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SCREENING OF INDIGENOUS BACTERIA FROM RHIZOSPHERE OF MAIZE (*ZEA MAYS* L.) FOR THEIR PLANT GROWTH PROMOTION ABILITY AND ANTAGONISM AGAINST FUNGAL AND BACTERIAL PATHOGENS

U. Farooq and A. Bano

Plant Physiology Lab, Department of Plant Sciences, Quaid-i-Azam University, 4600, Islamabad, Pakistan.
Dean of faculty of Biological Sciences, Quaid-i-Azam University Islamabad
Corresponding author E-mail: banoasghari@gmail.com

ABSTRACT

During present investigation, bacterial strains were isolated from rhizosphere of healthy and infected fields of maize grown at Yousafwalla, Pakistan. Fourteen isolates were screened for their antibacterial and antifungal activities. Three isolates showed the antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Staphylococcus aureus* and *Klebsiella pneumoniae* were found to be resistant to all the rhizobacteria except YCH1 which inhibits the growth of *Staphylococcus aureus* by 47%. All the strains exhibited maximum inhibition against *Fusarium moniliforme*. The Yys and YiBa were found to be most potent antagonist against *Fusarium moniliforme* showing maximum inhibition by *74%*. The bacterial isolates were not much effective in inhibition of the *Aspergillus flavus* and *Helminthosporium sativum* used in this study. It was found that the bacterial isolates which showed siderophore production also exhibited higher antifungal activity. All the isolates were catalase and oxidase positive solubilize phosphorus and produce bacteriocin. Bacterial strains were further evaluated as bioinoculant on maize (*Zea mays* L). The four selected isolates showed significant ($P < 0.05$) increase in dry matter production, plant height and root length of maize. The present study suggests the implication of PGPR isolates YDYs, Yys, YiH and Yipe as bioinoculant for biofertilizers.

Key words: Antifungal, Antibacterial, Phosphorus soloubilization, bacteriocin production.

INTRODUCTION

The rhizosphere is a complex system in which beneficial plant microbe interactions play vital role in agriculture to sustain the plant growth and productivity. Plant growth promoting rhizobacteria (PGPR) exert the positive effect on plant growth through various mechanisms either directly or indirectly (Joseph *et al*., 2007). The direct promotion of plant growth has been attributed by increased uptake of nitrogen (Kennedy *et al*., 2004) phytohormones synthesis (Arkhipova *et al*., 2005; Hayat *et al*. 2008 a, b) solubilization of phosphorus and siderophore production (Pidello, 2003). A large number of researchers have reported significant increases in productivity of important agronomic crops by inoculation with PGPR (Bashan *et al*. 2004). Wu *et al*., (2005) and has demonstrated the application of PGPR inoculants for the improved plant growth, increased rate of germination, resistance against environmental and pathogenic stress. Therefore, Introduction of certain bacterial strains to enhance the plant growth has gained considerable importance all over the world. Their application is promising alternative along with chemical fertilizers for increasing the yield with less negative impact on ecosystem (Compant *et al*., 2005).

The indirect effect of PGPR on plant growth is exerted by preventing harmful effects of plant pathogens by the production of secondary metabolites including HCN (Owen and Zlor, 2001), ammonia, antibiotics, and volatile metabolites. Plant growth promoting rhizobacteria actively colonize in plants rhizosphere and prevent the deleterious effects of phytopathogens (Rangajaran *et al*., 2003; Saikia *et al*., 2005). Biological control of a large array of phytopathogens by the induction of systemic resistance has received much importance in last few decades (Jetiyanon *et al*., 2003; Zhang *et al*., 2004). The ability of the antagonistic rhizobacteria is highly influenced by their morphological characteristics to inhibit the pathogens. High morphological activities results in production of more secondary metabolites to suppress the pathogens (Sullivan, 2004). The diversity of rhizobacteria in the suppressive soils is high as compared to conductive soil. In this respect the suppressive soil provide more chances to screen the antagonistic microbe that can be a potent biocontrol agent (Grabeva *et al*., 2004)

By keeping the above constrain in view, the objective of the present study was the comparative evaluation of the microbial diversity, potential biocontrol agent from the rhizosphere of maize fields infested with (conductive soil) and without stalk rot disease (suppressive soils). Furthermore to evaluate their plant growth promoting activities in order to reduce the conventional use of commonly applied fertilizers and pesticides.

MATERIALS AND METHODS

The present study was conducted to isolate native strains of rhizobacteria from maize growing areas of Yousafwalla. This place is situated in Sahiwal, Punjab, Pakistan. Its geographical position coordinates are 30˚ 40' 0" North, 73 6' 0". It has hot semi-arid climate as classified by Koppen climate classification.

Collection of soil samples and Isolation of rhizospheric bacteria: Rhizosphere soil was collected from samples maize fields uninfected and infested with stalk rot at anthesis stage. Each sample was taken in polythene bag, labeled and stored in refrigerator till further processing.

For the isolation of native rhizobacteria 1g of soil was suspended in 90 ml distilled autoclaved water. Serial dilution agar plate method was used for further processing of the prepared soil suspension Suitable dilutions (10^{-2}, 10^{-4}, and 10^{-8}) were plated on Luria Bertani (LB) medium to isolate rhizobacteria.

All the plates (in three replicates) were incubated for 2 days at 28˚C (Aneja, 2002). Well isolated colonies were purified by streaking on fresh LB plates.

Morphological characteristics: Morphological characteristics of the colony of each isolate were examined on LB agar plates after 3 days of incubation.

Antimicrobial activity of Isolated Bacteria: The bacterial strains were grown in LB broth and incubated for 48 h in a shaker at 125 rpm. The grown bacterial culture was centrifuged and the supernatant was filtered through 0.22µm Millipore filters. The cell free supernatant was used to determine antifungal and antibacterial activity as per Sumathi *et al*., (2012).

Antifungal activity: Antifungal activity of bacterial isolates was determined by agar tube dilution method (Washington and Sutter, 1980).

Cell free supernatant (67 µl) of each bacterial strain was loaded in autoclaved tubes containing sabouraud dextrose agar after autoclaving. These tubes were placed in slanting position and allowed to solidify. The tubes were inoculated with 4 mm diameter piece of fungal plug from 7 days old culture of fungus. The test tubes were incubated at 28°C for 7days. Reading was recorded by measuring the linear length (cm) of fungus in slant. Percentage inhibition of fungal growth for cell free supernatant of each bacterial isolate was calculated as

$$\% \text{ inhibition} = \frac{100 - \text{Linear growth in test (cm)}}{\text{Linear growth in control (cm)}} \times 100$$

Antibacterial activity: Antibacterial activity was screened by agar well diffusion method against selected clinical bacterial strains as described by Irobi *et al*., (1994) and Okeke *et al*., (2001). Nutrient agar plates were swabbed (by a sterile cotton swabs) with 24 hours old broth culture of selected bacterial strain to get a confluent growth. Bores were made by a 6 mm sterile cork borer. Afterwards, 100µl of cell free supernatant of each isolated bacteria was added in triplicate. Plates were placed at room temperature for 2h and incubated at 37°C. The LB broth was served as negative control. Simultaneously the standard antibiotics (as positive control) were tested against the pathogens. After 24h zones of inhibition was observed in plates. These results were compared with the zone of inhibition of positive control (penicillin a standard drug).

Determination of relative percentage inhibition: The relative percentage inhibition of the cell free supernatant of isolated bacteria with respect to positive control was determined by the following formula (Kumar *et al*., 2010; Ajay *et al*., 2003):

$$\text{Relative \% inhibition of the bacterial isolates} = \frac{(X-Y)}{(Z-Y)} X100$$

Where,

X: total area of inhibition of cell free supernatant of isolated bacteria

Y: total area of inhibition of the LB broth

Z: total area of inhibition of the standard drug.

Siderophore Production: Siderophore production was assayed by spot inoculation of bacterial isolates in the CAS agar medium (Clark and Bavoil, 1994). The plates were incubated at 28°C for 5 days. Siderophore production was observed by the development of orange halo around the colonies.

Detection of the Phosphate Solubilizing Activity: Phosphate solubilizing activity was determined by spot inoculation of the bacterial isolates on Pikovskaya agar medium plates. After incubation at 28°C for 7d, the clear zone around the colonies was considered positive for phosphate solubilization activity (Katznelson, 1959)

$$\text{Solubilization Index} = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

Catalase activity: Bacterial cultures were grown in LB agar medium for 24h to detect the catalase activity. Few drops of H_2O_2 (30%) were added to the culture on a glass slide and evaluation of oxygen as bubbles indicated the presence of catalase (Schaad, 1992).

Assay for protease production: Extracellular protease production was assayed according to Maurhofer *et al*. (1995). Each bacterial isolate was spot inoculated on skim milk agar plate and incubated for 24h. Development of halo zone around bacterial colony was considered as positive for protease production.

Bacteriocin production: The indicator strain VF 39 was grown in Tryptone yeast agar media (TY) medium to determine the bacteriocin production. It is diluted up to 10^{-2} and one ml of diluted TY media was mixed with 25 ml of molten TY agar (0.6% w/v) supplemented with 5mM Ca^{+2}. The bacterial isolates to be tested were stab inoculated after solidification of media (within 2h) and incubated for 48h (Oresenil *et al*., 1999).

Oxidase activity: Oxidase activity was determined by using filter paper spot method (Gerhardt *et al*., 1981). Kovács oxidase reagent (1-2 drops) was added to 24h old culture on a small piece of filter paper. Change in color to dark purple within 60 to 90s was considered as oxidase positive.

Screening of Rhizobacteria for Plant Growth Promotion In vivo

Preparation of inocula: Bacterial isolates were grown in 250 ml conical flasks containing 100 ml of LB broth for 48 h on a rotary shaker at 28 °C. Cells were taken by centrifugation at 10,000 g for 10 min at 4°C. The pellet was re-suspended in 100 ml of sterile distilled water (density measured as 1 at 600λ).

Seed treatment: Maize seeds (cv. Islamabad Gold) were obtained from Crop Research Institute, National Agriculture Research Centre, Islamabad (NARC) were surface sterilized with 95% ethanol followed by 10% chlorox for 3 min and rinsed with sterile water. Subsequently, seeds were soaked in bacterial inoculum for 2 to 4h with occasional shaking. Thereafter, seeds were shade dried for 30 min and used for sowing (Nandakumar *et al*., 2001). For control treatment seeds were soaked in sterilized distilled water.

Pot culture study: Seeds of maize (Islamabad Gold) were sown in sterilized pots measuring 8cm x 8cm filled with soil and sand in the ratio 3:1. All the pots arranged in completely randomized design in the green house of Quaid-e-Azam University. Autoclaved water was used for irrigation as and when required. Seedlings were harvested after 15 days for measurement of physiological parameters.

Data analysis: Data were statistically analyzed by analysis of variance technique and comparison among means was made by Least significant difference (LSD) using statistix 1.8. The least significant difference test at the 5% level was used for comparison of the means.

RESULTS AND DISCUSSION

During the present study a number of morphologically different colonies were obtained from maize rhizosphere on LB agar media, out of which 14 bacterial isolates were screened against *Aspergillus flavus, Fusarium moniliforme and Helminthosporium sativum* further their potential was evaluated as bioinoculant on maize.

Present studies revealed that all the bacterial isolates showed strong antifungal activity against *F. moniliforme* (fig. 1). However, some of them showed antagonistic activity for *A. flavus* and *H. sativum.* Five Isolates (YDYs, YCH1, Y3, YiPe, YiH, YiBs) showed more than 60%, and three isolates (YDY, YCC1, YiC) more than 50 % while, three isolates (Y4, YiLy, Yi16) showed 40% inhibition against the growth of *F. moniliforme.* The Yys and Yiba were found to be most potent antagonist against *F. moniliforme* showing maximum inhibition of *74%*. Isolate YiH was found to be an efficient antagonist against all the three phytopathogens tested. Highest inhibition was shown for *F. moniliforme*. These results are in accordance with the finding of Charles *et al*., (2001) who demonstrated that rhizobacteria inhibit the growth of F. *moniliforme.* Pal *et al*., (2001) and Shalini and Srivastava, (2008) reported the antagonistic activity of some metabolites produced by fluorescent *Pseudomonas* and *Bacillus* sp against *F. moniliforme.* The isolates YDY, Y4, YiC and Yi16 exhibited more than 40% inhibition of mycelia growth of *H. sativum (fig. 2)* whereas YiLy and YiBs exhibited the inhibition of *A. flavus* by 61.85 and 54.92 % respectively (fig. 3). Mushtaq *et al.,* (2010) have shown successful control of *A. flavus* by antagonistic bacteria. During present investigation four isolates exhibited more than 40% inhibition of mycelia growth of *H. sativum (fig. 2)*. The isolated bacterial strains were not as effective in inhibition of the *A. flavus* and *H. sativum* used in this study. The isolates isolated from the soil infested with stalk rot disease showed significantly more antifungal activity against *A. flavus* (36- 51%) and *H. sativum* (32 – 61 %) as compare to the isolates from the soil of healthy maize. According to Grabeva *et al*., (2004) and Singh and Singh (2008) the suppressive soil is dominant by antagonistic microbes as compare to the conductive soil. Shalini and Srivastava (2008) screened the antifungal activity of *P. fluorescence* against phytopathogenic fungi. Other studies have shown the successful control of *A. flavus* by antagonistic bacteria (Jeffrey *et al*., 2006; Mushtaq *et al*., 2010). Previously, antifungal potential of *Bacillus*, Pseudomonas and *Streptomyces* sp. has also been reported to inhibit the mycelial growth of many species of *Aspergillus*, and *Fusarium* (Nourozian *et al*., 2006).

In the present investigation YDYs, YCC1 and YiBa showed the antimicrobial behavior against *Pseudomonas aeruginosa* while YDYs, YCH1 and YiBs hampered the growth of *Bacillus subtilis*. *Staphylococcus aureus* and *Klebsiella pneumoniae* were found to be resistant to all the rhizobacteria except YCH1 which inhibits the growth of *S. aureus* by 47% (Table. 3). Inhibition of *Bacillus subtilis* by rhizobacteria was also

reported by Zaidi *et al* (2007). Saikia *et al*., (2011) reported that most of the fluorescent Pseudomonads isolate strongly inhibited the growth of gram-positive bacterium *S. aureus* and gram-negative bacteria *E. coli* and *K. pneumonia*. The rhizobacteria with strong antimicrobial activity have strong ability to colonize the crop plants (Espinosa-Urgel, 2000).

Phosphorus is one of the most important nutrients for plant growth. It is present in insoluble form in soil, rhizobacteria with phosphate solubilizing activity convert it in available form (Supraja *et al*., 2011). Out of 14 isolates tested for solubilization index eight isolates were positive for phosphate solubilizing potential (Table. 2). Tilak *et al*., (2005) reported highest frequency of phosphate solubilizing bacteria in the rhizoplane followed by rhizosphere and root free soil. The phosphate solubilizing bacteria are used as bio-inoculat to improve the growth of plants by enhancing the uptake of phosphorous (Chen *et al*., 2006). Bacillus species helps in plant growth promotion by solubilization of mineral phosphate (Chakraborty *et al*., 2006).

Siderophore production is another important trait of PGPR for the growth and survival in competitive ecosystem where iron is a limiting factor (Khan *et al*., 2006; Dimkpa *et al*., 2009). Five bacterial isolates showed the siderophore production (Table. 2). The production of siderophore by rhizobacteria has been confirmed by previous studies (Noori and Saud 2012; Raval and Desai 2012). A direct correlation was found to exist between siderophore production and antifungal activity. These results are in accordance with the finding of earlier researchers (Idris *et al*., 2010; Raval and Desai 2012). Rhizobacteria induce the systemic resistance (ISR) and enhance of the growth of plants by inducing the competition for nutrition for phytopathogens (Garcia *et al*., 2004). Siderophores are also important for some pathogenic bacteria for their acquisition of iron (Whipps, 2007).

Presence of cell wall degrading enzymes is pre-requisite for prohibiting the entrance of the host plant. The production of protease enzyme has been detected by seven isolates (Table. 1). Evaluation of Protease activity was observed by the formation of halo zone on skim milk agar plates. The isolates with the protease enzyme activity showed comparatively higher antifungal activity. Earlier studies reported that microorganisms secretes the extra cellular enzyme including proteases that inhibit various bacterial (Johansen *et al*., 2002) and fungal communities (Girlanda *et al*., 2001). Rakh *et al*., (2011) demonstrated that protease enzyme is effective for biocontrol of fungal pathogens directly or indirectly. Other enzymatic activities such as oxidase and catalase of the bacterial isolates were also determined in vitro. Out of 14 isolates, 8 of them showed oxidase activity and catalase activity (Table. 1). The isolates with oxidase activity also showed higher antifungal activity. These results are in accordance with the finding of other researchers (Ramyasmruthi *et al*., 2012). According to Joseph *et al*., (2007) catalase activity of rhizobacteria enables them extremely resistant to different types of stresses. Rhizosphere bacteria inhibit highly competitive environments as they are constantly competing for nutrients and ecological space. For the survival in such a competitive niche bacteria has devised several offensive tools like the production of lytic enzymes, bacteriocins and extracellular antimicrobial assist substances. The bacteriocins are the most potent and important class of biocontrol agents. YDYs, YCH1 and YiBs exhibited the bacteriocin production in the present study (Table. 2). As reported previously that various strains of fluorescent Pseudomonas spp. Isolated from the rhizosphere of a number of crops (banana, rice, wheat, maize) produce different types of bacteriocins (Parret *et al*., 2003). These isolates also showed the antibacterial activity (Table 3). It has been reported earlier that bacteriocins can be used for the suppression of bacterial pathogens (Lavermicocca *et al*., 2002).

Two isolates YDYs and Yys from found to be solubilizing phosphate and produce siderophore. They are also capable to be a potent antagonist against the Fusarium sp. and produce lytic enzymes including protease, catalase and oxidase. Further they produce bacteriocin that make them biocontrol agent against different pathogenic bacteria. YiPe and YiH isolated from infested soil also exhibit the above mentioned characteristic except that the production of bacteriocins, oxidase enzyme by YiPe and the protease enzyme by

Table. 1. Protease, catalase and oxidase activity of bacterial isolates from the rhizosphere soil of maize grown in fields at Yousafwalla (Sahiwal).

Bacterial isolates	Protease activity	catalase activity	oxidase activity
YDY	+	+	-
YDYs	-	+	+
YCC1	-	+	-
YCH1	+	+	+
Y4	-	+	-
Yys	+	+	+
Y3	+	+	+
YiPe	+	+	-
YiBa	-	+	-
YiLy	-	+	+
YiC	-	+	-
YiH	+	+	+
YiBs	+	+	+
Yi16	-	+	+

- stands for negative in test + stands for positive in test.

Table. 2. Solubilization index, Siderophore production and bacteriocin production (against VF39 strain of *Rhizobium leguminosarum*) of bacterial isolates.

Bacterial isolates	Siderophore production	Solubilization Index	Bacterocin Production
YDY	-	-	- - -
YDYs	+	1.46a	- 11.5
YCC1	-	-	- -
YCH1	-	-	- -
Y4	-	1.70b	- -
Yys	+	1.44c	- -
Y3	-	1.46a	- -
YiPe	-	1.34b	- -
YiBa	-	-	- -
YiLy	-	1.38b	- 9.7
YiC	-	-	- 8.9
YiH	+	1.12c	- -
YiBs	+	-	- -
Yi16	+	1.81a	- -

Means sharing same letter are not significantly different
According to Duncan's Multiple Range Test (P=0.05)
- stands for negative in test
+ stands for positive in test

Table. 3. Antibacterial activity of bioactive metabolite of bacterial isolates from the rhizosphere soil of maize grown in fields at Yousafwalla (Sahiwal).

	Relative Percentage Inhibition			
Bacterial Isolates	**S. aureus**	**P. aeruginosa**	**K. pneumonia**	**B. subtilis**
YDY	-	-	-	-
YDYs	-	37.83	-	57
YCC1	-	61.47	-	-
YCH1	47.21	-	-	38.42
Y4	-	-	-	-
Yys	-	-	-	-
Y3	-	-	-	-
YiPe	-	-	-	-
YiBa	-	67.55	-	-
YiLy	-	-	-	-
YiC	-	-	-	-
YiH	-	-	-	-
YiBs	-	-	-	63.15
Yi16	-	-	-	-

Table. 4. Morphological characteristics of 3 day old colonies of bacterial isolates from the rhizosphere soil of maize grown in fields at Yousafwalla (Sahiwal)

Bacterial isolates	Shape	Size (mm)	odor	Color	Elevation	Surface	Margins	Cell shape	Arrangement	Grams Test
YDY	Round	1.5	Odorless	Yellowish	Raised	Smooth shiny	Entire	Round	Paired	-
YDYs	Round	punctiform	Odorless	Yellowish	Raised	Smooth shiny	Entire	Round	Scattered	+
YCC1	Round	1.5	Odorless	White	Raised	Smooth shiny	Entire	Round	Paired	-
YCH1	Round	1	Odorless	Pink	Raised	Smooth shiny	Entire	Round	Paired	-
Y4	Irregular	4	Odorless	Yellow	Raised	Smooth	Undulate	Rod	Scattered	-
Yys	Round	1	Odorless	Yellow	Raised	Smooth shiny	Entire	Rod	Paired	-
Y3	Round	3.5	Odorless	White	Raised	Smooth	Entire	Round	Paired	-
YiPe	Round	4	Odorless	orange	Raised	Smooth shiny	Entire	Rod	Scattered	-
YiBa	Round	1	Odorless	Brown	Raised	Smooth shiny	Entire	Rod	Paired	-
YiLy	Round	3.5	Odorless	pale	Raised	Smooth shiny	Entire	Rod	Paired	+
YiC	Round	1.6	Odorless	White	Raised	Rough	Entire	Round	Scattered	+
YiH	Round	1	Odorless	White	Raised	Smooth shiny	Entire	Rod	Paired	-
YiBs	Round	Punctiform	Odorless	Golden brown	Raised	Smooth shiny	Entire	Round	Paired	-
Yi16	Irregular	4	Odorless	Yellow	Flat	Smooth shiny	Undulate	Rod	Scattered	-

YiH. Similar results were observed in earlier studies (Ali *et al*., 2010; Idris *et al*., 2010; Dastager *et al*., 2011). It is inferred that the YDYs, Yys, YiPe and YiH are able to produce plant growth promoting substances and antimicrobial substances. They could be potential candidates for the development of biofertilizer and bioinoculants for crop plants (Gupta *et al*., 1998; Noori and Saud, 2012).

These Bacterial isolates were further evaluated for the assessment of their effect on growth of maize (*Zea mays* L.). Survival efficiency (measured as cfu/g soil) of the bacterial strains isolated from the maize fields with healthy plants revealed higher values than those of isolated from conductive rhizosphere soil. All isolates showed significant increase in shoot and root length of maize as compared to un-inoculated control. Maximum increase (63 % of un-inoculated control) was observed with two isolate YDYs and Yys on root length of maize. Bacterial isolates YDYs, YYs and Yipe significantly increased shoot length by 40% over control (Fig. 6).

Most of the isolates significantly increased shoot and root fresh weight of maize seedlings. All bacterial isolates were stimulatory to fresh weight of shoot and root of maize seedlings as compared to un-inoculated control (fig. 5). The effect of inoculation with isolate YDYs in increasing shoot and root fresh weight of maize was found more pronounced (52 and 61 % respectively over untreated control) Significant difference ($P<0.05$) were recorded between isolate for this potential to increase of shoot fresh weight, maximum increase being 56 and 59%. Similar to fresh weight, all inoculated isolates showed increase in shoot dry weight. The increase in root dry weight with different treatments ranged from 25-69% (Fig. 4), the isolates YDYs and Yys being the most effective isolates which exhibited 69 and 59% increase in root dry weight compared with un-inoculated control.

The inoculation with YDYs, YYs (isolates from the healthy maize fields) were more effective in increasing plant root and shoot dry weight, and length of maize seedlings as compared to YiPe and YiH (the bacterial isolates from infested soil).

The increased root and shoot dry weight and fresh weight by the PGPR bio-inoculants clearly revealed the positive and advantageous role of these rhizobacteria. The results of present study suggest that rhizobacteria screening for the plant growth promotion is an efficient tool to select effective plant growth promoting rhizobacteria in the development of bio-inoculants for crop plants.

Table. 5. Ability of Bacterial isolates to colonize the rhizosphere of maize (*Zea mays* L.) under greenhouse conditions.

Bacterial Isolates	CFU/g soil
YDYs	1.92±0.88
Yys	2.42±0.57
YiPe	1.44±0.88
YiH	1.1±0.57
Control	0.577±0.58

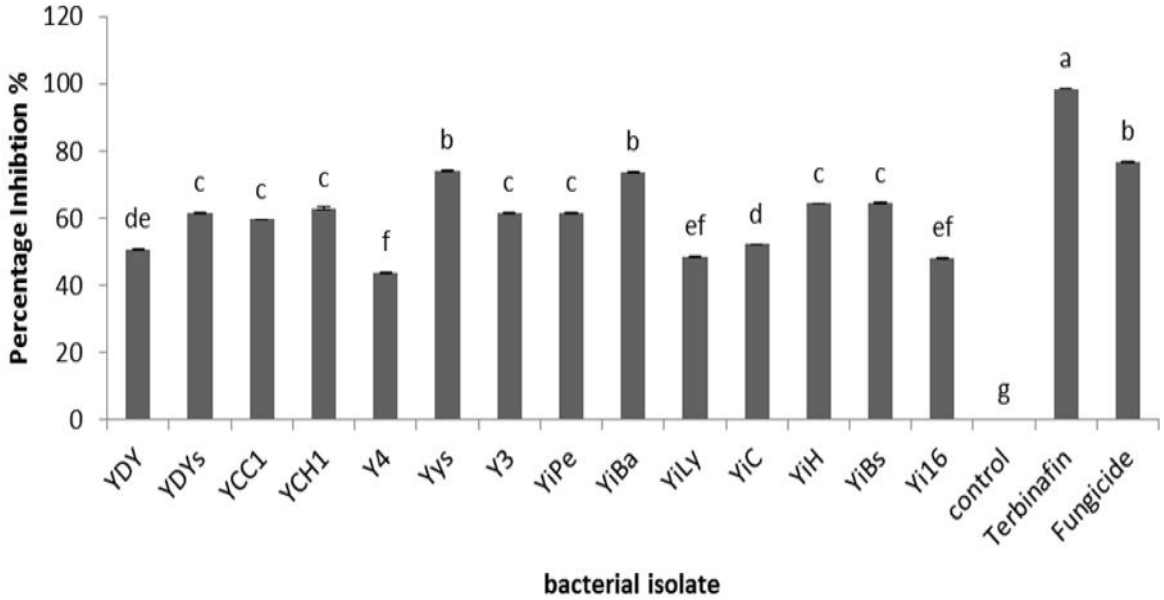

Fig. 1. Antifungal activity of bacterial isolates against *Fusarium moniliforme.*

Each bar represents the average of three independent measurements. Means with same letter are not significantly different at $P < 0.05$

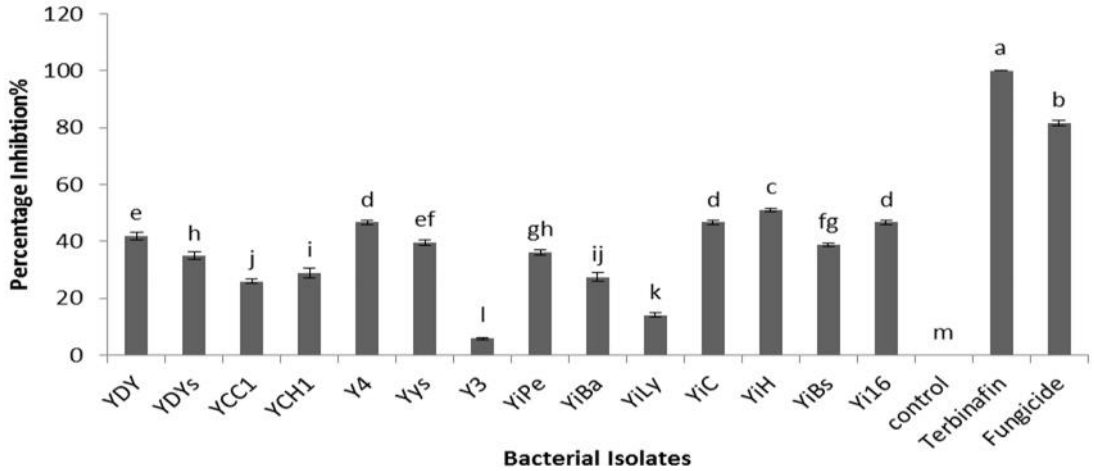

Fig. 2. Antifungal activity of bacterial isolates against *Helminthosporium sativum*

Each bar represents the average of three independent measurements. Means with same letter are not significantly different at $P < 0.05$

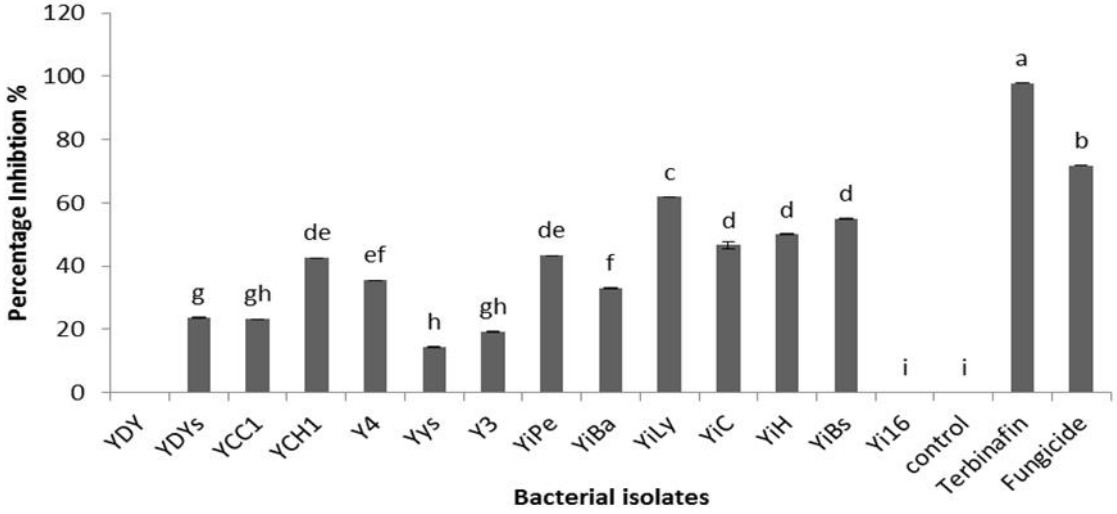

Fig. 3. Antifungal activity of bacterial isolates against *Aspergillus flavus*

Each bar represents the average of three independent measurements. Means with same letter are not significantly different at $P < 0.05$

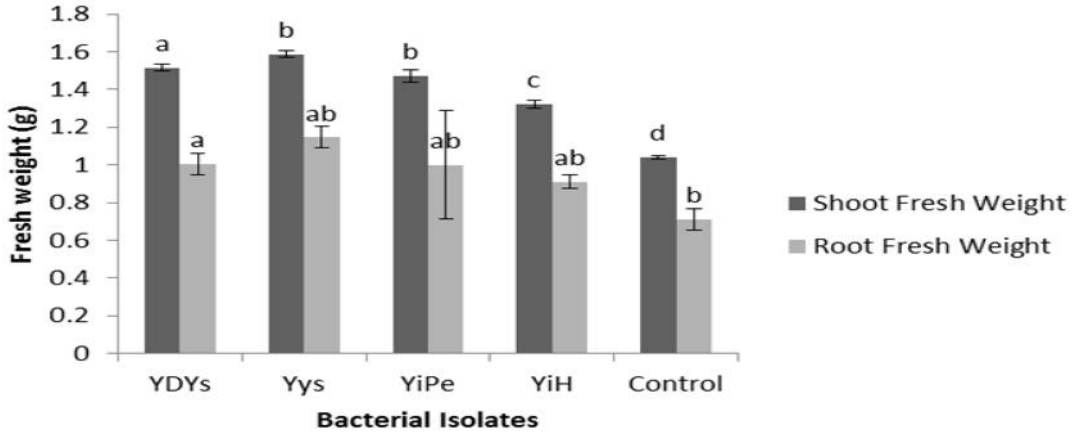

Fig. 4. Effect of bacterial isolates on shoot and root dry weight of maize (*Zea mays* L.)

Each bar represents the average of three independent measurements. Means with same letter are not significantly different at $P < 0.05$

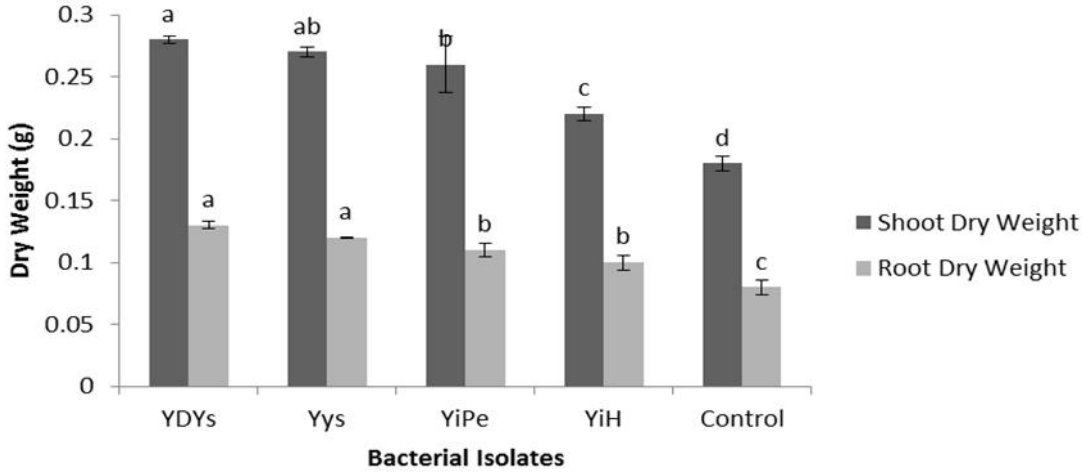

Fig. 5. Effect of bacterial isolates on shoot and root fresh weight of maize (*Zea mays L.*)

Each bar represents the average of three independent measurements. Means with same letter are not significantly different at $P < 0.05$

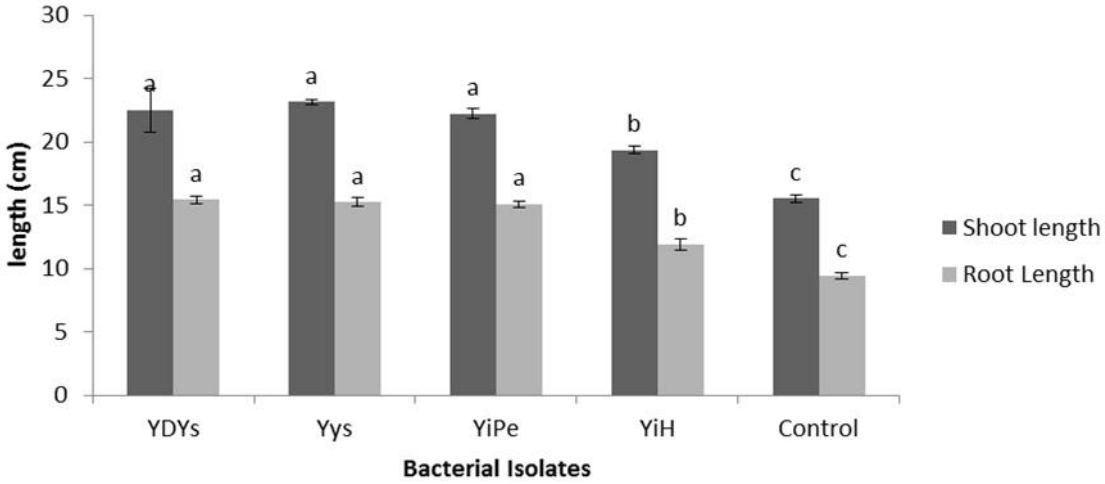

Fig. 6. Effect of Bacterial Isolates on shoot and root length of maize (*Zea mays L.*)

Each bar represents the average of three independent measurements. Means with same letter are not significantly different at $P < 0.05$.

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