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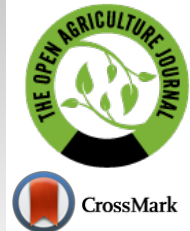


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RESEARCH ARTICLE

Impacts of Microbial Inoculants on the Growth and Yield of Maize Plant

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Abstract:

Background:

The use of microbial inoculants holds a great promise to improve crop yield without the negative environmental and health hazard associated with chemical fertilizer.

Aim:

To investigate if *Pseudomonas* spp. (*Pseudomonas kilonensis* F113 and *Pseudomonas protegens* CHA0 strains) have promoting effects on vegetative growth and yield of different maize genotypes (viz. AFLATOXIN SYN 4W, TZB-SR, AFLATOXIN R SYN 2Y, AFLATOXIN SYN 3W and AFLATOXIN SYN-2Y) under different soil types.

Methods:

Both pot and field experiments were employed. Bacterialized seeds were sown (2 seeds/pot/stand).

Results:

Pot experiment showed that both the bacterial species significantly stimulated the growth of maize shoot length, stem girth, leaf length, root length and root weight. The effect of genotypes AFLATOXIN SYN 4W, TZB-SR, AFLATOXIN R SYN 2Y and AFLATOXIN SYN 3W are not significantly different from one another but AFLATOXIN SYN-2Y showed a significantly lower increase in the measured parameters. No significant difference was observed according to soil types. AFLATOXIN SYN 4W showed a significantly higher root weight while AFLATOXIN R SYN 2Y showed a significantly higher root length compared to the other maize genotypes. Moreover, *Pseudomonas* significantly increased maize growth and yield under field experiment. AFLATOXIN R SYN 2Y and AFLATOXIN SYN 4W showed a significantly higher yield than the other maize genotypes studied.

Conclusion:

We concluded that *Pseudomonas kilonensis* F113 and *Pseudomonas protegens* CHA0 are potential biofertilizers.

Keywords: Biotechnology, Biofertilizers, Maize genotype, Microbial inoculants, *Pseudomonas* spp., Soil.

Article History

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1. INTRODUCTION

Maize (*Zea mays* L.) is a common cereal that is intensively cultivated worldwide [1]. Maize genotypes differ in starch structures and composition of maize kernels (content of amylase and/or amylopectin), grain filling rate, type of endosperm, *i.e.* floury (dent) vs. horny (flint) and in earliness and rate of maturation [2]. Maize genotypes can also differ in a

number of plant growth characters [3] or drought stress resistance ability [4].

Numerous agricultural soils worldwide are deficient in plant nutrients. Hence, significant fertilizer requirement is a major challenge for sustainable food production. Previously, these plant nutrients were provided solely in the form of synthetic chemical fertilizers. Such chemical fertilizers are quite expensive and increase crop production cost. In addition, chemical fertilizers lead to soil degradation and pose health hazard to both human and farm animals [5]. It is therefore imperative

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to improve soil fertility, while at the same time preventing associated negative environmental effects of chemical fertilizers.

Microbial inoculants are of growing interest for their potential role in improving soil fertility and enhancing an increase in crop yields and their nutrient contents. Microbial inoculants are the formulations composed of beneficial microorganisms that play important role in every ecosystem. When applied to seeds, soil or seedlings, microbial inoculants improve directly or indirectly the nutrient availability to the host plant and promote plant growth [6, 7]. They hold a great promise to improve crop yield (Isfahani and Besharati 2012). In the present agricultural practices, there is a number of beneficiary soil microorganisms used as inoculants. They include *Pseudomonas* sp., *Azospirillum*, *Azotobacter* and *Phosphobacterium* among others [8, 9]. Microbial inoculants improve plant growth through a number of mechanisms which include the production of plant hormones, the supply of nutrients and the suppression of various crop pests [10].

Pseudomonas is a predominant group of rhizosphere soil colonizing bacteria, which have been reported to promote plant growth [11]. *Pseudomonas* spp. rhizosphere strains have been described as effective phosphate solubilizers [12]. Verma et al. [13], noted that *Pseudomonas putida* strain BHUPSB04 solubilized tricalcium phosphate in soluble form by producing organic acid. Likewise, co-inoculation of *Galega orientalis* Lam. with root-colonizing *Pseudomonas* species (*Pseudomonas trivialis* strain 3Re27 and *Pseudomonas extremorientalis* strain TSAU20) and *Rhizobium*, resulted in enhanced root and shoot mass [14]. However, there has been very little reports on the impact of plant genotypes and soil types on the growth promoting potential of *Pseudomonas* spp.

In this investigation, we therefore aimed to determine if *Pseudomonas* spp. (*Pseudomonas kilonensis* and *Pseudomonas protegens* strains) have promoting effects on vegetative growth and yield of different maize genotypes under different soil types through the analysis of some indicating factors, such as root length, plant height, root weight and yield of maize plants.

2. MATERIALS AND METHODS

2.1. Plant Material

Seeds of maize cultivars (Aflatoxin SYN 4W, TZB-SR, Aflatoxin R SYN-2Y, Aflatoxin SYN 3W and Aflatoxin SYN-2Y) used in this investigation were obtained from the In-

ternational Institute of Tropical Agriculture, Ibadan, Nigeria. The seeds were sterilized with 70% ethanol for 2 minutes and washed with sterile distilled water.

2.2. Microbial Inoculant Preparation

P. kilonensis and *P. protegens* isolates selected for this study were obtained from the Microbial Ecology laboratory (C. Prigent-Combaret). The bacterial isolates were cultured for 48 hrs in Luria-Bertani Broth (LB) medium, and then cells were harvested by centrifugation at 12000g for 10 minutes. Each of the *P. kilonensis* and *P. putida* was suspended in sterile distilled water and the concentration adjusted to give 10^8 cells/ml [15].

Ten grams of pectin were suspended in 1 L each of *P. kilonensis* and *P. protegens* suspensions, which were shaken for 10 min on a magnetic stirrer plate. Maize seeds were imbibed at the ratio of 500 g/L microbial solutions for 16 hrs.

The bio-inoculated seeds were then air-dried on filter paper for 1 hr before sowing. Another group of surface-sterilized maize seeds (70% ethanol for 2 min) were prepared as control treatments [16].

2.3. Collection of Soil Samples

Soil samples were collected randomly from Mafikeng and Itsoseng areas in South Africa. Mafikeng is located at 25°50'S, 25°38'E. The soil was grey loamy sand with 84% sand, 3% clay and 13% classified as Hutton and Itsoseng is located at 26°08'S, 25°86'E [17]. The soil was red sandy loam with 6.5% silt, 9.8% clay 83.6% sand classified as Ferrallic Arenosols [17]. Using the random sampling method, auger samples (5kg) were collected from each sampling units at 0-15 cm. The soil samples collected from each of the sampling sites were bulked and transported to the laboratory in well labelled polyethylene bags. The core samples were then air dried for 3 days and passed through 2 mm sieve in the preparation for analysis.

2.4. Determination of Physical and Chemical Properties of Soil of Study Sites.

The physical and chemical properties measured include: pH using Kent pH metre model 7020, organic matter content using the wet oxidation method as described by [18]. The hydrometer method of Gee and Or. was employed in the determination of particle size [19]. Total nitrogen was estimated by the macro Kjeldahl method [20], available phosphorus (P) was determined by Bray-1 extraction method [21]. To determine E.C.E.C. the method of Chapman was employed [22]. These are shown in Table 1.

Table 1. Physical and chemical characteristics of the soils.

Physical and Chemical Properties of Soils	Itsoseng Soil	Mafikeng Soil
pH (H ₂ O)	6.44± 0.11	6.81± 0.03
OM (%)	0.79± 0.03	1.66± 0.06
P (mg/kg)	5.72± 0.04	1.39±0.04
N (%)	0.06± 0.01	0.11±0.02
Na ⁺ (cmol(+)/kg)	2.80± 0.03	2.60±0.02
K ⁺ (cmol(+)/kg)	3.25± 0.03	3.25±0.07
Ca ²⁺ (cmol(+)/kg)	2.60± 0.02	2.55± 0.02

(Table 1) contd....

Physical and Chemical Properties of Soils	Itsoseng Soil	Mafikeng Soil
Mg ⁺ (cmol(+)/kg)	1.20± 0.01	0.20±0.02
Exchangeable acidity (cmol(+)/kg)	3.68± 0.08	2.24±0.02
ECEC (cmol(+)/kg)	13.53± 0.1	10.84± 0.03
% Silt	6.5± 0.03	13.0±0.27
% Clay	9.88± 0.04	2.88± 0.02
% Sand	83.62± 0.04	84.12±0.03
Class	Sandy Loam	Loamy Sand

Table 2. Phenotype characteristics of maize seed used.

Genotype	Grain Colour	Grain Size	Type
Aflatoxin Syn-2Y	Yellow	Small	Flint
Aflatoxin SYN 4W	White	Very small	Flint
TZB-SR	White	Small	Flint
Aflatoxin R Syn-2Y	Yellow	Small	Flint
Aflatoxin SYN 3W	White	Small	Flint

2.5. Plant Growth Promotion Studies Under Screen House

A pot experiment was laid in a Randomized Complete Block Design (RCBD) split plot arrangement. The main plot was *Pseudomonas* spp. (*P. kilonensis*, *P. protegens* CHAO and non-inoculated control) applied as seed treatment while the sub plots were maize genotypes (AFLATOXIN SYN 4W, TZB-SR, AFLATOXIN R SYN 2Y, AFLATOXIN SYN 3W and AFLATOXIN SYN-2Y 4W, TZB, AR, 3W and 2Y) and 2 soil types (Itsoseng soil and Mafikeng soil). Bacterialized seeds were sown (2 seeds/pot) in 35cm-diameter pots filled with 12 Kg of sterilized soil. Soil was wet sterilized in an autoclave at 121°C for 20 minutes. This is to ensure that only the inoculant introduced influence the sown seed. Only one plant per pot was maintained after germination. All treatments were in triplicates. Plants were irrigated daily at 6.00 hr and 18.00 hr using a watering can. Neither inorganic fertilizer nor pesticide was applied. Physical and chemical properties of the studied soils are shown in Table 1. While the phenotype characteristics of seeds used are recorded in Table 2.

2.6. Growth Parameter Data Collection

Growth parameters (plant height (cm) using a measuring tape, leaf length (cm) using a measuring tape, plant girth (cm) using a thin thread whose length was measured on a ruler, root length (cm) *i.e.* length of the longest root using a measuring tape and root dry weight (g) per plant) were recorded at 12 weeks after planting. To obtain the root dry weight, the below ground portion of the plant was cut and washed to remove attached soil particles. The root was then air dried to a constant weight and the weight was recorded.

2.7. Assessing Plant Growth Promotion of *Pseudomonas* Spp. Under Field Condition

A field experiment was conducted between September, 2015 and February 2016 at North-West University, Mafikeng, (25°50'S, 25°38'E) South Africa. The *Pseudomonas* spp. tested were *P. kilonensis* F113 and *P. protegens* CHAO. Maize genotypes used include Aflatoxin SYN 4W, TZB-SR, Aflatoxin R SYN 2Y, Aflatoxin SYN 3W and Aflatoxin SYN-2Y. The soil

of the study site was a loamy sand soil, slightly acidic with organic matter value of 1.66%, available phosphorus 1.39PPM, total nitrogen was 0.11 and effective cation exchange capacity was 10.84 (Table 1).

2.8. Experimental Layout

The experimental design was a randomized complete block (RCBD) split plot arrangement with three replications. The main plot was *Pseudomonas* spp. (*P. kilonensis* F113, *P. protegens* CHAO and non-inoculated control) applied as seed treatment, while the sub plots were maize genotypes (Aflatoxinsyn 4w, TZB-SR, Aflatoxin R SYN 2y, Aflatoxin SYN 3W and Aflatoxin syn-2y). Plot size was 2.2 m². Interplant and row distance were 50 cm X 50 cm respectively. This gave a plant population of 8 plants per plot which is equivalent to 40,000 plants per hectare. Maize was sown at 3 seeds per hole. This was thinned to two per stand after germination and allowed to grow to maturity. Irrigation was solely by rainfall. Mafikeng had an average annual rainfall of 300 to 700 mm and temperatures range between 22°C and 34°C. No fertilizer was applied. Weeding was carried out manually using hoe at 4 weeks, 8 weeks and 12 weeks after planting. Pest was controlled by the microbial inoculant. No chemical pesticide was applied. The maize varieties matured at different date however harvesting was carried out same day as maize were left on the stalk in the field to dry.

2.9. Growth Parameter and Yield Data Collection

Growth parameters (plant heights (cm) using a measuring tape, leaf length (cm) using a measuring tape, stem girth (cm) using a thin thread whose length was measured on a ruler) were recorded at maturity. Dry maize cobs from each treatment were harvested threshed and the threshed maize seeds were weighed on a weighing scale and recorded as yield (tonnes/ha).

2.10. Statistical Analysis

Data collected from field and pot experiments were subjected to two way Analysis of Variance (ANOVA) using IBM SPSS statistical package 21. Means were compared using Duncan Multiple Range Test ($p < 0.05$).

Table 3. Effects of microbial inoculants, soil types and maize genotype on the growth of maize planted in pots.

Variables	Plant Height (cm)	Leaf Length (cm)	Plant Girth (cm)
Soil Type			
Itsoseng	95.01 ^a	73.22 ^a	6.11 ^a
Mafikeng	95.47 ^a	73.53 ^a	6.23 ^a
Inoculants			
<i>Pseudomonas kilonensis</i> F113	99.38 ^a	74.60 ^a	6.48 ^a
<i>Pseudomonas protegens</i> CHA0	96.36 ^b	71.09 ^b	6.01 ^b
Non inoculated	90.29 ^c	74.60 ^a	6.09 ^b
Maize Genotype			
Aflatoxin SYN 4W	95.37 ^a	74.04 ^a	6.29 ^a
TZB SR	96.67 ^a	74.40 ^a	6.17 ^{ab}
Aflatoxin R SYN 2Y	96.41 ^a	74.03 ^b	6.19 ^{ab}
Aflatoxin SYN 3W	97.52 ^a	74.74 ^a	6.28 ^a
Aflatoxin SYN -2Y	90.74 ^b	69.93 ^b	6.04 ^b
Treatment Effects			
Soil Type	0.958	0.948	0.064
Inoculant	0.000	0.000	0.000
Maize genotype	0.006	0.005	0.011
Soil Type × Inoculant	0.224	0.955	0.319
Soil type × maize genotype	0.001	0.008	0.000
Inoculant × maize genotype	0.000	0.000	0.000
Soil type × Inoculant × maize genotype	0.502	0.508	0.000

3. RESULTS

3.1. Effects of Microbial Inoculants, Soil Types and Maize Genotype on the Growth of Maize Planted in Pots

The results in Table 3 show that plant height, leaf length and plant girth on Itsoseng soil were not significantly different from those on Mafikeng soil. Both *P. kilonensis* F113 and *P. protegens* CHA0 significantly increased the plant height. *P. kilonensis* F113 significantly increased plant girth. However, *P. protegens* CHA0 decreased leaf length significantly. Also the growth parameters measurement significantly differed according to maize genotypes ($P < 0.05$).

Combined effect of soil type × maize genotype and microbial inoculant × maize genotype increased significantly the plant height, leaf length and plant girth, while soil type × inoculant × maize genotype significantly increased the plant girth. However, combined effect of soil type × microbial inoculant was not significant on plant height, leaf length and plant girth as reported in Table 3.

Means followed by the same letter along the column for a factor are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT).

3.2. Effects of Microbial Inoculants, Soil Types and Maize Genotype on Root Weight and Root Length of Maize Planted in Pots.

The effects of microbial inoculants, maize genotypes and soil type on root weight and root length of maize planted in pot was shown in Table 4. Maize plants inoculated with *P. kilonensis* F113 significantly increased root weight and root length.

Soil from Mafikeng produced a significantly heavier and longer root system than soil from Blaawbank. Maize genotype Aflatoxin SYN 4W produced a significantly higher root weight while maize genotype Aflatoxin R SYN 2Y had a significantly higher root length compared to the other genotypes. Interactions of inoculant × maize genotype, and soil type × inoculant × maize genotypes had significant effect on the both root weight and root length of maize while soil type × inoculant and soil type × maize genotype had significant effect on root length but not on the root weight. Microbial inoculation thus resulted into elongation of stems and roots (Fig. 1).

3.3. Effects of Microbial Inoculants on the Growth and Yield of Maize Planted on the Field

Table 5 shows the effects of *Pseudomonas* inoculants on the growth and yield of maize planted on the field. Microbial inoculants had very high significant improved effect on growth and yield performance of maize plant. Both *P. kilonensis* F113 and *P. protegens* CHA0 significantly increased the plant height, leaf length and the yield of maize (Fig. 2 and Table 5). However, plants inoculated with *P. kilonensis* F113 had a higher height than those inoculated with *P. protegens* CHA0. This experiment evidence that the maize genotypes were not significantly different in growth but Aflatoxin SYN 4W and Aflatoxin SYN -2Y produced significantly higher yield followed by TZB SR and Aflatoxin SYN 3W while Aflatoxin R SYN -2Y has the least yield. There was no significant difference between maize genotypes for both growth and yield of maize. Similarly, the effect of maize genotype × inoculant on the growth and yield of maize is not significant. The growth and yield of all the genotypes tested were increased by the inoculants ($P = 0.05$).

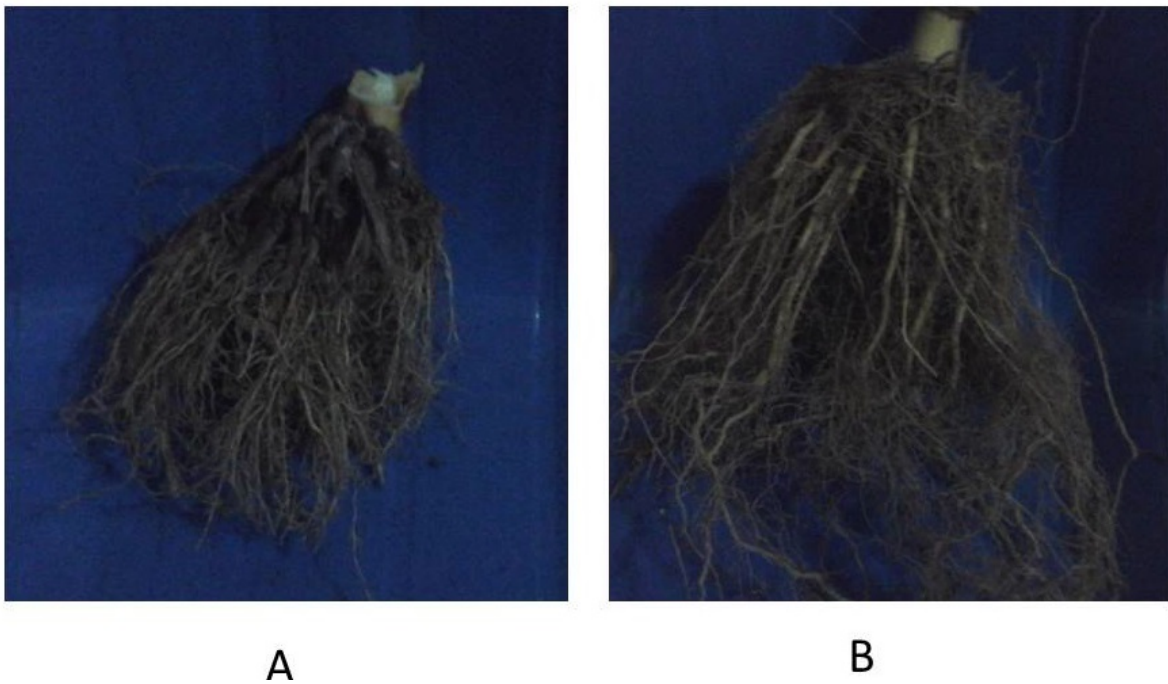


Fig. (1). Effect of microbial inoculant on root of maize. **(A)** Maize not inoculated with *Pseudomonas kilonensis* F113 **(B)** Maize inoculated with a *Pseudomonas kilonensis* F113.

Table 4. Effects of microbial inoculants, maize genotypes and soil type on root weight and root length of maize planted in pots in Screen house.

Variables	Root Weight (g)	Root Length (cm)
Soil Type		
Itsoseng	8.08 ^b	46.16 ^b
Mafikeng	17.65 ^a	50.16 ^a
Inoculants		
<i>Pseudomonas kilonensis</i> F113	20.20 ^a	53.93 ^a
<i>Pseudomonas protegens</i> CHA0	9.90 ^b	45.53 ^b
Non inoculated	13.28 ^b	47.00 ^b
Maize Genotype		
Aflatoxin SYN 4W	23.71 ^a	46.67 ^b
TZB SR	10.77 ^b	46.19 ^b
Aflatoxin R SYN 2Y	14.21 ^b	53.26 ^a
Aflatoxin SYN 3W	10.41 ^b	48.19 ^b
Aflatoxin SYN 2Y	13.19 ^b	49.81 ^{ab}
Treatment Effects		
Soil Type	.000	.014
Inoculant	.000	.000
Maize genotype	.000	.004
Soil Type × Inoculant	.000	.203
Soil type × maize genotype	.000	.436
Inoculant × maize genotype	.000	.000
Soil type × Inoculant × maizegenotype	.000	.013

Means followed by the same letter along the column for a factor are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT).

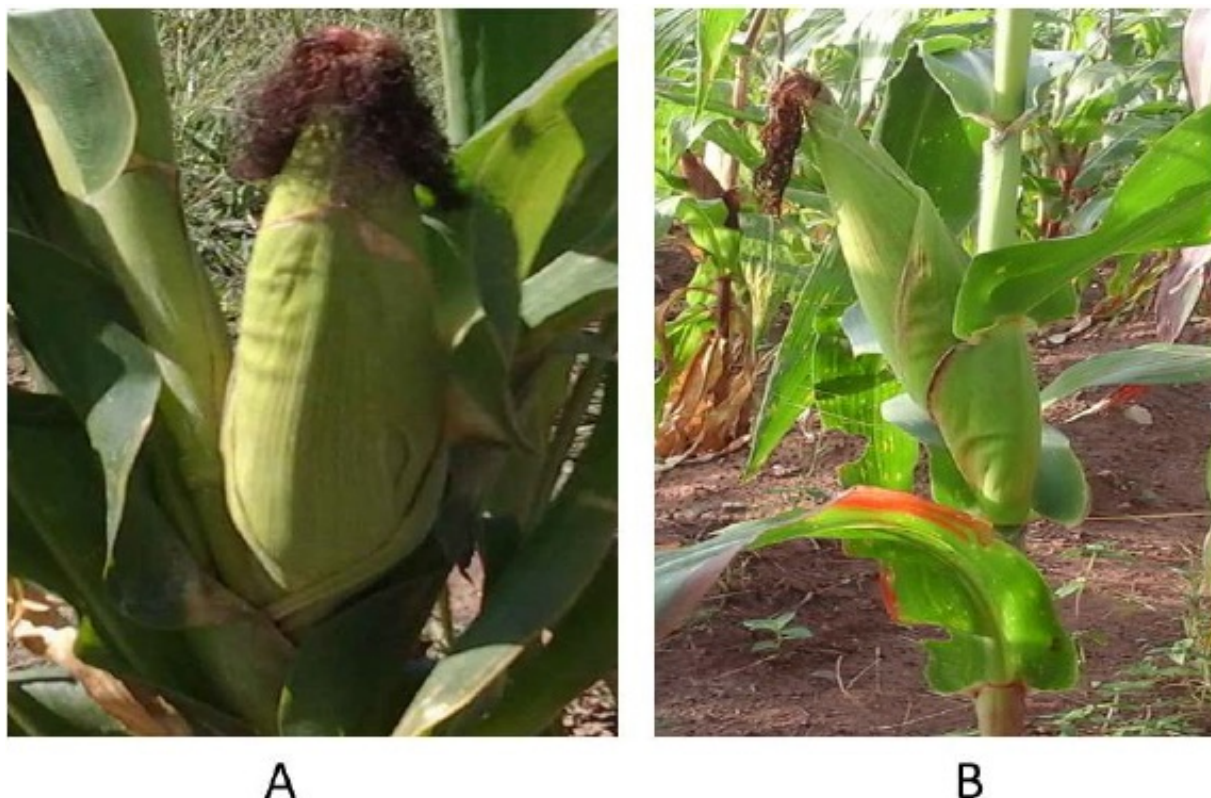


Fig. (2). Biofertilizer effect of microbial inoculant on maizen. **(A)** Maize (TZB SR) inoculated with pseudomonas kilonensis F113 **(B)** Maize (TZB SR) not inoculated with a *Pseudomonas kilonensis F113*.

4. DISCUSSION

The results of this research agree with some other studies where microbial inoculation improved plant growth. Application of microbial inoculants as revealed by Akladiou and Abbas caused increase in all measured growth parameters of maize plant [23]. Inoculation of maize seeds with *P. putida* strain R-168, *P. fluorescens* strain R-93, *P. fluorescens* DSM 50090, *P. putida* DSM291 significantly increased plant height in a field experiment [24]. The growth and yield of maize DMRESRY and EV99-MRP were differentially influenced by the application of bio-fertilizer *P. fluorescens* [25]. According to Oufdou *et al.* the plant genotype could also influence the phosphorus uptake released by phosphorus solubilizing bacteria from insoluble phosphate [26]. *P. fluorescens* has also shown ability to adapt to survival in soil and colonize the roots of plants [27] and to protect plant against drought stress [4]. *Pseudomonas protegens* was reported to produce biocontrol compounds [28]. Likewise Stockwell *et al.* and Yasmin *et al.* reported biocontrol potential of *Pseudomonas* spp., against a host of plant disease organisms [29,30].

Isfahani and Besharati also reported an increase in root weight of cucumber inoculated with *Pseudomonas* sp. soil type, microbial inoculant, maize genotype have significant positive effect on both root weight and root length of maize [31].

Table 4 confirms the report by Babalola that microbial

activity in the rhizosphere for nutrient acquisition affects root morphology and/or physiology [32]. The stimulation of root growth can enhance plant nutrient uptake [33]. Microbial inoculants often increase plant growth by promoting root development and alter root architecture *via* the production of phytohormones like Indole-3-Acetic Acid (IAA). This results in increased root length, root surface area numbers of root tips and volume [34,35]. Such stimulation of roots can aid plant defence against pathogens and can also relate to Induce Systemic Tolerance (IST) [36]. Since sites of nutrient uptake include root surfaces and root tips, it is likely that one mechanism by which microbial inoculants lead to increased nutrient uptake is *via* stimulation of root development [37]. A study to investigate effect of *P. fluorescens* on plant growth promotion under *in vitro* and *in situ* conditions conducted by Katiyar and Goel revealed that *P. fluorescens* produce a significant increase in root (30 and 20%) and shoot length (20 and 24%) of mung bean (*Vigna radiata*) *via* phosphorus solubilisation by acid production which results in a decrease in pH [38].

Babalola and Glick reported increased plant growth as one of the benefits of microbial inoculants [39]. A similar result was reported by Botelho [40]. They showed that *Pseudomonas fluorescens* strain BR-5 stimulated the growth of maize in a natural soil. Microbial inoculants improve plant growth and increase productivity as well as the host plant nutrient status [33].

Table 5. Effects of *Pseudomonas* inoculants on the growth and yield of maize planted on the field.

Variables	Yield (t/ha)	Plant Height (cm)	Leaf Length (cm)
Inoculants			
<i>Pseudomonas kilonensis</i> F113	555 ^a	111.27 ^a	68.38 ^a
<i>Pseudomonas protegens</i> CHA0	555 ^a	104.49 ^a	68.38 ^a
Non inoculated	122 ^b	41.27 ^b	32.40 ^b
Maize genotype			
Aflatoxin SYN 4W	473 ^a	90.93 ^a	61.16 ^a
TZB SR	416 ^{ab}	87.89 ^a	56.00 ^a
Aflatoxin R SYN 2Y	309 ^b	76.66 ^a	57.18 ^a
Aflatoxin SYN 3W	362 ^{ab}	77.51 ^a	50.83 ^a
Aflatoxin SYN -2Y	498 ^a	95.39 ^a	57.18 ^a
Treatment Effects			
Inoculant	0.000	0.000	0.000
Maize genotype	0.90	0.489	0.538
Inoculant × maize genotype	0.34	0.521	0.435

Means followed by the same letter along the column for a factor are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT).

Significant difference in plant growth and yield were observed among inoculated and un-inoculated treatments in all the maize genotypes tested. *Pseudomonas kilonensis* F113 and *Pseudomonas protegens* CHA0 increased maize plant height, plant girth, and leaf length in both screen house and field experiments with *Pseudomonas kilonensis* F113 performing the best. The root length and root weight were also enhanced by these organisms in the screen house experiment. *Pseudomonas kilonensis* F113 also produced the highest yield under field experiment. The impact of microbial inoculants on maize growth and yield were influenced by soil type and crop genotype. Aflatoxin SYN 4W had the best performance. *Pseudomonas kilonensis* F113 and *Pseudomonas protegens* CHA0 are potential biofertilizers for maize plants.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Decalred none.

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