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Effect of Plant Growth-Promoting Bacteria (Pgpb) and Chemical Fertilizer Co-Inoculation on Teff Growth, Yield, and Grain Nutrient Uptake Under Greenhouse Condition

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ABSTRACT

Application of native PGPB as bio inoculant is an alternative sustainable agricultural practice to enhance crop productivity, grain quality, and soil fertility also as conserve biodiversity. In this view, a study was to evaluate the effect of PGPB and chemical fertilizer co-inoculation on growth, yield, and grain nutrient uptake of teff varieties. A factorial experiment was laid out in a Completely Randomized Design (CRD) and replicated fourfold. A complete of 20 treatments were utilized in this study. The results revealed that the analysis of variance among treatment showed significant differences ($P \leq 0.001$) on most of the agronomic traits and grain P uptake of teff varieties while also significant differences ($P \leq 0.01$) for GYPP, grain Mg and Fe uptake. Whereas, interaction effects of two factors (TM*VT) were significant differences ($P \leq 0.01$) for PH and GYPP. Individual treatments mean comparison results showed that inoculation of native PGPB consortium significantly affected most of the PGP traits at ($P \leq 0.05$). The maximum traits like PH (189 cm), PL (66.7 cm), and NFT (4) were observed from Dz-01-196. Likewise, co-inoculation of native PGPB consortium with a half dose of chemical fertilizer significant difference at ($P \leq 0.05$) and markedly increased grain yield (5.25 g), SDBM (10.4 g), RDBM (2.91 g), grain N (1.99%), P (3.83%), and Ca (0.18%) uptake on Dz-01-974. Correlation analysis of GYPP among other PGP traits was showed that the grain yield per plant has a strong positive association with RDBM (r=0.86***). The present study showed that the half dose of chemical fertilizer inoculated with the consortium of native PGPB inoculants was advantageous to teff varieties production under limited chemical fertilizer inputs. Thus, the consortium of native PGPB might be used as inoculants to enhance growth, yield, yield-related traits, and grain nutrient uptake of both teff varieties also as save 50% chemical fertilizer by application of those strains with a half dose of chemical fertilizer. Further field evaluation would be necessary to form a conclusive recommendation of the study result.

INTRODUCTION

Teff *(Eragrostis tef (Zucc.) Trotter)* is an indigenous tropical cereal crop of Ethiopia and has been cultivated for thousands of years in Ethiopian highlands. It is a daily staple food for about 50 million Ethiopians accounting for 14% of all calories consumed. Teff is used to make injera, a delicious traditional fermented pancake. Its straw is extremely valued and used as feed for animals [1] besides the straw is incorporated with mud to strengthen and is used for plastering walls of homes. Teff has an excellent nutritional profile, being high in dietary fiber, iron, calcium, and carbohydrate and also has high levels of phosphorus, copper, aluminum, barium, thiamine, and excellent composition of amino acids essential for humans [2] Research has also shown that teff is free from gluten and may provide an alternate food source for people with celiac disease [3] .It is preferred both by farmers and consumers. Consumers prefer teff not only because it makes good quality "injera", but also because of its high protein and mineral content [4]. Farmers prefer cultivating teff to other cereals since it's more resilient to environmental stresses like poor soil drainage during moisture scarcity. Teff is adapted to the various environmental conditions and is widely grown from sea level up to 2800 m above sea level under various rainfalls, temperatures, and soil conditions [5]. In sustainable agricultural systems, it's imperative to use renewable inputs to improve crop productivity, ecological benefits, and minimize environmental hazards. The utilization of PGPB inoculants constitutes a biotechnological tool to enhance plant nutrition and mitigating the negative impact

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of conventional chemical fertilization [6]. Its application within the rhizosphere can increase plant-growth by the solubilization of phosphate, exudation of plant hormones, production of siderophores, production of the secondary metabolites, and production of lytic enzymes.

These organisms can improve the availability of deficient or immobile nutrients in soils after solubilizing their mineral forms to increase plant growth, yield, and nutrient uptake reported that teff seed inoculated with Phoshourous solubilizing bacteria significantly improve yield and nutrient uptake under greenhouse condition [7]. Similarly, indicated that teff seed inoculated with nitrogen-fixing bacterial isolates significantly increase growth, yield, and yield-related parameters [8]. Maize seeds were treated with *Bacillus megatherium, Pseudomonas sp., Burkholderia ambifaria, Enterobacter cloacae,* and *Pantoea ananatis*, getting to stimulate plant growth, and maintain or increase yields while reducing the need for nitrogen (N) fertilization [9]. Reported beneficial effects of bacterial consortia are an increase in plant yield, root biomass, water retention capacity, and nutrient availability [10]. The combination of appropriate rates of chemical fertilizer with microbial inoculants can have an enormous positive impact on soil quality and crop yield improvement [11]. However, there is no study previously conducted on the consequence of the chemical fertilizer and PGPB co-inoculation on plant growth, yield, and yield-related parameters, and also grain nutrient uptake of teff varieties under greenhouse conditions. Therefore, it's necessary to assess the impact of the PGPB and chemical fertilizer co-inoculation in crops to optimize the fertilization-growth and yield relationship. Supported the above target of this work was to assess the effect of either individual or consortia native PGPB inoculation with a half dose of chemical fertilizer to improve plant growth, yield, and yield-related traits also as grain nutrient uptake of teff varieties under greenhouse experimental condition.

MATERIALS AND METHODS

Study area

A greenhouse experimental trial was conducted at Debreziet Agricultural Research Center (DzARC) in Oromia National Regional State during 2019. The experimental site is located at 08° 44' N and 38° 58' E at an altitude of 1900 meters above sea level [12].

Soil sample collection

For greenhouse experimental trails composites soil samples were collected from the Boset district of the teff cultivated field at a depth of 0-20 cm using an auger. The collected soil samples were bagged, properly labeled, and transported to the DARC laboratory, the soil science department.

Soil laboratory analysis

The soil samples were analyzed for soil physical (texture) and chemical properties (pH, electrical conductivity, available phosphorous, organic matter, and total nitrogen) following standard laboratory analysis methods. Soil texture was determined by the hydrometer method [13]. Soil pH was determined by using a pH meter. Electrical conductivity of the soil extract was determined using an EC meter. The organic matter content of the soil was estimated from the organic carbon content which was determined using Degtjareff method [14]. Soil total Nitrogen determination was made by the macro Kjeldahl method [15]. Olsen method was used to determine the available phosphorous content of the soil [16].

Materials Used for experimental trail

The seed of two teff varieties named Magna (DZ-01-1960 and Dukem (Dz-01-974) were taken from DZARC. Three Potential PGP bacterial strains (*Pseudomonas fluorescens biotype G, Enterobacter cloacae ss disolvens, and Serratia marcescens ss marcescen*) and also chemical fertilizers (Urea and DAP) were used for greenhouse experimental trails.

Selection of potential PGP bacterial strains

PGPB strains selection was carried out based on three criteria such as plant growth-enhancing properties, biotic and a biotic stress tolerance performance during laboratory experimental evaluation.

Compatibility test

Compatibility among three PGPB strains was tested to formulate bacterial consortia. The method with slight modifications was used for in-vitro bacterial compatibility testing[17]. PGPB cultures were streaked on nutrient agar plates in such a way that for every single bacterial culture in the center of the plate, other cultures are streaked radiating from the center. The plates were incubated at 30°C for 48 h and the zone of inhibition was observed and recorded. The streaks did not produce an inhibition zone on nutrient agar medium shows that compatibility between PGP bacterial strains.

Bacterial inoculant preparation

Nutrient broth medium amended with 1% Carboxyl Methylcellulose (CMC) was prepared and inoculated with the selected three potential PGP bacterial strains alone or in a consortium and shake for 48 hrs in a rotary shaker. After shaking, the density

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of the culture was measured using a turbidimeter, microbial cell concentration of 10^6 to 10^8cfu mL^{-1}. Then the bacterial cultures were used for teff seed inoculation.

Seed surface sterilization and bacterial inoculation

Teff seeds were surface sterilized with 70% alcohol for 3 min and followed with 1% hypochlorite for 5 minutes and rinsed 5 times with sterile distilled water. The grown PGPB strains either single or in the consortium were mixed with surface-sterilized seeds of two teff varieties.

Treatment and experimental design

The treatment of the greenhouse experiment has consisted of three potential PGP bacterial strains as individual or consortium inoculated with or without chemical fertilizers (Phosphourous and Nitrogen). P. chemical fertilizer was applied at planting time and N. fertilizer was applied by splitting the dose into two half at planting and the remaining half at a mid tillering time. The experiments were laid out in factorial Complete Randomized Design (CRD) arrangement, replicated four times and form the following 20 treatments (Table 1).

Table 1: Different treatments used for greenhouse pot experiment.

T24 (Dz-01-196)	T36 (Dz-01-974)	T53 (Dz-01-1961)	TBCS (Dz-01-9741)
T36 (Dz-01-196)	T53 (Dz-01-974)	TBCS (Dz-01-1961)	FDCF (Dz-01-196)
T53 (Dz-01-196)	TBCS (Dz-01-974)	T24 (Dz-01-9741)	FDCF (Dz-01-974)
TBCS (Dz-01-196)	T24 (Dz-01-1961)	T36 (Dz-01-9741)	NI1 (Dz-01-196)
T24 (Dz-01-974)	T36 (Dz-01-1961)	T53 (Dz-01-9741)	NI2 (Dz-01-97)

Greenhouse pot experiment

The pot experiment was carried out under greenhouse conditions at the DzARC from May to July 2019. The minimum and maximum mean temperatures inside the greenhouse during the study period were 20 and 37 oc, respectively. Soil sample of about 150 kg was collected from local farm plots of Boset (115 km), where teff was produced for the past several years. The collected soil was sieved and sterilized at 121 oC and 15-pascal pressure. The soil was filled into the surface-sterilized plastic pots (18 cm, 9 cm). Surface sterilized seeds were inoculated with one of the selected bacterial strains as single and consortium during the time of sowing at a rate of 106 to 108 (1 ml) for every treatment. The treated seeds were shade dried and four seeds from each variety were then sown into each of the 80 pots. The number of seedlings in each pot was thinned down to one plant after ten days of emergence to reduce computation for different resources. All pots were watered using sterile distilled water regularly until the plants completely physiologically matured.

Agronomic data collection and measurement

At the physiological maturity plant growth, yield and yield component traits, and other data were collected before and after harvesting according.

Plant Height (PH)

Plant height was measured at physiological maturity from the ground level to the tip of the panicle from five randomly selected teff varieties in each plot.

Panicle Length (PL)

It is the length of the panicle from the node where the first panicle branches emerge to the tip of the panicle which was determined from an average of five randomly selected teff varieties per plot.

The Number of Fertile Tillers (NFT)

The number of fertile tillers was determined by counting the tillers.

Grain yield (Kg/ha-1)

Grain yield was measured by harvesting the crop from each pot.

GY = Grain yield plot (kg) × 10,000

Plot size (m2)

Methods of data analysis

All collected data were analyzed using the R software version 3.6 statistical analysis system following the appropriate procedures of Complete Randomized Design (CRD) in a factorial experiment. Analysis of variance (ANOVA) was conducted to test the significance levels of variables at $p<0.05$. A comparison of means was performed using the Least Significant Difference

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(LSD). Pearson's, multiple linear correlation coefficient (r) values were computed to examine the magnitude and direction of the relationship between grain yield per plant and other agronomic traits as well as grain nutrients uptake.

RESULTS

Soil analysis for physical and chemical properties

The data is presented in Table 1 showed that the composite soil samples used for the greenhouse experimental trial were clay, loam, and clay loam textures. The pH of the soils was found between 7.20 to 8.0 ranges, which are safe for teff cultivation. Soil E.C was also found between 0.04 to 0.15 dsm-1, which is a favorable range for all crop production. Total N of the soils was used for the greenhouse experimental trial found between 0.04 to 0.15 % ranges. The experimental soil of available phosphorus was found between 5 to 9.7 ranges. Also, the soil samples were categorized under low (0.47) and medium (2.17) organic carbon contents (Table 2).

Table 2: Physiochemical properties of soil used for greenhouse experimental trails.

Districts	Soil types	pH	E.C	TN	AV.P	% OC
Boset	Clay	7.20-7.30	0.04-0.05	0.04-0.05	5.00-5.12	1.16-1.33
	Loam	7.92-8.0	0.10-0.11	0.11-0.15	6.15-7.52	0.74-1.58
	Clay loam	7.40-7.97	0.03-0.13	0.12-0.15	6.10-9.76	0.46-1.33
	Silt loam	7.80-8.02	0.13-0.15	0.11-0.14	28.06-8.17	0.49-0.56
	Sandy loam	7.33-7.95	0.03-0.11	0.10-0.13	8.47-9.37	0.47-2.17

Selection of identified PGP microbial species for greenhouse evaluation

About 852 bacterial pure colonies were isolated and characterized for their plant growth-promoting traits (P-solubilization, ammonia production, and IAA production), bio-control properties (lytic enzymes production, hydrogen cyanide, and extra polysaccharide production), and a biotic stress tolerance ability (salinity, pH and temperature) Table 3. Three bacterial strains that fulfilled at least two PGPB properties during laboratory screening were selected for greenhouse experimental trials. Consequently, *Pseudomonas fluorescens biotype G, Enterobacter cloacaess disolvens,* and *Serratia marcescens ss marcescen* were selected.

Table 3: PGP traits of the Potential bacterial strains selected for greenhouse experiment.

S. No	Code of bacterial isolates	Plant growth-promoting properties			Biocontrol properties			A biotic stress tolerance properties		
		PS	IAA	NF	Pro	HCN	EPs	SL	pH	TP
1	Serretia marcescens ss marcescen (2^{nd}F(S))	+++	+++	+	+++	+	++	5	4,5,7 and 9	40
2	Pseudomonas fluorescent biotype G (2^{nd}F(E)	+++	+++	++	++	+++	+++	10	5,7 and 9	30
3	Enterobacter cloacae ss disolvens (2^{nd}F(S)	++++	++	+++	+++	+	+++	15	5,7	40
Note: ++++ (very strong), +++ (strong), ++ (medium), + (low) for PGP traits										

Effect of treatment and teff variety on teff agronomic traits (growth, yield, and yield-related parameter)

The result of the two-way analysis of the variance (ANOVA) showed that in Table3. Teff plant height, panicle length, shoot dry biomass, root dry biomass, and grain yield significantly affected by treatment (PGPB inoculants and chemical fertilizer) at 0.1% probability, while fertile tillers also significantly influenced at 5 % probability. However, the effect of teff variety statistically significant difference for grain yields per plant at 1%, while panicle length was significantly different at 5% probability. Treatment '*' variety interaction significantly influenced plant height and grain yield per plant at 1% probability, and also panicle length significantly affected at 5% probability.

Effect of treatment and teff variety on growth-related parameters Plant Height (PH)

The individual treatments' mean comparison result is presented in Table 4 showed that the height of both varieties (Dz-01-196 and Dz-01-974) were significantly influenced by the inoculation of PGP bacteria strains (*Serratia marcescens ss marcescens, Pseudomonas fluorescens biotype G,* and *Bacterial consortium*) over uninoculated control. The longest plant height (189.0 cm) was recorded from Dz-01-196 inoculated with the bacterial consortium, and the shortest height (163.3 cm) was recorded from uninoculated Dz-01-974. Similarly, effects of single or consortia of PGPB inoculants and chemical fertilizer co-inoculation result is presented in Table 5 revealed that the height of both varieties was also significantly influenced by *Serratia marcescens ss marcescens*, *Pseudomonas fluorescens biotype G*, and Bacterial consortium co-inoculated with a half dose of chemical fertilizer over uninoculated control. The longest height (187.3 cm) was recorded from Dz-01-974 co-inoculated with the bacterial consortium and half dose of chemical fertilizer, and the shortest height (163.3 cm) was recorded from uninoculated Dz-01-974.

Table 4: Mean square of treatment, variety, and its interaction effects on teff growth, yield, and yield-related parameters.

Source of variation	DF	Growth, yield, and yield-related parameters					
		PH	PL	NFT	SDBM	RDBM	GYPP
RP	2	15.2^{NS}	77.9^{NS}	0.35^{NS}	0.58^{NS}	0.31^{NS}	0.16^{NS}
TM	8	313.2***	334.3***	1.93*	9.5***	2.9***	7.8***
VT	1	10.7NS	93.4*	0.85NS	0.81NS	0.14Ns	1.7**
TM*VT	8	98.2**	38.8*	0.19Ns	0.56Ns	0.13NS	0.6**
Error	36	23.7	13.2	0.37	0.32	0.12	0.16
Note: *, **, ***: statistically significant at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001 probability level, respectively; NS: Not Significant							

Panicle Length (PL)

Based on individual treatment mean comparison result is presented in Table 4 showed that the PL of Dz-01-196 was significantly increased by inoculation of all PGP bacterial strains either alone or in a consortium, while PL of Dz-01-974 was significantly influenced by the inoculation of *Pseudomonas fluorescens biotype G, Enterobacter cloacae ss dissolvens* and bacterial consortium over the control. The longest PL (66.7 cm) was recorded from Dz-01-196 inoculated with the PGPB consortium and, the shortest PL (44.5 cm) was recorded from uninoculated Dz-01-974. Likewise, co-inoculation of either single or consortia of PGPB strains with a half dose of chemical fertilizer is given in Table 5. Treatments mean comparison result suggested that the PL of both varieties was significantly affected by *Serratia marcescens ss marcescens*, *Pseudomonas fluorescens biotype G*, and bacterial consortium co-inoculated with half-dose of chemical fertilizer over control. The longest PL (61.7 cm) was recorded from Dz-01-974 co-inoculated with the PGP bacterial consortium and half-dose chemical fertilizer and, the shortest PL (44.5 cm) was recorded from uninoculated Dz-01-94.

Table 5: Means of treatment and variety effects on teff growth-related characteristics.

PH	Teff growth-promoting traits					
	PH		PL		N FT	
	Magna	Dukem	Magna	Dukem	Magna	Dukem
Control	166.3^{c}	163.3^{c}	44.8^{c}	44.5^{c}	2.3^{c}	2.3^{b}
Serratia marcescens ss marcescens	179.7^{b}	175.3^{b}	58.0^{b}	48.3^{bc}	3.7^{ab}	3.3^{a}
Pseudomonas fluorescens biotype G	183.3^{ab}	176.7^{b}	61.3a^{b}	57.0^{a}	2.7^{bc}	3.0^{ab}
Enterobacter cloacae ss dissolvens	172.3^{c}	177.7^{b}	58.7^{b}	53.0^{ab}	3.0^{abc}	2.7^{ab}
Bacteria consortium	189.0^{a}	185.0^{a}	66.7^{a}	61.0^{a}	4.0^{a}	3.3^{a}
LSD (0.05) %	6.42	6.47	6.45	8.06	1.15	0.94
Treatment	**PH**		**PL**		**N FT**	
	Magna	**Dukem**	**Magna**	**Dukem**	**magna**	**Dukem**
Control	166.3^{d}	163.3^{d}	44.8^{d}	44.5^{c}	2.3^{c}	2.3^{b}
100% NP	179.7^{c}	176.3^{c}	53.0^{bc}	53.0^{b}	3.3^{ab}	3.3^{ab}
Serratia marcescens ss marcescens + 1/2 dose NP	177.7^{bc}	177.3^{bc}	49.7^{cd}	50.7^{bc}	3.3^{ab}	3.0^{bc}
Pseudomonas fluorescens biotype G + 1/2 dose NP	181.0^{ab}	181.7^{cd}	56.7^{ab}	49.0^{bc}	3.7^{a}	3.3^{ab}
Enterobacter cloacae ss dissolvens + 1/2 dose NP	173.0^{cd}	181.0^{ab}	53.7^{bc}	55.0^{ab}	3.3^{ab}	3.0^{bc}
Bacteria consortium + 1/2 dose NP	185.7^{a}	187.3^{a}	60.0^{a}	61.7^{a}	3.3a^{b}	4.0^{a}
LSD (0.05) %	7.23	6.88	5.45	7.53	1.05	0.73
Note: Different letters indicate significant differences at P ≤ 0.05 according to the LSD test. And NS: Not Significant						

Number of Fertile Tillers (NFT)

According to the individual treatments mean comparison result is presented in Table 4 showed that the NFT of both varieties were significantly affected by *Serratia marcescens ss marcescens* and bacteria consortium inoculation. The maximum number of the NFT (4) was recorded from Dz-01-196 inoculated with the bacterial consortium, and the minimum number of the NFT (2.3) was recorded from two untreated varieties. Similarly, the NFTs of both varieties were significantly affected by the co-inoculation of all PGP bacterial treatments with a half dose of chemical fertilizer. The maximum number of the NFT (3.7) was recorded from

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Dz-01-196 co-inoculated with *Pseudomonas fluorescens biotype G* and half dose of chemical fertilizer, and the minimum number of the NFT (2.1) was recorded from Dz-01-974 co-inoculated with *Enterobacter cloacae ss dissolvens* and half dose of chemical fertilizer.

Effect of treatment and teff variety on yield and yield-related parameters Shoot Dry Biomass (SDBM)

Individual treatment means comparison results are presented in Table 5 showed that SDBM of Dz-01-196 was significantly improved by the inoculation of *Pseudomonas fluorescens biotype G, Enterobacter cloacae ss dissolvens*, and Bacterial consortium. Whereas the SDBM of the Dz-01-974 was significantly affected by the inoculation of all PGP bacteria strains either alone or in a consortium. The maximum SDBM (9.98 g) was obtained from Dz-01-196 inoculated with the bacterial consortium, and the minimum SDBM (5.40 gm) was obtained from uninoculated Dz-01-974. Similarly, the SDBMs of both varieties were significantly influenced by all PGPB inoculants co-inoculated with a half dose of chemical fertilizer. The maximum SDBM (10.4 gm) was obtained from Dz-01-196 co-inoculated with the bacterial consortium and half dose of chemical fertilizer, and the minimum (5.40 gm) was obtained from uninoculated Dz-01-974.

Root Dry Biomass (RDBM)

Individual treatment means comparison result is given in Table 5 revealed that the RDBM of Dz-01-196 was significantly affected by inoculation of all PGPB inoculants either alone or in a combined, whereas the RDBM of Dz-01-974 was significantly influenced by inoculation of *Enterobacter cloacae ss dissolvens* and bacterial consortium over control. The maximum RDBM (2.89 gm) was obtained from Dz-01-974 inoculated with the PGPB consortium, and the minimum (0.61 gm) was obtained from uninoculated Dz-01-196. Likewise, the RDBM of both varieties were significantly affected by *Enterobacter cloacae ss dissolvens*, and the PGPB consortium co-inoculated with a half dose of chemical fertilizer. The maximum RDBM (2.91 gm) was obtained from Dz-01-974 co-inoculated with the PGPB consortium and half dose of chemical fertilizer, and the minimum (0.61 gm) was obtained from uninoculated Dz-01-196.

Grain Yield Per Plants (GYPP)

Individual treatments mean comparison result is presented in Table 6 showed that the GYPPs of both varieties were significantly influenced by the inoculation of all PGPB strains either alone or in a consortium. The maximum GYPP (4.55 gm) was obtained from Dz-01-196 inoculated with the bacterial consortium, and the minimum (1.20 gm) was obtained from uninoculated Dz-01-974, which exceeds 279 % over uninoculated pots. Likewise, the GYPPs of both varieties were significantly influenced by co-inoculation of all PGPB strains either alone or in consortium along with a half dose of chemical fertilizer. The maximum GYPPs (5.25 gm) was obtained from Dz-01-974 co-inoculated with the PGPB consortium and half dose of chemical fertilizer, and the minimum (1.20 gm) was obtained from uninoculated Dz-01-974.

Table 6: Means of treatment and teff variety on yield, and yield-related parameters.

Treatment	Teff yield and yield-related parameters					
	SDBM		RDBM		GYPP	
	Magna	Dukem	Magna	Dukem	Magna	Dukem
Control	6.30^{c}	5.4^{d}	0.61^{d}	0.71^{c}	1.25^{e}	1 .20^{d}
Serratia marcescens ss marcescens	7.4b^{c}	6.7^{c}	0.98^{c}	0.94^{c}	1.98^{d}	2 .64^{c}
Pseudomonas fluorescens biotype G	8.5^{b}	7.9^{b}	1.31^{c}	1.15^{c}	3.64^{b}	3.57^{b}
Enterobacter cloacae ss dissolvens	7.9^{b}	8.4^{b}	1.76^{b}	1.65^{b}	3.14^{c}	3 .40^{b}
Bacteria consortium	9.98^{a}	9.90^{a}	2.10^{a}	2.89^{a}	4.55^{a}	4.54^{a}
LSD 5%	1.35	1.1	0.33	0.48	0.38	0.7
Treatment	**SDBM**		**RDBM**		**GYPP**	
	Magna	**Dukem**	**Magna**	**Dukem**	**Magna**	**Dukem**
Control	6.3^{e}	5.4^{e}	0.61^{c}	0.71^{c}	1.25^{d}	1.20^{d}
100 % NP	8.1^{bc}	8.1^{b}	0.94^{bc}	0.97^{bc}	3.0^{c}	2.01^{cd}
Serratia marcescens ss marcescens + ½ dose NP	7.9^{c}	7.3^{d}	0.87^{bc}	0.81^{bc}	2.93^{c}	2.49^{c}
Pseudomonas fluorescens biotype G + ½ dose NP	7.2^{d}	7.7^{c}	0.88^{bc}	0.91^{bc}	3.95^{b}	4.09^{b}
Enterobacter cloacae ss dissolvens + ½ dose NP	8.7^{b}	8.2^{b}	1.27^{b}	1.67^{bc}	2.92^{c}	4.73^{ab}
Bacteria consortium + ½ dose NP	9.99^{a}	10.4^{a}	2.60^{a}	2.91^{a}	4.83^{a}	5.25^{a}
LSD (0.05) %	0.79	0.25	0.76	0.85	0.57	0.96
Note: Different letters indicate significant differences at P≤0.05 according to the LSD test. And NS: no significant difference						

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Effects of treatment and teff variety on grain nutrient uptake

The result of the analysis of variance (ANOVA) is presented in Table 7 showed that the teff grain phosphorus uptake was significantly affected by treatment at 0.1% probability, while grain nitrogen and calcium uptake were significantly influenced at 1% probability. Whereas teff grain magnesium and iron uptake were significantly influenced by the teff variety at 1% probability, and also grain nitrogen, potassium, and zinc uptake were significantly affected by teff variety at 5 % probability.

Table 7: Mean square of treatment and variety effects on tef grain nutrient uptake.

SOV	D.F	N%	P%	K%	Mg%	Ca%	Zn%	Fe%
TM	8	0.04**	1.72***	0.002NS	0.002Ns	0.004**	0.0001NS	0.0003NS
VT	1	0.03*	0.27NS	0.01*	0.003**	0.0024NS	0.0002*	0.01**
Error	8	0.003	0.24	0.001	0.0001	0.0003	0.0001	0.0004
Note: *, **, ***: statistically significant at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001 probability level respectively; NS: Not Significant								

Effect of treatments on teff grain uptake

EThe experimental data are given in Table 8 showed that inoculation of all PGPB strains either alone or in combination was significantly influenced teff grains nitrogen uptake over control. The maximum grain nitrogen (1.91 %) uptake was recorded from a variety inoculated with *Enterobacter cloacae ss dissolvens*, and the minimum grain nitrogen (1.42 %) uptake was recorded from the uninoculated one. Similarly, co-inoculation of either single or combined PGPB with a half-dose of chemical fertilizer significantly affected grain nitrogen uptake. The maximum grain nitrogen (1.99 %) uptake was recorded from the variety co-inoculated with *Pseudomonas fluorescens biotype G* and half dose of chemical fertilizer, while the minimum grain nitrogen (1.42 %) uptake was recorded from uninoculated treatment.

Table 8: Means of treatment on tef grain nutrients content improvement.

Treatment	N%	P%	K%	Mg%	Ca%	Zn%	Fe%
Control	1.42 b	0.67c	0.44a	0.09a	0.06b	0.00a	0.00a
Serratia marcescens ss marcescens	1.78 a	2.44b	0.36a	0.12a	0.07b	0.00a	0.00a
Pseudomonas fluorescens biotype G	1.76a	2.18b	0.42a	0.14a	0.16a	0.01a	0.05a
Enterobacter cloacae ss dissolvens	1.92a	2.07b	0.31a	0.13a	0.09b	0.01a	0.00a
Bacteria consortium	1.86a	3.80a	0.45a	0.14a	0.15a	0.02a	0.05a
LSD (0.05)	0.17	0.6	0.17	0.09	0.09	0.01	0.01
Treatment	N%	P%	K%	Mg%	Ca%	Zn%	Fe%
Control	1.42 c	0.67c	0.44a	0.09a	0.06b	0.00a	0.04a
100% NP	1.68b	0.33c	0.38a	0.10a	0.04b	0.03a	0.01a
Serratia marcescens ss marcescens + ½ dose NP	1.82ab	2.44b	0.36a	0.10a	0.11b	0.00a	0.02a
Pseudomonas fluorescens biotype G+½ dose NP	1.87ab	2.78ab	0.44a	0.11a	0.17a	0.05a	0.05a
Enterobacter cloacae ss dissolvens +½ dose NP	1.89a	3.63b	0.43a	0.13a	0.07b	0.01a	0.05a
Bacteria consortium +½ dose NP	1.99 a	3.83a	0.47a	0.13a	0.18a	0.05a	0.06a
LSD (0.05)	0.19	1.2	0.1	0.05	0.09	0.01	0. 10
Note: Different letters indicate significant differences at P≤0.05 according to the LSD test. And NS: No Significant difference							

The grain nutrients analysis data is given in Table 8 suggested that the inoculation of PGPB either alone or in consortium significantly improved grain phosphorus uptake. The maximum grain phosphorus (3.80%) uptake was recorded from a variety of inoculated with bacteria consortium, and the minimum grain phosphorus (0.67%) uptake was recorded from uninoculated treatment. Similarly, co-inoculation of either single and consortium PGPB with a half dose of chemical fertilizer significantly influenced grain phosphorus uptake. The maximum grain phosphorus (3.85 %) uptake was recorded from variety co-inoculated with PGPB consortium and half dose of chemical fertilizer, and the minimum grain phosphorus (0.67 %) uptake was recorded from uninoculated pots.

The results are given in Table 8 showed that the grain calcium uptake was significantly affected by the inoculation of PGPB

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either alone or combined. The highest grain calcium (0.16 %) uptake was recorded from the variety inoculated with *Pseudomonas fluorescens biotype G*, and the minimum grain calcium (0.06 %) uptake was recorded from uninoculated pots. Similarly, grain calcium uptake significantly affected by *Serratia marcescens ss marcescens, Pseudomonas fluorescens biotype G*, and PGPB consortium co-inoculated with a half dose of chemical fertilizer. The maximum grain calcium (0.18 %) uptake was recorded from the variety co-inoculated with PGPB consortium and half dose of chemical fertilizer, and the minimum (0.06 %) uptake was recorded from uninoculated plots. Whereas also grain potassium, magnesium, zinc, and iron uptake was no significantly influenced by PGPB inoculation with or without chemical fertilizer in Table 8, and only grain calcium uptake significantly affected by co-inoculation of *Enterobacter cloacae ss dissolvens* with a half dose of chemical fertilizer.

Correlation of grain yield per plants among other agronomic traits

The relationship of teff grain yield per plant among plant-growth-promoting and yield-related traits is useful while selecting traits for yield improvement. To determine the association between grain yield per plant with growth and yield-related parameter we calculated the coefficient of correlation (r). Data is presented in Figure 1 showed that teff grain yield per plants had the strongest relation with shoot dry biomass (r=0.80***), root ground dry biomass (r=0.68***), panicle length (r=0.62***), plant height (r=0.60***), also found positive association with number of fertile tillers (r=0.34*).

DISCUSSION

Application of native PGPB as bio inoculant is an alternative sustainable agricultural practice to improve crop productivity, grain quality, and soil fertility also as conserve biodiversity. Their co-inoculation with a half dose of chemical fertilizer increased fertility of the rhizosphere soil and resulted in more efficient plant nutrient uptake from the soil. Our study correlates with the same trend of PGPB co-inoculation with chemical fertilizer to improve soil fertility as well as to increase the plant growth and its biomass [6]. Similarly reported that the integrated effect of plant growth-promoting rhizobacteria, phosphate solubilizing bacteria, and chemical fertilizers on the growth of Maize [18]. The effect of co-inoculation of nitrogen-fixing and phosphate is solubilizing microorganisms in combination with chemical fertilizers on the growth and development of rice [19]. Whereas, the side effects of chemical fertilizers on soil health could be mitigated by the use of PGPB inoculants with a half dose of recommended chemical fertilizer. Furthermore, in the present study, PGPB was used with a half dose of chemical fertilizer; thus, it is an environmentally-friendly technology that can minimize soil pollution and maximize crop returns. In this study, we used either a single or consortium of three potential native PGPB strains such as *Serratia marcescens ss marcescens*, *Pseudomonas fluorescens biotype G*, and *Enterobacter cloacae ss dissolvens* with or without amendment of chemical fertilizer to improve plant growth, yield, and grain nutrient uptake. They are the most studied and possess multiple plant growth-promoting properties like inorganic phosphate solubilization, nitrogen fixation, IAA production, ammonia production, hydrogen cyanide production, and tolerance of a biotic stress Table 2. Analysis variance result presented in Table 3 showed that inoculation of the native PGPB either alone or consortium significantly improved most of the plant growth, yield, yield-related traits, and grain nutrient uptake. Similarly, the treatment's mean comparison results in Table 4 showed that inoculated with consortia of native PGPB inoculants significantly increased plant growth-related characteristics over a single inoculated one. The maximum plant growth-related traits like PH (189 cm), PL (66.7 cm), and NFT (4) were observed from Dz-01-196. These significant enhancements of the plant growth-promoting traits of PGPB might be linked with their PGP traits recorded under the in vitro experiments in Table 2 as well as synergistic effects due to co-inoculation. Inoculation with a consortium of several bacterial strains superior to inoculation with individual strains [20]. It may be due to plant growth-regulating hormone-producing capability which might have led to enhancing cell division and cell elongation, resulting in higher plant growth parameters. *Pseudomonas fluorescens* release regulatory hormone-like IAA that significantly influences cell division and enhances cell enlargement in crop plants [21].

Likewise, the treatment's mean comparison result is presented in Table 5 showed that co-inoculation of native PGPB consortium along with a half dose of recommended chemical fertilizer was significantly improved yield, yield-related traits, and grain nutrient uptake of teff variety. The maximum SDBM (10.4g/p), RDBM (2.91g/p), GYPP (5.25 g/p), grain N (1.99%) and P (3.83%) uptake were obtained on Dz-01-974. This might be due to higher Phosphate solubilization and/or atmospheric nitrogen fixation by PGPB inoculants as well as synergistic effects of PGPB strains and chemical fertilizer. *Pseudomonas fluorescens biotype G* and *Serratia marcescens ss marcescens* would have caused more mobilization and solubilization of insoluble phosphate in the soil and improve the availability of phosphorus to plant [22]. Co-inoculation of plant growth-promoting rhizobacteria along with an economic dose of chemical fertilizer significantly increased wheat yield by 9.4%, biomass by 9.2% relative to the uninoculated control [23]. The results also revealed that the co-inoculation of plant growth- promoting rhizobacteria at different levels of chemical fertilizer had a significant role for different yield attribute characters of wheat [24]. The application of different microbial inoculants in combination with 50% recommended chemical fertilizer resulted in a 2 to 6% increase in yield [25]. An adequate combination of microbial inoculants with rock-based fertilizer improved grain yield in maize under conditions of glass-house [26]. Compared to 100% recommended chemical fertilizer, PGP bacterial strain either single or consortium co-inoculated with a half dose of chemical fertilizer significantly increased average grain yield per plant by 29% and 50% respectively. A high dose of recommended chemical fertilizer might be suppressing beneficial microbial activities in the rhizosphere. It is an opportunity for poor farmers with low investing capacity on fertilizer may optimize for the integrated use of PGPB inoculants with a lower rate of

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chemical fertilizers for getting a higher yield. Furthermore, in other section of this study, the result of the present study showed that teff variety co-inoculated with PGPB consortium along with a half dose of chemical fertilizer significantly increased grain Phosphorus (3.83%) uptake, nitrogen (1.99%), and calcium (0.18%) uptake over the uninoculated one. Wheat inoculated with different bacterial combinations increased plant N content from 40.7 to 97.7%, P content from 41.2 to 96.4%, and K content from 2.3 to 42.1% [27]. This might be due to the effect of the application of PGPB having properties such as atmospheric nitrogen fixation and phosphate solubilizing capability. Higher nutrient uptake in plants could be attributed to effective translocation of nutrients due to better biological nitrogen fixation and P solubilization by the introduced microbial inoculants which conform to the findings of Mohandas. Correlation analysis of GYPP among other PGP traits was showed that the grain yield has a strong positive association with RDBM (r= 0.86***). The present study showed that half the dose of chemical fertilizer inoculated with the consortium of PGPB inoculants was beneficial to teff varies cultivation under limited chemical fertilizer inputs. Thus, the consortium of native PGPB inoculants could be used as biofertilizer to improve growth, yield-related traits, yield, and grain nutrient uptake of both teff varieties also as save 50% chemical fertilizer by application of these strains. Further field evaluation would be necessary to make a conclusive recommendation.

CONCLUSION AND RECOMMENDATION

The result is present in the study indicated that inoculation of either individual or consortium PGPB strains along with a half dose of chemical fertilizer significantly increased plant height, panicle length, shoot dry biomass, grain yield and straw yield, N, P, S, Ca, and Fe contents of the over uninoculated and 100% recommended chemical fertilizer received treatment. This study suggested that the use of PGPB inoculants with a half dose of chemical fertilizer as inputs could be an efficient approach to save 50% chemical fertilizer application without affecting the environment and human health as well as biodiversity. Further investigation was conducted using these potential PGPB inoculants along with half-dose recommended chemical fertilizers under field conditions, and are needed to clarify its role as bio fertilizers that exert beneficial effects on plant growth and development also as improve grain quality.

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