



Perspectives of Bio-Organic Fertilizer for Growth Promotion of Sorghum and Maize Under Variably Irrigated Field Conditions

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Abstract

*The ability of specific plant growth-promoting rhizobacteria (PGPR) to alleviate biotic and abiotic pressures on crop productivity is largely attributed to their ACC-deaminase activity. This research investigated how the inoculation of *Pseudomonas fluorescens* and the application of rock phosphate-enriched organic fertilizer (OF) influenced the development of maize and sorghum during periods of drought. To simulate moisture stress, irrigation was withheld during vital growth phases. Experimental treatments included 2, 3, and 5 irrigation cycles for sorghum, and 3 and 5 cycles for maize, using the 5-irrigation setup as the ideal moisture baseline. Data showed that for plants treated exclusively with synthetic fertilizers (uninoculated), growth plummeted as water availability decreased. Conversely, the introduction of *P. fluorescens* significantly offset the growth inhibition caused by water scarcity. The synergy between PGPR and OF emerged as the most successful strategy for enhancing biomass in both crops, particularly when irrigation was limited to only 2 or 3 applications. Remarkably, inoculated plants subjected to water stress exhibited superior growth performance compared to well-watered (5 irrigations) plants that lacked microbial inoculation.*

1. Introduction

Sustainable agricultural systems rely heavily on a consistent water supply. In Pakistan, this resource is becoming increasingly scarce; the country is currently categorized as "water-stressed," with projected irrigation deficits rising from 29% in 2010 to an estimated 33% by 2025 (Ministry of Environment Report 2005). These challenges are compounded by global climate shifts that render rainfall patterns unpredictable, threatening future food security. Consequently, identifying innovative methods to maximize water use efficiency (WUE) is a critical priority. A key biological driver for enhancing WUE is the development of extensive root systems, which allow plants to extract moisture more effectively from the soil profile. The

phytohormone ethylene plays a central role in modulating plant physiological behavior, particularly during environmental hardships (Zarembinski and Theologis, 1994; Arshad and Frankenberger, 2002; Wang et al., 2002). Drought conditions typically trigger an overproduction of endogenous ethylene, which unfortunately suppresses root elongation (Graves and Gladen, 1985; Mayak et al., 2004a). This inhibition restricts the plant's ability to access deep-soil water and essential minerals. Therefore, managing ethylene levels within the root zone under drought could potentially sustain root expansion and neutralize the growth-stunting effects of water scarcity. Ethylene biosynthesis in higher plants is directly regulated by its immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (Arshad and Frankenberger, 2002). Research suggests that plant growth-

promoting rhizobacteria (PGPR) equipped with the enzyme ACC deaminase can shield crops from environmental stressors like drought (Mayak et al., 2004a). This enzyme functions by breaking down ACC into ammonia and alpha-ketobutyrate, thereby preventing its transformation into ethylene (Glick et al., 1994). While numerous laboratory-scale studies have shown that ACC deaminase-producing microbes can boost root development (Shaharoon et al., 2008; Arshad et al., 2009), there is a significant research gap regarding their practical utility for water conservation in real-world field settings. In addition to microbial intervention, phosphorus (P) is vital for improving plant water acquisition. However, the high cost of synthetic P fertilizers necessitates the exploration of more economical alternatives. Rock phosphate (RP) offers a low-cost P source, though its solubility in soil is naturally limited. Integrating RP with organic waste during the composting process can enhance its availability, as the resulting organic acids help solubilize the phosphorus. Based on these considerations, this study was designed to evaluate the efficacy of *Pseudomonas fluorescens* (an ACC deaminase-producing PGPR) in enhancing the productivity of maize and sorghum. The research specifically looks at the interaction between this microbial inoculant and rock phosphate-enriched organic fertilizer (OF) under varying irrigation regimes in a field environment.

2. Materials and Methods

2.1. Preparation of Inoculum and Seed Inoculation

Previously isolated and screened PGPR strain *Pseudomonas fluorescens* (ACC14) containing ACC deaminase was used for seed inoculation of sorghum and maize (Shaharoon et al., 2006a). Dworkin and Foster salt minimal medium (Dworkin and Foster, 1958) containing ACC as substrate (N Source) was used for preparation of inocula. The sterilized broth was inoculated with *P. fluorescens* and incubated at $28 \pm 1^\circ\text{C}$ for 48 hours under shaking (100 rpm) conditions.

Seeds of sorghum (var. Sibbi) and maize (var. S-2002) were inoculated by mixing 100 g of seeds with peat-based inoculum. The peat-based inoculum consisted of 50g sterilized peat, 50g sterilized clay (50:50), 10ml sugar solution (15%), 10ml cultured broth and 10ml distilled water. The bacterial density in inoculum was 10⁹-10¹⁰ cells per gram of peat. Inoculated seeds were kept overnight for drying.

2.2. Field Experiments

Two Field experiment were conducted on sorghum and maize in $\frac{1}{2}$ acre area each. Variable irrigations were applied to the field. In case of sorghum 2, 3 and 5 no. of irrigations were applied to sub plots while maize crops, 3 and 5 irrigations were applied. Three treatments including i) NPK control (120:90:50, N: P: K respectively), ii) inoculation with *P. fluorescens* plus NPK and iii) inoculation with *P. fluorescens* + NPK + OF were tested under each irrigation. In the (iii) treatment the N, P & K contents of organic fertilizers were adjusted in their respective amounts applied as chemical fertilizer. RP enriched Organic fertilizers were prepared as explained by Ahmad et al., (2010).

3. Results

3.1. Effect of inoculation and organic fertilizer on growth of Sorghum under variable number of irrigations

Data regarding plant height as influenced by inoculation with *P. fluorescens* in the presence of chemical fertilizers (CF) or CF + organic fertilizer (OF) under variable number of irrigations are summarized in Table 1. Comparison of uninoculated treatments (CF only) under variable number of irrigations revealed that plant height decreased as number of irrigations reduced from 5 to 2. However, inoculation with *P. fluorescens* reversed the negative impact of less than optimum no. of irrigations. Interestingly, inoculation + CF proved most effective in case where 2 irrigations were applied followed by 3 and 5 irrigations in decreasing order. Inoculation enhanced plant height by 71, 67 and 20% over their respective uninoculated control under 2, 3 and 5 irrigations, respectively. However combined application of inoculation + CF + OF further enhanced the plant height up to 117, 104 and 36% over their respective uninoculated control under 2, 3 and 5 irrigations, respectively. It was worth noting that plant height recorded in response to inoculation with or without OF under 2 and 3 irrigations was more than that of found in case of uninoculated control (CF only) where 5 irrigations were applied.

The significant influence of inoculation with *P. fluorescens* in presence of CF or CF + OF on stem diameter of sorghum under variable no. of irrigations is evident from the data given in Table 1. In comparison of uninoculated treatments (CF only), it was recorded that maximum stem diameter was produced in case of maximum no. of irrigations (5) while the lowest stem diameter was observed with minimum no. of irrigations (2 irrigations). However,

Table 1. Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on plant height and stem diameter of sorghum

Treatments	Plant height (cm)			Stem diameter (cm)		
	2 Irr	3 Irr	5 Irr	2 Irr	3 Irr	5 Irr
NPK (Control)	101.3 i	113.6 h	135.6 g	4.0 e	4.3 de	4.5 d
NPK + <i>P. fluorescens</i>	173.6 e (71%)	190.0 c (67%)	162.0 f (20%)	6.0 c (50%)	6.2 c (44%)	6.1 c (35%)
NPK + <i>P. fluorescens</i> + OF*	220.0 b (117%)	231.0 a (104%)	184.0 d (36%)	7.2 ab (80%)	7.4 a (72%)	6.9 b (53%)

* The N, P & K content of organic fertilizer were adjusted in N, P & K fertilizer application, Percent increase over NPK control is indicated in parentheses.

inoculation with *P. fluorescens* had positive impacts on stem diameter under all the three irrigation levels. Inoculation + CF enhanced stem diameter by 50, 44 and 35% over their respective uninoculated control (CF only) under 2, 3 and 5 irrigations, respectively. Like other growth parameters, the combined application of inoculation + CF + OF was the most effective treatment in increasing the stem diameter as this treatment increased the stem diameter up to 80, 72 and 53% over their respective uninoculated control under 2, 3 and 5 irrigations, respectively.

The impact of inoculation with *P. fluorescens* in the presence or absence of OF on leaf parameters (No., length and width) under different numbers of irrigation is evident from the data summarized in Table 2. Results revealed that all the three parameters were significantly promoted by the inoculation + CF, however inoculation + CF + OF was the most effective treatment under all three levels of irrigations (2, 3 and 5 irrigations). Interestingly, inoculation + CF and inoculation + CF + OF had positive effect on all the three leaf parameters under 2 and 3 irrigations while under 5 irrigations the effects of these treatments were relatively diluted.

Data given in Table 3 show the effect of treatments including CF (control), inoculation + CF and inoculation + CF + OF on root growth parameters (elongation and dry weight) of sorghum grown under 2, 3 and 5 irrigations. It was observed that inoculation plus CF significantly increased the root parameters of sorghum under all the levels of irrigation as compared to uninoculated control (CF only) while the treatment, inoculation + CF + OF resulted in maximum root growth parameters under all the irrigation levels used. However, the efficacy of inoculation was more visible in case of 2 and 3 irrigations as compared to 5 irrigations.

Data illustrated in Figure 1 revealed the effect of CF alone, inoculation plus CF and inoculation + CF + OF on biomass of sorghum under different no. of irrigations. It was observed that inoculation in the presence or absence of OF significantly increased the biomass of sorghum under all the levels of irrigations, however impact of treatments was more obvious in case of 2 and 3 irrigations as compared to 5 irrigations. Moreover, combined use of inoculation + CF + OF proved most promising followed by inoculation + CF in enhancing the biomass over uninoculated control (CF only).

Table 2. Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on no. of leaves, leaf length and leaf width of Sorghum

Treatments	No. of leaves			Leaf length (cm)			Leaf width (cm)		
	2 Irr	3 Irr	5 Irr	2 Irr	3 Irr	5 Irr	2 Irr	3 Irr	5 Irr
NPK (Control)	6.0 g	6.3 g	7.3 f	62.7 c	65.6 c	71.0 b	4.0 c	4.1 c	4.8 c
NPK + <i>P. fluorescens</i>	9.0 d (50%)	9.6 d (52%)	8.3 e (13%)	79.0 b (25%)	81.5 b (24%)	74.3 ab (5%)	6.8 b (70%)	7.0 b (70%)	6.7 b (39%)
NPK + <i>P. fluorescens</i> + OF*	11.0 a (83%)	11.6 b (84%)	10.3 c (41%)	85.0 a (35%)	90.0 a (37%)	83.0 a (17%)	7.5 a (87%)	7.8 a (90%)	7.2 a (50%)

* The N, P & K content of organic fertilizer were adjusted in N, P & K fertilizer application, Percent increase over NPK control is indicated in parentheses.

Table 3. Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on root length, root fresh weight and root dry weight of Sorghum

Treatments	Root length (cm)			Root dry weight (g)		
	2 Irr	3 Irr	5 Irr	2 Irr	3 Irr	5 Irr
NPK (Control)	29 g	30 g	33 f	1.8 e	1.9 e	2.1 d
NPK + <i>P. fluorescens</i>	46 c (58.6 %)	44 d (46 %)	39 e (18 %)	2.9 b (59 %)	2.8 b (44 %)	2.4 b (16 %)
NPK + <i>P. fluorescens</i> + OF*	60 a (106.9 %)	58 b (93 %)	47 c (42 %)	3.8 a (108 %)	3.6 a (90 %)	2.9 b (39 %)

* The N, P & K content of organic fertilizer were adjusted in N, P & K fertilizer application, Percent increase over NPK control is indicated in parentheses.

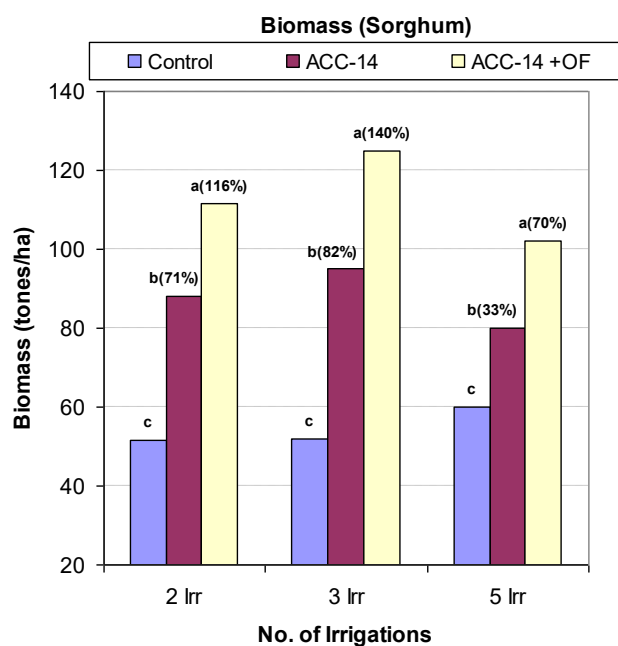


Figure 1. Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on biomass of Sorghum

3.2. Effect of inoculation and organic fertilizer on growth of maize under variable number of irrigations

The influence of inoculation with chemical fertilizer (CF) alone and inoculation + CF + OF on growth of maize under 3 and 5 irrigations, is evident from the data summarized in Table 4. The uninoculated treatments (CF only) under both the irrigations (3 & 5) showed less plant height in case of 3 irrigations compared to that recorded in 5 irrigations. However, this negative effect of less no. of irrigation was significantly diluted by inoculation with *P. fluorescens*. Inoculation was relatively more effective in promoting plant

height in case of 3 irrigations than in case of 5 irrigations. Inoculation enhanced the plant height by 46 and 17% over their respective uninoculated control (CF only) under 3 and 5 irrigations, respectively. However, the combined application of inoculation + CF + OF was more promising as it enhanced the plant height up to 71 and 50% over their respective uninoculated control under 3 and 5 irrigations, respectively. Interestingly, inoculated plants had more height under 3 irrigations than that of recorded under 5 irrigations in case of uninoculated control (CF).

Data summarized in Table 4 reflects the influence of inoculation with *P. fluorescens* with or without OF on stem diameter of maize under 3 and 5 irrigations. Comparison of uninoculated treatments (CF only) under 3 and 5 irrigations revealed that 3 irrigations resulted in minimum stem diameter compared to that recorded in 5 irrigations. This negative impact of less than optimum no. of irrigations was diluted by inoculation with *P. fluorescens* as it increased stem diameter by 47% and 23% over their respective uninoculated control under 3 and 5 irrigations, respectively.

However, combined application of inoculation + CF + OF further increased the stem diameter up to 70 and 41% over their respective uninoculated control under 3 and 5 irrigations, respectively.

Data regarding the impact of inoculation with *P. fluorescens* + CF in the presence or absence of OF on leaf parameters (No., length and width) under 3 and 5 irrigations are given in Table 5. Results showed that all the three parameters were significantly promoted by the inoculation both in presence of CF only and CF + OF, however inoculation + CF + OF was the most effective treatment under both the levels of irrigation in increasing the leaf parameters.

Table 4. Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on plant height and stem diameter of Maize

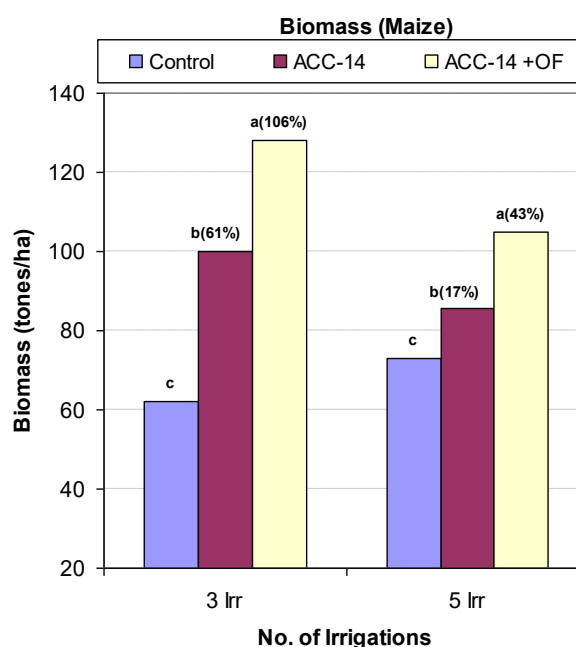
Treatments	Plant height (cm)		Stem diameter (cm)	
	3 Irr	5 Irr	3 Irr	5 Irr
NPK (Control)	137.3 c	145.0 c	4.6 c	5.1 c
NPK + <i>P. fluorescens</i>	201.0 b (46 %)	170.0 b (17 %)	6.8 b (47 %)	6.3 b (23 %)
NPK + <i>P. fluorescens</i> + OF*	241.0 a (71 %)	205.0 a (50 %)	7.8 a (70 %)	7.2 a (41 %)

* The N, P & K content of organic fertilizer were adjusted in N, P & K fertilizer application, Percent increase over NPK control is indicated in parentheses.

The effect of CF alone (control), inoculation + CF and inoculation + CF + OF on root growth parameters (elongation and dry weight) of maize under 3 and 5 irrigations is evident from the data given in Table 6. It was found that the root growth of maize was positively influenced by inoculation + CF under both 3 and 5 irrigations as compared to uninoculated control (CF only).

However, the combination of inoculation + CF + OF further enhanced root growth under both 3 and 5 irrigations. Interestingly, inoculation was more effective under 3 irrigations as compared to 5 irrigations.

The effect of chemical fertilizer (CF) alone, inoculation plus CF and inoculation plus CF plus OF on biomass of maize under 3 and 5 irrigations is evident from the data summarized in Fig. 2. Results revealed that inoculation + CF as well as inoculation + CF + OF significantly increased the biomass of maize under both 3 and 5 irrigations, however impact of treatments was more obvious in case of 3 irrigations as compared to 5 irrigations. Furthermore, the treatment, inoculation + CF + OF proved the most significant followed

**Figure 2.** Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on biomass of maize**Table 5.** Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on no. of leaves, leaf length and leaf width of Maize

Treatments	No. of leaves		Leaf length (cm)		Leaf width (cm)	
	3 Irr	5 Irr	3 Irr	5 Irr	3 Irr	5 Irr
NPK (Control)	7.0 c	8.0 c	65.0 c	69.0 c	4.5 c	5.2 c
NPK + <i>P. fluorescens</i>	10.0 b (42 %)	9.3 b (16 %)	82.0 b (26 %)	76.0 b (10 %)	6.8 b (51 %)	6.5 b (25 %)
NPK + <i>P. fluorescens</i> + OF*	12.0 a (71 %)	10.0 a (25 %)	92.0 a (41 %)	85.0 a (23 %)	7.9 a (75 %)	7.3 a (46 %)

* The N, P & K content of organic fertilizer were adjusted in N, P & K fertilizer application, Percent increase over NPK control is indicated in parentheses.

Table 6. Effect of inoculation with *P. fluorescens* and rock phosphate enriched organic fertilizer (OF) on root length, root fresh weight and root dry weight of Maize

Treatments	Root length (cm)		Root dry weight (g)	
	3 Irr	5 Irr	3 Irr	5 Irr
NPK (Control)	27 c	31 c	2.5 c	2.8 c
NPK + <i>P. fluorescens</i>	43 b (59 %)	39 b (25 %)	3.9 b (57 %)	3.5 b (25 %)
NPK + <i>P. fluorescens</i> + OF*	56 a (107 %)	46 a (48 %)	5.1 a (105 %)	4.2 a (47 %)

* The N, P & K content of organic fertilizer were adjusted in N, P & K fertilizer application, Percent increase over NPK control is indicated in parentheses.

by inoculation + CF in enhancing the biomass over control (CF only).

4. Discussion

Typically, plant growth is severely inhibited across various stress categories, with water-deficit stress being particularly detrimental to physiological development (Hussain et al., 2021). Drought conditions are closely linked to the accelerated biosynthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) and a subsequent surge in endogenous ethylene within the root system (Naneh Karan et al., 2022). This "stress ethylene" often results in suboptimal root architecture and a corresponding decline in shoot biomass (Rani et al., 2021).

Plant growth-promoting rhizobacteria (PGPR) possessing the enzyme ACC deaminase are capable of hydrolyzing ACC (the direct precursor of ethylene) into α -ketobutyrate and ammonia. This enzymatic cleavage effectively lowers ethylene concentrations in stressed tissues, thereby preventing growth inhibition (Glick, 2020; Gupta & Pandey, 2023). Our findings demonstrate that *P. fluorescens* containing ACC deaminase successfully enhanced the growth of maize and sorghum under diverse irrigation regimes. Notably, the synergy between *P. fluorescens* and organic fertilizer (OF) was more potent than inoculation in isolation. This treatment efficacy was most significant under deficit irrigation, whereas the impact was less distinct under full irrigation (5 cycles).

Enriched organic fertilizer represents a stable humic material derived from the composting of organic residues and nutrient fortification. Beyond providing essential nutrients and enhancing soil physical properties, OF has been shown

to amplify the stimulatory effects of microbial inoculants (Mahmood et al., 2021; Santoyo et al., 2021). Recent studies suggest that the efficacy of ACC deaminase-producing strains is significantly boosted when applied alongside organic amendments (Zhu et al., 2022; Amna et al., 2023). It is probable that the OF application not only fueled the metabolic activity of the inoculum but also enhanced nutrient bioavailability and overall soil health, leading to superior growth in maize and sorghum.

Furthermore, organic fertilizers modify the biological and chemical landscape of the soil, playing a pivotal role in maintaining long-term fertility and sustainable crop yields (Zhu et al., 2022). Recent literature confirms that compost-based products significantly improve soil water-holding capacity by optimizing pore size distribution and soil structure (Oelmann et al., 2021; Jiao et al., 2023). While the N, P, and K contributions of the OF were balanced against chemical fertilizer rates in this study, the biological and physical benefits of organic matter clearly provided an additive advantage under water-stressed conditions.

5. Conclusion

The field study demonstrates that inoculation with *Pseudomonas fluorescens* possessing ACC-deaminase activity significantly mitigates drought-induced growth suppression in maize and sorghum, particularly under reduced irrigation regimes. The combined application of PGPR with rock phosphate-enriched organic fertilizer consistently outperformed chemical fertilizer alone, even surpassing fully irrigated uninoculated controls. These findings confirm that integrating bio-organic fertilization with microbial inoculants is an effective and sustainable

strategy to enhance crop productivity and water-use efficiency under water-limited conditions.

6. Funding

This research received no external funding.

7. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

8. Authors' Contribution

Muhammad Rashid Waqas conceived the study and wrote the manuscript. Zia Shahid conducted the field experiments, while Muhammad Naeem Mushtaq performed the statistical analysis of the data. Sajid Mahmood Nadeem critically reviewed the manuscript, and Muhammad Shafique formatted the tables and assisted in data presentation.

9. SDGs Addressed

This study contributes to SDG 2 (Zero Hunger) by promoting sustainable crop productivity of maize and sorghum under water-limited conditions through bio-organic fertilization. It also supports SDG 6 (Clean Water and Sanitation) and SDG 13 (Climate Action) by improving water-use efficiency and enhancing crop resilience to drought stress using environmentally friendly agricultural practices.

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