at first (which was also evident from our study but data not mentioned) brought on by the breakdown of readily available organic matter, this pattern probably indicates that microbial activity increases first due to the decomposition of easily accessible organic matter and subsequently decreases when these substrates become scarcer. Our finding is similar to Vahdat *et al.* (2011), who had reported that, CO_2-C release, in untreated soils (controls) has shown very slow patterns meanwhile treated soils showed an initial rapid increase, followed by a slower, linear release. Incorporation of paddy residue had demonstrated a progressive rise over time, reaching a peak at 25.27 mg g^{-1} on day 45. This indicates sustained microbial activity, likely due to the slow decomposition, which might be due to high C: N ratio. This suggests that microbial activity has persisted, most likely as a result of the paddy residue's gradual breakdown. Our result was consistent with the findings of Kobke *et al*. (2018), who had reported that, legumes typically emit the highest cumulative CO_2 emissions, which decrease over time, while cereals exhibit lower emissions but higher stability. Incorporation of sunflower residue led to increase in soil respiration upto day 30, later there was a sharp decrease after. It falls to 1.15 by day 120, indicating that sunflower residue may break down rapidly and cause an early microbial substrate depletion. Cotton residue exhibits a similar pattern to paddy residue i.e., it had shown a gradual increase in respiration that peaks on day 30, then a notable decline by day 120, signifying a slower rate of decomposition.

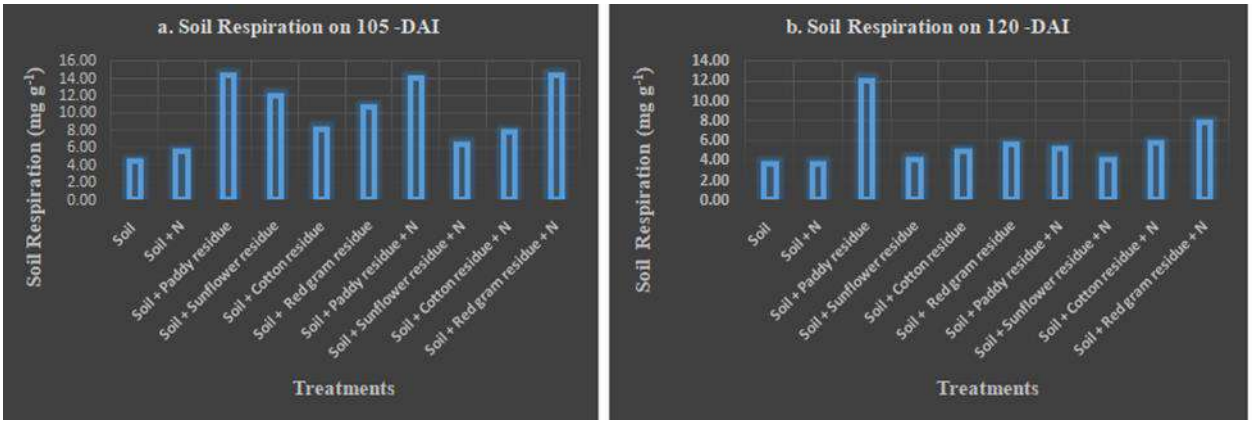


Fig. 4: Impact of different residues decomposition with or without in organic N on Soil Respiration at various days of incubation (105, 120 DAI).

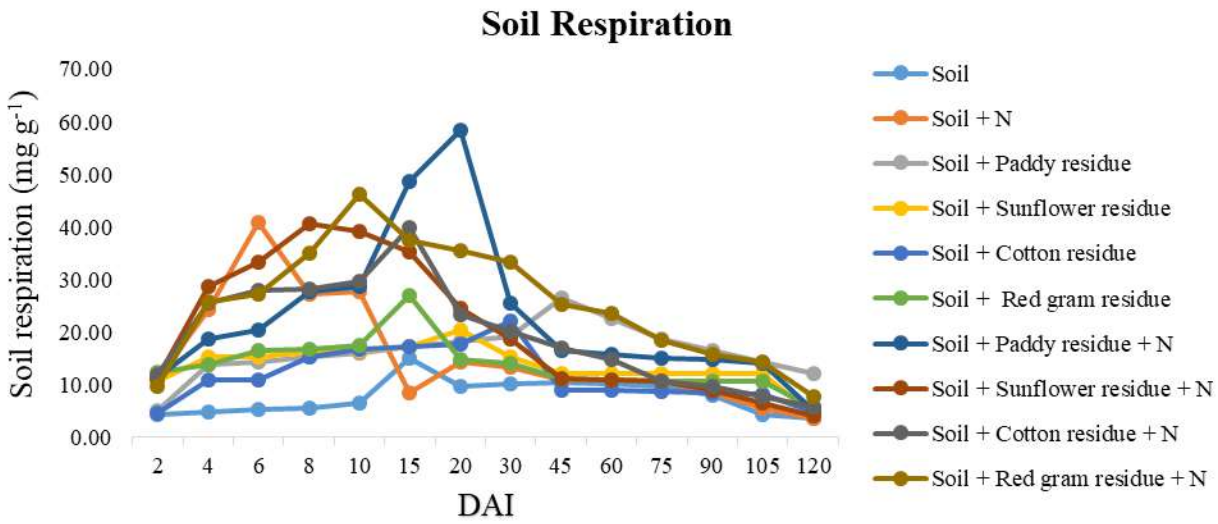


Fig. 5: Temporal dynamics of impact of crop residue decomposition on Soil Respiration

Rezgui *et al*. (2021) reported that the mineralization rate decreased due to the increasing recalcitrance of the remaining residues. Lignin content was negatively correlated with C mineralization due to its resistance to microbial breakdown. Incorporation of red gram residue showed a moderate rate of decomposition which peaked on day 15, followed by a steady fall in microbial activity which reflected in decreased respiration. Cogle *et al*. (1989) estimated that incorporation of straw hastened its decomposition rate only within the first 15 days, thereafter the decomposition rate was similar. Decomposition rate generally peaked between 4 and 15 days. The carbon source however was quickly exhausted. When residues are added once, respiration rates are initially high due to the decomposition of easily available compounds. Thereafter, respiration rates decrease as easily available compounds are depleted. Respiration rates are low in the later stages of decomposition when only more recalcitrant compounds such as lignin-encrusted cellulose and other macromolecules are left (Wang *et al*. 2004). It is well-known that incorporation of plant residues into the soil results in a rapid increase in microbial activity and biomass followed by gradual decrease (Wang *et al*. 2004). Sarma *et al*. (2013) reported that C:N ratio of the different residues were responsible for maximum mineralization of native soil organic matter and added crop residues by increasing microbial activity in soil environment at the particular day of incorporation. Soil + nitrogen treatment showed significant increase in respiration, which peaked on day 6 at 40.83. However, by day 15, respiration drastically drops, suggesting that the initial nitrogen-induced microbial boost is just temporary. On the other hand, respiration dramatically decreases by day 15, indicating that the initial nitrogen-induced microbial boost is transient. After day 20, respiration rates level off and resemble those found in the soil by themselves.

Paddy residue + N treatment, the respiration rate increases dramatically when nitrogen is introduced

to paddy residue, peaking at 48.57 on day 15, as compared to paddy residue alone. This suggests that nitrogen significantly accelerates the breakdown of rice residue. Sunflower residue + N treatment showed similar to the impact of nitrogen alone, *i.e*., respiration peaked early (on day 6), at 40.63. It does, however, decline significantly thereafter, indicating a quick initial breakdown followed by the exhaustion of accessible substrates. Cogle *et al*. (1989) estimated that incorporation of straw hastened its decomposition rate only within the first 15 days, thereafter the decomposition rate was similar. Sakala *et al*. (2000) reported rapid CO_2 evolution within the first 10 days. Alexander and Scow (1989) reported that, as the decomposition proceeds, the organic matter is not attacked as a whole. Some of the constituents are decomposed readily (sugar and starches), followed by proteins, cellulose, hemicellulose and finally lignin, waxes and tannins. Zeng *et al*. (2010) reported maximum degradation of hemicellulose as compared to lignin and cellulose during composting. In all the five trials, it was found that the amount of hemicellulose present in the compost at the end of 20 days was less than 10% indicating very rapid degradation of hemicellulose under microbial activity. This implies that nitrogen is essential for promoting cotton residue breakdown. Red gram residue + N treatment showed a peak on day 10, (respiration reaches 46.17), the greatest peak of all the combinations. The C: N ratio of crop residue is a key factor for its degradation. During the initial decomposition phase, low C: N ratio causes manifold increase in the decomposition rate (Golueke, 1992). This implies that red gram residue, when mixed with nitrogen, the result is in line with Wang *et al*. (2004) also found that addition of N fertilizer accelerated C mineralization during early stages. The C mineralization of residues in the early stage of decomposition is influenced by the amount of soluble C present in the added plant materials (Hadas *et al*., 2004). Muhammad *et al*. (2010) reported that, addition of N fertilizer to sugarcane, maize and sorghum residues to soil promoted CO_2 emissions significantly, compared to the unfertilized N treatment, during the first 10 days of incubation. Ravali *et al*. (2024) also reported that, C:N ratio of crop residue is a key factor for its degradation. During the initial decomposition phase, low C: N ratio causes manifold increase in the decomposition rate. Guhe and Deshmukh (1973) found that crop residues like wheat straw incorporated with fertilizer N in soil favorably enhanced the soil microbial ecology, microbial biomass and yield of legume crop. Gallardo and Merino (1993) studied the rate of organic material breakdown depends on the relative proportion of soluble sugars, cellulose, hemicellulose and lignin content.

4. Conclusion

Incorporating crop residues, particularly when combined with nitrogen, significantly enhances soil respiration. Soil with crop residues showed higher CO_2 emissions compared to soil alone. When crop residues were combined with nitrogen, it had further enhanced respiration rates. Paddy residue + nitrogen (T_7) exhibited the highest respiration rates, highlighting the role of N in accelerating organic matter decomposition. Crop residues with lower C:N ratio enhanced decomposition and increased nutrient availability. After incorporation, there was rise in CO_2 emissions upto 20 DAI, followed by decrease throughout the incubation period. The CO_2 emissions reflected carbon mineralization.

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Assessing morphological and physiological responses of local and exotic Rice varieties to salinity stress

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ABSTRACT

This study aimed to evaluate the differential morphological and physiological responses of local and exotic rice varieties growing in arid and semi-arid climates under soil salinity stress. The experiment was laid out in a Completely Randomized Design (CRD) with four replicates and conducted in a greenhouse under natural conditions at the Department of Soil and Environmental Sciences (lath house), Faculty of Agriculture and Environmental Sciences, MNS-University of Agriculture, Multan, Pakistan, during 2023. The pot experiment involved two exotic rice varieties (BR-61 and BR-47) and one local variety (Al-Khalid), tested under two salinity treatments: control (non-saline) and saline (EC = 10 dS m^{-1}). Data on various growth parameters - such as shoot and root length, shoot and root fresh and dry weights, chlorophyll content, and ionic concentrations (Ca^{2+}, Na^{+}, and K^{+}) in leaf tissues - were collected and analyzed statistically using factorial CRD. The results indicated that exposure to NaCl stress significantly reduced shoot and root growth while increasing Na^{+} concentration in the plants. Among the tested varieties, BR-61 exhibited the highest shoot and root dry weight (8.0 g and 1.0 g, respectively), while BR-47 showed the lowest root dry weight (5.6 g and 0.6 g). The maximum Na^{+} concentration (348.7 ppm) in leaf sap was observed in Al-Khalid, which was approximately twice the control, while the minimum (157.4 ppm) was found in BR-61. BR-61 also recorded the highest K^{+} concentration (77.1 ppm), while BR-47 had the lowest (62 ppm) under saline conditions. Based on the findings, the rice varieties were classified into salt-tolerant (BR-61) and moderately tolerant (BR-47 and Al-Khalid) categories at the 10 dS m^{-1} NaCl level. These insights could be valuable in formulating strategies to enhance salinity stress tolerance in rice varieties and optimize their physiological performance, particularly chlorophyll content, under saline conditions.

KEY WORDS: *Exotic varieties*; *Local variety*; *Rice*; *Salinity stress*; *Salt tolerance*

1. Introduction

Salt-affected agricultural lands are a major hurdle in the way of sustainable agriculture as well as food production (Syed *et al*., 2020). Consequently, saline soils and Sodic soils are the serious drawbacks of crop production in arid and semi-arid regions where the annual precipitation rate is very low in comparison of higher evaporation rates and the main causes of this problem are

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severe climate events along with use of brackish water for irrigation (Ahmad *et al*., 2013). About 955 Mha land around the globe is under salt stress out of which Pakistan's 6.68 Mha arable land is affected with soil salinity (Khan, 1998). Pakistan and other countries of the world are facing a change in food security and an increased demand of food for growing population. It has been reported that Pakistan is facing 14% irrigated land losses and 64% yield losses due to soil salinity, leaving behind only 23 Mha land suitable for agricultural production (Irum and Ehetisham-ul-Haq, 2017). Soil salinization has severely damaged about 2.5 Mha of total irrigated land area. Furthermore, it is also estimated that 4.5 Mha area of Pakistan out of its total geographical area of 79.61 Mha is under the effect of soil salinization (Aslam, 2016). Salinity is an abiotic stress condition which hampers the proper growth and production of plants (Parvaiz, 2014). Higher the salinity more will be the Na^+ or Cl^- ions uptake by the plant which results in injury, burning and premature death of cells (Wahome *et al*., 2001). Due to soil salinity seed germination, seedling growth and crop growth has been affected adversely and cause up to 70% yield loss of wheat, maize, barley and rice (Veatch-Blohm, 2007). Primarily, under salt stress water deficiency, osmotic pressure, ion toxicity and nutritional imbalance cause decreased growth rate. Although, there is sufficient water available in the soil but under salinity stress plant roots unable to absorb water from the soil solution (Sakina *et al*., 2016). All these effects of salinity on crop results in imbalance of nutrients, membrane leakage, inhibition of enzymatic activities, metabolic malfunctioning and inhibition of growth regulators and photosynthesis ultimately ends up with plant demise (Hasanuzzaman *et al*., 2013). Moreover, under high salinity stress, for the maintenance of water balance and protection of cell structures, plants biosynthesize of osmo-protectants and compatible solutes such as proline and glycine betaine (Mittal *et al*., 2012) and to counteract the oxidative stress due to ROS plants increase the synthesis of antioxidant enzymes (superoxide dismutase and catalase) and compounds (Gupta and Huang, 2014).

Production of imperative crops like rice is important to fulfill the food demand of the world as rice is a cereal crop (Shah *et al*., 2020). It is a crop that is widely consumed by global population after wheat and maize as three of them are major food crops of the world and proved to be a source of calories for about 3.5 Billion people. It has proved that a 100g of rice is a good source of carbohydrates (78.2g), protein (6.8g) and energy (345.0 Kcal) (Aykroyd *et al*., 1963). Rice is generally sensitive to soil salinity with the threshold level of 3.0 dSm^{-1}; however, it is moderately tolerant to soil sodicity (ESP 20-40%) (Maas and Hoffman, 1977). During vegetative stage and then at reproductive stage rice show sensitivity to salinity, while at germination stage it shows resistance to salinity up to 16.3 dSm^{-1} (Ali *et al*., 2014). Despite its sensitivity to salinity rice – wheat crop rotation is recommended during reclamation because of its ability to grow well under flooded conditions, and because the standing water in rice fields can help leach salts from the soil profile (Chand *et al*., 1978). A considerable genetic variability has been observed among and even within rice germplasm (Yeo and Flowers, 1980) and this variation can be exploited to select and develop salt tolerant rice varieties. Multiple research studies have shown that the intake and absorption of micro- and macro-mineral nutrients change when plants are subjected to high salinity levels, resulting in

higher Na transport to the shoots and reduced intake of K, Zn, and P (Razzaq *et al*., 2019). Similarly, it has been noted that an excess of salt leads to elevated reactive oxygen species (ROS) levels, resulting in considerable harm and eventual mortality in plants (Chawla *et al*., 2012). The production and build-up of compatible solutes such as proline, glycine betaine, trehalose, polyols are necessary to counteract the osmotic pressure caused by high salinity (Chen and Murata, 2002). The production of these compatible solutes is frequently linked to salt tolerance in rice (Zhao *et al*., 2014). Applying compatible solutes from external sources or increasing the expression of relevant genes related to osmolytes production can enhance rice's ability to tolerate salt (Garg *et al*., 2002). Therefore, existing local and exotic rice varieties can be screened to select and develop favorable salt tolerant varieties of rice to grow on salt affected lands with minimal yield reduction.

The objectives of this study were to classify promising exotic and local rice varieties based on different morphological and physiological traits and to assess the response of different growth parameters against salinity stress by establishing the relationship between ion concentration and salinity tolerance in order to manage salt-affected lands cost-effectively. This research aimed to bridge the knowledge gap in local and exotic varieties of rice response towards salinity stress.

2. Material and Methods

2.1 Experimental site, design and treatment plan

The reported experiment was conducted in Lath house without any environmental control at Department of Soil and Environmental Sciences, Faculty of Agriculture and Environmental Sciences, MNS-University of Agriculture, Multan, Pakistan during the year 2023. The coordinates of the location were 71.4° E Longitude and 30.2° N Latitude. The study was carried out as CRD with factorial arrangement in soil filled pots. There were 03 varieties of rice and 01 salinity level (Control (Non-saline), EC = 10 dS m^{-1}) in reported experiment. Randomization of the four replications of each variety was done in all the treatments.

2.2 Plant material

Varieties were signified as follows: (Local + Kernel) V1 = Al-Khalid, (Exotic + Coarse) V2 = BR-47 and V3 = BR-61. The seeds of rice were obtained from the Soil Salinity Research Institute (SSRI), Pindi Bhattian, Pakistan (Local) and International Center for Biosaline Agriculture (ICBA), Dubai (Kernel). The nursery of rice seeds was sown on 27th July, 2023 in the plug trays containing thoroughly washed fine river sand. The trays were placed on the bench top in the net house and watered regularly till transplantation.

2.3 Growth conditions and salinity development

Nursery of rice plants was grown in the sand culture. The seedlings of rice at the three leaf stage were transferred into the pots filled with 08 Kg soil (Sandy Loam). Cloth screens were placed in the bottom of the pots to prevent soil loss. A population of four plants per pot was maintained. One pot was considered as one replication, and each treatment contained four pots of each variety. Thus, a total of 24 pots were maintained. Transplanted seedlings were kept well irrigated in the greenhouse during the growth period. Soil was fertilized using an adequate dose of fertilizers (N, P_2O_5, K_2O, $ZnSO_4$ @ 150-90-50-7 (Kg ha^{-1})) at basal stage. The soil was salinized using NaCl salt

to develop an EC_e of 10 dS m^{-1} and puddled before transplanting of rice seedlings, whereas soil without any salt treatment (EC_e = 1.58 dS m^{-1}, pH = 8.2) was used as control. All cultural practices were carried out regularly throughout the growing period.

2.4 Data collection

Plants were harvested 90 days after exposure to (EC_e of 10 dS m^{-1}) NaCl salinity stress and separated into the shoots and roots. After harvesting, roots were thoroughly washed with distilled water to remove adhered soil and patted on tissue paper to remove excess water and shoots were patted on paper towel to remove dirt and excess moisture. The shoot length of each plant was measured in cm using meter rod from base to top and average of all replicates were calculated. Afterwards, roots were cut off from the shoot and lengths were measured in cm using measuring tape and means of every replicate were calculated. The fresh weight of roots and shoots were separately recorded in g by using a portable analytical balance immediately after harvesting. In order to measure dry weights, plants were placed in a drying oven at 65°C for 72 hours. After moisture removal, shoot and root dry weights were recorded separately in g by using analytical balance. For chlorophyll content determination in rice plants, SPAD meter (model: SPAD-502 Plus made by Konica Minolta, Europe) was used and SPAD values were recorded. For checking the variation in SPAD values chlorophyll content was checked twice with a gap of two weeks. Moreover, in order to determine the ions concentration (Na^+, K^+ and Ca^{2+}), 2nd fully expanded leaf from the top of the plant was taken and cell sap was extracted using centrifuge machine and then the sap of leaves was used to determine Na^+, Ca^{2+}, and K^+ concentration in ppm by using Flame photometer.

2.5 Statistical analysis

All the recorded data were analysed using Analysis of Variance (ANOVA) technique following the Completely Randomized Design (CRD) under factorial arrangement by using Statistix 8.1 (Taylor-Powell and Steele, 1996). The means were compared by using the least significant difference (LSD) test at a 5% probability level.

3. Results and Discussion

3.1 Effect of salinity on morphological parameters

Shoot length (cm)

The data related to variation in shoot length of different rice varieties under salinity stress was shown in (Fig. 1A) and clearly showed that variation in shoot length at salinity stress was highly significant (*P*= .05). Under saline conditions, all varieties experience a reduction in shoot length compared to control condition, however, variations in plant height of three rice varieties were may be due to their genetic variability. At salinity stress of EC = 10 dS m^{-1} maximum shoot length (57 cm) was recorded in variety BR-61 followed by BR-47 (54 cm), while minimum was found in Al-Khalid (52 cm), which accounts for lowest decrease of 11% in BR-61 variety followed by BR-47 with 23% and highest decrease of 25% in Al-Khalid variety when compared with control. Salinity stress impacted both the ultimate size of the cells and the speed at which new cells were produced, leading to a decline in plant height (Reddy *et al*., 2017).

Shoot fresh weight (g)

The (Fig. 1B) depicted the data related to changes in shoot fresh weights of different rice varieties which clearly shown that effect of salinity stress on three varieties of rice was highly significant (*P*= .05). NaCl stress noticeably affected the shoot fresh weights of all rice varieties when compared to non-saline condition, although variation among three varieties of rice had been observed markedly. At NaCl stress of 10 dS m^{-1}, highest shoot fresh weight was witnessed in variety BR-61 (15.2 g) and also followed by Al-Khalid (12.6 g) while lowest shoot fresh weight was exhibited by BR-47 (11.6 g) variety of rice. Hence, 14% of minimum reduction in fresh weight was observed in BR-47 followed by 24% reduction in variety BR-61, whereas maximum decrease of 28% was examined in rice variety Al-Khalid. Jamil *et al*. (2010) reported that the reason behind the decrease in shoot fresh weight in saline environments was due to reduced water absorption, toxicity of sodium and chloride in plant cells, and decreased photosynthesis.

Shoot dry weight (g)

The (Fig. 1C) represented the data about the effect of salinity stress on shoot dry weights of three rice varieties which markedly demonstrated that the response of all rice varieties and salinity level is statistically highly significant (*P*= .05). At salinity level of 10 dS m^{-1}, there was a decrease in shoot dry weight across all rice varieties compared to non-saline control, however, variation in shoot dry weights of all three varieties of rice was observed under NaCl stress. When exposed to salt stress, higher shoot dry weight (8.0 g) was recorded in variety BR-61 (reduced 23% from control) followed by Al-Khalid (6.6 g) variety (32% of highest reduction from control), while, lower shoot dry weight (5.6 g) was observed in BR-47 variety (24% reduction from control). Zeeshan *et al*. (2020) reported that the main cause of serious decline in shoot dry weight is excessive increase in salinity levels.

Root length (cm)

The data related to the root lengths variation of three different rice varieties is illustrated in the (Fig. 2A) which evidently shown the effect of salinity stress on different rice varieties is statistically significant (*P*= .05). When exposed to saline conditions, there was a significant reduction in root lengths for all rice varieties when compared to control, however, the variation among different varieties was may be due to genetically different rice varieties. At salinity stress of 10 dS m^{-1}, maximum root length was observed in BR-61 (10.3 cm) variety and followed by variety BR-47 (9.3 cm), while minimum root length was recorded in Al-Khalid (8.4 cm) variety. Furthermore, it was found that the minimum reduction in root length of varieties BR-61 and BR-47 was 17%, whereas, for variety Al-Khalid it was 18% as compared to control. The reason for the reduction in root lengths could be attributed to salt stress inhibiting photosynthesis, leading to a decrease in carbohydrate supply for growth, lower water potential due to reduced turgor, and disrupted mineral supply affecting growth (Sabagh *et al*., 2019).

Root fresh weight (g)

The following data related to root fresh weight of different varieties of rice under salinity stress is illustrated in (Fig. 2B) which visibly represented that the effect of added salts on root fresh weight of three rice varieties was highly significant (*P*=.05). When compared to control, there was significant reduction in root fresh weight of three

rice varieties at the exposure to excessive salts. At salinity stress (EC = 10 dS m^{-1}), minimum root fresh weight (1.5 g) was perceived in varieties BR-47 and Al-Khalid, representing a decrease of 33% for Al-Khalid and 28% for BR-47, while maximum root fresh weight (2.0 g) was recorded in BR-61 variety translating to a decrease of 28% when compared to control. The reasons for the decrease in root fresh weight in rice may include cell content reduction, diminished tissue development and differentiation, inadequate nutrition, membrane damage, and disrupted avoidance mechanisms (Saeedpour, 2014).

Root dry weight (g)

Variations in root dry weights of different rice varieties under salinity stress is shown in (Fig. 2C), while effect of different salinity levels on root dry weights of rice varieties was highly significant (*P*= .05). Salinity affected root dry weight of all the varieties of rice compared to controlled treatments. Due to salinity, root dry weight of all varieties was decreased compared to control. At salinity stress of 10 dS m^{-1}, maximum root dry weight (1.0 g) was observed in BR-61 accompanied by Al-Khalid variety (0.7 g); however minimum root dry weight (0.6 g) was recorded in BR-47 variety. Hence, 40% of minimum reduction in root dry weight was observed in BR-61 and BR-47, whereas maximum decrease of 42% was examined in rice variety Al-Khalid. Root dry weights hold the same reason of reduction as observed previously in case of root fresh weight of the plant.

3.2 Effect of salinity on physiological parameters

Chlorophyll content (SPAD value)

Variations in SPAD value (Soil Plant Analysis Development) of three varieties of rice under salinity stress is shown in (Fig. 3A) which evidently displayed the response of salinity levels on chlorophyll contents of rice was statistically significant (*P*= .05). Chlorophyll contents of all the varieties of rice are affected by salt stress compared to controlled treatments. The decrease in chlorophyll levels is likely caused by the hindering impact of various salt ions build-up on the production of different chlorophyll types (Djanaguiraman and Ramadass, 2004). When exposed to the high salinity condition, all three varieties experience a notable reduction in chlorophyll content compared to non-saline. Maximum chlorophyll contents (33.3) were observed in variety BR-61 (13% of minimum reduction from control), while minimum chlorophyll contents (27.5) were recorded in BR-47 (22% of reduction from control) and Al-Khalid (26% of highest reduction from control). Ali *et al.* (2004) reported that due to the membrane-bound chloroplast's dependence on the stability of the membrane, chlorophyll reduction was observed in high salinity conditions where the membrane stability is often compromised.

Na^+ concentration (ppm) in leaf sap

The data related to changes in Na^+ concentration of different rice varieties under salinity stress is shown in (Fig. 3B). It displayed that effect of different salinity levels on Na^+ uptake of rice is also statistically highly significant (*P*= .05). Salinity affected Na^+ uptake in all the varieties of rice compared to controlled treatments. However, variations in Na^+ concentrations were observed due to genetic variations in rice varieties. Due to salinity, Na^+ concentration of all varieties was increased compared to control. Na^+ levels in leaf tissues exhibited a proportional increase with

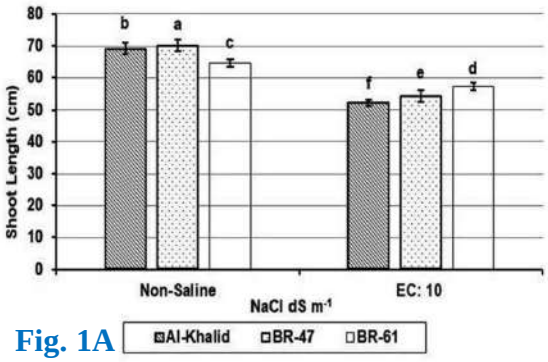

Fig. 1A

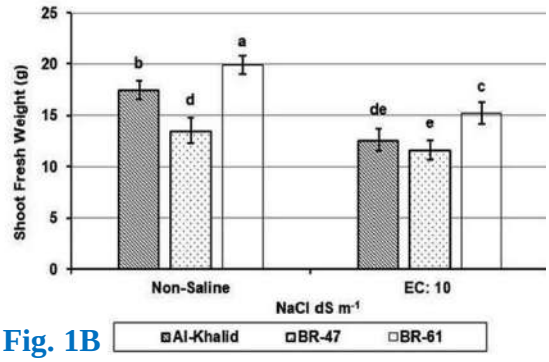

Fig. 1B

Fig.: Effect of treatment with 10 dS m^{-1} NaCl concentration compared to control condition after 90 days on shoot length (cm) (Fig. 1A) and shoot fresh weight (g $plant^{-1}$) (Fig. 1B) of three rice varieties

Mean ± SE (n = 4 biological replicates)

Data labeled by different lowercase letters are statistically significant at P < 0.05

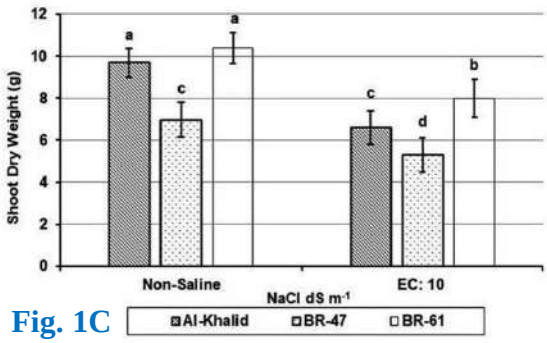

Fig. 1C

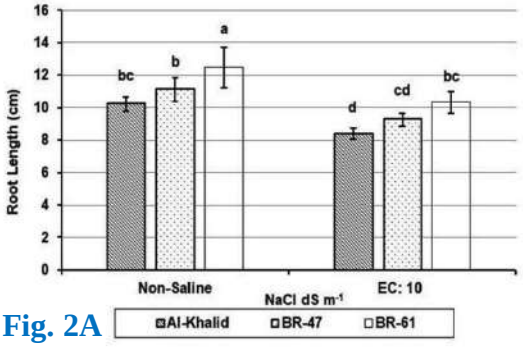

Fig. 2A

Fig.: Effect of treatment with 10 dS m^{-1} NaCl concentration compared to control condition after 90 days on shoot dry weight (g $plant^{-1}$) (Fig. 1C) and root length (cm) (Fig. 2A) of three rice varieties

Mean ± SE (n = 4 biological replicates)

Data labeled by different lowercase letters are statistically significant at P < 0.05

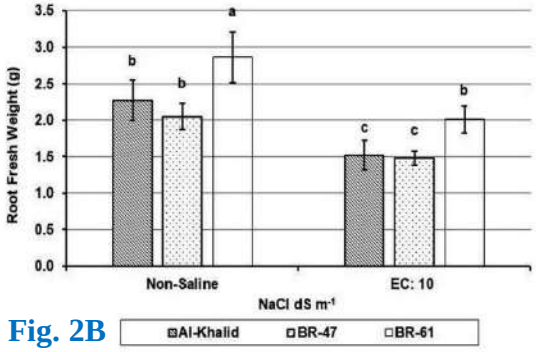

Fig. 2B

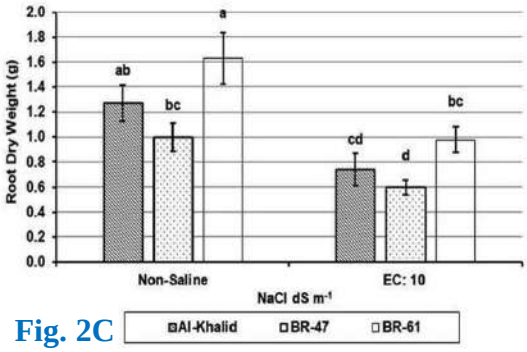

Fig. 2C

Fig.: Effect of treatment with 10 dS m^{-1} NaCl concentration compared to control condition after 90 days on root fresh weight (g $plant^{-1}$) (Fig. 2B) and root dry weight (g $plant^{-1}$) (Fig. 2C) of three rice varieties

Mean ± SE (n = 4 biological replicates)

Data labeled by different lowercase letters are statistically significant at P < 0.05

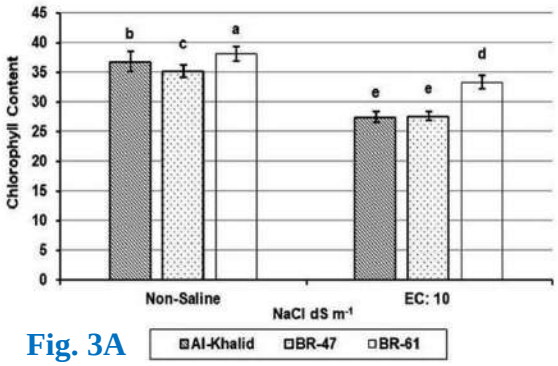

Fig. 3A

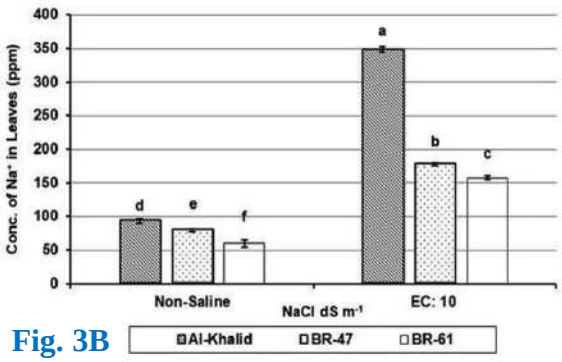

Fig. 3B

Fig.: Effect of treatment with 10 dS m^{-1} NaCl concentration compared to control condition after 90 days on chlorophyll content (Fig. 3A) and Na^+ concentration of leaf sap (ppm) (Fig. 3B) of three rice varieties

Mean ± SE (n = 4 biological replicates)

Data labeled by different lowercase letters are statistically significant at P < 0.05

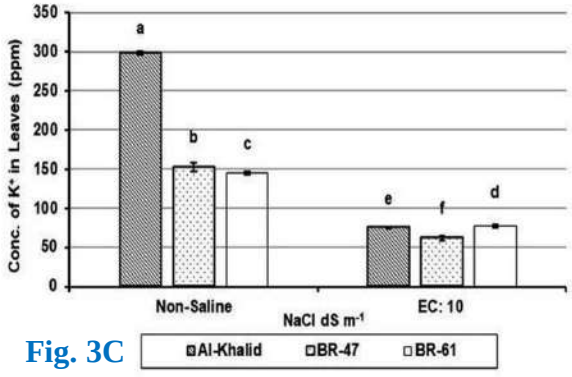

Fig. 3C

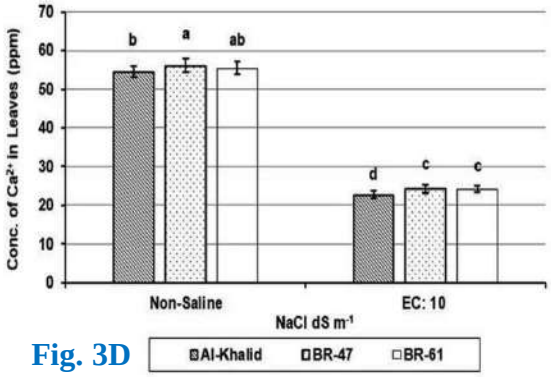

Fig. 3D

Fig.: Effect of treatment with 10 dS m^{-1} NaCl concentration compared to control condition after 90 days on K^+ concentration of leaf sap (ppm) (Fig. 3C) and Ca^{2+} concentration of leaf sap (ppm) (Fig. 3D) of three rice varieties

Mean ± SE (n = 4 biological replicates)

Data labeled by different lowercase letters are statistically significant at P < 0.05

varying levels of salinity (Hakim *et al*., 2014). At salt stress of 10 dS m^{-1}, maximum Na^+ concentration (348.7 ppm) with 3 folds increase from control was calculated in Al-Khalid variety. While minimum Na^+ concentration (177.4 ppm) with 2 folds increase from control for BR-47 and that for variety BR-61 was (157.4 ppm) with increase of 1.5 folds was recorded.

K^+ concentration (ppm) in leaf sap

Variations in K^+ concentrations of different varieties of rice under salinity stress is shown in (Fig. 3C) which undoubtedly shows that response of three rice varieties and effect of salinity level was statistically highly significant (*P*= .05). Salinity affected K^+ concentration in all the three rice varieties compared to controlled treatments.

However, changes in K^+ concentrations were observed due to genetic variations in rice varieties. Due to salinity, K^+ concentration of all genotypes was increased compared to control (Fig. 3C). At salt stress of (10 dS m^{-1}) NaCl, maximum K^+ concentration (77.1 ppm) with 47 % minimum increase was observed in BR-61 variety followed by Al-Khalid (74.6 ppm) with a maximum increase of 75% as compared to control. While, minimum K^+ concentration (62 ppm) with 59% increase was recorded in rice variety BR-47. Hakim *et al.* (2014) described that plants with more K^+ion accumulation was less affected with Na^+ ions and decrease in K^+/Na^+ ratio concentration due to salinity stress.

Ca^{2+} concentration (ppm) in leaf sap

The information associated to variations in Ca^{2+} concentration of diverse rice varieties under salinity stress is shown in (Fig. 3D) which clearly exhibits that response of salinity levels on Ca^{2+} concentration of rice was also statistically highly significant (P= .05). Salinity affected Ca^{2+} concentration in all the varieties of rice compared to controlled treatments. Due to salinity, Ca^{2+} concentration of all varieties was decreased compared to control (Fig. 3D). At NaCl stress of 10 dS m^{-1}, the BR-47 and BR-61 varieties showed equal concentrations (24.3 ppm) indicated no significant difference between them under saline stress and showed the reduction of 57% when compared to control, while Al-Khalid variety had the lowest Ca^{2+} concentration under saline conditions (22.8 ppm) with 58% maximum reduction compared to non-saline control. The results align with the discoveries of Razzaque *et al.* (2009), Summart *et al.* (2010). Ca^{2+} is crucial in the formation of new cell walls, especially in the middle lamellae that divide newly created cells, therefore the rice membrane experienced increased permeability because Ca^{2+} was displaced and Na^+ levels rose in the phospholipids binding sites, leading to damage (Momayezi *et al.*, 2009).

4. Conclusion

It can be concluded from the above experiment that among the three rice varieties tested, BR-61 proved to be the most tolerant and a potential variety for cultivation in the saline areas, and it exhibited maximum SPAD values and expressed higher root-shoot fresh/dry weights and root-shoot lengths under 10 dS m^{-1} NaCl stress. Contrastingly, other varieties like; BR-47. Al-Khalid being on the local maturity group, exhibited moderate adaptability and represented lower values for all parameters as expressed to salinity stress, however it could be enhanced by further breeding exercises. The content is the key factor which clearly signifies the tolerant and sensitive varieties. Furthermore, the tolerant rice varieties effectively restricted the entry of Na^+ into their leaves, however, efficiently maintained K^+ concentration and vice versa. This implies that management and breeding of rice types should focus on improving on the salinity tolerance in order to reduce its effects on the yield of rice. Hence, future studies should be directed towards establishing the particular molecular; biochemical and physiological factors that give the tolerance to these varieties and which should lay the basis for the production of new varieties of rice that are salt-tolerant.

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6. Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Improved forage as supplemental feed source and its utilization system in Yem special Woreda of Central Ethiopia

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ABSTRACT

This study was conducted to assess the improved forage production and Utilization. For this study, three kebeles were selected purposively based on their livestock potential and 160 households were selected from selected kebeles randomly. The major feed resource in the study area was crop residue and pasture land grazing. Desho and elephant grass were the dominant forage species adopted in the area. Feed shortage was the primary problem or livestock production. Majority of the households (66%) were in the active productive age (31-45) about 60% of household heads were literate (primary school and above).The mean cultivated land holding was 0.25 ha in study area. Crop production is the main source of cash income (1st rank) followed by cattle (2nd rank) and sheep (3rd rank) production. Almost all householders in study area have experience to produce improved forage specifically elephant grasses and desho which are most common in the study area. The major constraints in the study area that related to livestock production is disease (1st rank) followed by feed shortage (2nd rank) and water shortage (3rd rank), poor breed performance. Among major feed sources identified, grazing, crop residue and desho grass were given 1st, 2nd and 3rd rank in the study area respectively.

KEY WORDS: *Bread wheat*; *Correlation*; *Disease severity*; *Yellow rust disease*; *Yield component*

1. Introduction

Livestock population of south nation national people region state (SNNPR) estimated to 12,404,963 cattle, 4,735,604 sheep, 4,819,573 goats, 292,496 horses 305,089 donkey, 7,0365 mules, and 7,347,205 poetry (CSA, 2020). Despite the large livestock population in the country with high potential for meat and milk production, the contribution of the sector is well below its biological potential due to various reasons. Feed Shortage and disease, fewer effects in introducing the appropriate improved livestock technologies, cross breeds, improved feeds management practices feed scarcity is indicated as a factor responsible for lower production, reproductive and growth performance of animal especially during the dry season (Hurissa and Legesse, 2008).

During dry season, in adequacy of grazing resources result for animals not to be able to meet even their maintenance requirements and lose substantial amount of their weight. Livestock feed resources are classified as natural pasture, crop residue, improved pasture, forage, agro industrial by product and other by products like food and vegetable refusal, with the first two contribute the largest feed type (Mengistu, 2003). Animal

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depends mainly on natural pasture for their feed requirements. However, natural pastures which provide more than 90% are very poorly managed.

The important of natural pasture is gradually declining and expansion of crop production in to grazing lands also redistribution of common lands to the landless and land degradation (Berhanu *et al*., 2009). Though increased utilization of agro-industrial products has been reported, they are not available, affordable or feasible for most of the small holder farmers in Ethiopia. Hence, animals are allowed to graze natural pasture or crop stubbles around homestead supplemented with weed was the major feeding practice and it is now shifting to zero grazing because of continuing shrinkage of grazing.

Thus, there is no doubt that evaluating the current potential and identification of challenges that threatening this potential is mandatory in order to keep and exploit the current potential and tackle the threatening problems. Identification of feed resources and opportunities and constraints associated to livestock feeding are therefore, preconditions.

This study was, therefore, initiated to assess the feed resources utilization system and improved forage production status in the study area.

2. Material and Methods

2.1 Description of the study area

The study was conducted in Yem special woreda, located in the north-western apex of the Southern Nations, Nationalities and Peoples Regional State of Ethiopia. The administrative center of Yem special woreda (Saja city) is located at 247 km from Addis Ababa Southwest Ethiopia. Yem is bordered on the west and north by the Oromia Region, and separated from Gurage on the northeast and Hadiya on the east by the gibe River. Yem occupies a surface area of 724.5 km^2. The woreda lies within elevations of 920–2939 meters above sea level (MASL) and has three agro climatic zones; namely, Dega (cool highlands) (18.4%), WeynaDega (tropical highlands) (57.6%) and Kolla (lowlands) (24.0%). It receives a mean annual rainfall of 900 – 2200 mm in a bimodal pattern, from mid-February to April, and June to September. The mean annual temperature is in the range of 12– 30°C. The topography of Yem district is characterized by rolling mountains, long gorges, steep slopes and flat to undulating plateaus. The physiographic features of the woreda are characterized by high peaks and mountains and partly by deep gorges of Gibe River to the east. The total human population of the woreda as per 2007 population census is estimated to be 80,647 of which 50.3% are male and 49.7% female (CSA, 2011) and the population density is 111.3 persons/km^2. The major livestock production system in the woreda is cattle in Mixed crop-livestock farming comprising more than 43.7% (n = 107,201) of regional livestock population.

2.2 Data collection techniques

All relevant primary and secondary data source was employed for this study. Both qualitative and quantitative data from primary and secondary sources was used. The primary data was collected from sampled households, Woreda agricultural offices, from site development agents (DA) and others who have adequate information about the existing situation of the research area. Structured questionnaire which was filled by respondents, focus group discussion with farmer groups and an in depth interview was conducted to collect the

primary data. On the other hand qualitative data type was collected through focus group discussion and informal discussions with administrators and personal observations. Secondary data was collected from records of the woreda agricultural offices, and related literature prepared by government and nongovernmental organization. Such data sources include journal, research works, articles, statistical report, and official world-wide web sites for literature review and information about the study area.

Key informant interview was carried out to collect required primary data that lead to discussion with concerned bodies to obtain information about the issue related to the study objectives and description of study area. The key informant of this particular study was livestock directorate experts and kebeles level extension agents. The interview was recorded by using checklist.

2.3 Sample size

According to the data obtained from the Yem special woreda agriculture and natural resource development office, there are 32 rural kebeles and 5 urban centers in the woreda. Two-stage sampling procedure was employed in this specific study. In the first stage, the 3 representative kebeles was selected purposively based on the livestock production potential. These selected kebeles were Daritegu, Ediya and Oyakepo. Secondly, within the 3 kebeles, 135 households were selected using random sampling methods.

2.4 Research design

The study applied cross sectional survey method that both qualitative and quantitative design was employed to address the proposed study objectives. Quantitative methods aim to classify features, count them, and create statistical methods and explain observations, interview and use of questionnaires. Qualitative methods aim for a complete, detailed description of observations, including the context of events and circumstances. In order to achieve the objective of the research, considering the nature of the problem and the type of the assessment, this study was using both qualitative and quantitative research approaches.

2.5 Methods of data analysis

Data was analyzed with reference to the purpose or to the objective of the study, and was in referring to the research problem at hand or the hypothesis. The process of data analysis includes steps like categorization, coding, statistically adjusting the data and tabulation. Finally SPSS (Version 20.0) was used to analyze data and the results were presented in the form of tables and figures. Descriptive statistics was applied to describe the collected data using mean, standard deviation, percentages, and graphs.

3. Results and Discussion

3.1 Household characteristics

Information on family size, age and educational level of household's were indicated in Table 1. Average family size of a household is (Medium family size (4-6)) 45. Male and female headed households were 80% and 20%, respectively. Majority of the households (66%) were in the active productive age of 31-45 and 60% of household heads were literate (primary school and above). Average family size of a household was 6.56.

Table 1: Age, Sex, Educational level and Family size of a household

Description	Frequency	Percent
Household sex		
- Male	108	80
- Female	27	20
Household age		
- 18-30	18	13.3
- 31-45	90	66.7
- 46-65	18	13.3
- >65	9	6.7
Education level		
- Illiterate	0	0
- Primary (1-4)	45	33
- Primary (5-8)	81	60
- Secondary (9-10)	9	7
- Preparatory (11-12)	0	
Family size		
- ≥3	12	8
- 4-6	62	45
- 7-10	50	35
- 11-12	12	8

Average family size of a household (Medium family size of 4-6) in the current study is in agreement with previous assessment report (7.3) (Biruk *et al*., 2014) conducted in the same location from different agro ecology and in Anelemo district (Salo *et al*., 2017). In most rural part of Ethiopian, family members are the main source of household labor. Hence, large family size could be taken as an opportunity with regard to accomplishing laborious farm activities. However, large family size could have negative impact on the livelihood of the family if economic activities and income sources are limited (Abba, 2010). The presence of large family size might be attributed to labor demanding agricultural activities in the area (Yadessa, 2015) and/or lack of awareness on proper family planning methods.

Education on the other hand plays great role in transferring technology and in initiating farmers' willingness to adopt different technologies. Accordingly, in this study, about 60% of household heads were literate (primary school and above) that can be considered as an opportunity to easily disseminate different technologies through strengthened trainings. Majority of the household head being in the range of active working age groups is also a big opportunity to undertake multiple tasks.

3.2 Farming characteristics and land holding

Farming activities and land holding was presented in Table 2. Major farming activities in the study area were cultivation/cropping and rearing livestock (86%) followed by both Farming and labor (24%). The mean land holding of the area was 0.25 ha.

Table 2: Major occupation and land holdings

Description	Frequency	Percent
Major occupation		
Farming	117	86
Both farming and trading	0	0
Farming and labor	18	24
Farming, trading and labor	0	0
Land holding	Mean	
Cultivated land	0.52	52
Grazing land	0.16	16
Wood land and settlement	0.2	20
Fodder land (cultivated)	0.12	12

The total average land holding per household in the study areas in the current study were similar with reports for average land holding (0.52 ha) in Anelemo district (Salo *et al*., 2017) and for Doyogena district (0.5-1 ha) (Mekonnen *et al*., 2014). However, the value in the current study was lower than a report for Burie district (Yenesew, 2013), and 2.98 reported for enset based farming system in Enorworeda of Gurage

zone (Adem, 2024). Family size could have an impact on the livelihood of the farmers. The situation further will exacerbate the problem unless development options are arranged for landless groups, and intensive and wise land resource use practices are applied. There was no significant variation in land holdings among farmers.

Major occupation in the district being farming followed by both farming and petty trading sites indicated that farming (both crop and livestock), is the main means of living in the study area.

3.3 The contribution of agricultural activities to the household income

Income contributions of different agricultural activities are indicated in Table 3. Farmers in the area had different sources of income, where crop production is the main source of cash income (1st rank) followed by cattle (2nd rank) and sheep (3rd rank) production. Forages feed was also to lesser extents (5th rank) serve as income source.

This result is in consistent with report for Lemu district of Hadiya zone. The lower contribution of small ruminant compared to cattle was related with small number small ruminant holding of the area.

3.4 Livestock production challenges

Challenges for livestock production in the study area were shown in Table 4. The major constraints in the study area that related to livestock production was disease (1st rank) followed by feed shortage (2nd rank) and shortage of water (3rd rank), poor breed performance and others.

These problems were in line with reports for Horro and Guduru districts (Gurmessa, 2015). Moreover, local breeds are resistant to disease and can perform better under limited feed availability and easy management condition (Getahun, 2008).

3.5 Livestock feed shortage and coping mechanisms

Specific feed shortage, time of shortage and coping mechanisms are presented in Figure 1. About 96% households in the study areas were responded as they are suffering from feed shortage. Majority (80%) of farmers faced the challenge during dry season starting from December to May and farmers were responded as they use different coping mechanisms to alleviate the problem of feed shortage. Purchasing grass and concentrate, and feeding non-conventional feed resources like kitchen wastes and enset leaf are most adopted coping mechanisms in the study site which is in line with reports for several highland parts of Ethiopia (Deribe, 2015).

The feed shortage problem observed during dry seasons in the study area was related with moisture stress that resulted in low herbage growth on existing grazing land as similar reports

Table 3: The contribution of different agricultural activities to the household income

Income source	Primary choice						Rank
	1st	2nd	3rd	4th	T-sum	PI	
Crop	63	19	6	0	88	35	1
Cattle	0	0	23	26	49	19	2
Sheep	0	0	23	26	49	19	3
Poultry	0	3	23	14	40	16	4
Forage	2	23	4	1	30	12	5

Table 4: Major livestock production challenges

Income source	Primary choice						Rank
	1st	2nd	3rd	4th	T-sum	PI	
Disease	85	23	10	17	135	25	1
Feed shortage	35	58	20	13	103	19	2
water shortage	23	15	38	55	131	24	3
Poor breed performance	50	43	5	6	104	20	4
Market problem	0	0	43	17	60	11	5

that supports these results are exists from different location of Ethiopia having similar agro ecology. Purchasing grasses which are serving as the main source of roughage during dry season (Salo *et al*., 2017), but low in their nutrient content (Deribe, 2015).

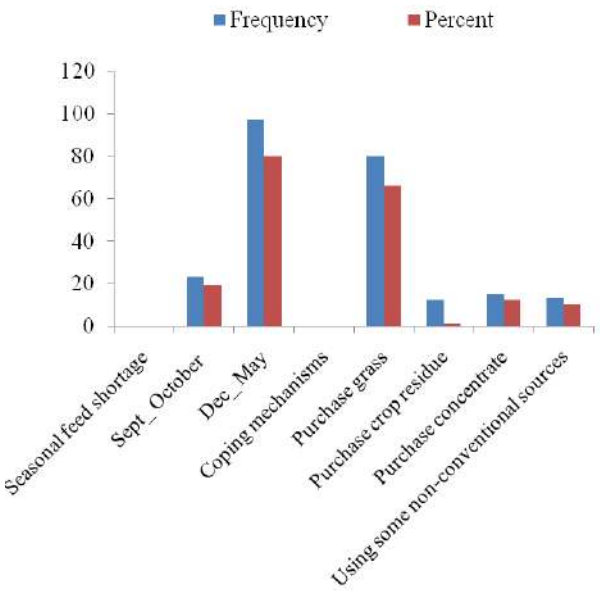

Fig. 1: Feed shortage problems and coping strategy % of respondent (N=135)

3.6 Livestock feed sources

The major feed resources used for cattle in the area are indicated in Table 5. Among major feed sources identified, grazing, crop residue and desho grass were given 1st, 2nd and 3rd rank in the study area respectively.

The results relating to feed resources show the dominancy of grazing, crop residue and local grass in the study area which is similar with reports for Anelemo district of Hadiya zone (Salo *et al*., 2017). Concentrate feed utilization in the study area was high during dry period with the objective of supplementing poor quality roughages that are available during dry season. As a result of grazing land shortage that resulted in fewer animals stocking rate, majority of farm households practice day time controlled/tethered grazing and night time feeding in individual.

3.7 Seasonal livestock feed availability and utilization

The seasonal availability and utilization of existing feed resource are presented in Table 6. Crop residues, Grazing, Elephant grass and Desho grass were the major feed resources available and utilized in the study areas. Among these, Crop residues, Grazing aftermath Elephant grass, Local grass and Desho grass were the major feeds and frequently utilized in dry seasons. On the other hand, Grazing on pasture, Elephant grass, Local grass (cut and carry system) and Desho grass were the major feeds and frequently utilized in wet seasons. Improved forages were mostly utilized in both dry and wet season of the year as supplement.

Table 5: Major cattle feed sources in the district (N=135)

Feed type	Primary choice						Rank
	1st	2nd	3rd	4th	T-sum	PI	
Local grass (harvested)	25	34	39	33	131	26	1
Desho grass	20	21	54	32	127	25	2
Grazing	55	10	25	15	105	20	3
Crop residue	30	18	12	23	83	16	4
Elephant grass	5	`12	23	0	28	5	5
Concentrate	10	0	0	10	20	3	6
Enset leaf	5	2	`21	2	9	1	7

3.8 Livestock feeding practice

Seasonal animal feeding practices identified are given on Fig. 2. Most of the farmers were practicing individual night time feeding and controlled grazing during day time. Free grazing feeding practice were the most commonly used in the study area.

Majority of the householders of the study area has experience to use several supplementary feeds like Atela' (local brewery by products), wheat bran, and others (food grains, enset corm, food wastes, root crop tubers, sweet potato vine).

3.9 Improved forage production

The major improved forage produced in the area is presented in Table 7. Almost all householders in study area (100%) have experience to produce improved forage specifically elephant grasses and desho which are most common in the study area. Satariya and Guatemala grasses are newly introduced forage species.

Improved forage production is believed to be remedies for overcoming feed shortage but is constrained by many challenges including small land holding, encroachment of food crop production, lack of forage seeds, and limited knowledge on forage species and their production systems. This situation was exacerbated by absence of improved forage seed provision and transfer system in the area. In contrary to this result, land was reported as primary constraint in Anelemo and Robi district (Salo *et al.*, 2017) and (Yadessa, 2015).

3.10 Constraints for improved forage production

Major constraints that hampered improved forage productions are presented on Fig. 3. Majority of the householders responded that they have

Table 6: Seasonal availability and utilization of feed resources (N=135)

Dry season							Wet season						
Feed resources	Primary choice					Rank	Feed resources	Primary choice 4th					Rank
	1st	2nd	3rd	4th		2nd		1st	2nd	3rd	4th		
Crop residue	68	3	3	0	133	1	Grazing on pasture	111	12	9	0	132	1
Desho grass	30	13	32	32	107	4	Desho grass	78	4	5	8	95	4
Elephant grass	25	20	49	30	124	3	Elephant grass	12	40	46	9	107	3
Grazing	47	40	43	2	132	2	Crop residue	23	45	55	0	123	2

Source: Field Survey, (2021)

shortage of Land and Financial problem as primary constraints followed by lack of awareness; with shortage of land were highly prominent (93%) in the study area.

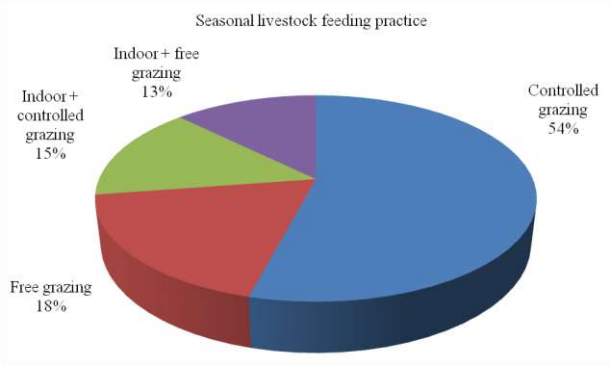

Fig. 2: Seasonal livestock feeding practice

Table 7: Improved forage producers and forage type produced in three kebeles (N=135)

Description	Frequency	Percent
Use of improved forage		
Forage type /ha/	Hectare	% of respondents
Desho grass/ha/	380	79
Elephant grass/ha/	76.85	16
Satariya	14.25	2.9
Guatemala grass/ha/	7,125	1.4

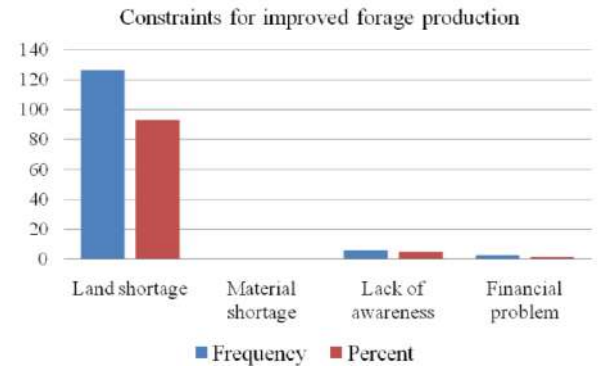

Fig. 3: Major constraints for improved forage production in the area (N=135)

Lack of awareness on different improved forages and its production strategies together with the shortage of land and improved forage seeds had hindered the scaling up of improved forage technologies. Hence, this situation calls attention for application of different forage development strategies and introduction of legume forages that can be integrated with other cropping system.

4. Conclusion

The major feed resources in the area were natural pasture which is shrinking from time to time as a result of converting lands to food crop production. Besides, the quality of available feed from pasture land is not substantial to livestock need, because its quality and quantity was highly fluctuated to the season of the year. Hence feed shortage was recorded as primary constraints in the study area following seasonal fluctuation. Accordingly, feed shortage season started from end of December to May. The observed feed shortage was exacerbated by lack of supplementary feed like improved forage production in the area. As a result, purchased feeds and several locally available by products like enset leaf were used as a coping mechanism against feed shortage. Though it was not at adequate level, these were starting points for improved forage adoption in the area. So introducing of improved forage like desho grass, vetch, elephant grass, sesbania, and lecuceana were becoming as common practice in the study area. Farmers gave particular emphasis to lactating cows, pregnant cows, fattening cattle and calves in utilizing this improved forage. Shortage of land, lack of awareness and the increased piece of forage seed were the main constraints that hinder the adoption of improved forage.

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6. Conflicts of interests

Authors declare that there is no conflict of interest exists.

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Evaluation of phosphorus solubilising bacterial strain on growth and yield of Maize (*Zea mays* L.)

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ABSTRACT

A field experiment was conducted to evaluate the effectiveness of a phosphate-solubilising bacterium (PSB), designated as isolate PSB S-2, on the growth and yield parameters of maize (*Zea mays* L.). The isolate, initially screened and confirmed for its high phosphate solubilising efficiency under in vitro conditions, was selected for seed inoculation. The study comprised nine treatments arranged in a randomized block design with three replications. PSB S-2 was applied as a bioinoculant in combination with varying levels of recommended dose of fertilizers (RDF). Among the treatments, the application of PSB S-2 along with 75% RDF significantly enhanced plant growth parameters, including the number of leaves per plant and chlorophyll content. It also resulted in notable improvements in yield attributes such as the number of rows per cob, grains per row, total grains per cob, grain weight per cob, test weight, cob length, cob weight, and the number of cobs per plant. In contrast, the control treatment without any inoculant recorded the lowest values in all observed parameters. The findings suggest that PSB S-2, when combined with a reduced dose of chemical fertilizer, can effectively improve maize growth and productivity under field conditions, promoting sustainable agricultural practices.

KEY WORDS: *Zea mays*; *PSB*; *Growth and Yield*; *Recommended Dose of Fertilizers*; *Bioinoculant*

1. Introduction

Maize (*Zea mays* L.) is one of the world's most important cereal crops, cultivated extensively across a broad range of agro-climatic zones due to its remarkable adaptability (Shiferaw *et al*., 2011). It serves as a staple food in many regions and provides raw material for industrial and livestock applications. In countries like India, more than 85% of maize produced is consumed directly as human food, with grains processed into flour and incorporated into a variety of traditional dishes (Sharma and Misra, 2021). Nutritionally, maize grains are rich in carbohydrates and contain considerable amounts of vitamin A, niacin, riboflavin, and vitamin E, although the protein component, zein, is deficient in lysine and tryptophan - two essential amino acids required for human health (Vasal, 2000).

Plant nutrition, which involves the study of essential mineral elements required for plant growth and reproduction, plays a fundamental role in crop productivity. Macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) are vital for various physiological processes, including cell division, photosynthesis, and energy transfer (Marschner and Marschner, 2012).

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Among these, phosphorus ranks next to nitrogen in importance for crop growth. It plays a critical role in nucleic acid synthesis, energy metabolism via adenosine triphosphate (ATP), and the regulation of enzymatic processes (Tisdale *et al*., 1993). An adequate supply of phosphorus in early plant growth stages is essential for root development and the initiation of floral primordia, ultimately influencing reproductive success (Sharma *et al*., 2022). However, phosphorus availability is often limited in soils due to its strong fixation with calcium, iron, and aluminum compounds, especially in alkaline and acidic soils (Hinsinger, 2001). This results in widespread phosphorus deficiency in many agricultural systems, leading to symptoms such as stunted growth, delayed maturity, and purple leaf pigmentation (Rashid *et al*., 2004).

To address this issue sustainably, the application of phosphate-solubilising microorganisms (PSMs) has gained attention. These include diverse bacterial and fungal genera capable of transforming insoluble phosphates like tricalcium phosphate, hydroxyapatite, and rock phosphate into bioavailable forms through acidification, chelation, and enzymatic mechanisms (Rodríguez and Fraga, 1999; Liu *et al*., 2020). Among these, phosphate-solubilising bacteria (PSB) such as species of *Bacillus*, *Pseudomonas*, and *Rhizobium* have been widely studied for their efficiency and multifunctional roles in promoting plant growth (Khan *et al*., 2009; Sharma *et al*., 2021). These bacteria not only enhance phosphorus availability but also contribute to improved nutrient uptake, biomass accumulation, and yield through the secretion of plant growth-promoting substances like indole acetic acid (IAA), siderophores, and enzymes like ACC deaminase (Vessey, 2003).

The integration of PSBs into fertilization regimes offers a promising alternative to reduce the overuse of chemical fertilizers and supports environmentally sound agricultural practices. Particularly, combining PSBs with reduced levels of chemical phosphorus fertilizers has shown potential to maintain or even enhance crop yield while minimizing environmental degradation (López-Arredondo *et al*., 2014; Singh and Prasanna, 2020).

The present investigation focuses on the field evaluation of an efficient phosphate-solubilising bacterial isolate (PSB S-2), which had previously demonstrated high solubilisation potential under in vitro conditions. The study aimed to assess its impact on the growth and yield attributes of maize when applied as a seed inoculant, in combination with different levels of recommended dose of fertilizers (RDF), to determine its utility in integrated nutrient management under field conditions.

2. Material and Methods

2.1 Experimental site and soil characteristics

A field experiment was conducted to evaluate the effectiveness of phosphate solubilising bacterial (PSB) inoculants on the growth and yield performance of maize (*Zea mays* L.) under black cotton soil conditions. The study was carried out at the experimental farm of the College of Agriculture, Raichur, Karnataka, India.

The experimental site is located in the northern dry zone of Karnataka, characterized by black cotton soil (Vertisol) with good moisture retention capacity.

2.2 Procurement of bacterial isolates

Two efficient PSB strains—one locally isolated and the other a standard reference strain—were obtained from the Department of Agricultural Microbiology, College of Agriculture, Raichur. These isolates had previously been screened and characterized for phosphate solubilisation potential under *in vitro* conditions.

2.3 Preparation of PSB inoculants

The bacterial cultures were grown in Pikovskaya's liquid medium. The broth was prepared in 250 mL Erlenmeyer flasks, sterilized at 121°C for 30 minutes, and subsequently cooled to ambient temperature. Each flask was inoculated with 1 mL of standardized bacterial suspension (10^{-8} CFU mL^{-1}) and incubated at 37°C on a rotary shaker at 120 rpm for 72 hours. After the incubation period, the broth cultures were mixed thoroughly with sterilized lignite powder in a 1:1 ratio (w/v) to serve as a carrier material. The formulation was cured under shade for 24 hours and packed into sterile low-density polyethylene (LDPE) bags at 200 g per packet. This formulation was used as the seed treatment bioinoculant.

2.4 Seed treatment and field layout

Maize seeds (cv. Hema hybrid) were surface-sterilized using 0.1% mercuric chloride solution, rinsed with sterile distilled water, and air-dried. Treated seeds were coated uniformly with PSB inoculants using 10% jaggery solution as an adhesive.

The experiment was laid out in a randomized complete block design (RCBD) with nine treatments replicated thrice. Each treatment represented a combination of PSB inoculation and graded levels of recommended dose of fertilizer (RDF).

Treatments details

T_1 – Control
T_2 – PSB
T_3 – Reference PSB
T_4 – RDP 100%
T_5 – RDP 100% + PSB
T_6 – RDP 75%
T_7 – RDP 75% + PSB
T_8 – RDP 50%
T_9 – RDP 50% + PSB

2.5 Growth parameter observations

Plant growth observations were recorded at 30, 60, and 90 days after sowing (DAS) and at the harvest stage to evaluate the influence of phosphate solubilising bacterial inoculants on maize development. Five plants were randomly selected and tagged in each plot for detailed measurements. The number of fully developed green leaves per plant was counted manually to assess vegetative vigor. Leaf chlorophyll concentration was measured using a SPAD-502 chlorophyll meter (Konica Minolta, Japan). For each plant, SPAD readings were taken from the uppermost fully expanded leaf at three different positions, and the average was considered as the representative value. These measurements were conducted consistently across all growth stages to monitor the photosynthetic status of the plants throughout the crop cycle.

2.6 Yield attributes and harvest observations

At the time of harvest, several yield-related parameters were assessed to determine the effect of PSB inoculation on reproductive performance and productivity of maize. The number of cobs produced per plant was recorded, followed by measurements of cob length using a standard ruler. The number of kernel rows per cob and the

number of grains per row were counted to estimate grain setting efficiency, and the total number of grains per cob was calculated accordingly. Grain weight per cob was determined after manual threshing and drying to uniform moisture content. The 100-seed weight (test weight) was measured from a randomly selected grain sample and adjusted to 12% moisture content to ensure accuracy and comparability. Finally, the grain and stover yields were determined from the net plot area and extrapolated to a per hectare basis. These comprehensive yield measurements helped establish the relationship between bacterial inoculation, plant nutrition, and crop productivity under field conditions.

3. Results and Discussion

3.1 Effect of phosphate solubilising bacteria on number of leaves

The application of efficient phosphate solubilising bacterial (PSB) isolates had a notable and statistically significant effect on the number of leaves produced by maize plants at different stages of growth. Among the treatments, the maximum number of leaves per plant was recorded at 30 days after sowing (DAS) in the treatment receiving inoculation with the efficient PSB isolate PSB-S-2 combined with 75% of the recommended dose of phosphorus (RDP), which recorded 7.47 leaves per plant. This was significantly higher than both the treatment involving inoculation with the reference PSB strain (5.60 leaves per plant) and the uninoculated control (5.40 leaves per plant). The enhanced leaf development trend continued consistently across later growth stages and was evident up to the harvest **(**Table 1**)**.

This increase in leaf production can be attributed to the role of PSB in improving phosphorus availability in the rhizosphere. Phosphorus is one of the key macronutrients influencing root development and photosynthetic activity, which in turn supports enhanced vegetative growth. Ahmad *et al*. (2009) also observed similar improvements in plant height, number of leaves, and pod formation in chickpea due to PSB inoculation. Enhanced leaf production in cowpea plants was similarly reported by Nagaraju *et al*. (1995), particularly when PSB inoculants were applied in conjunction with rock phosphate (RP), leading to greater solubilisation of unavailable phosphorus forms and improved nutrient uptake. Tomar *et al*. (1993) also reported increased branching and foliage development due to enhanced rhizospheric P availability facilitated by P-solubilising microorganisms. According to Jat and Mali (1992), phosphorus application stimulates meristematic activity and photosynthetic efficiency, both of which are essential for leaf initiation and expansion. These earlier findings corroborate the present study's observation that PSB-S-2, when combined with partial RDP, creates an optimal nutrient environment conducive to vigorous vegetative growth.

3.2 Chlorophyll content of maize leaves

Chlorophyll content is a direct indicator of the photosynthetic potential and physiological health of the plant. In the present investigation, the treatment involving PSB inoculation along with 75% RDP (T_7) exhibited a significant increase in leaf chlorophyll concentration, especially at 60 DAS. The SPAD values in this treatment were statistically superior to those recorded in the uninoculated control and the treatment with reference PSB alone **(**Table 1**)**. The improved chlorophyll content is likely the result of enhanced

Table 1: Influence of efficient strain of phosphate solubilising bacteria on the Number of leaves plant^{-1}, Chlorophyll content of maize leaves at different growth stages

Treatments	Number of leaves plant^{-1}				Chlorophyll content (SPAD value)			
	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T_1 - Control	21.82	22.60	23.75	22.37	5.40	10.47	11.07	11.13
T_2 - PSB	22.82	24.54	25.63	24.03	5.73	11.07	11.87	11.93
T_3 - Reference PSB	22.26	24.28	24.78	23.55	5.60	10.53	11.43	11.50
T_4 - RDP 100%	29.80	29.87	30.59	29.15	7.32	13.87	14.07	14.13
T_5 - RDP 100% + PSB	30.70	30.76	31.00	30.29	7.33	14.07	14.40	14.47
T_6 - RDP 75%	25.41	25.71	28.45	25.90	6.47	11.67	12.80	12.87
T_7 - RDP 75% + PSB	31.17	31.82	32.30	31.33	7.47	14.13	14.87	14.93
T_8 - RDP 50%	24.50	25.24	26.59	24.75	6.27	11.47	12.33	12.47
T_9 - RDP 50% + PSB	26.22	26.40	28.88	26.17	6.53	12.40	13.53	13.60
S.Em±	0.79	0.95	1.21	1.19	0.22	0.35	0.39	0.40
C.D at 5%	2.30	2.77	3.53	3.49	0.63	1.04	1.13	1.18

Note: Values are mean of three replications, PSB - Phosphate Solubilising Bacteria, DAS – Days After Sowing

phosphorus availability, which plays a key role in energy transfer and photosynthesis. Phosphorus is involved in the synthesis of ATP and nucleic acids, and its availability directly influences the production of photosynthetic pigments.

These results align with the findings of Madhaiyan *et al.* (2004), who reported increased photosynthetic activity in plants inoculated with beneficial microbes, including PSB. Their study demonstrated that such inoculations could increase chlorophyll concentration, the number of stomata, and the accumulation of organic acids like maleic acid, which support photosynthetic function and overall plant vigor. Improved chlorophyll content in the present study suggests that PSB inoculation not only supports nutrient acquisition but also enhances physiological processes related to plant productivity.

3.3 Influence of PSB inoculation on yield parameters

A significant improvement in yield attributes was observed due to the inoculation of the efficient PSB isolate PSB-S-2 in combination with 75% RDP. This treatment resulted in the highest values for several key yield components. The number of kernel rows per cob was 18.91, and the number of grains per row reached 35.47, resulting in an average of 543.33 grains per cob. The grain weight per cob was also enhanced (121.00 g), along with a test weight of 26.47 g. The same treatment recorded an average of 1.17 cobs per plant, cob length of 19.27 cm, and cob weight of 98.43 g per plant, all of which were significantly higher than other treatment combinations, including the uninoculated control and the treatment receiving only reference PSB.

Interestingly, although the treatment involving 100% RDP with or without PSB inoculation also performed well, it was statistically on par with T_7, indicating that the combination of PSB-S-2 with 75% RDP was sufficient to match the performance of full-dose fertilizer applications. This demonstrates the potential of bioinoculants like PSB to reduce the need for chemical

fertilizers while maintaining or even enhancing crop productivity.

The results are in agreement with Wu *et al*. (2005), who documented significant increases in maize yield and improvement in soil properties, including organic matter content, following the application of PSB. Similarly, Balsubramanian and Subramanian (2006) observed enhanced grain yield in rice due to silicate solubilising bacterial inoculation, where treated plots yielded 5218 kg ha^{-1} compared to 4419 kg ha^{-1} in control treatments. These findings reinforce the present study's outcomes, highlighting that microbial inoculants not only support plant nutrition but also positively influence the soil microbiome and nutrient cycling, resulting in improved yield (Table 2).

Increased phosphorus availability from PSB activity enhances root development and nutrient uptake, which are critical during the reproductive stages of maize. The resulting improvements in cob formation, grain setting, and grain filling demonstrate the cascading benefits of microbial biofertilizers on crop performance. Furthermore, the use of PSB in combination with reduced levels of chemical fertilizers offers a sustainable and eco-friendly approach to crop production, reducing input costs and environmental impact.

4. Conclusion

The present study clearly demonstrates that the inoculation of maize seeds with an efficient phosphate solubilising bacterial isolate (PSB-S-2), in combination with 75% of the recommended dose of phosphorus, significantly enhanced plant growth and yield attributes under field conditions in black cotton soil. The results indicated that this treatment notably improved critical growth parameters such as the number of leaves and chlorophyll content at various crop stages, highlighting improved vegetative vigour and photosynthetic capacity. Furthermore, yield-contributing traits including number of rows per

Table 2: Number of rows cob^{-1}, Number of grains row^{-1}, Number of grains cob^{-1}, Grain weight cob^{-1} and Test weight, Number of cobs plant $^{-1}$, Cob length, Cob weight of maize as influenced by the application of PSB

Treatments	Number of rows cob^{-1}	Number of grains row^{-1}	Number of grains cob^{-1}	Grain weight cob^{-1} (g)	Test weight (g)	Number of cobs $plant^{-1}$	Cob length (cm)	Cob weight (g/plant)
T_1 – Control	17.36	28.92	475.33	98.67	24.21	1.00	15.40	71.43
T_2 – PSB	17.51	29.67	498.33	108.00	24.96	1.00	16.85	78.40
T_3 – Reference PSB	17.43	29.28	482.67	104.00	24.55	1.00	16.55	74.43
T_4 – RDP 100%	18.57	33.27	540.00	119.33	26.12	1.10	18.39	96.83
T_5 – RDP 100% + PSB	18.80	34.20	542.33	120.00	26.23	1.13	18.78	97.23
T_6 – RDP 75%	17.73	31.80	521.67	114.67	25.67	1.00	17.13	86.30
T_7 – RDP 75% + PSB	18.91	35.47	543.33	121.00	26.47	1.17	19.27	98.43
T_8 – RDP 50%	17.69	30.07	517.67	112.00	25.59	1.00	16.99	82.53
T_9 – RDP 50% + PSB	17.87	32.91	530.67	117.00	25.70	1.13	17.30	88.23
S. Em ±	0.29	0.79	1.27	0.61	0.22	0.05	0.66	0.71
C.D at 5%	0.83	2.32	3.70	1.78	0.64	NS	1.92	2.08

Note: Values are mean of three replications

cob, grains per cob, cob weight, test weight, and grain yield were significantly increased compared to uninoculated control and even the reference PSB strain.

The ability of PSB-S-2 to enhance phosphorus availability in the rhizosphere contributed to better nutrient uptake, which translated into improved physiological and agronomic performance. The treatment with PSB-S-2 + 75% RDP was on par with 100% RDP, suggesting that the use of efficient PSB can partially substitute chemical phosphorus fertilizers, offering a more sustainable and cost-effective alternative. These findings reinforce the potential of using microbial inoculants like PSB as a viable component of integrated nutrient management strategies for maize, ensuring higher productivity with reduced dependence on synthetic fertilizers while maintaining soil health and environmental sustainability.

5. Conflicts of interests

Authors declare that there is no conflict of interest exists.

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Effects of irrigation water level on Bread Wheat at Qadalle scheme, Yabello district of Borana zone, Ethiopia

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ABSTRACT

The study conducted on the response of bread wheat yield and water use efficiency to six levels of irrigation ranging from 50% to 100%. The bread wheat variety, ETW9578, was used as the test crop. The experimental design consisted RCBD with three replications. The 12 m^2 net plot size was received 100 kg ha^{-1} of NPS, and 150 kg ha^{-1} of urea. The relationship between water levels and the bread wheat yield was analyzed through various components including grain yield, plant height, tiller number, and above-ground biomass. This is supported by the results of an ANOVA, which provides statistical evidence to confirm the significant impact of irrigation levels on wheat production. Accordingly, the max (4751.46 kg ha^{-1}) and min (2101.87 kg ha^{-1}) yield recorded at 100% ETc and 50% ETc irrigation level respectively. These findings demonstrate that as irrigation approaches full crop water requirements, bread wheat are better able to meet their physiological needs, resulting in optimal growth and productivity. The number of productive tillers is crucial as it directly influences the number of grains per plant. Enhanced irrigation results in greater biomass accumulation due to improved photosynthesis rates facilitated by healthier plants. The data presented indicates that different irrigation levels have varying impacts on the thousand seed weight (TSW) of bread wheat. The highest TSW was observed at 60% ETc and 90% ETc irrigation treatments. This suggests that these levels provide sufficient moisture to support optimal seed development. However, there was no significant variation in yields among 70% and 80% ETc treatments. This indicates that both levels may be similarly effective for achieving satisfactory yields. Conversely, reducing the irrigation level from 90% ETc to 70% ETc does not significantly reduce yield but offers advantages in terms of water savings. This finding is critical as it suggests that farmers can maintain competitive yields while using less water, which is essential for sustainable agriculture. Based on the findings, applying an irrigation level (70% ETc) is recommended for optimum returns in wheat. This recommendation balances yield performance with efficient water usage, making it a practical approach for farmers aiming to enhance sustainability while maintaining productivity.

KEY WORDS: *Irrigation*; *Water Use Efficiency*; *Water Level*; *Yield*

1. Introduction

Irrigated agriculture consumes the majority of the existing fresh water. Irrigation accounts for approximately more than 70% of total water abstraction and 60-80% of overall water usage (Ingrao *et al*., 2023). In order to feed 8 billion people by 2025, the irrigated agriculture should be

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doubled by more than 20% and the yield of irrigated crops improved by at least 40% (Senbeta and Worku, 2023). Improving agricultural water use efficiency is crucial to achieving this goal. Many studies have been undertaken to gather experience in watering crops to enhance performance, efficiency, and profitability, and water saving irrigation investigation will continue.

Agriculture's long-term water management and utilization has become a headache for scientists as well as for users. Acceptance of methods for saving irrigation water while keeping acceptable yields may help to preserve this increasingly scarce resource. In locations where water is a scarce resource, farmers may find that improving water productivity is more beneficial than boosting crop output. As a result, research should be established and carried out with the goal of increasing agricultural water productivity through various water-saving strategies in integration with the use of the correct scheme and farm structures (Yakubu *et al*., 2019).

The demand for wheat in Ethiopia is increasing faster than for any other food crops, particularly in urban areas. The gap between demand and supply is widening because of quickly increasing population number and changing preferences towards wheat-based food items. Conscious to the aforementioned facts, the Government of Ethiopia has already identified key priority intervention areas to increase productivity of small-scale farmers and expand large-scale commercial production of wheat. The top priorities identified include: development of small and large-scale irrigation schemes, financing effectual supply of agricultural inputs, improving agricultural production methods using mechanization, post-harvest loss reduction and natural resources management. Even though, government planned need to produce wheat crop by irrigation is high, there was no or little research work on irrigation water levels that gain high net return.

Among many restrictive factors water is the most common limiting elements in the agricultural production system, which is crucial for wheat growth and productivity. However, the optimal irrigation is not well identified, particularly in the study area. Therefore, this study was undertaken to determine the required irrigation water level that optimize wheat yield in Yabello district of Borana zone.

2. Material and Methods

2.1 Description of the study area

The study was carried out in Qadalle scheme, Yabello district of Borana zone of Oromia National Regional State. Yabello is the capital town of the Borana zone and situated south to Addis Ababa at a distance of 570 km. The Borana lowland is usually known as the southern rangelands. The Qadalle scheme is made from micro earthen dam collecting runoff water. Geographically, it is situated at 4°53'00"N to 5° 55' 09"N latitude and 38°5′ 00" Eto 40° 8′ 01"longitudes with an elevation ranging from 1550m to 1970m above sea level. The minimum and maximum temperature 19 to 24°C and annual mean rainfall is and 300 to1000 mm, respectively. The annual precipitation distribution is bimodal with 60% falling from April to May and 30% from October to November.

2.2 Experimental design and layout

The experiment was conducted for three consecutive years, at Qadalle irrigation scheme. The experimental field was ploughed, harrowed

and human labor. The Experimental designed a factorial RCBD with three replications. The irrigation levels include (100% ETc, 90% ETc, 80% ETc, 70% ETc, 60% ETc and 50% ETc). The experimental field was divided in to 18 with the plot size of 3.6m × 5m ($18m^2$) to accommodate 6 furrows with spacing of 60cm and having 4m length, consisting four ridges and five furrows for each plot. The blocks and plot had a buffer zone of 1.2m and 1.5m respectively from each other to eliminate influence of lateral flow of water. Field canal was constructed for each block to irrigate the field.

The bread wheat (ETW9578) variety seed was sown by drilling on both sides of the rows at a rate of 120 kg ha^{-1}. For data collection and measurement, four central rows with a net plot size of 12 m^2 were used. At time of seeding, all plots were given 100 kg ha^{-1} of NPS. 150 kg ha^{-1} was applied in splits form, half at 20 days after sowing and remaining half at 48 days after sowing. All cultural practices, other than treatment variables, were carried out according to the standard practices recommended for the area.

2.3 Discharge measurement

The irrigation water applied for each experimental plot was measured by 3-inch Parshall flume installed 10m away from the nearest plot along field canal. The calculated gross irrigation was finally applied to each experimental plots based on the treatments proportion. Volume of water applied for every treatment was determined from plot area and depth of gross irrigation requirement. Time required to irrigate each treatment was calculated from the ratio of volume of applied water to the discharge-head relation of 3-inch Parshall flume. Since discharge level might vary at field condition, time required was calculated at 15 cm head levels. The time required to deliver the desired depth of water into each furrow was calculated using the following equation.

$$t = \frac{A * dgross}{Q} - - - - - - \rightarrow \quad 1$$

Where: dg = gross depth of water applied (mm), t = application time (sec), A = plot Area (m^2) and Q = flow rate (l/s).

2.4 Agronomic data collection

Plant height and dry matter were measured at harvest. The plant heights of 10 randomly selected plants per plot were measured from soil surface to the top covering of the plant. The same 10 random plant samples were oven dried for 72 hour at 68°C and the dry weight was recorded. The spike length excluding awns was also recorded. As the kernels matured, the crop in the harvest area of 6.3 m^2 per plot (excluding border rows) was harvested and collected in clean sacks to record the weight. The harvested crop was sun-dried for three days and threshed separately using wooden sticks and finally the seed was separated, cleaned and weighed to record the grain yield for each plot.

The yield data used in this study were estimated using yield sampling methods. This was achieved by using the crop cut methods by demarcating a plot 3m × 3m in size. Then, followed by harvesting the produce from the plots, threshing, winnowing, and drying the produce, and later determining the dry weight. The weight of one thousand seeds per plot was recorded to 3 decimal places using a sensitive weighing balance.

2.5 Water Use Efficiency

The water use efficiency (WUE) was also calculated. The following Equation 1 was used to compute water use efficiency as a ratio of grain yield to total crop evapotranspiration (ETc) during the course of the growing season (Zwart and Bastiaanssen, 2004).

$$WUE = \frac{Y}{ETc} \quad \longrightarrow \quad 2$$

Where, WUE is water use efficiency (kg m^{-3}), Y is crop yield (kg ha^{-1}) and ETc is the seasonal crop water consumption by evapotranspiration (m^3/ha).

2.6 Data analysis

Analysis of variance for the collected parameters was performed as per the methods described by Allison, (2001) using SAS computer software for randomized complete block design and treatment mean comparison is done by Fisher's list significance difference (LSD) at 5%.

3. Results and Discussion

3.1 Soil physico-chemical properties of the experimental site

To identify some of the chemical and physical properties of the soil, representative composite soil samples were collected from the experimental site at the depths of 0–20 cm using an auger and core samplers. Some of the physical properties of the soil are given in Table 1. The soil in the experimental field was classified mainly as sandy loam textured. The soil water holding capacity at field capacity (FC) and the permanent wilting point (PWP) were on average 37.7 and 12.1%, respectively. The average soil bulk density (BD) was 1.38 g cm^{-3} hence, the average total available water (TAW) by volume percentage is also estimated as 97.10 mm/m.

3.2 Irrigation water applied for each treatment

As indicated in Table 2, that reducing irrigation based on actual crop needs (as represented by varying levels of ETc) can lead to substantial water savings while still meeting crop requirements effectively. The analysis shows that reducing irrigation based on varying levels of evapotranspiration (ETc) can lead to substantial water savings ranging from 0 mm at full (100% ETc) to as much as 294.1 mm at reduced levels (50% ETc).

Table 1: Some selected soil physico-chemical analysis of experimental site (0–120 cm).

Soil parameters	Values	Texture/Rating	Reference
Sand (%)	65.12	-	USDA
Silt (%)	17.12	-	
Clay (%)	17.76	-	
Textural class	Sandy Loam	-	
Soil pH	7.24	Slightly alkaline	Jones (2003)
Electrical conductivity (dS/m)	0.77	Non-saline	Hazelton and Murphy (2007)
Organic matter (%)	0.9	Low	Tekalign (1991)
Bulk density (g cm^{-3})	1.38	Loose	Clout and Manuel (2015).
Field capacity vol. (%)	27.7	Low	Cong *et al.* (2014)
Permanent wilting point vol. (%)	12.1	Ideal	
TAW (mm/m)	97.10	High	

Table 2: Water applied and water saved from each treatment (mm)

Treatments	Growth stage				IRg (mm)	Saved water (mm)
	Initial	Dev	Mid	Late		
100% ETc	154.7	141.3	206.6	71.5	574.0	-
90% ETc	139.1	127.2	185.9	64.3	516.5	57.5
80% ETc	123.6	113.1	165.2	57.2	459.1	114.9
70% ETc	108.2	98.9	144.6	50.1	401.7	172.3
60% ETc	92.7	84.7	123.9	42.9	344.3	229.7
50% ETc	77.3	63.6	103.3	35.8	279.9	294.1

3.3 Effect of different irrigation levels on yield and yield component of bread wheat

The study of the bread wheat variety "ETW9578" highlights the significant impact that irrigation water levels have on various growth parameters of the plant (Table 3). The findings indicate that under normal conditions, this variety has a total growth period of 94 days from germination to harvest. The research found that irrigation water levels had a highly significant effect ($p < 0.001$) on plant height. Specifically, treatment T_6 resulted in the highest plant height among all treatments. This suggests that adequate irrigation is crucial for maximizing the vertical growth of wheat plants. Similar to plant height, spike length was also significantly affected by irrigation levels. This parameter is essential as it directly relates to the potential yield of the wheat crop. Above ground biomass, which includes all parts of the plant above soil level, was also positively influenced by higher irrigation levels. The results indicated that increased water availability led to greater biomass accumulation, which is critical for overall crop productivity. Grain yields were significantly impacted by irrigation treatments as well, reinforcing the importance of proper water management in achieving optimal yields. Interestingly, while other parameters showed significant differences due to varying irrigation

Table 3: Effects of water levels on grain yield and yield components for three cropping seasons

Treatments	Water L (%ETc)	PH (cm)	TN	SL (cm)	BM (Kg ha^{-1})	TSW	GY (Kg ha^{-1})
T_1 (50% ETc)	50	51.75^{b}	7.83	5.5^{c}	3152.812^{d}	36.2250	2101.87^{d}
T_2 (60% ETc)	60	65.75^{a}	7.16	8.58^{ab}	3905.50^{dc}	37.1750	2603.67^{c}
T_3 (70% ETc)	70	63.75^{ab}	7.33	8.33^{b}	6339.37^{b}	35.9375	4226.25^{b}
T_4 (80% ETc)	80	62.00^{ab}	7.50	8.6^{ab}	6242.57^{b}	34.3750	4361.71^{b}
T_5 (90% ETc)	90	64.33^{a}	8.75	8.47^{ab}	6737.10^{ab}	37.1250	4491.40^{ab}
T_6 (100% ETc)	100	73.16^{a}	7.83	9.0^{a}	7277.10^{a}	35.3000	4751.46^{a}
LSD(0.05)		12.02	ns	0.56	640.98	ns	427.32
CV (%)		12.57	14.34	4.59	7.58	6.18	7.58

*PH = Plant height; TN = Tiller number; SL = Spike length; BM = Biomass in Kilogram; TSW = Thousand seed weight and Kg ha^{-1} = Kilogram per hectare

*ns = non-significant; *** = significant at p<0.001; **= significant at p<0.01 and *= significant at p<0.05

levels, tiller number and TSW did not exhibit such effects in this study. This could imply that these two factors may be less sensitive to changes in water availability compared to other growth parameters. The findings align with previous research conducted by Mehdizadeh *et al*., (2013), which reported that water stress negatively affects dry matter accumulation in wheat plants. Furthermore, studies by Mansoor *et al*., (2023) corroborate these results, indicating that different irrigation levels can influence plant height and above ground biomass by approximately 20% when compared with well- watered treatments. On the other hand, the study shows that the highest grain yield was recorded at 100% ETc, achieving a yield of 4751.46 kg ha^{-1}. This suggests that full irrigation is optimal for maximizing wheat production. Conversely, the lowest yield was observed at 50% ETc, with a yield of only 2101.87 kg ha^{-1}. This stark contrast highlights the detrimental effects of deficit irrigation on wheat productivity. The findings indicate specific percentage increases in grain yield associated with various treatments (T_1 to T_5) compared to a control treatment (T_6). The increases were quantified as follows: T_1 (9.33%), T_2 (11.55%), T_3 (18.75%), T_4 (19.35%), and T_5 (19.93%). These percentages reflect how incremental increases in irrigation correlate with improved yields.

Table 4: Effects of water levels on wheat water use efficiency

Treatments	GY (Kg ha^{-1})	Water used (mm)	WUE (Kg ha^{-1} mm^{-1})
T_1 (50% ETc)	2101.87^{d}	562.6	12.61^{a}
T_2 (60% ETc)	2603.67^{c}	506.34	11.92^{ab}
T_3 (70% ETc)	4226.25^{b}	450.1	11.47^{b}
T_4 (80% ETc)	4361.71^{b}	393.82	11.11^{b}
T_5 (90% ETc)	4491.40^{ab}	337.56	10.02^{bc}
T_6 (100% ETc)	4751.46^{a}	281.3	8.9^{d}
LSD (0.05)	427.32		1.55
CV (%)	7.58		15.9

Notably, treatments T_3, T_4, and T_5 did not show significant differences among themselves, suggesting a plant effect where further increases in irrigation beyond a certain point do not lead to proportional gains in yield. The results align with previous studies conducted by Wang *et al*., 2019, and Mohammed and Kadhem, 2017, which also reported similar trends regarding the positive impact of adequate irrigation on wheat yields.

The highest yield of 4751.46 kg ha^{-1} was obtained in T_6 under full irrigation conditions, while the lowest yield of 2101.87 kg ha^{-1} occurred in T_1 under deficit irrigation regimes (Table 3).

3.4 Effect of irrigation water level on water use efficiency

Water use efficiency (WUE) is a critical metric in agricultural practices, particularly in the context of irrigation management. It quantifies the amount of crop yield produced per unit of water used, which is essential for optimizing water resources in agriculture. The findings presented indicate that irrigation levels significantly influence WUE, with statistical significance noted at $p < 0.05$.

The study highlights that different irrigation treatments were applied, specifically focusing on 50%, 60%, 70%, 80%, 90% ETc and 100% ETc. The results showed that the highest WUE was achieved with the 50% ETc treatment, yielding an impressive 12.61 kg ha^{-1} mm^{-1}. In contrast, the full irrigation treatment at 100% ETc resulted in a lower WUE of 8.9 kg ha^{-1} mm^{-1} (Table 4). The findings align with previous studies conducted on

Table 5: Effects water application levels water use efficiency of wheat for each treatments

Water/L (ETc %)	GY(Kg ha^{-1})	Water used (mm)	WUE (Kg ha^{-1} mm^{-1})	Saved water (mm)	Additional GY (Kg ha^{-1})
T_1 (50 % ETc)	2101.87^{d}	562.6	12.61^{a}	294.1	2208.5
T_2 (60 % ETc)	2603.67^{c}	506.34	11.92^{ab}	229.7	1737.04
T_3 (70 % ETc)	4226.25^{b}	450.1	11.47^{b}	172.3	1812.75
T_4 (80 % ETc)	4361.71^{b}	393.82	11.11^{b}	114.9	1091.62
T_5 (90 % ETc)	4491.40^{ab}	337.56	10.02^{bc}	57.5	500.01
T_6 (100 % ETc)	4751.46^{a}	281.3	8.9^{d}	0	0
LSD (0.05)	427.32		1.55		123.5
CV (%)	7.58		15.9		8.15

wheat by Pradhan *et al*., 2014, which similarly demonstrated that reductions in irrigation levels positively impacted WUE for irrigated wheat crops.

Deficit irrigation is a strategy where water is applied at levels below full crop water requirements. The goal is to optimize water use while still achieving acceptable yields. In this case, applying 70% ETc allows farmers to conserve water while still maintaining reasonable crop productivity. The statement indicates that by utilizing saved water from these strategies, farmers could increase their water productivity by approximately 42.89%. This significant increase suggests that farmers can achieve more with less water, which is crucial in regions facing water scarcity. The potential yield increase mentioned is up to 1812.75 kg ha^{-1} when using the optimal irrigation level of 70% ETc. This figure highlights not only the effectiveness of the irrigation strategy but also its economic viability for farmers, as higher yields typically translates into higher profits (Table 5). The study by Kidane *et al*., 2023 corroborates these findings by reporting a similar level of water use efficiency (1.4 kg m^{-3}) under comparable conditions and agroecology. This consistency across different studies strengthens the validity of the results regarding optimal irrigation practices.

4. Conclusion

Different water application levels had significant effect on grain yield, yield related and WUE of bread wheat "ETW9578" variety. Interestingly, while higher irrigation levels improved grain yield, they also resulted in reduced water use efficiency. The highest observed WUE was at the 50% ETc irrigation level, which recorded a value of 12.61 kg ha^{-1} mm^{-1}. This indicates that at this level of irrigation, the plants were able to produce more grain relative to the amount of water consumed compared to other irrigation levels.

The findings also revealed that reducing irrigation from 100% ETc to 70% ETc did not significantly decrease yield; rather, it allowed for substantial water savings without compromising productivity. Therefore, it can be concluded that a 70% ETc irrigation level strikes an optimal balance between maintaining acceptable yields while conserving water resources.

Based on these findings, it is recommended that farmers within the Qadalle irrigation scheme adopt a 70% ETc irrigation strategy. This

approach not only enhances production but also promotes sustainable agricultural practices by conserving valuable water resources.

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6. Conflicts of interests

Authors declare that there is no conflict of interest exists.

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Cultural and morphological characterization of *Curvularia geniculata* causing leaf spot of Orchid in Indo-gangetic plain of West Bengal

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ABSTRACT

Orchids are one of the most diverse and extensively evolved groups of angiosperms with 25,000 to 35,000 species belonging to 600-800 genera, that is why Orchids occupy the largest family of flowering plants. In India, orchids comprise 158 genera and 1331 species. Asia now becoming the primary source of orchids for the entire world market. Orchids, like any other crop, are exposed to various biotic and abiotic stresses. Very recently more than 130 diseases are reported in orchids caused by fungi, bacteria, nematodes and viruses. In the Indo-gangetic plain of West Bengal, the orchid growers/farmers are facing problems mainly due to the leaf spots like diseases of orchids. Among which *Curvularia geniculata* produce necrotic leaf spot symptoms. But very less information is available in the literature on these new diseases of orchids and their favourable conditions as well as on management strategies. So this study is based on pathogen isolation, identification and morphological characterization of pathogen. Through morphological (*i.e.*, fruit body, mycelial properties, conidiogenous cell, conidial morphology) it was confirmed that the pathogens were similar to *Curvularia geniculata*. *Curvularia geniculata* produced maximum growth on Potato dextrose agar medium and Oat meal agar medium, whereas it least grew on Host extract agar medium. It produced black to brownish colour powdery texture colony. Zonation is present on the colony of Potato sucrose agar medium, Oat meal agar medium, whereas no zonation present on the colony of Potato dextrose agar medium. The growth rate of *Curvularia geniculata* showed that maximum 7 days after inoculation at Potato dextrose agar (26.33 mm/day) followed by Carrot agar media (21.67 mm/day) and Potato sucrose agar (19.67 mm/day).

KEY WORDS: *Orchid*; *West Bengal*; *Leaf spot*; *Curvularia geniculata*

1. Introduction

Orchids are one of the most diverse and extensively evolved groups of angiosperms. With 25,000 to 35,000 species belonging to 600-800 genera, orchids occupy the largest family of flowering plants, covering 6.8% of the total flowering plants in India (Yonzone and Kamran, 2008). They are treasured for their diverse sizes, shapes, forms and colours (Pant *et al.*, 2012). The majority of them are from Central and South America's tropical wet forests, as well as India, Sri Lanka, Burma, China, Thailand, Malaysia, the Philippines, New Guinea, and Australia (Karthigeyan *et al.*, 2014).

In India, Orchids comprise with 158 genera and 1331 species, which grow up to an elevation of

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5000 m. In North-Western India, orchids are commonly found under tree shades of humas, rich moist soil and Western-Ghats harbor the small flowered orchids (Hegde, 2014). In India, native genera such as *Cymbidium, Paphiopedilum, Vanda, Arachnis,* and *Dendrobium* are cultivated for cut flower production on a huge scale (Pant *et al.*, 2013).

Orchids, like any other crop, are impacted by a variety of biotic and abiotic variables. More than 130 plant diseases caused by fungi, bacteria, nematodes, and viruses have been identified in different genera of orchids (Pant *et al.*, 2012). Among fungal diseases, black rot (*Phytophthora palmivora, P. parasitica, Pythium ultimum* and *P. splendens*), anthracnose (*Colletotrichum gloeosporioides*), Orchid wilt (*Sclerotium rolfsii*), petal blight (*Botrytis cinerea*), rust (*Uredo* sp.), leaf blight (*Fusarium oxysporum*), *Sclerotinia* white rot (*Sclerotinia sclerotium*) and leaf spot (caused by species of *Fusarium, Cercospora, Alternaria, Pestalotia* and *Haplosporella*) are most common (Pant *et al.*, 2013).

Curvularia geniculata is the most important leaf spot-causing fungi. *Curvularia geniculata* (*Botryosphaeriaceae, Dothideomycetes*) is a fast-growing anamorphic fungus. *Curvularia* sp. is a soil-born, seed-borne as well as airborne fungus that's mostly found in tropical areas and has been reported in different hosts such as rice (Kusai *et al.*, 2016), maize (Manzar *et al.*, 2021), tomato (Rao *et. al.*, 2020), pearl millet (Khatal *et. al.*, 2019) etc. But in orchids pathogens belong to *Curvularia* sp. are either endophytes or saprophyte in nature. The presence of a necrotic lesion on the leaves is one of the most typical indications of leaf spot (Kusai *et al.*, 2016).

Based on the abovementioned information, it can be concluded that the orchid is one of the most important popular high-value flower crops contributing in different regions of India and exploring new areas for cultivation. But due to the climate changing scenario, the orchid growers are facing problems which may cause mild to severe losses if not reported at the right time. But very less information is available in the literatures on these diseases of orchid, keeping this research gap on mind, the present investigation was carried out with the objective of isolation, identification, morphological and cultural characterization of Orchid leaf spot disease causing pathogen *Curvularia geniculata.*

2. Material and Methods

The laboratory experiments were carried out in the "Survey Selection and Mass Production (SSMP) of Nodule Bacteria" laboratory at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal.

The orchid leaf showing typical leaf spot symptoms were first scrapped with sterilized teasing needle. The scrapped bits were placed on clean glass slide in a drop of Lactophenol, covered with cover slip and examined under microscope for the presence of mycelium/spores if any, for identification of pathogen associated with the disease symptoms.

Fungal mycelia and spores were observed under a Light microscope and photographed. Conidia were measured using a light microscope with a micrometer at 40X magnification. The isolates were identified initially by comparing morphological and cultural characteristics (*i.e.*, size of conidia, color, number of cells and number of apical appendages, formation of pycnidia etc.).

2.1 Culture media preparation

Several types of culture media were prepared and used for the isolation, maintenance, and experimental evaluation of fungal growth and antagonism studies.

Potato Dextrose Agar (PDA): was used as a general-purpose medium for growth studies, inhibition assays, and culture maintenance. To prepare PDA, 200 g of peeled potatoes were boiled in 500 ml of distilled water to obtain an extract. After filtration, 20 g of dextrose was added to the potato extract. Separately, 20 g of agar was dissolved in 500 ml of distilled water by heating. Both solutions were combined, and the final volume was adjusted to 1000 ml with distilled water. The medium was sterilized by autoclaving at 15 psi for 15–20 minutes.

Corn Meal Agar (CMA): was prepared by boiling 40 g of corn meal in 600 ml of distilled water. Separately, 20 g of agar was dissolved in 400 ml of distilled water. After complete dissolution, both mixtures were combined, and the final volume was adjusted to 1000 ml. The medium was autoclaved at 15 psi for 15 minutes to ensure sterility.

Oat Meal Agar (OMA): was prepared by suspending 60 g of oatmeal and 12 g of agar in distilled water, and the final volume was adjusted to 1000 ml. The mixture was heated until fully dissolved and then autoclaved under standard conditions at 15 psi for 15–20 minutes.

Potato Sucrose Agar (PSA): 200 g of potatoes were boiled in distilled water, and the extract was mixed with 20 g of sucrose and 20 g of agar. The final volume was brought up to 1000 ml using distilled water. This medium was also sterilized by autoclaving at 15 psi for 15–20 minutes.

Carrot Agar Medium: was formulated by extracting juice from 200 g of fresh carrots boiled in distilled water. After filtration, 10 g of agar was added, and the volume was made up to 1000 ml with distilled water. The medium was then autoclaved at 15 psi for 15 minutes.

Host Leaf Extract Agar: was prepared using 200 g of host leaves extracted in 500 ml of distilled water. To this extract, 20 g of dextrose was added, and the solution was gently heated to ensure dissolution. Separately, 20 g of agar was melted in 500 ml of distilled water and combined with the leaf extract. The final volume was adjusted to 1000 ml, and the medium was sterilized at 15 psi for 15–20 minutes.

2.2 Experimental design and statistical analysis

All laboratory experiments were conducted following a Completely Randomized Design (CRD). The data recorded from various experiments were subjected to statistical analysis using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). One-way Analysis of Variance (ANOVA) was employed to assess the significance of the treatment effects. Where significant differences were detected, means were separated using Tukey's Honest Significant Difference (HSD) test at a 5% level of significance ($P = 0.05$).

3. Results and Discussion

Curvularia geniculata is one of the most important leaf spot-causing pathogen in tropical region. In recent years these pathogen become an emerging threat in Indo-gangetic plain of West

Bengal. Initially, symptoms arise as light brown to yellow spots from the tip of leaves or terminal portion of leaf blades. Spots were surrounded by a light green chlorotic margin. With advances of disease dark brown necrotic spots developed in the infected region. At severe condition, entire lesions were turn into turn into necrotic, gradually dark brown with a yellow halo and leaves become fall off (Fig. 1).

Fig. 1: Typical *Curvularia geniculata* symptom produce in leaf

3.1 Isolation of pathogens

To isolate present microorganisms from the collected samples, different parts of the infected plant leaves were used. Diseased samples were cut into small specimens, washed of impurities dirt under running water before disinfecting with a solution of with 0.1% $HgCl_2$ solution for 20 seconds and then rinsed 5 times with sterile distilled water. The sterilized specimens were cut into small pieces of 0.5 × 0.5 cm (for the leaves). Diseased portion contain with a small healthy portion of initial nutrient source. These pieces were cultured on the plates of PDA and incubated at 27°. When mycelial growth and spores were observed, further isolation was carried out into PDA plate for preparing pure culture. The microbial isolates were identified by morphological structures observed under a microscope with a magnification of x10 and x40.

3.2 Pathogenicity test

In case of *Curvularia geniculata* symptom developed five days after inoculation. After symptom expression, isolation was carried out to confirm the genus identification with subsequent pathogen re-isolation in PDA medium to fulfil Koch's postulates and to identify the species morphologically.

To comply with Koch's postulates, lesions were resembling with the initial symptoms observed on leaf five days after inoculation of *Curvularia geniculata* in all the orchid leaves. No symptoms were observed in control leaves. *Curvularia geniculata* first isolated from field after its natural occurrence and re isolated from artificially inoculated leaves were identical (Fig. 2).

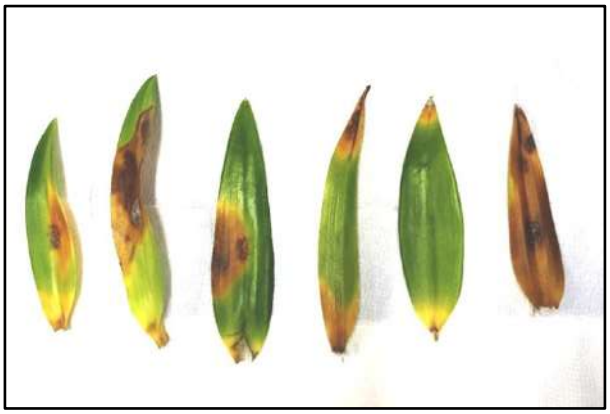

Fig. 2: Pathogenicity text of *Curvularia geniculata* on orchid leaves

3.3 Morphological identification of pathogen

In *Curvularia geniculata*, fungal colonies are dark brown colour, zonation present (Fig. 3). Hyphae also brown to black colour, brown conidiophore. The conidia are geniculata to almost straight and boat shaped with disproportional enlargement at the third cell from the base. They are straw coloured to dark brown and sometimes paler coloured at both ends. Conidia contain with 4 distosepta with geniculated or inflated at the middle part. Length of conidia varies from 9.56 to 11.79 µm (x = 10.84 µm; S.D.= 1.01), Width of conidia varies from 4.32 to 5.38 µm (x = 4.76 µm; S.D.=0.40), Area ranges from 41.29 to 58.19 µm^2 (x= 51.67 µm^2; S.D.= 6.56) (Fig. 4).

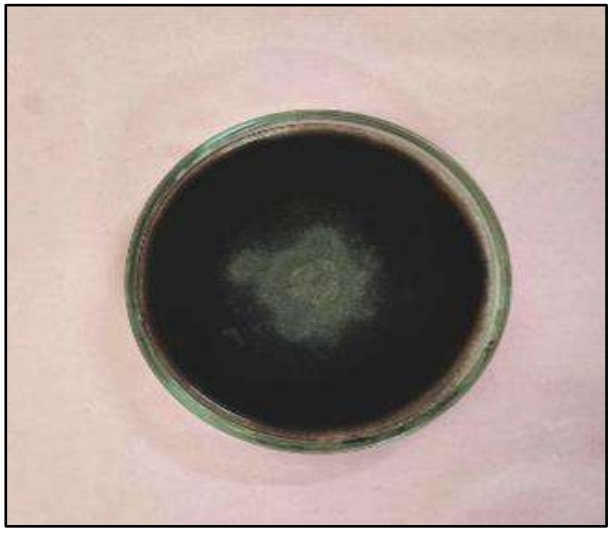

Fig. 3: Cultural character of *Curvularia geniculate* on petri plate

Qi *et al.*, (2022) observed fluffy greyish green fungal colonies with white aerial mycelium. Manzar *et al.*, (2021) observed similar dark greyish to black fungal colonies. Spindle to elliptical in shape and light brown conidia, with 3 to 4 septa with an enlarged central cell. Conidial size ranged from 10.0 to 14.1 µm wide and 19.3 to 26.2 µm long. Sumangala *et al.*, (2010) observed large colonies that had a very dark, blue–black appearance on the reverse and produced brown, floccose aerial mycelium on the obverse. Hyphae brown to black with curved shape, smooth wall, brown colour conidia.

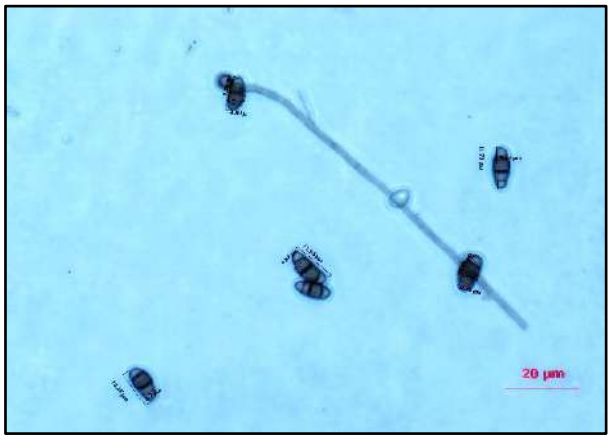

Fig. 4: Conidia of *Curvularia geniculata*

3.4 Cultural characteristic of pathogen

Curvularia geniculata when cultured on six different media it showed a variation in their colony characteristic (Table 1; Fig. 5). The colony shape was varied round in Potato dextrose agar media, filamentous in Potato sucrose agar media and Carrot extract agar media, circular in Host extract agar media, Irregular in Oat meal agar media and Corn meal agar media. The surface colour of the colony was mostly black in Potato dextrose agar media, Potato sucrose agar media, Oat meal agar media, Carrot extract agar media whereas light black in Host extract agar media and Corn meal agar media. The elevation showed umbonate for Potato sucrose agar media, Potato dextrose agar media and Carrot extract agar media whereas flat on Host extract agar media, Oat meal agar media and Corn meal agar media. The margin of culture plate was entire on Potato

Table 1: Colony morphology of *Curvularia geniculata* on different medium

Media	Colony Shape/Form	Surface Colour	Elevation	Margin	Reverse Plate Colour	Growth	Texture	Special Character	Time Taken for Full Plate
PDA	Round	Black	Umbonate	Entire	Black	Rapid	Powdery	No	7 days
PSA	Filamentous	Black	Umbonate	Undulate	Black	Rapid	Powdery	Zonation Present	8 days
HEA	Circular	Light Black	Flat	Filiform	Brown	Very Slow	Powdery	No	12 days
OMA	Irregular	Black	Flat	Filamentous	Black	Moderate	Powdery	Zonation Present	9 days
CMA	Irregular	Light Black	Flat	Undulate	White	Slow	Powdery	Zonation Present	10 days
CEA	Filamentous	Black	Umbonate	Filiform	Black	Slow	Powdery	Zonation Present	10 days

Note: PDA – Potato Dextrose Agar, PSA – Potato Sucrose Agar, HEA – Host Extract Agar, OMA – Oat Meal Agar, CMA – Corn Meal Agar, CEA – Carrot Extract Agar, Umbonate - Elevated at the center flatted at the margin

dextrose agar media; filiform on Host extract agar media and Carrot extract agar media; undulate on Potato sucrose agar media and Corn meal agar media whereas filamentous on Oat meal agar media. Reverse plate colour showed black on Potato dextrose agar media, Potato sucrose agar media, Oat meal agar media and Carrot extract agar media; brown on Host extract agar media and white on Corn meal agar media. Rapid growth rate was observed on Potato dextrose agar media and Potato sucrose agar media; moderate growth rate was observed on Oat meal agar media; slow growth rate was observed on Corn meal agar media and Carrot extract agar media; whereas very slow growth rate was observed on Host extract agar media. The textures of all the culture media were powdery. Zonation as a special characteristic was present in Potato sucrose agar media, Oat meal agar media, Carrot extract agar media, Corn meal agar media whereas zonation absent in Potato dextrose agar media and Host extract agar media.

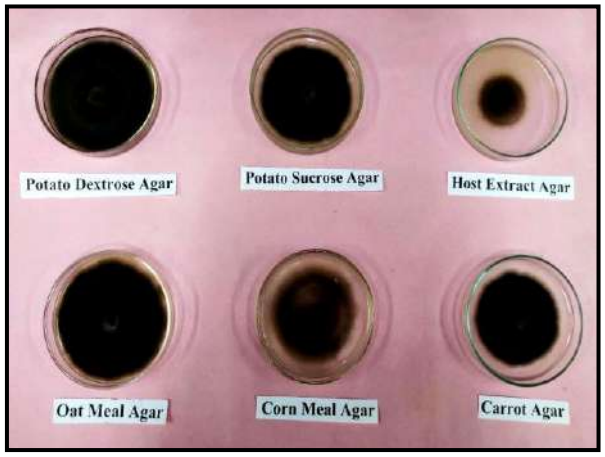

Fig. 5: Growth of *Curvularia geniculata* on different growth medium

Curvularia geniculata growth on 6 different media *i.e.* Potato dextrose agar (PDA), Potato sucrose agar (PSA), Host extract agar (HEA), Oat meal agar (OMA), Corn meal agar, Carrot extract agar media showed differential result on different days after inoculation from 2 days after inoculation to 9 days after inoculation and differences were statistically significant (Table 2; Fig. 6). The data indicated that all the media supported the growth of *Curvularia geniculata*

with statistically significant variation in radial growth. At 2 days after inoculation, it was resulted that maximum growth was obtained in Potato dextrose agar (1.90 cm) followed by Potato sucrose agar (1.67 cm) statistically at par with Corn meal agar (1.60 cm), Oat meal agar (1.56 cm) and Carrot extract agar media (1.56 cm). At 4 days after inoculation the growth was increased and maximum was noticed in Potato dextrose agar (3.00 cm) statistically at par with Potato sucrose

Table 2: Radial growth (cm) of *Curvularia geniculata* on different medium

Different Medium	Radial growth (cm)					
	2 DAI	4 DAI	6 DAI	7 DAI	8 DAI	9 DAI
Potato Dextrose Agar	1.90^{a}	3.00^{a}	4.06^{b}	6.70^{a}	8.10^{a}	9.00^{a}
Potato Sucrose Agar	1.67^{b}	2.90^{a}	4.10^{b}	6.06^{b}	7.70^{a}	8.60^{a}
Host Extract Agar	1.10^{e}	2.13^{e}	3.03^{e}	4.10^{e}	4.70^{e}	5.26^{e}
Corn Meal Agar	1.60^{b}	2.60^{b}	3.67^{c}	5.40^{c}	6.23^{c}	7.00^{c}
Oat Meal Agar	1.56^{b}	2.93^{a}	4.67^{a}	6.06^{b}	7.56^{a}	9.00^{a}
Carrot Extract Agar	1.56^{b}	2.67^{b}	4.20^{b}	6.36^{a}	7.06^{b}	7.53^{b}
S.E.(m)±	0.06	0.08	0.12	0.21	0.28	0.32
C.D. at 5%	0.18	0.21	0.35	0.58	0.79	0.91

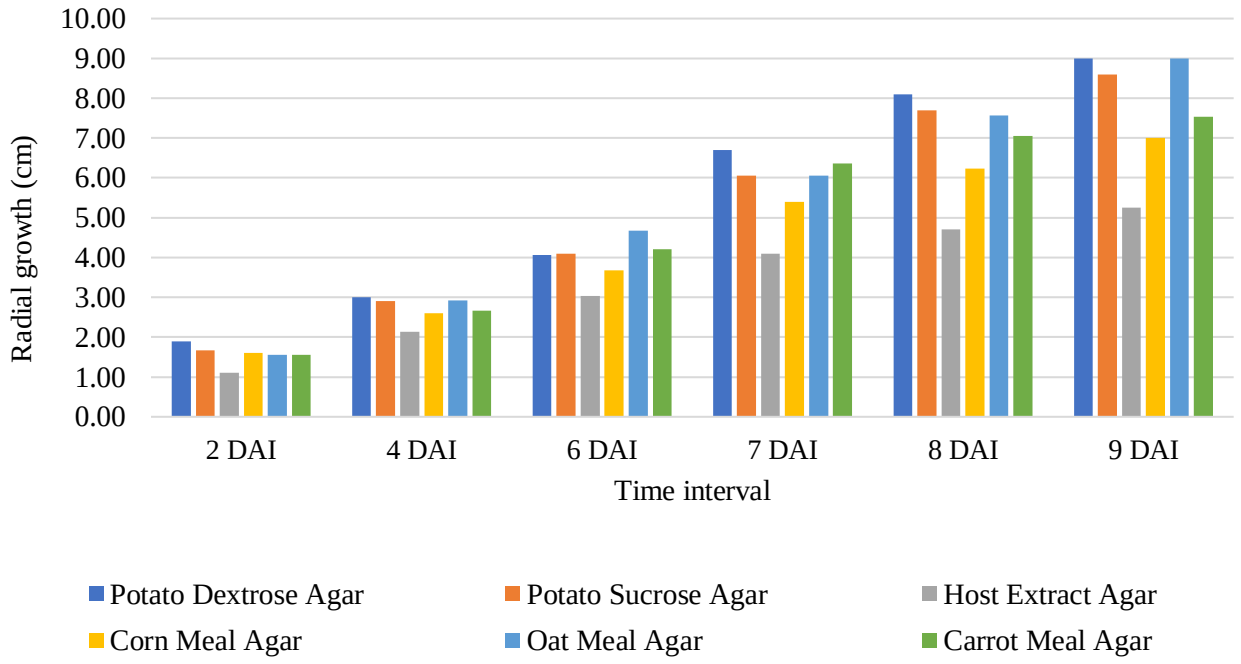

Fig. 6: Graphical representation of Radial growth (cm) of *Curvularia geniculata* at different medium

agar (2.90 cm) and Oat meal agar (2.93 cm). Whereas minimum was observed in Host extract agar (2.13 cm) followed by Corn meal agar (2.60 cm) and their difference were not statistically at par. Similarly at 6 days after inoculation maximum growth was notice at Oat meal agar (4.67 cm) followed by Potato sucrose agar (4.10 cm) and Potato dextrose agar (4.06 cm) and minimum in Host extract agar (3.03 cm) followed by Corn meal agar (3.67 cm) and their difference was not statistically at par. At 7 days after inoculation similar type of observation was noticed and maximum was observed on Potato dextrose agar (6.70 cm) statistically at par with

Carrot extract agar media (6.36 cm) and minimum in Host extract agar (4.10 cm) followed by Corn meal agar (5.40 cm) and their differences were statistically at par. At 8 days after inoculation maximum radial growth was noticed in Potato dextrose agar (8.10 cm) statistically at par with Potato sucrose agar (7.70 cm) and Oat meal agar (7.56 cm), whereas lowest in Host extract agar (4.10 cm) followed by Corn meal agar (6.23 cm) and their differences were not statistically at par. At 9 days after inoculation *i.e.* at final day maximum growth was noted at Potato dextrose agar (9.00 cm) stastically at par with Oat meal agar (9.00 cm) and Potato sucrose agar (8.60 cm) whereas minimum in Host extract agar (5.26 cm) followed by Corn meal agar (7.00 cm) and their differences were not statistically at par.

This result therefore indicated that Potato dextrose agar media has maximum induce the growth of *Curvularia geniculata* followed by Oat meal agar and Potato sucrose agar. Although they were statistically at par with each other. Similar type of observation was also noticed by Sumangala and Patil (2010). They reported that *Curvularia geniculata* produce maximum growth and sporulation on Potato dextrose agar and followed by Oat meal agar media. Majumdar and Mondal (2019) also reported that *Curvularia geniculata* produce highest growth on Potato dextrose agar media after 7 days of inoculation. Bhatt and Kumar (2018) reported that *Curvularia geniculata* produce maximum radial growth on Potato dextrose agar media followed by Oat meal agar and Czapeck's dox agar and minimum on Corn meal agar media at 30 °C temperature.

The growth rate of *Curvularia geniculata* showed that maximum 7 days after inoculation at Potato dextrose agar (26.33 mm/day) followed by Carrot agar media (21.67 mm/day) and Potato sucrose agar (19.67 mm/day) though Carrot extract agar media and Potato sucrose agar media were

Table 3: Growth rate (mm/day) of *Curvularia geniculata* on different media

Different Medium	Growth rate (mm/day)					
	2 DAI	4 DAI	6 DAI	7 DAI	8 DAI	9 DAI
Potato Dextrose Agar	9.50^{a}	5.50^{c}	5.33^{d}	26.33^{a}	14.00^{b}	9.00^{b}
Potato Sucrose Agar	8.33^{b}	6.67^{a}	5.50^{d}	19.67^{b}	16.33^{a}	9.00^{b}
Host Extract Agar	5.50^{e}	5.17^{c}	4.50^{e}	10.67^{e}	6.00^{d}	5.67^{d}
Corn Meal Agar	8.00^{b}	5.00^{d}	5.33^{d}	17.33^{c}	8.33^{c}	7.67^{c}
Oat Meal Agar	7.80^{b}	6.83^{a}	8.67^{a}	14.00^{d}	15.00^{a}	14.33^{a}
Carrot Extract Agar	7.83^{b}	6.00^{b}	7.17^{b}	21.67^{b}	7.00^{d}	4.67^{e}
S.E.(m)±	0.31	0.21	0.362	1.267	1.025	0.796
C.D. at 5%	0.88	0.59	1.02	3.58	2.90	2.25

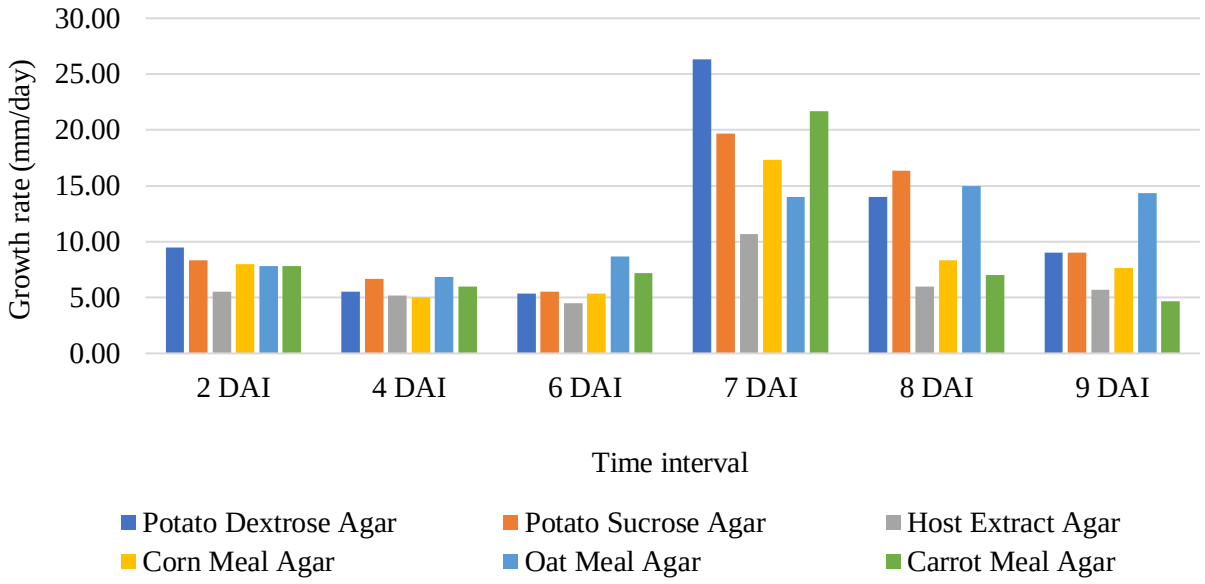

Fig. 7: Graphical representation of Growth rate (mm/day) of *Curvularia geniculata* at different media

statistically at par with each other with regards to growth rate of *Curvularia geniculata.* Whereas minimum growth rate was noticed 9 days after inoculation in every growth media and here also maximum was noticed in Oat meal agar media (14.33 mm/day) and minimum in Carrot agar media (4.67 mm/day). It was noticed that 4 days after inoculation and 6 days after inoculation the growth rate was reduced in comparison to 2 days after inoculation and it was observed in all growth media (Table-3; Fig. 7**).**

Whereas this result contradict with the result of Majumdar and Mondal (2019), There *Curvularia lunata* shows maximum growth rate on 6 days after inoculation. This result may be due to induction of enzyme for utilization of substrate. Zhao and Shamoun (2006) reported that the type of culture media and their chemical composition significantly affect the mycelial growth, growth rate and conidial production of other pathogen like *Phoma exigua*.

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Assessment of herbicide-induced changes in beneficial microflora of Bajra (*Pennisetum glaucum* L.) rhizosphere

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ABSTRACT

The present study investigates the impact of various herbicides on beneficial microbial populations in the rhizosphere of *Pennisetum glaucum* (Bajra), focusing on *Azotobacter*, *Azospirillum*, and phosphate-solubilizing bacteria (PSB). Herbicidal treatments significantly influenced microbial populations at different crop growth stages, with a peak observed at 60 days after sowing (DAS). Among pre-emergence herbicides, Pendimethalin consistently supported the highest populations of all three microbial groups, while Oxyfluorfen exhibited suppressive effects. Phenaxoprop-ethyl emerged as the most favorable post-emergence herbicide, enhancing microbial proliferation, whereas Imazethapyr consistently reduced microbial abundance. Sequential application of Pendimethalin + Propaquizafop-ethyl maintained the highest microbial populations, while the combination with Imazethapyr was the least favorable. Microbial populations declined slightly by harvest, though treatment trends remained consistent. The findings highlight Pendimethalin and Phenaxoprop-ethyl as microbiologically compatible options, while Imazethapyr poses potential risks to rhizospheric health. These results emphasize the importance of selecting herbicides not only for weed management efficacy but also for their compatibility with beneficial soil microflora crucial for nutrient cycling and plant productivity. The study underscores the ecological benefits of integrating herbicide strategies with microbial sustainability, recommending Pendimethalin + Propaquizafop-ethyl for promoting rhizospheric microbial health in sustainable bajra cultivation.

KEY WORDS: *Herbicides*; *Rhizosphere microflora*; *Bajra*; *Pennisetum glaucum*

1. Introduction

Bajra (*Pennisetum glaucum* L.), commonly known as Pearl Millet, is an essential cereal crop grown primarily in the arid and semi-arid regions of Africa and Asia. This resilient crop is known for its ability to thrive in harsh environmental conditions such as low rainfall, high temperatures, and poor soils, making it a vital food source for millions of people in these areas. Bajra is highly nutritious, containing carbohydrates (67%), protein (11.6%), and minerals (2.7%), which make it an important dietary staple for both humans and livestock (Tejagouda, 2012). It is particularly significant in regions with limited water resources, where its drought-tolerant nature ensures food security and sustenance. However, despite its hardiness, Bajra productivity is often constrained by factors such as weed competition, which can substantially reduce crop yield.

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Weed control in Bajra cultivation is commonly achieved through the use of herbicides. Herbicides offer a convenient and effective means of managing weed populations, which are known to reduce yields by up to 50% in some regions (Dobrovoljskiy and Grishina, 1985). However, the increased reliance on herbicides in modern agriculture raises concerns regarding their potential impact on soil health and microbial communities. Herbicides are designed to target and control weeds, but their application may also affect non-target organisms, particularly the beneficial microorganisms present in the soil. Soil microorganisms, including nitrogen-fixing bacteria, mycorrhizal fungi, and other beneficial microflora, play a crucial role in nutrient cycling, organic matter decomposition, and overall plant health. These microorganisms contribute to plant growth by enhancing nutrient uptake, suppressing soilborne pathogens, and improving soil structure. Any disturbance in their populations due to herbicide application can adversely affect soil fertility, plant growth, and crop yield (Ayansina *et al*., 2003).

The residual effects of herbicides on soil microflora, particularly beneficial microorganisms, remain an area of concern in agricultural research. Some herbicides, especially those with low biodegradability, can persist in the soil for extended periods, leading to long-term alterations in microbial diversity and activity. These herbicide residues may interfere with the natural soil processes, resulting in reduced microbial populations or shifts in microbial community composition. Such changes can negatively influence soil health, nutrient cycling, and the availability of essential nutrients for plants (Ashim *et al*., 2008). Furthermore, herbicides may disrupt the balance between beneficial and harmful microorganisms, potentially leading to the dominance of pathogenic microbes that can further hinder plant growth (Amakiri, 1982).

The present study focuses on the impact of herbicides on beneficial soil microflora in the rhizosphere of Bajra. Specifically, it aims to evaluate how residual herbicide toxicity affects the populations of beneficial microorganisms that are critical for soil fertility and plant health. By investigating the effects of herbicides on the microbial diversity and activity in the Bajra rhizosphere, the study seeks to contribute to a better understanding of the long-term consequences of herbicide use on soil microbial communities. The findings will provide valuable insights into how herbicide-induced changes in soil microflora can affect Bajra growth, nutrient availability, and crop productivity. Ultimately, this research aims to support the development of more sustainable herbicide management practices that minimize harm to beneficial microorganisms and enhance overall soil health, ensuring the continued productivity of Bajra in arid and semi-arid regions (Hutsch, 2001).

2. Material and Methods

2.1 Study site and experimental design

A field experiment was conducted to investigate the residual effects of herbicides on soil microbial communities, including beneficial microflora, and their subsequent influence on the growth and yield of *Pennisetum glaucum* L. (Bajra) under rainfed conditions during the rainy season. The experimental site was part of a long-term cropping sequence, wherein various herbicidal treatments were applied to a preceding chickpea (*Cicer arietinum* L.) crop. The objective was to assess the residual impact of these herbicides on the

rhizospheric soil microbiota and subsequent crop performance.

The study employed a randomized complete block design (RCBD) comprising 18 treatments and three replications. Treatments included both pre-emergence and post-emergence herbicides applied to the chickpea crop. Pre-emergence herbicides were administered on the day of sowing, whereas post-emergence herbicides were applied 20 days after sowing (DAS).

Each plot measured 3 m × 4 m, with row spacing maintained at 45 cm and intra-row spacing at 15 cm. The hybrid variety ICTP-8230 of Bajra was sown in all experimental plots following standard agronomic practices. The treatment details consisting of mode of application and dosage are provided in Table 1.

2.2 Herbicide Treatments

Herbicidal treatments included both individual and combined formulations to simulate practical field conditions. Table 1 summarizes the herbicides employed, their application stages (pre- or post-emergence), and dosages. These treatments were selected to reflect real-world usage patterns and to assess the persistence and subsequent effects of herbicidal residues in soil.

Table 1: Treatments imposed on previous chickpea crop using different herbicides

Treatments	Herbicides	Mode / method of application	Dosage
1	Pendimethalin	Pre emergence	3.3 ml/l
2	Pendimethalin (xtra formulation)	Pre emergence	2.3 ml/l
3	Chlormuron Ethyl	Pre emergence	7.5 g/l
4	Oxyfluorfen	Pre emergence	2.0 ml/l
5	Quizalofop Ethyl	Post emergent	1.5 ml/1
6	Phenaxoprop Ethyl	Post emergent	0.1 ml/l
7	Propaquizafop Ethyl	Post emergent	1 ml/l
8	Oxyfluorfen	Post emergent	1 ml/l
9	Imazethapyr	Post emergent	1 ml/l
10	Pendimethalin+Quizalofop Ethyl	$T_1 + T_6$	3.3 ml/l + 1.5 ml/1
11	Pendimethalin + Phenaxoprop Ethyl	$T_1 + T_7$	3.3 ml/l + 0.1 ml/l
12	Pendimethalin + Propaquizafop	$T_1 + T_8$	3.3 ml/l + 1 ml/l
13	Pendimethalin + Oxyflorofen	$T_1 + T_9$	3.3 ml/l + 1 ml/l
14	Pendimethalin + Imazethapyr	$T_1 + T_{10}$	3.3 ml/l + 1ml/l
15	Weedy check	Weedy check	Control
16	Weed free (Hand weeding)	Herbicides were not imposed	Hand weeding
17	Pendimethalin + Intercultivation	Pre emergence + Intercultivation	3.3 ml/l + intra cultivation
18	Valore -32	Pre emergence	Pendimethalin 3.3 ml + Imazethapyr 2.0 ml was added to l liter of water and the volume was made to 10 liters

Source: Raghavendra, K. S. (2013)

2.3 Soil sampling and physicochemical analysis

Soil samples were collected at four time points: before sowing of Bajra, and at 20, 45, and 60 DAS, as well as at crop harvest. Samples were obtained from the rhizosphere zone at a depth of 0–15 cm using a soil auger, composited, and processed for analysis.

Soil pH was determined in a 1:2.5 soil-to-water suspension using a glass electrode pH meter. Electrical conductivity (EC) was measured using a conductivity bridge, following the protocol outlined by Jackson (1967).

2.4 Enumeration of soil microbial populations

Total soil microbial populations, including key beneficial microorganisms such as *Azotobacter*, *Azospirillum*, and phosphate solubilizing microorganisms (PSM), were assessed using the standard serial dilution and plating technique on selective culture media. General bacterial populations were enumerated on nutrient agar, while specific microbial groups were isolated and quantified using their respective selective media. *Azotobacter* was cultured on Waksman No. 77 medium (Allen, 1953), *Azospirillum* was grown on nitrogen-free malate medium, and PSM were isolated using Pikovskaya's agar medium (Bunt Rovira, 1955). After inoculation, plates were incubated under optimal laboratory conditions, and microbial colonies were counted and expressed as colony-forming units (CFU) per gram of dry soil.

2.5 Statistical analysis

All collected data, including microbial counts and soil chemical parameters, were subjected to analysis of variance (ANOVA) using the statistical procedure described by Gomez and Gomez (1984). The F-test was applied to determine the significance of treatment effects at a probability level of $P \leq 0.05$. Wherever applicable, treatment means were compared using the least significant difference (LSD) test to determine statistically significant differences among treatments.

3. Results and Discussion

3.1 Effect of herbicides on *Azotobacter* population in Bajra rhizosphere

The application of herbicides significantly affected *Azotobacter* populations in the rhizospheric soil of *Pennisetum glaucum* across various growth stages (Table 2). Across all treatments, the population of *Azotobacter* consistently peaked at 60 days after sowing (DAS), suggesting enhanced microbial activity during this phase.

Among pre-emergence herbicides, Pendimethalin consistently supported the highest *Azotobacter* populations across all stages, with a maximum of 4.40×10^4 cfu g^{-1} soil at 60 DAS, whereas Oxyfluorfen showed the most suppressive effects (2.84×10^4 cfu g^{-1}). Post-emergence treatments followed a similar trend: Phenaxoprop-ethyl favored microbial proliferation (4.50×10^4 cfu g^{-1}), while Imazethapyr exhibited inhibitory effects (2.84×10^4 cfu g^{-1}). Sequential applications of Pendimethalin + Propaquizafop-ethyl consistently maintained higher microbial counts, peaking at 4.48×10^4 cfu g^{-1} at 60 DAS. Conversely, Pendimethalin + Imazethapyr reduced the population to 2.79×10^4 cfu g^{-1}.

Table 2: Residual effect of herbicides on *Azotobacter* population at different growth stages of Bajra

Treatments	× 10^4 cfu g^{-1} of soil				
	Before sowing	20 DAS	45 DAS	60 DAS	At harvest
T_1 - Pendimethalin (PRE)	2.02	2.68	2.94	4.40	3.42
T_2 - Pendimethalin (Xtra formulation) (PRE)	1.81	2.57	2.73	4.26	3.30
T_3 - Chlormuron Ethyl (PRE)	1.88	2.56	2.82	4.01	3.11
T_4 -Oxyfluorfen (PRE)	1.49	2.15	2.31	2.84	2.43
T_5 - Quizalofop Ethyl (POE)	1.91	2.60	2.79	4.27	3.35
T_6 - Phenaxoprop Ethyl (POE)	2.06	2.70	2.91	4.50	3.39
T_7 - Propaquizafop Ethyl (POE)	1.81	2.55	2.75	4.37	3.48
T_8 -Oxyfluorfen (POE)	1.53	2.20	2.36	2.90	2.50
T_9 - Imazethapyr (POE)	1.49	2.10	2.26	2.84	2.43
T_{10} - Pendimethalin + Quizalofop Ethyl	1.96	2.56	2.85	4.35	3.27
T_{11} - Pendimethalin + Phenaxoprop Ethyl	1.79	2.51	2.78	4.30	3.32
T_{12} - Pendimethalin + Propaquizafop Ethyl	2.11	2.66	2.97	4.48	3.45
T_{13} - Pendimethalin + Oxyfluorfen	1.60	2.57	2.39	2.95	2.55
T_{14} - Pendimethalin + Imazethapyr	1.45	2.16	2.22	2.79	2.38
T_{15} - Weedy check (WC)	1.88	2.47	2.61	4.22	3.24
T_{16} - Weed free check (WF)	2.26	2.80	3.10	4.65	3.57
T_{17} - Pendimethalin + Intercultivation (IC)	1.85	2.21	2.63	4.21	3.32
T_{18} - Valore-32	1.85	2.39	2.61	4.11	3.23
S.Em±	0.05	0.03	0.04	0.04	0.03
C.D at 5%	0.14	0.08	0.11	0.12	0.10

Note: DAS = Days after sowing, NS = Non significant, PRE = Pre-emergence herbicide, POE = Post-emergence herbicide

Following 60 DAS, a decline in population was observed at harvest. However, the relative trends persisted, with Pendimethalin and Phenaxoprop-ethyl continuing to sustain the highest *Azotobacter* counts and Imazethapyr remaining the most inhibitory.

3.2 Effect of herbicides on *Azospirillum* population in Bajra rhizosphere

Azospirillum populations also demonstrated significant sensitivity to herbicidal influence (Table 3). Population densities peaked at 60 DAS across all treatments, indicating this stage as optimal for *Azospirillum* proliferation, likely due to heightened root exudation and favorable soil conditions.

Pendimethalin once again emerged as the most supportive pre-emergence herbicide, with the *Azospirillum* population reaching 4.38 × 10^6 cfu g^{-1} soil at 60 DAS. Oxyfluorfen, in contrast, suppressed microbial abundance (2.86 × 10^6 cfu g^{-1}). Among post-emergence herbicides, Phenaxoprop-ethyl resulted in the highest count (4.36 × 10^6 cfu g^{-1}), while Imazethapyr was consistently detrimental (2.82 × 10^6 cfu g^{-1}).

Table 3: Residual effect of herbicides on *Azospirillum* population at different growth stages of Bajra

Treatments	× 10^6 cfu g^{-1} of soil				
	Before sowing	20 DAS	45 DAS	60 DAS	At harvest
T_1 - Pendimethalin (PRE)	2.03	2.30	3.00	4.38	3.94
T_2 - Pendimethalin (Xtra formulation) (PRE)	1.86	2.19	2.83	4.28	3.82
T_3 - Chlormuron Ethyl (PRE)	1.85	2.18	2.70	4.11	3.65
T_4 - Oxyfluorfen (PRE)	0.96	1.45	2.04	2.86	2.48
T_5 - Quizalofop Ethyl (POE)	1.80	2.25	2.90	4.26	3.84
T_6 - Phenaxoprop Ethyl (POE)	2.05	2.36	3.14	4.36	3.96
T_7 - Propaquizafop Ethyl (POE)	1.88	2.26	2.95	4.24	3.84
T_8 -Oxyfluorfen (POE)	1.02	1.49	2.18	2.90	2.50
T_9 - Imazethapyr (POE)	0.98	1.16	2.13	2.82	2.36
T_{10} - Pendimethalin + Quizalofop Ethyl	1.80	2.21	2.94	4.22	3.86
T_{11} - Pendimethalin + Phenaxoprop Ethyl	1.68	2.22	2.91	4.24	3.82
T_{12} - Pendimethalin + Propaquizafop Ethyl	1.96	2.31	3.20	4.34	4.00
T_{13} - Pendimethalin + Oxyfluorfen	1.12	1.50	2.15	2.95	2.64
T_{14} - Pendimethalin + Imazethapyr	1.08	1.18	2.07	2.79	2.43
T_{15} - Weedy check (WC)	1.76	2.18	2.90	4.12	3.75
T_{16} - Weed free check (WF)	2.22	2.47	3.35	4.48	4.15
T_{17} - Pendimethalin + Intercultivation (IC)	1.88	2.14	2.80	4.18	3.70
T_{18} - Valore-32	1.94	2.16	2.88	4.20	3.69
S.Em±	0.05	0.03	0.05	0.03	0.04
C.D at 5%	0.16	0.09	0.14	0.09	0.11

Note: DAS = Days after sowing, NS = Non significant, PRE = Pre-emergence herbicide, POE = Post-emergence herbicide

Sequential application of Pendimethalin + Propaquizafop-ethyl supported the highest population (4.34 × 10^6 cfu g^{-1}), while Pendimethalin + Imazethapyr was the least favorable (2.79 × 10^6 cfu g^{-1}).

At harvest, populations declined slightly, in line with senescing roots and declining nutrient levels. However, relative treatment efficacy remained constant, reaffirming the safety of Pendimethalin and Phenaxoprop-ethyl.

3.4 Effect of herbicides on phosphate solubilizing bacteria (PSB) in Bajra rhizosphere

Phosphate solubilizing bacteria (PSB) populations followed a similar trend (Table 4), with maximum proliferation at 60 DAS. Among pre-emergence herbicides, Pendimethalin again yielded the highest population (4.01 × 10^3 cfu g^{-1}), while Oxyfluorfen was least favorable (2.77 × 10^3 cfu g^{-1}). Phenaxoprop-ethyl facilitated the highest PSB counts among post-emergence herbicides (4.20 × 10^3 cfu g^{-1}), whereas Imazethapyr markedly inhibited PSB (2.65 × 10^3 cfu g^{-1}).

Sequentially, Pendimethalin + Propaquizafop-ethyl promoted the highest PSB count (4.11×10^3 cfu g^{-1}), whereas Pendimethalin + Imazethapyr had a suppressive effect (2.64×10^3 cfu g^{-1}).

At harvest, a reduction in microbial activity was evident, yet the relative performance of the treatments remained consistent, emphasizing the persistent effects of herbicide combinations on microbial ecology.

The results clearly indicate that herbicide applications substantially influence beneficial microflora in the rhizosphere of *Pennisetum glaucum*, with Pendimethalin and Phenaxoprop-ethyl showing minimal to no adverse effects on *Azotobacter*, *Azospirillum*, and PSB populations. In contrast, Oxyfluorfen and particularly Imazethapyr consistently demonstrated microbial suppressiveness, regardless of timing or combination. This variation in microbial response aligns with previous reports that certain herbicides, depending on their chemical nature, persistence, and mode of action, can selectively inhibit or promote microbial populations (Cycoń

Table 4: Residual effect of herbicides on phosphate solubilizing bacteria (PSB) population at different growth stages of Bajra

Treatments	× 10^3 cfu g^{-1} of soil				
	Before sowing	20 DAS	45 DAS	60 DAS	At harvest
T_1 - Pendimethalin (PRE)	1.48	2.18	2.66	4.01	3.30
T_2 - Pendimethalin (Xtra formulation) (PRE)	1.25	2.08	2.48	3.77	3.14
T_3 - Chlormuron Ethyl (PRE)	1.25	1.86	2.54	3.86	2.96
T_4 - Oxyfluorfen (PRE)	1.07	1.15	2.07	2.77	2.48
T_5 - Quizalofop Ethyl (POE)	1.29	2.14	2.54	4.08	3.28
T_6 - Phenaxoprop Ethyl (POE)	1.60	2.25	2.65	4.20	3.33
T_7 - Propaquizafop Ethyl (POE)	1.36	2.15	2.57	4.02	3.16
T_8 -Oxyfluorfen (POE)	1.11	1.27	2.12	2.80	2.53
T_9 - Imazethapyr (POE)	1.04	1.25	2.10	2.65	2.47
T_{10} - Pendimethalin + Quizalofop Ethyl	1.30	2.23	2.48	3.99	3.30
T_{11} - Pendimethalin + Phenaxoprop Ethyl	1.18	2.19	2.64	3.86	3.27
T_{12} - Pendimethalin + Propaquizafop Ethyl	1.41	2.35	2.75	4.11	3.40
T_{13} - Pendimethalin + Oxyfluorfen	1.18	1.30	2.26	2.72	2.54
T_{14} - Pendimethalin + Imazethapyr	1.04	1.27	2.11	2.64	2.47
T_{15} - Weedy check (WC)	1.18	2.15	2.51	3.89	3.25
T_{16} - Weed free check (WF)	1.85	2.46	2.85	4.35	3.51
T_{17} - Pendimethalin + Intercultivation (IC)	1.23	2.14	2.43	4.06	3.18
T_{18} - Valore-32	1.22	2.15	2.47	4.04	3.19
S.Em±	0.08	0.03	0.03	0.04	0.03
C.D at 5%	0.23	0.09	0.08	0.11	0.09

Note: DAS = Days after sowing, NS = Non significant, PRE = Pre-emergence herbicide, POE = Post-emergence herbicide

and Piotrowska-Seget, 2015; Walia *et al*., 2017). The superior microbial performance at 60 DAS across all treatments is likely attributed to increased root biomass, exudation, and active rhizospheric interactions (Mishra *et al*., 2013).

Importantly, the sequential combination of Pendimethalin + Propaquizafop-ethyl emerged as the most microbiologically favorable treatment, supporting high populations across all three microbial groups, thus suggesting its compatibility with sustainable crop management strategies. In contrast, the Pendimethalin + Imazethapyr combination consistently recorded the lowest microbial counts, raising concerns about its long-term impact on soil health. These findings underscore the necessity of selecting herbicides not solely based on weed control efficacy, but also on their ecological compatibility with soil microbial communities vital to nutrient cycling and plant growth promotion.

4. Conflicts of interests

Authors declare that there is no conflict of interest exists.

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Ethnobotanical investigation of plants used in the treatment of Prostatitis in the prefecture of Boke

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ABSTRACT

Within the framework of the valorization of medicinal plants traditionally used in the treatment of prostatitis in the prefecture of Boké, we carried out an ethnobotanical survey from April 06 to July 02, 2018. During this survey, we met 35 healers, mostly herbalists and marabouts, including 23 men and 12 women. These tradi therapeutes practiced a variety of activities, and for the most part had acquired their knowledge through family ties. The majority of these healers were elderly, aged between 55 and 70. The most frequently cited plants were *Phyllanthus muellerianus* (10 times), *Smilax anceps* (8 times) and *Carica papaya* (4 times). The mode of acquisition was through family ties (68.57%), followed by community and dreams (11.43%). Recipes were prepared mainly by infusion (60%) and maceration (28.57%). In view of these results, we feel it is imperative to extend this study to all prefectures and regions of Guinea, with a view to strengthening data banks in this field, for the benefit of future technologies for the pharmaceutical industry.

KEY WORDS: *Prostate*; *Prostatitis*; *Ethnobotany*; *Ethno-medicines*; *Boke*

1. Introduction

The use of medicinal plants is still important in African traditions. Living beings have always sought to use plants to ensure their survival, and to derive remedies from them to treat their illnesses (Mahomoodally, 2013). Indeed, primary health care relies heavily on medicinal plants and the local knowledge associated with them (Fyhrquist, 2007). Ethnobotany, the science of how communities in a given region use plant species for food, clothing and medicine, plays a fundamental role in understanding how communities perceive, use and conserve plant resources (Aiyeloja and Bello, 2006; Betti, 2004). It enables popular know-how to be translated into scientific knowledge (Tahiri *et al*., 2012). However, in our tropics, most ethnobotanical studies in general and ethno-medicinal studies in particular, focus on the inventory and analysis of plant uses, without sufficiently highlighting the

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associated practices, which are thus overlooked (Malan and Neuba, 2011).

In Guinea, as elsewhere in Africa, traditional medicine is a very important component of cultural heritage, deeply rooted in the history, culture and beliefs of the people, and determines their aptitudes and behaviour in the face of the personal, family and social events of daily life (Banza *et al*., 2020). Until the middle of the 20th century, medicinal plants still held an important place in people's therapeutic arsenals, and there was no resistance to their use for both benign and serious illnesses (Banza *et al*., 2020).

In Africa, 80% of the population use medicinal plants to satisfy their health needs, either out of cultural habit or financial necessity (Banza *et al*., 2020). Some sexually-transmitted infections are bacterial in origin and can be cured by medicinal plants. It is therefore to be hoped that prostatitis can also be treated. By the age of 80, one man in nine (1/9) will have developed prostatitis (Krieger *et al*., 2008).

With this in mind, and faced with the high cost of conventional medicines and the risk of counterfeiting, more and more people are turning to herbal remedies. Traditional medicine is thus becoming a credible alternative worth exploring in depth.

We therefore decided to carry out an ethnobotanical survey in the Boké region, in order to identify a number of anti-prostatitis plants and, finally, to carry out a phytochemical studies of a number of species with prostatitis properties, hence the choice of the theme "Ethnobotanical investigation of plants with prostatitis properties in the Prefecture of Boke".

2. Material and Methods

2.1 Presentation of the study area

The prefecture of Boké was used for the ethno-botanical investigations. Located on Guinea's maritime coast, Boké covers an area of 334 km^2. It is located in the North-Western part of Guinea, known as maritime Guinea, and more specifically in Baga country. The Boké region borders Senegal to the north, Guinea-Bissau to the west and the Gaoual prefecture to the northwest. To the south-west, the region borders the Atlantic Ocean (Fig. 1). It had a population of 108,145 in 2014, with a density of 35 inhabitants/km^2. The natives who built Boké were initially Landoumas, after which the Bagas settled on the Boké plateau, extending as far as Guinea-Bissau to the north and approaching the plains of the Rio Kapatchez to the south.

Close to Guinea Bissau, a tarmac road allows you to cover the 250 km distance to Conakry quickly. At regional level, a tarred road takes you 50 km from Kamsar, and tracks lead to Sangarédi or Gaoual.

The town of Boké is the most cosmopolitan in Guinea, with Nalous living along the coast, Landoumas, Bagas, Soussous, Peuls, Kissis,

Fig. 1: Boke Card

Diakankés (Baralandés and Coréah) and Mikiforês. The "Camara" Fulani were the first from Fouta to settle in Kakandé (IRD, 2001).

2.2 Ethno-botanical survey

Study setting: The prefecture of Boké served the ethnobotanical investigation work, which took place from 06 April to 02 July 2018.

2.3 Working materials

Plant material: The plant material consisted of: leaves, roots and stem barks of the twelve (12) plants (Fig. 2 and Fig. 3) selected during the ethnobotanical survey. The different parts of these plants were collected from 06 April to 02 July 2018 in the prefecture of Boké. Botanical identification was carried out at the National Herbarium of Guinea.

Harvesting equipment: Daba, Cutters, Polyethylene bag, Cardboard folder; Scissors; Pruning shears.

2.4 Working method

Type and period of study: This was a prospective cross-sectional survey conducted from April 6 to July 2, 2018.

Study population: Our investigations focused on traditional practitioners in the prefecture of Boké recognized by the population in the treatment of prostatitis.

2.5 Method of analysis

The results are presented in tabular form, analyzed and interpreted. They were processed manually, and entered using computer software.

Inclusion and exclusion criteria:

Inclusion criteria

- Traditherapeutes living in Boké and recognized by the community, able to treat prostatitis
- Agree to participate voluntarily in the study.

Exclusion criteria

- Any healer also using modern prostatitis treatment.

2.6 Contact with the authorities

After receiving the mission order issued by the Department of Chemistry, we travelled to the prefecture of Boké to carry out the surveys. We then received orders from the prefectural and communal authorities.

2.7 Contact with healers

In the interests of maintaining confidentiality and safeguarding the family heritage, we had difficulty gaining access to information. Guided by an intermediary, we were able to meet the healers. This approach to our introduction to the healers involved the presentation of colas and sums of money, depending on the case.

2.8 Data collection

The data collection method used was interactive. It consisted in questioning healers about their art, in the form of an interview and on the basis of a survey form, a model of which can be found in the appendix.

In order to respect the confidentiality surrounding the practice of traditional medicine, we opted for individual contact, using the survey forms drawn up by the Institut de Recherche et de Développement des Plantes Médicinale et

Halieutique. They contained the following information;

- Survey location;
- Information on the healer;
- Part of the plant used;
- Method of preparation;
- Dosage;
- Duration of treatment.

2.9 Drug harvesting

The drugs were harvested from plant parts such as leaves, roots and stem bark. The drugs were then transported to the city (to our home) and then to the organic chemistry laboratory of the Gamal Abdel University in Conakry for drying. This takes place in the shade, followed by packaging in polyethylene bags. At the end of this operation, the samples were transported to the Organic Chemistry Laboratory of the Chemistry Department at Conakry's Gamal Abdel Nasser University.

2.10 Healer's diagnosis

- Pain on urination;
- Pus on urination;
- Blood in urine;
- Lumbar pain.

3. Results and Discussion

Our work focused on the ethnobotanical investigation and monograph of plants used in the treatment of prostatitis in traditional Guinean medicine in the prefecture of Boké, which took place from 06 April to 02 July 2018.

In total we met 35 healers including 23 men and 12 women. This predominance of men could be explained by the masculine nature of this disease. The majority of these healers were herbalists (68.57%), followed by marabouts (25.71%), planters (2.86%), and farmers (2.86%). The age of the traditherapeutes ranged from 35 to 80, with an average age of 60.

The most represented age group was 55 to 70 (45.71%) and the least represented was under 40 (22.85%) (Table 1). Judging by these results, the percentage of young people taking up the profession of traditherapeutes is low. This is why promoting traditional medicine must be a priority.

Of the 12 plants listed, *Phyllanthus muellerianus* (10 times), *Smilax anceps* (8 times), *Carica papaya* (4 times), *Pterocarpus erinaceus'* (3 times), *Dioscorea bulbifera* and *Vitex doniana* (2 times), and the other 6 only once (Table 2). Moreover, according to current literature, *Pterocarpus erinaceus* is of great importance in the treatment of a number of diseases.

It is used in North America and Europe to treat chronic diarrhoea. Decoctions or infusions of bark or roots are used to treat bronchial infections, toothache, painful menstruation, anaemia, post-partum haemorrhage, tapeworm infections, leprosy, wounds, tumours and ulcers; they are also used for their anti-emetic, purgative and tonic properties. Root-based preparations are administered as enemas to treat venereal diseases. A decoction of the leaves is used to treat fever and syphilis.

Carica papaya has many medicinal virtues. Phytotherapy uses the fruit, leaves, seeds, latex and roots. Depending on the part used, papaya is a purgative, an anti-inflammatory or an agent with positive effects on digestion, the treatment of sciatica caused by herniated discs; laxative;

Table 1: Socio-professional distribution of healers

Sl. No.	First and last names	Gender	Age	Profession	Address	Plant vernacular name (Sousou)	Part used	Galenic form
1	Banaro Maimouna	F	55	Herbalist	Dabis	Toumbégbely	R	Decoction
2	Camara Abdou	M	72	Herbalist	Sansalé	Welenwelendji	f, ET	Infusion
3	Bangoura Adama	F	80	Herbalist	Boke center	Koumissossè	f	Infusion
4	Camara Oumar	M	55	Planter	Boke center	Guésséfouté	f	Maceration
5	Manè Fatoumata	F	45	Herbalist	Boke center	Toumbégbély	R	Decoction
6	Sylla Issa	M	58	Herbalist	Boke center	Toumbégbély	f	Infusion
7	Tambasa Salématou	F	45	Herbalist	Malapouyah	Welenwelendji	f	Infusion
8	Camara Fatoumata	F	40	Herbalist	Boké center	Föfia	f	Decoction
9	Camara Moussa	M	39	Marabout	Boké center	Sougni	f	Maceration
10	N'tiasso mahawa	F	60	Herbalist	Dongol	Toumbégbély	f, ET	Infusion
11	Sylla Abdoulaye	M	70	Marabout	Dongol	Welenwelendji	R	Infusion
12	Diallo Mamadou	M	36	Herbalist	Dongol	Khary	ET	Infusion
13	Diallo Mouctar	M	49	Herbalist	Dongol	Firy forêt	f	Maceration
14	Diassy Mamadouba	M	70	Marabout	Tassimbo	Welenwelendji	f	Infusion
15	Tambassa Kadiatou	F	38	Herbalist	Tassimbo	Föfiya	f	Infusion
16	Fofana Mohamed Lami	M	38	Marabout	Tassimbo	Khary	f	Maceration
17	Fofana Soriba	M	35	Marabout	Tassimbo	Woulonyi	f	Maceration
18	Camara Oumar	M	60	Herbalist	Tassimbo	Bamba	f	Maceration
19	Sylla Bintia	F	75	Herbalist	Koffiya	Toumbégbély	f	Infusion
20	Camara Bintou	F	70	Herbalist	Koffiya	Föfiya	f	Maceration
21	Camara Alsény	M	65	Cultivator	Dibya	Toumbégbély	f	Infusion
22	Koumbassa A. Diouldé	M	35	Marabout	Tanènè	Firy forêt	f	Maceration
23	Koumbassa Mariama	F	60	Herbalist	Baralandé	Welenwelendji	f	Infusion
24	Dramé Bamba	M	58	Marabout	Baralandé	Toumbégbély	R	Infusion
25	Keita Salifou	M	65	Herbalist	Baralandé	Toumbégbély	R	Infusion
26	Sambou M'mahawa	F	57	Herbalist	Sansalé	Welenwelendji	f	Infusion
27	Savané Fatoumata	F	61	Herbalist	Kissassi	Sougni	f	Maceration
28	Khalissa Mamdouba	M	80	Marabout	Kissassi	Piya	f	Infusion
29	Koumbassa Aboubacar	M	40	Marabout	Tanènè	Föfiya	F	Infusion
30	Chérif yalatif	M	59	Herbalist	Boké Centre	Koussou	f, ET	Infusion
31	Koumbassa Alsény	M	65	Herbalist	Kolaboui	Toumbégbély	f	Infusion
32	Sylla Boubacar	M	68	Herbalist	Kolaboui	Welenwelendji	f	Decoction
33	Keita Alsény	M	78	Herbalist	Kolaboui	Toumbégbély	f	Infusion
34	Soumah Boubacar	M	80	Herbalist	Kolaboui	Khary	f	Maceration
35	Bangoura Alya	M	70	Herbalist	Kolaboui	Welenwelendji	f	Infusion

Legend: F: Female; M: Male f: Leaf; R: Root; ET: Stem Bark.

Table 2: Frequency of citing the plant

Common names of plants	Scientific names	Frequency
Toumbégbély	*Phyllanthus muellerianus*	10
Welenwelendji	*Smilax anceps*	8
Föfiya	*Carica papaya*	4
Khary	*Pterocarpus erinaceus*	3
Firyforêt	*Dioscorea bulbifera*	2
Sounyi	*Vitex doniana*	2
Koumissossè	*Premna hispida*	1
Guésséfouté	*Gossipium barbadense*	1
Piya	*Persea americana*	1
Koussou	*Anacardium occidentale*	1
Woulonyi	*Daniellia oliveri*	1
Bamba	*Cassia sieberiana*	1
	Total	35

vermifuge; purgative; analgesic, relaxant; papaya protects against the induction of cotton cancer.

Papaya has a de-infiltrating and anti-inflammatory effect. Papaya is recommended for the treatment of peptic ulcer.

Infections from the leaves can be used to treat bloating and other digestive problems.

Cassia siberiana's purgative action can be attributed to its anthraquinones. Flavones promote diuresis and have antibacterial and anti-inflammatory activity. A test for antiviral activity against Herpes simplex virus type 1 (HSV-1) showed that *Cassia sieberiana* extracts had significant activity against this virus. In vitro tests showed only weak activity against trypanosomes. Leaf extracts were found to be active against *Staphylococcus lutea*, *Mycobacterium phlei*, *Bacilllus subtilis* and *Proteus* sp, (Kamanzi *et al*., 2002).The majority of these plants were prepared by infusion (60%), followed by maceration (28.57%) and decoction (11.43%) (Table 3).

Table 3: Breakdown of recipes by galenic form

Sl. No.	Galenic forms	Citation frequencies	Percentage (%)
1	Infusion	21fois	60.00
2	Maceration	10fois	28.57
3	Decoction	4fois	11.43
	Total	35	100

With regard to the method of acquiring knowledge, our work shows that 68.57% of traditherapeutes acquired their knowledge through their families, respectively 22.86% (father), 20% (grandfather), 17.14% (grandmother) and 8.57% (mother).

The least common was acquisition through professional experience (2.86%) (Table 4).

Table 4: Different ways in which healers acquire knowledge

Method of acquisition		Number of healers	Percentage	
Family Parental	Father	8	22.86	68.57
	Grandfather	7	20.00	
	Grandma	6	17.14	
	Mother	3	8.57	
	Community	4	11.43	
	Dream	4	11.43	
	Learning	2	5.71	
	Personal experience	1	2 .86	
	Total	35	100	

Inheritance was the most common method of acquisition, followed by community and dreams. This could be explained by the long African tradition according to which art is passed down

from generation to generation (from grandfather to father or grandmother to mother).

The most commonly used parts are: leaves (60%); roots (31.43%), followed by stem bark (Table 5).

Table 5: Breakdown of recipes according to the part of the plant used

Organs	Frequencies	Percentage
Leaves	28	73.68
Roots	6	15.79
Stem bark	4	10.53
Total	38	100.00

Twelve (12) plants were identified for the treatment of prostatitis. The most frequently cited plants belong to the Euphorbiaceae family (10); Smilaceae (8); Caricaceae (4); Leguminoseae-Cesalpinoideae (3) (Table 6).

4. Conclusion

Today, with the help of scientific and technological progress, African countries must fight to bring the positive contributions of traditional medicine into the mainstream of modern medicine. In Guinea, Traditherapeutes are the first port of call for the treatment of urinary tract infections. Thus this botanical survey conducted from 06 April to 02 July 2018 in the prefecture of Boké identified 35 Traditherapeutes including 12 women and 23 men.

Botanically, 12 plant species were identified. The most frequently cited plant species were *Phyllanthus muellerianus* (10 times) and *Smilax anceps* (8 times). All the medicines were prepared with water, and the recipes were presented in different forms: Infusion 60%, Maceration 28.57%, and Decoction 11.43%.

Table 6: List of anti-prostatitis plants found in the prefecture of Boké

Family	Scientific names	Common names			Part used
		Sussu	Pular	Maninka	
Euphorbiaceae	*Phyllanthus muellerianus*	Toumgbegbely	--	Tri	R, f
Smilacaceae	*Smilax anceps*	Welenwelendji	--	--	R, f
Caricaceae	*Carica papaya*	Fofiya	Popo	Yiridjé	R, f
Leguminosae-Cesalpinoideae	*Pterocarpus erinaceus*	Khary	Bani	Ben	ET
Discoreaceae sp	*Dioscorea bulbifera*	Firy forêt	Pouri-balé	Dan-dan	F
Verbenaceae	*Vitex doniana*	Sounyi	Doukoumé	Sounsoun	F
Verbenaceae	*Premna hispida*	Koumissosso		Bilankourou fida	F
Malvaceae	*Gossypium barbadense*	Guéssefouté	Diarounde	Tourouba	F
Lauraceae	*Persea americana*	Piya	Piya	Piyah	F
Anacardiaceae	*Anacardium occidentale*	Koussou	Yalaguè	Somo	f, ET
Leguminosae-Cesalpinoideae	*Daniellia oliveri*	Woulougni	Kévé	Sandan	F
Leguminosaea-MimosoÏdeae	*Cassia sieberiana*	Bamba	Sindia	Sindjan	F

Legend: f: Leaf; R: Root; ET: Stem Bark.

Phyllanthus muellerianus

Smilax anceps (Wild)

Carica papaya L

Pterocarpus erinaceus (Pear)

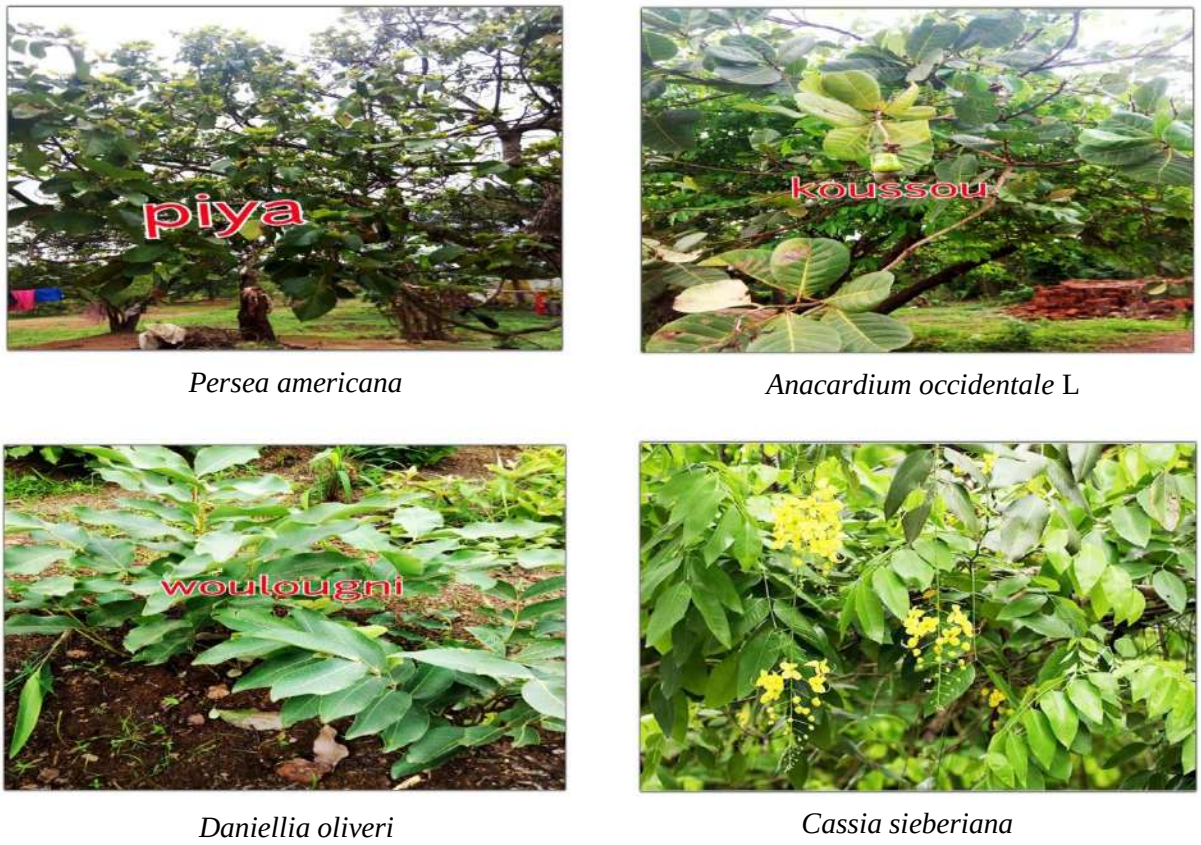
Dioscorea bulbifera L

Vitex doniana (Sweet)

Premna hispida

Gossypium barbadense L

Fig. 2: Representative images of the plant species observed during the survey



Persea americana *Anacardium occidentale* L

Daniellia oliveri *Cassia sieberiana*

Fig. 3: Representative images of the plant species observed during the survey

The most frequently cited parts are root, leaf and stem bark. Our studies show that this knowledge is held almost exclusively by the elderly.

5. Conflicts of interests

Authors declare that there is no conflict of interest exists.

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Development of cost-effective entomopathogenic fungal biopesticides using cereal and pulse-based substrates for sustainable agricultural practices

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ABSTRACT

The present study was conducted to assess the biological efficacy and economic viability of six agro-industrial substrates - rice, chickpea husk, maize, sorghum, wheat bran, and ragi - for the mass production of entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana*, and *Lecanicillium lecanii*. All substrates were evaluated under standardized treatments, and spore yields ($\times 10^9$ spores g^{-1}) were quantified. Among the substrates, rice and chickpea husk (both treated with crushed + yeast extract) supported the highest sporulation, with *B. bassiana* reaching 3.47 and 3.25 spores g^{-1}, *M. anisopliae* yielding 2.25 and 2.12 spores g^{-1}, and *L. lecanii* producing 1.74 and 1.53 spores g^{-1}, respectively. These values were significantly higher (CD: 0.466, 0.800, and 0.393, respectively) than those of lower-performing substrates such as ragi, which showed minimal sporulation across all fungi. Economic evaluation indicated that chickpea husk was the most profitable substrate, with a net income of ₹ 116.50 kg^{-1} and the highest benefit-cost (B:C) ratio of 4.70, followed by wheat bran and rice. In contrast, ragi incurred a slight loss (₹ -0.25 kg^{-1}), with a B:C ratio of 1.00. Overall, chickpea husk emerged as the most promising substrate for large-scale production of entomopathogenic fungi, combining high spore output with superior economic returns, making it ideal for use in microbial biopesticide development.

KEY WORDS: *Entomopathogens*; *Beauveria bassiana*; *Lecanicillium lecanii*; *Metarhizium*; *Pulse*

1. Introduction

The increasing concern over the environmental and health hazards associated with chemical pesticides has led to growing interest in sustainable pest management strategies. Among these, the use of entomopathogenic fungi (EPF) such as *Metarhizium anisopliae*, *Beauveria bassiana*, and *Lecanicillium lecanii* has emerged as an effective, eco-friendly alternative for controlling various insect pests in agriculture (Goettel and Jaronski, 2007; Jackson and Roberts, 2011). These fungi infect and kill insect hosts through cuticle penetration, ultimately leading to insect death by toxicosis and tissue degradation (Ball *et al*., 1994). Their effectiveness, however, depends significantly on the quality and quantity of viable spores produced, which is influenced by the substrate used during mass production (Sahayaraj and Namasivayam, 2008).

Selection of an appropriate substrate is crucial for maximizing the efficiency and cost-effectiveness

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of EPF production. Traditional grains like rice and maize have been widely used for fungal propagation due to their nutritional richness, but their higher costs can limit large-scale applications (Shankar *et al*., 2016). Agro-industrial by-products such as chickpea husk and wheat bran offer a low-cost alternative and are readily available in many agricultural regions. Studies have demonstrated that these substrates can support satisfactory conidial yields when supplemented with nutrients like yeast extract (Ranganayaki and Lakshmanan, 2020). Additionally, preparation methods, including boiling or crushing, play a vital role in enhancing the substrate's suitability for fungal colonization and sporulation (Vimala *et al*., 2002).

Despite the growing research in this domain, comparative studies evaluating both the biological efficiency and economic feasibility of different substrates remain limited. Therefore, the present investigation was undertaken to evaluate six commonly available substrates - rice, chickpea husk, maize, sorghum, wheat bran, and ragi - under two different treatment methods for their ability to support spore production of *M. anisopliae*, *B. bassiana*, and *L. lecanii*. The study also included an economic analysis to determine the benefit-cost ratio (B:C) and net income per kilogram of production, aiming to identify a cost-effective, high-yield substrate suitable for commercial-scale fungal biopesticide production. The findings are expected to provide valuable insights for sustainable mass production strategies of EPF in integrated pest management programs.

2. Material and Methods

The experiment was conducted in the Department of Agricultural Microbiology, UAS, Raichur. Three fungal species - *Metarhizium anisopliae*, *Beauveria bassiana*, and *Lecanicillium lecanii* were procured from the microbial culture bank and maintained on SMAY (Sabouraud Maltose Agar with Yeast extract) medium.

Substrates selected for evaluation included rice, maize, sorghum, chickpea husk, and wheat bran based on their local availability and economic feasibility. Each substrate was subjected to three treatments: (1) whole boiled grains, (2) crushed grains, and (3) crushed grains with 1% yeast extract. One kilogram of each substrate was partially boiled for 5 minutes and shade dried. For treatments requiring crushing, substrates were ground coarsely. Each treatment was packed in autoclavable polyethylene bags (250 g per bag), sterilized at 121°C for 30 minutes, and inoculated with 6 mm agar discs from two-week-old fungal cultures. Bags were incubated at 25 ± 2°C and 75 ± 5% RH for 20 days. Uninoculated bags served as controls.

After incubation, fungal biomass was dried at 60°C to constant weight. Spore load was estimated by suspending 1 g of colonized substrate in 10 mL sterile water with 0.05% Tween 80, and counting was performed using a Neubauer hemocytometer. Data were analyzed using ANOVA and mean separation was done using CRD with 5% significance level.

3. Results and Discussion

3.1 Spore production on different substrates

The efficacy of various agro-industrial substrates in supporting the growth and sporulation of three entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana*, and *Lecanicillium lecanii* was evaluated based on spore counts (expressed as $\times 10^9$ spores g^{-1}). The treatments involved two

processing methods: Crushed + YE (yeast extract) and Whole Boiled, applied to substrates like rice, chickpea husk, maize, sorghum, wheat bran, and ragi. The spore production of fungal entomopathogens on different substrates is given in Table 1.

For *Metarhizium anisopliae*, rice (2.25×10^9 spores g^{-1}) and chickpea husk (2.12×10^9 spores g^{-1}) produced the highest spore counts. When compared with the calculated Critical Difference (CD = 0.466), the difference between rice and chickpea husk is not statistically significant, but both are significantly superior to maize (1.88), sorghum (1.70), wheat bran (1.55), and ragi (0.95), which fall below the CD threshold. Ragi, with the lowest value, was significantly inferior to all other substrates. The trend indicates that crushed substrates supplemented with yeast extract are more conducive to fungal growth than whole-boiled ones.

For *Beauveria bassiana*, a similar pattern was observed. Rice supported the maximum sporulation (3.47×10^9 spores g^{-1}), closely followed by chickpea husk (3.25). These values are significantly higher than those for maize (2.65), sorghum (2.14), and wheat bran (1.96), when assessed against the CD of 0.800. Ragi (1.38) was again the least effective substrate and significantly different from rice, chickpea husk, and maize. These findings suggest that *Beauveria bassiana* sporulation is significantly influenced by substrate type, with rice and chickpea husk providing optimal nutrient profiles.

In the case of *Lecanicillium lecanii*, the overall spore counts were lower compared to the other two fungi. However, rice (1.74) and chickpea husk (1.53) again led the list. The CD value here is 0.393, and both these substrates are significantly superior to maize (1.06), sorghum (1.01), wheat bran (0.89), and ragi (0.72). Notably, differences among the lower-yielding substrates (maize to ragi) are not significant, as they fall within the CD range.

In summary, rice and chickpea husk under the Crushed + YE treatment consistently supported significantly higher spore production across all three fungal species (Fig. 1). Conversely, ragi (Whole Boiled) was the least effective substrate. The results strongly suggest that both substrate type and processing method play a critical role in optimizing entomopathogenic fungal growth for biocontrol applications.

3.2 Economic feasibility of substrate usage

The economic feasibility of using various agro-

Table 1: Spore production of fungal entomopathogens on different substrates

Substrate	Treatment	*Metarhizium anisopliae* ($\times10^9$ spores g^{-1})	*Beauveria bassiana* ($\times10^9$ spores g^{-1})	*Lecanicillium lecanii* ($\times10^9$ spores g^{-1})
Rice	Crushed + YE	2.25	3.47	1.74
Chickpea Husk	Crushed + YE	2.12	3.25	1.53
Maize	Crushed + YE	1.88	2.65	1.06
Sorghum	Crushed + YE	1.70	2.14	1.01
Wheat Bran	Crushed + YE	1.55	1.96	0.89
Ragi	Whole Boiled	0.95	1.38	0.72
S.Em±		0.190	0.326	0.160
CD		0.466	0.800	0.393

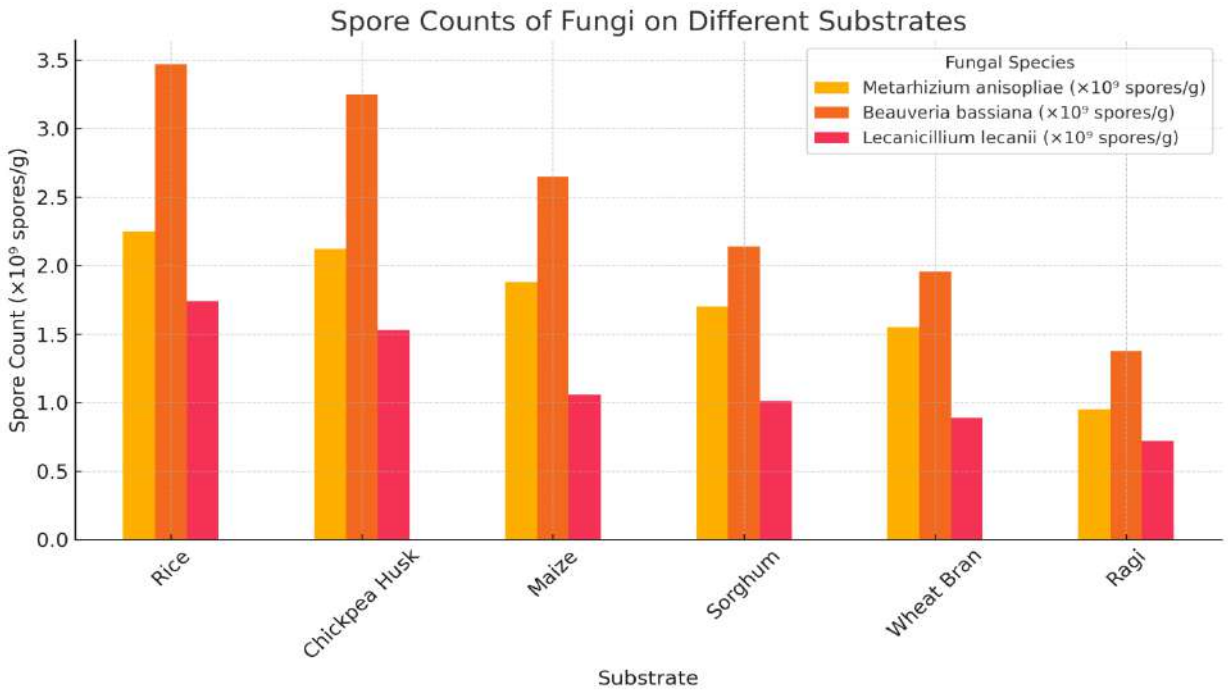

Fig. 1: Spore counts of entomopathogenic fungi on different substrates

industrial substrates for entomopathogenic fungal production was analyzed based on grain cost, gross income, production cost, net income, and the benefit-cost (B:C) ratio. The analysis revealed significant differences in profitability among the substrates and the values obtained are presented in Table 2.

Chickpea husk emerged as the most cost-effective substrate. With the lowest grain cost (₹ 9.00 kg^{-1}) and a gross income of ₹ 148.00 kg^{-1}, it yielded the highest net income of ₹ 116.50 kg^{-1} and a B:C ratio of 4.70. This reflects an excellent economic return, likely due to its low raw material and processing costs coupled with high spore productivity. The findings are in agreement with earlier reports that emphasize the value of inexpensive and nutrient-rich substrates for low-cost fungal production (Sahayaraj and Namasivayam, 2008).

Rice, though relatively expensive (₹ 26.00 kg^{-1}), resulted in the highest gross income (₹ 207.00 kg^{-1}) and a net income of ₹ 116.00 kg^{-1}, closely following chickpea husk. However, due to its high input cost and higher production cost (₹ 91.00 kg^{-1}), its B:C ratio was lower at 2.27. This still represents a viable option, especially when premium fungal quality is desired, as rice is traditionally considered a rich medium for fungal growth (Goettel and Jaronski, 2007).

Wheat bran, like chickpea husk, also had a low grain cost (Rs. 9.00 kg^{-1}) and offered a relatively good net income of ₹ 62.50 kg^{-1} with a B:C ratio of 2.98, making it the third most economical choice. Maize and sorghum, though moderately priced, yielded much lower net incomes of ₹ 52.50 kg^{-1} and ₹ 47.50 kg^{-1}, respectively, with B:C ratios below 2.00, indicating limited profitability.

Table 2: Economic analysis of substrates used for mass production of entomopathogenic fungi.

Substrate	Grain cost (₹/kg)	Gross income (₹/kg)	Production cost (₹/kg)	Net income (₹/kg)	B:C Ratio
Rice	26.00	207	91.00	116.00	2.27
Chickpea Husk	9.00	148	31.50	116.50	4.70
Maize	17.00	112	59.50	52.50	1.88
Sorghum	15.00	100	52.50	47.50	1.90
Wheat Bran	9.00	94	31.50	62.50	2.98
Ragi	21.50	75	75.25	-0.25	1.00

In contrast, ragi was economically unviable. Despite its mid-range cost (₹ 21.50 kg^{-1}), the production cost (₹ 75.25 kg^{-1}) slightly exceeded the gross income (₹ 75.00 kg^{-1}), resulting in a negative net income (-0.25 ₹ kg^{-1}) and a B:C ratio of 1.00, reflecting a break-even scenario. This makes ragi unsuitable for commercial-scale fungal production under the current conditions (Fig. 2).

Overall, chickpea husk and wheat bran proved to be the most profitable substrates, followed by rice. These findings support the use of locally available, low-cost agro-industrial byproducts for economical mycoinsecticide production.

4. Conclusion

The current study assessed the biological performance and economic viability of six agro-industrial substrates in supporting the mass production of entomopathogenic fungi - *Metarhizium anisopliae*, *Beauveria bassiana*, and

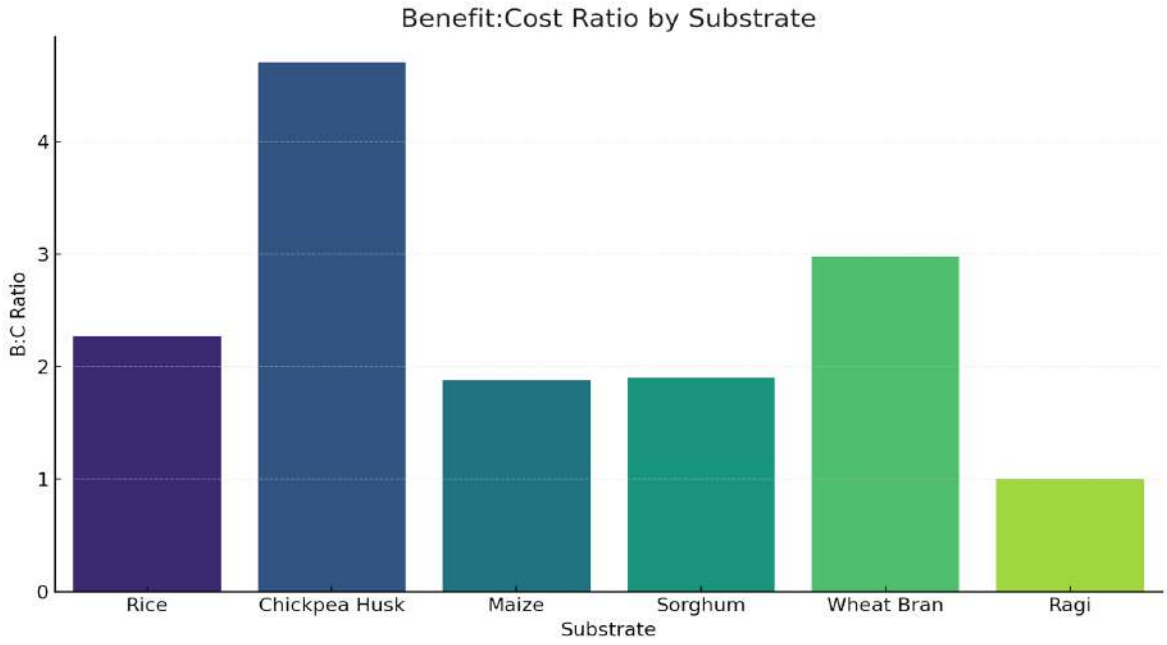

Fig. 2: Benefit cost ratio of different substrates used for fungal biopesticide production

Lecanicillium lecanii. Results demonstrated that substrate type and treatment method significantly influenced both spore yield and production economics.

Biologically, rice and chickpea husk (under Crushed + YE treatment) consistently supported higher sporulation across all three fungal species. The spore counts for these substrates exceeded the calculated critical difference (CD) thresholds, confirming statistically significant differences when compared to lower-performing substrates such as ragi and wheat bran. This highlights the importance of selecting nutrient-rich and well-processed substrates to optimize fungal biomass and spore production, as previously reported by Rombach *et al.* (1988) and Goettel and Jaronski (2007). Substrates like ragi, particularly when used in whole-boiled form, were found to be biologically and economically inferior.

Economically, chickpea husk stood out as the most viable substrate, offering the highest benefit-cost (B:C) ratio of 4.70 and a net income of ₹ 116.50 kg^{-1}, owing to its low raw material cost and high biological yield. Wheat bran and rice also demonstrated favorable economics, with B:C ratios of 2.98 and 2.27, respectively. In contrast, ragi resulted in a net loss, underlining its unsuitability for commercial-scale production.

In conclusion, chickpea husk offers an ideal combination of high fungal yield and cost-effectiveness, making it the most promising substrate for mass production of entomopathogenic fungi. This integrated biological and economic evaluation provides a practical guide for selecting substrates in fungal biopesticide industries, supporting the shift toward more sustainable and affordable bio-control solutions.

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Biodiversity and functional role of Potassium Solubilizing Bacteria (KSB) in sustainable crop production: A review

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ABSTRACT

The Potassium (K) is a vital macronutrient required for plant growth, photosynthesis, enzyme activation, and overall crop productivity. Despite its abundance in soils, more than 90% of potassium is locked in insoluble mineral forms such as feldspar, mica, and illite, making it unavailable for plant uptake. Potassium Solubilizing Bacteria (KSB) represent an eco-friendly alternative to chemical fertilizers, capable of mobilizing mineral-bound potassium through the production of organic acids, chelating compounds, and exopolysaccharides. These microbes enhance soil fertility, promote plant growth, and improve nutrient uptake efficiency. This review explores the diversity of KSB, mechanisms of potassium solubilization, and their effects on crop yield and soil health. It also examines the synergistic effects of co-inoculation with phosphate solubilizing bacteria (PSB), the influence of environmental factors on KSB activity, and recent advances in formulation technologies for field application. Understanding the bioefficacy and adaptability of KSB is essential for developing sustainable, low-cost biofertilizers to address potassium deficiency in agricultural systems. The review concludes by identifying key challenges and future research directions to harness the full potential of KSB for global food security and environmentally sustainable farming practices.

KEY WORDS: *Potassium Solubilising Bacteria*; *Bacillus*; *Rhizosphere*; *Biofertilizer*

1. Introduction

Potassium (K) is a critical macronutrient that plays a pivotal role in numerous plant physiological processes, including enzyme activation, photosynthesis, osmoregulation, protein synthesis, and translocation of assimilates. It is the third most essential macronutrient for plants, following nitrogen and phosphorus (Cakmak, 2005). Despite the abundant presence of potassium in most soils—often exceeding 20,000 ppm—the majority of it is found in forms that are not directly available to plants. Only a small fraction of potassium (approximately 0.1–2%) is present in plant-available forms such as exchangeable or solution potassium in the soil water phase (George and Micheal, 2002). The bulk of potassium is trapped in mineral matrices like feldspar, mica, and illite, from which it is not readily accessible to plants (Buchholz and Brown, 1993). This limitation in potassium availability, coupled with the high cost of conventional potassium fertilizers such as muriate of potash (KCl), has sparked a growing interest in

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sustainable alternatives to traditional fertilizers, particularly in regions like India, where the domestic production of potassium falls short of agricultural demand (Mala, 2013).

In response to this challenge, Potassium Solubilizing Bacteria (KSB) have emerged as promising biological agents capable of converting insoluble forms of potassium into more accessible, plant-available forms. These microorganisms, primarily from genera such as *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Acidothiobacillus*, mobilize potassium through a variety of mechanisms, including acidolysis, complexolysis, and the production of organic acids like citric, tartaric, and oxalic acid (Sheng and Huang, 2002; Friedrich *et al*., 1991). In addition to solubilizing potassium, KSB enhance plant growth through the production of phytohormones such as indole-3-acetic acid (IAA), phosphate solubilization, and siderophore-mediated nutrient acquisition (Sheng and Huang, 2001; Hu and Boyer, 1996).

The integration of KSB into agricultural systems offers a range of agronomic and ecological benefits. Studies in both field and controlled environments have shown that inoculation with KSB strains such as *Bacillus edaphicus* and *Paenibacillus glucanolyticus* can enhance dry matter content, root and shoot elongation, and overall yield in crops such as cotton, black pepper, groundnut, and tea (Sheng, 2005; Sangeeth *et al*., 2012; Bagyalakshmi *et al*., 2012). For example, the inoculation of *Paenibacillus glucanolyticus* has been shown to significantly improve potassium uptake and biomass accumulation in black pepper grown in potassium-deficient soils treated with wood ash (Sangeeth *et al*., 2012). These benefits are particularly notable in soils with low available potassium, where KSB help to alleviate nutrient deficiencies and boost crop productivity.

In addition to the direct benefits of KSB inoculation, these bacteria exhibit synergistic effects when co-inoculated with other beneficial microorganisms, such as phosphate-solubilizing bacteria (PSB). Han *et al*. (2006) demonstrated that dual inoculation with KSB and PSB in pepper and cucumber plants resulted in greater nutrient uptake and biomass accumulation than single inoculations or conventional mineral applications. The synergistic interaction between KSB and other microbial species offers significant potential in integrated nutrient management (INM) strategies, reducing reliance on chemical fertilizers while maintaining or even enhancing crop productivity. This highlights the importance of microbial consortia in modern agricultural practices, where multiple microbial functions can be harnessed to improve soil health and plant nutrition.

Despite the promising potential of KSB, several biotic and abiotic factors can influence their effectiveness in field conditions. Soil pH, moisture, temperature, mineral composition, and the availability of organic carbon are among the key factors that can impact the performance of KSB in soil (Parmar and Sindhu, 2013). For instance, extreme pH values, both acidic and alkaline, can hinder microbial activity and potassium solubilization. Furthermore, the availability of organic carbon and other nutrients can affect the growth and activity of KSB, particularly in nutrient-deficient soils. Understanding these environmental variables is essential for optimizing the use of KSB as biofertilizers in diverse agroecosystems.

The formulation and field application of KSB biofertilizers present additional challenges. While microbial inoculants such as nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi have been extensively studied and commercialized, KSB-based formulations are relatively underexplored. The formulation of KSB products must account for factors such as microbial stability, shelf-life, and cost-effectiveness. Advances in biotechnology and microbial ecology are being employed to develop stress-tolerant and multifunctional KSB strains, with a focus on improving their shelf-life, viability during storage, and efficacy under field conditions. Additionally, the development of suitable carrier materials and the integration of KSB into existing agricultural practices, such as combined use with other microbial inoculants or chemical fertilizers, remains a critical area of research.

Moreover, the commercialization of KSB biofertilizers is impeded by regulatory hurdles. The lack of standardized testing protocols, quality control measures, and certification processes in many regions has led to concerns about the consistency and effectiveness of KSB products available in the market. The absence of clear regulatory frameworks also complicates the widespread adoption of KSB-based products, as farmers may be hesitant to invest in biofertilizers that are not well-regulated or certified. Thus, there is a need for the development of robust regulatory mechanisms that ensure the quality and efficacy of KSB biofertilizers before they can be widely adopted.

Given the growing body of research on KSB, significant knowledge gaps remain in areas such as the molecular mechanisms of potassium solubilization, strain-specific interactions with host plants, and the long-term field performance of KSB under diverse agro-climatic conditions. Understanding these mechanisms will be crucial for improving the efficiency and stability of KSB-based biofertilizers. In particular, research into the genetic regulation of potassium solubilization processes, as well as the role of microbial communities in enhancing nutrient cycling, will open new avenues for optimizing KSB strains for different environmental conditions.

This review aims to provide a comprehensive overview of the current state of research on KSB, focusing on the taxonomy, mechanisms of potassium solubilization, their effects on plant growth and nutrient uptake, and their interactions with other soil microbes. It also evaluates the challenges related to the commercialization and formulation of KSB biofertilizers and discusses potential future directions for advancing KSB research. Through this review, we aim to consolidate the existing knowledge on KSB and highlight the key areas that require further investigation to maximize their potential as sustainable alternatives to chemical fertilizers.

2. Soil potassium status and its limitations

Potassium (K) is one of the most abundant mineral nutrients in soil and plays a fundamental role in plant physiology, including enzyme activation, photosynthesis, osmoregulation, translocation of assimilates, and resistance to abiotic stress (Cakmak, 2005). Despite its high total concentration in most agricultural soils, potassium is predominantly present in forms that are not directly available for plant uptake. More than 90% of the total soil potassium is structurally bound within silicate minerals such as feldspar, mica, and illite. These forms are highly stable and

cannot be absorbed by plant roots without prior solubilization.

Soil potassium exists in four interrelated pools that vary in their plant availability. The largest portion is mineral or structural potassium, which is tightly bound in the crystal lattices of primary minerals. This fraction is virtually inert in the short term and requires extensive weathering to be released. A second fraction, termed non-exchangeable or fixed potassium, is held between layers of 2:1 clay minerals, particularly illite and vermiculite. This pool can contribute potassium to the soil solution over time, albeit slowly, and is influenced by soil moisture, cation exchange processes, and microbial activity. More readily available forms include exchangeable potassium, which is adsorbed onto the surfaces of clay particles and soil organic matter and can be rapidly taken up by plant roots. Finally, the smallest fraction, soil solution potassium, exists as free K^+ ions in the soil water and represents the form most directly accessible to plants (George and Micheal, 2002; Friedrich *et al*., 1991).

However, the availability of these pools is not static. A critical issue is potassium fixation, a phenomenon wherein K^+ ions added through fertilizers or released from non-exchangeable sources become trapped between clay layers, especially in soils rich in illite and vermiculite. This fixation limits the immediate bioavailability of potassium and often necessitates repeated fertilization to meet crop demands. Additionally, potassium is highly mobile in certain soil textures. In sandy or low cation-exchange-capacity soils, it is prone to leaching beyond the root zone, particularly under conditions of heavy rainfall or over-irrigation. Such losses reduce nutrient use efficiency and contribute to environmental degradation.

The extraction of potassium through crop harvest is another major depletion pathway. High-yielding and intensively cultivated systems remove substantial quantities of potassium from the soil annually, further widening the gap between natural potassium supply and crop requirement. This is especially problematic in developing countries where potassium fertilizers are either unaffordable or heavily reliant on imports, such as in India where more than 29,000 tonnes of potassium are imported annually (Mala, 2013).

Several environmental and soil factors modulate potassium availability. Soil pH, for instance, plays a key role; acidic conditions may enhance leaching, while high pH may reduce microbial-mediated solubilization. Soil moisture and temperature also influence potassium diffusion and uptake by affecting root metabolism and microbial activity. Texture and mineralogy determine potassium retention capacity, with clay-rich soils generally holding more potassium in exchangeable form than sandy soils. Biological factors, especially the composition and activity of rhizosphere microorganisms, contribute significantly to the release of potassium from mineral forms (Sheng and Huang, 2002).

Among these biological agents, potassium solubilizing bacteria (KSB) have shown promise in mobilizing unavailable potassium through mechanisms such as acidolysis, chelation, and polysaccharide production. Certain strains of *Bacillus mucilaginosus*, *Paenibacillus glucanolyticus*, and *Bacillus edaphicus* have been shown to effectively solubilize potassium-bearing minerals and improve K uptake in various crops. These microbial processes offer a natural and

sustainable means of increasing potassium bioavailability, thereby reducing dependence on chemical fertilizers.

Despite these benefits, the consistent performance of KSB in field conditions remains influenced by soil heterogeneity, competition with native microflora, and formulation constraints. Therefore, a detailed understanding of the soil potassium pools, their dynamics, and the environmental context is crucial for optimizing the use of KSB in sustainable agricultural systems.

3. Mechanisms of potassium solubilization by Potassium Solubilizing Bacteria (KSB)

Potassium (K) is an essential macronutrient required for a variety of physiological processes in plants, including enzyme activation, protein synthesis, and osmoregulation. However, a large proportion of potassium in soils is present in mineral forms (e.g., feldspar, mica) that are insoluble and not readily available to plants. Potassium solubilizing bacteria (KSB) play a crucial role in enhancing the bioavailability of potassium by converting insoluble forms into soluble ones, thereby improving plant nutrition. Several mechanisms have been identified through which KSB solubilize potassium, including organic acid production, proton excretion, enzyme secretion, and ion exchange.

3.1 Organic acid production

One of the primary mechanisms through which KSB solubilize potassium is the production of organic acids. These acids lower the pH of the surrounding soil environment, leading to the dissolution of potassium-bearing minerals. The production of acids, such as citric acid, oxalic acid, lactic acid, and acetic acid, is widely recognized as a key factor in K solubilization (Saharan & Nehra, 2011). These organic acids are capable of chelating potassium ions and breaking the mineral structures that bind potassium, thus making it more accessible to plants.

The acidification of the soil through organic acid production can also enhance the solubility of other essential nutrients, such as phosphorus and calcium, further promoting plant growth (Khan *et al*., 2012). For instance, the bacterium *Bacillus mucilaginosus* is known to produce citric acid, which effectively solubilizes potassium from feldspar, a common potassium-containing mineral in soils (Swarup *et al*., 2013). Similarly, *Enterobacter* species have been shown to produce a combination of organic acids that facilitate the solubilization of potassium as well as other macro- and micronutrients.

3.2 Proton excretion

In addition to organic acid production, KSB also solubilize potassium through proton excretion. The secretion of protons (H+) by bacteria into the surrounding environment causes acidification, which helps in the breakdown of potassium-bearing minerals. The acidification process not only dissolves minerals but also enhances the release of potassium ions into the soil solution (Rodríguez *et al*., 2006). This mechanism is particularly important in alkaline soils where potassium availability is naturally limited due to the higher pH and reduced solubility of potassium minerals.

Bacteria such as *Bacillus* and *Pseudomonas* spp. are known for their ability to secrete protons, effectively lowering the pH of the rhizosphere.

The presence of protons in the soil promotes the dissociation of potassium from mineral particles, making it available for plant uptake (Gaur & Pathak, 2003).

3.3 Production of enzymes

KSB also produce enzymes that contribute to potassium solubilization. These enzymes include phosphatases, proteases, and cellulases, which can break down organic materials in the soil, releasing minerals including potassium. The enzymatic degradation of minerals in the soil matrix is an essential process for mineral weathering and the subsequent release of potassium (Kandeler *et al*., 2000).

For example, *Bacillus* spp. and *Pseudomonas* spp. produce various extracellular enzymes that catalyze the release of potassium ions from insoluble mineral sources. Additionally, some KSB also secrete exopolysaccharides, which enhance mineral dissolution by forming a protective biofilm around the bacterial colony, thereby increasing the efficiency of potassium solubilization in the rhizosphere (Barka *et al*., 2004).

3.4 Ion exchange and chelation

Potassium solubilization by KSB can also occur through ion exchange and chelation. KSB can exchange cations, such as calcium and magnesium, with potassium ions from the mineral surfaces. This ion exchange process effectively releases potassium into the soil solution, increasing its availability for plant uptake. In addition, KSB can produce chelating agents, which bind to potassium and release it from mineral particles. Chelation plays a vital role in the solubilization of potassium from complex minerals, especially in soils with high cation exchange capacity (CEC).

A study by Glick *et al*. (2007) demonstrated that *Pseudomonas putida* and other KSB can mobilize potassium through a combination of ion exchange and chelation. These bacteria have the ability to use organic compounds as chelating agents, which not only solubilize potassium but also enhance the uptake of other essential nutrients like phosphorus and magnesium.

3.5 Role of phytosiderophores and other metabolites

Some KSB, particularly those in the genus *Pseudomonas*, can produce metabolites known as phytosiderophores. These are compounds that specifically bind to metal ions, such as potassium, facilitating their transport into the plant roots (Loper and Buyer, 1991). Phytosiderophores play a crucial role in solubilizing potassium in soils where it is otherwise bound to mineral particles or organic matter, thus enhancing the bioavailability of potassium to plants.

In addition to phytosiderophores, KSB may secrete other secondary metabolites that can assist in potassium solubilization. These include siderophores for iron, which often work in tandem with potassium solubilization mechanisms, improving the overall nutrient availability in the rhizosphere (Bais *et al*., 2006).

3.6 Interactions with plant roots

The interaction between KSB and plant roots is another important factor in potassium solubilization. KSB can establish symbiotic or associative relationships with plant roots, leading to enhanced potassium uptake. In return for

potassium and other nutrients, plants provide organic carbon sources to the bacteria. The rhizosphere is a dynamic environment where KSB can alter the soil chemistry, making potassium more available to plants. For example, the exudation of organic acids and enzymes by plant roots can stimulate KSB activity, thereby promoting the solubilization of potassium from minerals (Etesami and Beattie, 2018).

4. Effects of KSB on plant growth and nutrient uptake

4.1 Enhancement of potassium availability by KSB

Potassium (K) is essential for plant growth (Fig. 1), yet its availability in many agricultural soils is limited due to its fixation in mineral forms such as feldspars, micas, and illite, which plants cannot directly absorb. Potassium solubilizing bacteria (KSB) are recognized for their unique ability to mobilize these unavailable forms through biochemical mechanisms, thus enhancing the potassium nutrition of crops in a sustainable manner.

One of the principal mechanisms by which KSB enhance potassium availability is through the production of organic acids such as citric, oxalic, gluconic, and malic acids. These acids lower the soil pH and chelate metal ions (like Al^{3+}, Fe^{3+}, Ca^{2+}), thereby releasing potassium ions into the soil solution (Sheng and He, 2006). For example, *Bacillus mucilaginosus* was reported to solubilize up to 32% of the total potassium content from feldspar by secreting oxalic and citric acids (Zhang and Kong, 2014).

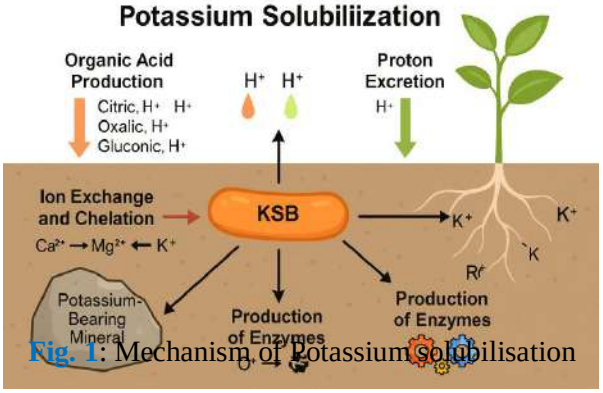

Fig. 1: Mechanism of Potassium solubilisation

A comparative study by Parmar and Sindhu (2013) demonstrated that inoculation with potassium-solubilizing strains of *Bacillus* and *Pseudomonas* significantly increased the available potassium content in both clay and loamy soils, outperforming untreated controls by 20–40%. In a pot experiment with tomato (*Solanum lycopersicum*), these strains increased soil available K from 58.2 mg kg^{-1} to 93.6 mg kg^{-1} and improved the K uptake by 42%.

Moreover, Pramanik *et al*. (2019) isolated a novel strain of *Bacillus pseudomycoides* from the rhizosphere of tea plants that enhanced potassium release from mica waste, leading to a 1.5-fold increase in tea biomass in greenhouse trials. Similarly, Meena *et al.* (2016) found that co-application of *Frateuria aurantia* (a known KSB) with vermicompost improved available potassium in the root zone by 27% in wheat and maize under field conditions.

The mode of action of these microbes is not restricted to acid production alone. They also release extracellular enzymes and polysaccharides that modify the micro-environment of the rhizosphere, promoting mineral dissolution. An investigation by Ahmad *et al*. (2021) found that KSB could bio-weather silicate minerals like

biotite and muscovite, enhancing potassium mobilization up to 35% in 60 days.

Further, KSB activity also improves soil structure and microbial diversity. Ramesh *et al.* (2022) observed that repeated KSB inoculation led to higher microbial biomass carbon and dehydrogenase activity, contributing to improved potassium cycling in the rhizosphere.

In summary, the capacity of KSB to transform non-exchangeable and mineral-bound potassium into plant-available forms is well-supported by various studies. Their application as biofertilizers not only improves K nutrition but also reduces dependency on chemical potassium fertilizers, thus offering a sustainable approach to nutrient management.

4.2 Promotion of Plant Growth Parameters

The influence of potassium solubilizing bacteria (KSB) on plant growth extends beyond mere enhancement of potassium availability. These microbes also exert significant effects on a wide range of plant growth parameters including germination rate, root and shoot elongation, biomass accumulation, chlorophyll content, and overall vigor. This multifaceted plant growth promotion is the result of a combination of mechanisms including nutrient solubilization, phytohormone production, siderophore release, and stress mitigation.

Several studies have reported that KSB strains, particularly from genera like *Bacillus*, *Frateuria*, and *Pseudomonas*, stimulate seed germination and seedling vigor. For instance, Basak and Biswas (2010) demonstrated that inoculation of rice seeds with *Bacillus mucilaginosus* significantly improved seedling length (by 28%) and dry weight (by 34%) over uninoculated controls, attributed to increased K availability and enhanced production of indole-3-acetic acid (IAA), a plant growth-promoting hormone.

Similarly, *Frateuria aurantia*, a known KSB species, was tested on tomato plants by Meena *et al*. (2016). The results indicated not only a 20–25% increase in shoot length and leaf number but also a substantial increase in chlorophyll content and flowering rate. These improvements were linked to the bacterium's dual ability to mobilize potassium and produce cytokinins, which modulate cell division and shoot development.

Potassium plays a pivotal role in osmoregulation, enzyme activation, and photosynthesis. Therefore, its enhanced availability via KSB activity can significantly improve physiological parameters. In a greenhouse study, Sheng and He (2006) reported that wheat plants inoculated with *Bacillus edaphicus* exhibited higher stomatal conductance, photosynthetic rate, and relative water content. These physiological enhancements translated into a 35% increase in dry matter accumulation compared to uninoculated controls.

Moreover, KSB have shown synergistic effects when applied with other plant growth-promoting rhizobacteria (PGPR). For instance, Parmar and Sindhu (2013) observed that co-inoculation of KSB with phosphorus-solubilizing bacteria (PSB) in maize resulted in a 45% increase in plant height and a 30% increase in leaf area index compared to either inoculant alone. This suggests that nutrient solubilization by KSB creates a conducive environment for overall microbial synergism, boosting plant growth.

In a field experiment, Ahmad *et al*. (2021) applied a KSB-based biofertilizer to potato crops. The

treated plots not only yielded 18% more tubers but also showed significant increases in root volume and shoot-to-root ratio. Furthermore, these plants had higher potassium content in leaf tissues, which correlated positively with photosynthetic efficiency and biomass productivity.

Some strains of KSB also contribute to abiotic stress alleviation. Under saline or drought conditions, potassium acts as an essential osmoprotectant. Khan *et al.* (2015) reported that plants treated with *Enterobacter* sp. KSB isolates under salt stress maintained higher relative water content and exhibited less electrolyte leakage. This was partly due to improved K^+/Na^+ balance facilitated by microbial solubilization and uptake regulation.

Beyond growth metrics, KSB influence reproductive development. Experiments on sunflower by Prasad *et al.* (2020) revealed that application of *Frateuria aurantia* increased flower diameter, seed set percentage, and 1000-seed weight, leading to an overall 22% yield increase. Such findings emphasize the potential of KSB not only as growth enhancers but also as yield boosters.

4.3 Impact of KSB on nutrient uptake efficiency

Potassium solubilizing bacteria (KSB) have been increasingly recognized not only for their capacity to mobilize potassium but also for their broader impact on improving the overall nutrient uptake efficiency in plants. This includes enhanced absorption of macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as several important micronutrients like magnesium (Mg), zinc (Zn), and iron (Fe). By improving root growth, increasing root surface area, and altering the rhizosphere chemistry, KSB help plants acquire more nutrients efficiently, even in nutrient-poor soils.

A landmark study by Meena *et al.* (2016) demonstrated that inoculation of maize with *Frateuria aurantia* significantly increased uptake of N (by 23%), P (by 31%), and K (by 45%) compared to uninoculated controls. This improvement in nutrient uptake was attributed to the enhanced root length and surface area induced by microbial activity as well as better mobilization of nutrients in the soil matrix. The researchers observed a corresponding increase in biomass accumulation and yield parameters.

Similarly, Parmar and Sindhu (2013) reported that co-inoculation of KSB with nitrogen-fixing and phosphate-solubilizing bacteria in wheat led to improved uptake of all three major nutrients (NPK), with potassium uptake showing the highest improvement of 50%. The synergistic microbial interactions appeared to trigger a cascade of beneficial changes in soil enzymatic activity and rhizosphere bioavailability of essential ions.

KSB also impact micronutrient uptake through indirect mechanisms such as siderophore production, pH modification, and chelation of soil minerals. For example, Basak and Biswas (2010) observed a marked increase in Fe and Zn uptake in rice following treatment with *Bacillus mucilaginosus*. The bacteria secreted siderophores and organic acids that helped in chelating Fe^{3+} and releasing Zn^{2+} from insoluble complexes in soil, thus improving micronutrient availability.

The influence of KSB on nutrient uptake is not restricted to controlled conditions. In a field experiment conducted by Ramesh *et al*. (2022) on

chickpea, KSB inoculation led to a 38% increase in phosphorus uptake and a 47% increase in potassium uptake compared to standard fertilizer application alone. Soil analysis showed a significant rise in available P and exchangeable K, likely due to organic acid secretion and root-induced changes in rhizosphere pH.

Another study by Adhikari *et al*. (2018) investigated the combined impact of KSB and compost on nutrient uptake in mustard plants. Results showed that microbial inoculation significantly enhanced the availability and plant uptake of nitrogen (by 19%), phosphorus (by 24%), and potassium (by 36%), suggesting that organic matter and microbial synergy can further improve the nutrient acquisition process. Interestingly, this study also noted enhanced sulfur and magnesium concentrations in treated plants, further illustrating the broad-spectrum benefit of KSB.

Moreover, some strains of KSB can modulate nutrient transporters in plants. Ahmad *et al*. (2021) demonstrated that inoculation with a strain of *Enterobacter cloacae* upregulated the expression of K^+ and NO_3^- transporter genes in maize roots, resulting in improved nutrient translocation to aerial parts. This kind of plant–microbe signaling indicates that the benefits of KSB go beyond solubilization and extend to influencing plant metabolic and transport processes.

In legumes, potassium is especially important for nodule function and biological nitrogen fixation. A recent study by Narayanasamy *et al*. (2023) on soybean demonstrated that KSB inoculation increased both nodule number and nitrogenase activity, thereby boosting nitrogen uptake efficiency. This illustrates the indirect but critical role of K in facilitating N_2 fixation and overall plant nutrition.

4.4 KSB mediated stress tolerance and yield enhancement

In addition to improving nutrient availability, potassium solubilizing bacteria (KSB) play a crucial role in enhancing plant resilience to abiotic stresses such as drought, salinity, and nutrient deficiency, ultimately contributing to improved crop yields. This multifaceted benefit arises from the combined action of enhanced potassium uptake, modulation of stress-responsive genes, production of plant growth-promoting compounds, and improvement in soil health parameters. Potassium is a key osmolyte and plays a significant role in regulating plant water balance, enzyme activation, and stomatal functioning. Thus, any increase in its bioavailability through microbial action can greatly influence plant adaptation to stress conditions.

Several studies have highlighted the influence of KSB on drought tolerance. For instance, Verma *et al*. (2017) demonstrated that *Bacillus mucilaginosus* inoculation in wheat under limited irrigation led to increased root biomass, relative water content, and improved chlorophyll stability index. This was attributed to the enhanced K uptake facilitated by microbial activity, which allowed better osmotic regulation and stomatal conductance. The study also observed a 25% increase in grain yield under stress conditions, underlining the practical significance of KSB in real-world agriculture. Similarly, Singh and Reddy (2020) observed that maize plants inoculated with a consortium of KSB and arbuscular mycorrhizal fungi showed improved

drought resistance through enhanced antioxidant enzyme activity and reduced membrane damage, leading to yield stability under water-limited regimes.

Salinity stress, another major yield-limiting factor, can also be mitigated by the action of KSB. Potassium plays a pivotal role in maintaining ionic balance by reducing sodium uptake and maintaining high K^+/Na^+ ratios within cells. In a controlled pot experiment, Rahi *et al.* (2019) reported that tomato plants inoculated with *Frateuria aurantia* exhibited significantly higher potassium content and reduced Na^+ accumulation in leaves under salinity stress, leading to enhanced biomass production and fruit quality. The bacterial strain produced high levels of gluconic and citric acid, facilitating K solubilization from feldspar and mica in saline soils. Furthermore, Sharma *et al*. (2021) reported that the application of KSB in rice improved electrolyte balance and osmotic potential, ultimately mitigating the adverse effects of salt stress.

Beyond abiotic stress, the role of KSB in boosting crop yield in normal agronomic conditions has also been substantiated through multiple field trials. Ahmad *et al*. (2018) conducted multi-season trials in chickpea and observed that the use of KSB inoculants increased grain yield by 20–30% compared to uninoculated controls. This increase was directly linked to enhanced nutrient uptake, increased photosynthetic efficiency, and better reproductive development. The biofertilizer treatment also improved root-to-shoot ratio, which is often associated with efficient nutrient translocation. In another study, Ghosh and Banerjee (2022) found that sugarcane plots treated with a mixture of KSB and vermicompost showed 18% higher cane yield and improved sugar recovery, highlighting the commercial relevance of microbial potassium mobilization.

The ability of KSB to produce secondary metabolites such as indole-3-acetic acid (IAA), gibberellins, and ACC deaminase also contributes to stress tolerance and yield improvement. These phytohormones stimulate cell elongation, lateral root proliferation, and delay senescence during stress episodes. For example, an isolate of *Paenibacillus* sp. reported by Thakur *et al.* (2020) exhibited dual functionality of K solubilization and IAA production, which significantly enhanced the root architecture and nutrient acquisition potential in soybean. This resulted in a 22% increase in pod yield under suboptimal potassium availability.

Interestingly, KSB-mediated improvements in soil health parameters such as soil structure, microbial biomass carbon, and cation exchange capacity also indirectly contribute to plant growth and yield. Long-term experiments in wheat by Dwivedi *et al*. (2019) demonstrated that repeated use of KSB inoculants enhanced the soil aggregate stability and maintained higher levels of exchangeable K, even after successive cropping seasons. The authors suggested that the build-up of beneficial microbial populations and enhanced nutrient cycling played a vital role in sustaining crop productivity.

4.5 Synergistic interactions of Potassium-Solubilizing Bacteria (KSB) with other soil microorganisms

Potassium-solubilizing bacteria (KSB) play a pivotal role in enhancing plant nutrient uptake by converting insoluble potassium forms into bioavailable forms. Their interactions with other soil microbes, such as nitrogen-fixing bacteria,

phosphate-solubilizing bacteria (PSB), and mycorrhizal fungi, can lead to synergistic effects that further promote plant growth and soil health. This section delves into these interactions, highlighting their mechanisms and implications for sustainable agriculture.

Interaction with Nitrogen-Fixing bacteria

Nitrogen-fixing bacteria, such as *Rhizobium*, *Azotobacter*, and *Acinetobacter* species, convert atmospheric nitrogen into ammonia, a form usable by plants. When co-inoculated with KSB, these bacteria can synergistically enhance plant growth by simultaneously improving nitrogen and potassium availability. For instance, a study demonstrated that the combined application of *Acinetobacter guillouiae* (a nitrogen fixer) and *Acinetobacter calcoaceticus* (a potassium solubilizer) significantly improved shoot and root length, biomass, and chlorophyll content in onion plants compared to individual inoculations or control treatments .

Similarly, Basak and Biswas (2010) investigated the co-inoculation of *Bacillus mucilaginosus* (KSB) and *Azotobacter chroococcum* (nitrogen-fixing bacteria) on sudan grass grown in Typic Haplustalf soil. The study revealed that this microbial consortium, in the presence of waste mica as a potassium source, led to significantly higher biomass accumulation and nutrient acquisition compared to single inoculations or control treatments. These findings underscore the potential of integrating KSB with nitrogen-fixing bacteria to enhance plant growth and nutrient uptake.

Interaction with Phosphate-Solubilizing Bacteria

Phosphorus is another essential macronutrient often limited in soils. PSB, such as *Bacillus megaterium*, solubilize inorganic phosphate compounds, making phosphorus available to plants. The co-inoculation of KSB and PSB has been shown to enhance the availability of both potassium and phosphorus. In maize, dual inoculation with KSB and PSB led to increased microbial populations and enzyme activities in the rhizosphere, resulting in enhanced nutrient availability and plant growth. Similarly, in chamomile, the combined application of *Bacillus megaterium* (PSB) and *Frateuria aurantia* (KSB) with reduced fertilizer doses improved plant height, flower number, and dry weight, indicating the potential of such combinations in sustainable agriculture.

Han *et al*. (2006) evaluated the effect of co-inoculation with PSB (*Bacillus megaterium* var. phosphaticum) and KSB (*Bacillus mucilaginosus*) on pepper and cucumber. The study demonstrated that this microbial combination significantly increased the availability of phosphorus and potassium in the soil, leading to enhanced nutrient uptake and plant growth. These results highlight the synergistic potential of PSB and KSB in improving nutrient dynamics and crop performance.

Interaction with mycorrhizal fungi

Mycorrhizal fungi form symbiotic associations with plant roots, extending the root system and enhancing nutrient uptake, particularly phosphorus. While specific studies on the direct interaction between KSB and mycorrhizal fungi are limited, it's well-established that mycorrhizal associations enhance nutrient uptake, including potassium. The presence of KSB in the

rhizosphere can complement mycorrhizal functions by increasing the availability of potassium, which mycorrhizal fungi can then transport to the plant, suggesting a potential synergistic relationship that warrants further research.

Combined effects on plant growth and soil health

The integration of KSB with other beneficial microbes not only improves nutrient solubilization but also enhances overall soil health. For example, the co-inoculation of KSB and PSB in maize resulted in increased enzyme activities like dehydrogenase, urease, and phosphatase in the rhizosphere, indicating a more active and healthy soil microbial community. Such microbial consortia can reduce the reliance on chemical fertilizers, promoting sustainable agricultural practices.

4.6 Formulation, commercialization, and application of KSB biofertilizers

The effectiveness of potassium-solubilizing bacteria (KSB) in sustainable agriculture depends not only on their isolation and mechanistic potential but also significantly on their formulation, commercialization, and application methods. Formulating KSB as stable, viable, and scalable products is essential for their successful integration into agricultural practices.

Formulation involves incorporating live microbial cells into carriers that ensure their survival during storage and field application. Several studies have demonstrated the importance of carrier materials in maintaining microbial viability. For example, Basak and Biswas (2010) found that peat-based formulations preserved *Bacillus mucilaginosus* viability for over 180 days at ambient temperatures, whereas charcoal and vermiculite showed lower efficacy. In another study, Gopalakrishnan *et al*. (2015) used talc as a carrier and noted improved shelf life and colonization efficiency of KSB strains when used in chickpea rhizospheres, promoting growth and potassium uptake. Such results underscore the importance of optimizing carrier type and moisture content to enhance microbial fitness and functional performance.

Encapsulation technology has emerged as a superior approach to bioformulation. Pandey and Maheshwari (2013) developed sodium alginate-based beads to encapsulate *Frateuria aurantia*, a known KSB, and observed a higher survival rate and controlled release of viable cells in rhizosphere soil. This technique improved both microbial stability and colonization efficiency under field conditions. Similarly, Adesemoye *et al*. (2015) reported that encapsulated formulations of *Bacillus aryabhattai* not only extended shelf-life but also improved nutrient acquisition in tomato plants, suggesting that encapsulation can be a game-changer for biofertilizer commercialization in regions with erratic climate and storage challenges.

The commercialization of KSB-based products has gained momentum in recent years, especially in India and China, where the demand for low-cost, environmentally safe fertilizers is growing. Companies like IFFCO and Krishak Bharati Cooperative (KRIBHCO) have launched KSB-based formulations such as *Frateuria aurantia* biofertilizer and potassium mobilizing consortia targeting rice and wheat systems. According to Sharma *et al*. (2021), these products have shown promising results in multi-location field trials, increasing K uptake by 25–30% and yield by 12–

20% compared to control plots. Nevertheless, the success of these products hinges not only on microbial efficacy but also on awareness, farmer training, and policy support to encourage adoption.

In terms of application, KSB biofertilizers can be delivered through multiple methods, including seed coating, seedling root dipping, soil broadcasting, and fertigation. Each technique has specific advantages depending on crop type, soil texture, and agroecological conditions. For instance, Pindi and Satyanarayana (2012) reported that seed coating of chili (*Capsicum annuum*) with a talc-based *Bacillus mucilaginosus* formulation enhanced early root colonization and significantly increased fruit yield. In contrast, soil drenching methods were more effective for perennial crops like banana and citrus, where root surface exposure is limited.

Field experiments conducted by Verma *et al*. (2013) demonstrated that a dual inoculation approach - combining KSB with phosphate-solubilizing bacteria - resulted in synergistic effects, improving nutrient uptake, soil fertility, and plant biomass in maize. This highlights the potential of integrating KSB within consortia-based biofertilizer products. However, large-scale application also requires compatibility with existing farming practices and agricultural inputs, including irrigation methods, chemical fertilizers, and organic amendments. Compatibility studies, such as those by Ahmad *et al*. (2016), showed that KSB strains could withstand co-application with low doses of muriate of potash, indicating flexibility in use.

Despite these advancements, there remain challenges in maintaining microbial viability during transport, achieving consistent field performance, and registering microbial strains for legal commercialization. Regulatory frameworks in many countries, especially in Africa and Latin America, lack standardization, which hampers the global scale-up of KSB-based biofertilizers. Therefore, further research into strain stability, formulation science, and product validation under diverse climatic zones is essential for widespread adoption.

5. Challenges and Future Directions

The implementation of potassium-solubilizing bacteria (KSB) in sustainable agriculture presents considerable promise, yet several persistent challenges restrict their widespread use, efficacy, and market penetration. A primary issue lies in the variability of KSB performance under field conditions. Numerous studies have demonstrated that the efficiency of KSB observed in vitro or under greenhouse conditions does not always translate reliably to open-field scenarios. For instance, Basak and Biswas (2010) reported significant potassium solubilization and plant growth enhancement by *Bacillus mucilaginosus* under controlled conditions, but noted only marginal improvements under natural field settings, attributing the discrepancy to factors such as competition with indigenous microbial populations, climatic variations, and soil heterogeneity. Such findings highlight the pressing need for context-specific selection and testing of microbial strains.

Another major obstacle is the formulation and shelf-life stability of KSB-based products. The efficacy of biofertilizers is highly dependent on their ability to maintain viable cell counts over time without loss of functional properties.

Conventional formulations using talc or peat often suffer from rapid viability loss during storage and transportation, particularly in tropical climates. Gopalakrishnan *et al*. (2015) explored talc-based formulations and found a significant decline in viable cell counts beyond 90 days under ambient storage, limiting the utility for large-scale commercial distribution. While encapsulation techniques using alginate or starch matrices have shown potential in prolonging shelf-life and ensuring controlled release (Pandey and Maheshwari, 2013), such technologies remain cost-intensive and are yet to be widely adopted by small-scale biofertilizer producers.

Moreover, the absence of well-defined regulatory frameworks for microbial biofertilizers, especially in developing countries, has emerged as a substantial barrier to commercialization. In many regions, KSB products are marketed without mandatory validation of microbial content, efficacy, or strain identity, leading to inconsistent quality and skepticism among end-users. Sharma *et al*. (2021) emphasized that several commercially available KSB products in India contained lower-than-claimed viable cell counts or included unverified strains, pointing to the urgent need for stringent quality control mechanisms, certification processes, and legislative oversight.

From a scientific standpoint, incomplete understanding of the molecular mechanisms underlying potassium solubilization also hampers the development of more efficient KSB strains. While it is well-established that organic acid production plays a key role in mobilizing potassium from insoluble minerals, the genetic regulation of this process remains inadequately explored. Recent genome analyses of *Bacillus aryabhattai* and *Frateuria aurantia* have revealed potential gene clusters associated with acidogenesis and mineral dissolution, yet their functional characterization remains incomplete (Ahmad *et al*., 2016). Targeted research utilizing transcriptomics and proteomics could elucidate stress-responsive pathways and identify markers for strain selection under specific agroecological conditions.

Another concern is the compatibility of KSB with prevailing agricultural practices. KSB strains often exhibit reduced survival when applied in fields treated with chemical pesticides or excessive mineral fertilizers. Verma *et al*. (2013) demonstrated that the combined application of KSB and potassium chloride (KCl) at moderate doses improved plant growth and nutrient uptake in maize. However, higher doses of KCl suppressed microbial activity and neutralized the solubilizing effects, underscoring the need for precise integration within existing nutrient management systems.

Looking forward, research must focus on developing genetically robust and ecologically adaptable KSB strains through advanced biotechnological interventions such as CRISPR-based genome editing and adaptive evolution techniques. Additionally, low-cost, locally available carrier materials - such as sugarcane bagasse, fly ash, or rice husk ash - should be explored to design stable and farmer-friendly bioformulations. Furthermore, widespread farmer awareness campaigns and extension services must be deployed to bridge the knowledge gap between scientific research and practical application. Lastly, region-specific consortium-based formulations that combine KSB with other beneficial microbes, such as nitrogen fixers and phosphate solubilizers, could offer synergistic

effects that enhance overall soil fertility and crop productivity.

Addressing these multifaceted challenges through coordinated scientific, technological, and policy efforts will be pivotal for translating the laboratory success of KSB into field-level agricultural benefits, thereby contributing to long-term soil health and global food security.

6. Conclusion

Potassium solubilizing bacteria (KSB) represent a promising solution for enhancing potassium availability in soils, which is crucial for sustainable agricultural practices. The review presented above has discussed various facets of KSB, starting with the underlying biogeochemical processes that govern their function in soil. KSB play a key role in mobilizing potassium from insoluble mineral sources, thereby making this essential nutrient more accessible to plants. The diversity and taxonomy of these microorganisms reveal a vast array of bacterial species with different mechanisms for potassium solubilization, which are yet to be fully understood at the molecular level.

In terms of their application, KSB have demonstrated substantial benefits in greenhouse and controlled-environment studies, improving plant growth and yield in a variety of crops. However, translating these findings to the field remains a challenge due to environmental factors such as soil type, microbial community interactions, and climate variability. The performance of KSB biofertilizers often varies under different field conditions, highlighting the need for careful selection and optimization of microbial strains based on local agricultural practices.

Formulation, commercialization, and application of KSB biofertilizers have faced hurdles such as formulation stability, shelf-life, and regulatory issues. Several studies have indicated that current formulations, such as talc-based carriers, are not always effective in maintaining the viability of KSB during storage and application. Advances in formulation technologies, such as encapsulation and the use of organic materials, show promise in enhancing product stability, but these solutions still require cost-effective scaling for mass production. Furthermore, the regulatory landscape for KSB-based products remains underdeveloped, which may restrict the widespread use of these biofertilizers in global markets.

The integration of KSB into integrated nutrient management systems is a promising direction for future research. Studies suggest that combining KSB with other beneficial microbes, such as nitrogen-fixing bacteria and phosphate-solubilizing microorganisms, can lead to synergistic effects that further enhance soil fertility and crop productivity. Nevertheless, challenges related to microbial competition, environmental stress, and the lack of detailed molecular understanding of KSB mechanisms need to be addressed for more effective applications.

Future research should prioritize advancing the molecular understanding of KSB through genomics and proteomics, which will allow for the development of more efficient and resilient strains. Additionally, strategies aimed at improving the compatibility of KSB with conventional farming practices, such as chemical

fertilizers and pesticides, must be explored. Strain improvement and formulation innovation will be critical in overcoming the current limitations of KSB biofertilizers and enabling their widespread adoption by farmers.

In conclusion, while significant progress has been made in understanding and utilizing KSB for sustainable agriculture, challenges remain that must be overcome to realize their full potential. A concerted effort involving scientific research, technological innovation, regulatory development, and farmer education is essential to ensure the successful application of KSB in modern agriculture, ultimately contributing to more sustainable and resilient farming systems worldwide.

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