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The effect of inoculating plant growth promoting microorganisms on rice production

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Abstract

Plant Growth Promoting Microorganisms (PGPMs) include bacteria and fungi that have the ability to enhance plant growth through several mechanisms. Plant growth is influenced by the interaction between plants and microbes. The role of PGPMs includes solublization of Phosphorus (P), increasing Nitrogen (N) uptake and synthesizing phytohormones such as auxin. The inorganic fertilizer used to increase rice production cause environmental hazards necessitating for an alternative source of fertilizer supplement. The research aimed to evaluate the efficiency of selected microorganisms in improving the yield performance of Basmati 317 rice. The study was conducted in the experimental farm of Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. A split plot experiment arranged in randomized complete block design with two factors; microbial concentrations and inoculants was replicated four times. The concentrations at three levels (10^9cfu spores^{-1} ml^{-1}, 10^7cfu spores^{-1} ml^{-1} and 10^5cfu spores^{-1} ml^{-1}) comprised the main plots while microbial inoculants (16 and two controls) were the sub-plots. Data was collected on yield attribute parameters such as number of panicles, panicle length and 100 grain weight. Results revealed that the performance of plants inoculated with high microbial concentrations was better in terms of the yield attributes compared to those treated with low microbial concentrations. It was also evident that the number of tillers, panicle length and 100 grain weight of plants treated with species of *Brevudimonas, Bacillus, Enterobacter, Trichoderma* and *Aspergillus*, were better than for the control plants. It was concluded that PGPMs inoculants improved the performance of the rice plants' growth and production.

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Introduction

Rice (*Oryza sativa* L.) which is a staple food for 70 % of the world population is a very important cereal crop (Britto and Kronzucker, 2004). It is ranked amongst the top cereals in Kenya. Worldwide, the demand for rice is increasing as a result of increasing human population. Increasing rice production requires improved soil fertility with high-input farming practices being employed and these have generated environmental problems and natural resource degradation. The continuous use of inorganic fertilizer decreases soil fertility in that it damages the physical, chemical and biological properties of the soil (Havlin *et al*., 2005). To overcome the problems caused by the use of inorganic fertilizer, an alternative solution leading to increased rice yields while maintaining high resource sustainability needs to be applied. An organic fertilizer such as bio-fertilizer is a better substitute.

In recent times to ease the detrimental effects of high-input farming, there has been a rising interest in sustainable agriculture which involves the use of bio-fertilizers. Sustainable rice production depends mostly on chemical fertilizers. The continuous use of inorganic fertilizer not only affects the activities of soil microorganisms but also lowers rice production. The role of microorganisms in agriculture includes promoting plant nutrient circulation while reducing the use of chemical fertilizer. The soil plant continuum constitutes crucial microbial population involved in framework of interactions affecting the development of plants (Oliveira *et al*., 2009). The beneficial microorganisms important in rice production such as plant growth promoting bacteria (PGPB) and plant growth promoting fungi (PGPF) have a major role to play in maintaining ecosystem functions (Hermosa *et al*., 2011).

The essential nutrients required for normal plant growth and development include N and P. Cereals including rice require large quantities of N for their growth, development as well as grain production (Baset Mia and Shamsuddin, 2010).

As an essential nutrient for plant growth and development, P has a defined role in plant metabolism which includes root development, photosynthesis and nutrient transportation within the plant (Rasipour and Asgharzadeh, 2007). The exogenous Indole acetic acid (IAA) produced by microorganisms has a role in increasing plant growth through root elongation initiation, cell expansion, vascular differentiation and flower initiation (Brandl *et al*., 1996). However these elements are not readily available to the plants due to among other reasons fixation and precipitation. The rice eco-system comprises a diversity of microorganisms which play a major role in sustainable agriculture. The mechanisms applied by the microbes to effect plant productivity include nitrogen fixation (Velasco *et al*., 2013), phosphate solubilization (Bakhshandeh *et al*., 2014) and phytohormones production (Cassán *et al*., 2014) amongst others.

The application of indigenous rhizospheric microorganisms can increase rice production. Nitrogen fixation, nutrients solubilization and growth hormone production are some of the advantages accrued by applying bacteria as bio-fertilizer for growth promotion (Thakuria *et al*., 2004). Vessey (2003) defined bio-fertilizer as a microbial life containing substance which on application to plants stimulates growth, improve soil fertility and increase production of crops without causing detrimental effects to the environment. Rhizospheric N fixing and P solubilizing microorganisms have been utilized in non-leguminous crops including maize and wheat in addition to rice. Recently, various microorganisms of the genera *Pseudomonas, Bacillus, Enterobacter, Acinetobacter* and *Azospirillum* amongst others were applied in several crops (Ahemad and Kibret, 2014). Bashan *et al*. (2004) reported significant increase in total plant biomass, plant height and root length amongst other parameters of cereal plants after application of *Azospirillum* sp. In response to Plant Growth Promoting Rhizobacteria (PGPR) application, Sharma *et al*. (2014) in their experiment indicated an improvement in rice growth resulting in an increase in rice yield.

While evaluating the effects of different PGPR's Cong *et al.* (2011) reported an increase in the paddy grain yield in response to PGPR's application compared to the control.

Despite the advancements in the use of indigenous microorganisms as bio-fertilizers to improve crop production, their use in Kenya in the agricultural sector is very minimal. Therefore, this study was conducted to evaluate the effect of selected PGPMs on rice production.

Materials and methods

Isolation and identification of the microorganisms

Soil samples were collected from the paddy fields of Mwea, Western and Coastal regions of Kenya. The samples were taken to a depth of 0 – 15 cm from different points of the rhizosphere and kept separately in the refrigerator at 4^{o}C during the experimental period. To isolate the microorganisms, serial dilution method was performed for bacteria on nutrient agar (NA) and for the fungi on potato dextrose agar (PDA). To obtain pure cultures, individual bacterial colonies and fungal structures were selected based on their morphological characters, picked and re-cultured on fresh media for purification. The microscopic characterization of the bacterial and fungal isolates was done in accordance to Cappuccino and Sherman (2002) and Barnett and Hunter (1987) respectively. The isolates were identified to the Genus level.

Screening of microorganisms for plant growth promoting activities

The bacterial and fungal isolates were further screened for their physiological properties as plant growth promoters. The plate assay using the National Botanical Research Institute's phosphate (NBRIP) growth medium as recommended by Nautiyal, (1999) was used to detect the phosphate solubilization ability of the isolates. To detect the IAA producing ability of the isolates, the procedure described by Brick *et al.*, (2004) was applied whereas the ability of the bacterial isolates to reduce acetylene was evaluated by the nitrogen fixation ability assay which uses the nitrogen-free (NFb) semi-solid media. Based on these properties, nine bacterial and four fungal isolates were selected to be used as rice seed inoculants.

Inocula preparation and seed inoculation

The selected potential bacterial and fungal isolates were rejuvenated, inoculated into their respective liquid media and incubated for 24 hours at 37^{o}C. Basmati 317 rice seeds were treated with three different microbial suspension (inocula) concentrations (10^{-5}cfu spores^{-1} ml^{-1}, 10^{-7}cfu spores^{-1} ml^{-1}and 10^{-9}cfu spores^{-1} ml^{-1}). Nine bacterial strains, four fungal strains, three mixed strains (all nine bacterial strains (MB), all four fungal strains (MF) and a mix of all the bacterial and fungal strains (MBF) and two controls (natural condition (C) and farmers' practice (C2) formed the treatments. The seeds were aseptically soaked into the appropriate PGP broth (inocula) for 30 minutes until uniformly coated. Seeds were then air-dried and sown immediately.

Plant growth experiment

A pot experiment was conducted during the period 2013 - 2014 at JKUAT experimental field. The site which is 1530 m above sea level lies between latitudes 3^{o} 35" and 1^{o} 45"S and longitudes 36^{o} 35" and 37^{o} 25" E (Batjes, 2006) is located 36 km N - E of Nairobi city in Kenya. The experimental design used was split-plot based randomized complete block design (RCBD) replicated four times. Main plots were microbial concentrations at three levels (10^{-5}cfu spores^{-1} ml^{-1}, 10^{-7}cfu spores^{-1} ml^{-1}and 10^{-9}cfu spores^{-1} ml^{-1}) and sub-plots were the different microbial strains (treatments). Six treated seeds were sown into two kg plastic pots (15 cm diameter) containing well sieved vertisols soil. After emergence, only four homogenous seedlings were left per pot. Pots containing uninoculated seeds (C) and farmers practice (C2) acted as controls. Two doses of fertilizers were applied for the C2 pots and these were; Di-ammonium phosphate (DAP) and Muriate of potash (MOP) at the rate of 125 kg / ha and 75 kg / ha respectively three weeks after planting as the first application. Seven weeks after planting the second application of Sulphate of ammonium (SA) 250 kg / ha was done. The pots were maintained in paddy condition (Fig. 1) and weeds were hand-controlled throughout the experimental period.

Harvesting was done at physiological grain maturity stage and the parameters evaluated were; the number of panicles/plant, panicle length and 100 grain weight. Data were analyzed by GENSTAT ™ statistical software where the treatment means were compared relative to the controls (uninoculated and the farmers practice) by Duncan's multiple-range test (DMRT) and were considered significant at 5 % level (p<0.05).

Results

Isolation and culture purification

In the present study the tested bacteria and fungi were isolated from three rice growing regions of Kenya.

After serial dilution and incubation of the samples in their respective media and conditions, numerous cultures were obtained (Fig. 2a and 2b).

Based on their morphological characteristics, single bacterial colonies and fungal structures after several sub-cultures resulted into pure cultures (Fig. 3a and 3b).

Thereafter pure bacterial isolates were identified microscopically (Table 1) according to Cappuccino and Sherman (2002) whereas after morphological characterization (Table 2), the pure fungal isolates based on spores and spore bearing structures were identified based on classification by Barnett and Hunter (1987).

Table 1. Microscopic observation and identification of bacterial isolates.

No. of isolates	Cell shape	Motility	Gram reaction	Genus
82	Rod/cocci	Motile	Positive	*Bacillus* spp.
8	Rod	Motile	Negative/positive	*Pseudomonas* spp.
8	Rod/cocci	Motile	Positive	*Enterobacter* spp.
6	Rod	Motile	Positive	*Lysinbacillus* spp.
6	Rod/cocci	Motile	Positive	*Staphylococcus* spp.
3	Rod	Motile	Negative	*Micrococcus* spp.
3	Rod	Motile	Positive/negative	*Streptomyces* spp.
3	Rod/cocci	Motile	Negative/positive	*Brevundomonas* spp.
2	Rod	Motile	Positive	*Acinetobacter* spp.
2	Rod	Motile	Positive	*Serratia* spp.
2	Rod	Motile	Positive	*Vagococcus* spp.
2	Rod	Motile	Negative	*Exiguobacterium* spp.
1	Rod	Motile	Positive	*Alcaligenes* spp.
1	Rod	Motile	Positive	*Brevibacillus* spp.
1	Rod	Motile	Positive	*Advenella* spp.

Table 2. Colony characteristics and microscopic identification of fungal isolates.

No. of isolates	Surface colour	Reverse colour	Periphery colour	Genus
47	Dark green	Yellow/brown	Whitish	*Penicillium* spp.
29	Sulphur yellow	Creamish	Whitish	*Aspergillus* spp.
24	Grey	Yellow	Creamish	*Trichoderma* spp.
9	Grey/Green	Yellow	Whitish/Creamish	*Eupeniccilium* spp.
6	Dark green	Creamish	Dark green	*Isaria* spp.
3	Whitish	Creamish	Whitish	*Leptosphaerulina* spp.
1	Fiesta green	Creamish	Fiesta green	*Hypocrea* spp.
1	Pink	Red	Pink	*Fusarium* spp.

Characterization and identification of the bacterial and fungal isolates

Table 1 shows that a total of 130 bacterial isolates were obtained from the rice rhizospere samples.

All the isolates were rod shaped and motile with only four exhibiting either rod or cocci shape. Most of the isolate (82) reacted positively to the Gram test and were identified to belong to the genus *Bacillus*.

All the remaining isolates reacted positively to the Gram test with the exception of five (Table 2). Apart from the *Bacillus* spp. other genera which were dominant include *Pseudomonas*, *Enterobacter*, *Lysinbacillus* and *Staphylococcus* spps.

The fungal isolates had a wide variation in terms of surface colour ranging from white to dark green with majority of them (47 out of 120) featuring the dark green surfaces (Table 2).

All except one isolate had their reverse colour creamish- yellow. The periphery colour of most of the isolates (112) ranged from white to creamish with the exception of six isolates which were dark green and one each fiesta green and pink. The dominant fungal spps. were *Penicillium*, *Aspergillus* and *Trichoderma*.

Table 3. Plant growth promoting properties of the bacterial and fungal isolates.

Genus	P solubilization	N fixation	IAA production
Bacterial isolates			
Micrococcus ssp.	Present	Present	Present
Brevudimonas ssp.	Present	Present	Present
Acinetobacter ssp.	Present	Present	Absent
Staphylococcus ssp.	Present	Present	Present
Enterobacter ssp.	Present	Present	Present
Pseudomonas ssp.	Present	Present	Absent
Bacillus ssp.	Present	Present	Present
Bacillus ssp.	Present	Present	Present
Enterobacter ssp.	Present	Present	Present
Fungal isolates			
Penicillium ssp.	Present	Absent	Present
Trichoderma ssp.	Present	Absent	Absent
Penicillium ssp.	Present	Absent	Absent
Aspergillus ssp.	Absent	Absent	Present

Phosphate solubilization, Nitrogen fixation and IAA production

Table 3 present the plant growth promoting properties of the selected bacterial and fungal isolates used for the experiment. As shown in Table 3, all the selected bacterial isolates had the ability to solubilize P and fix N. All except two bacterial isolates (*Acinetobacter* and *Pseudomonas* spps.) induced the production of IAA. Fungi lack the ability to fix N hence isolates were not tested for this property. Three (two *Penicillium* and one *Trichoderma* spps.) out of the four tested isolates solubilized P. For the tested properties, *Aspergillus* spp. was positive only in the induction of IAA production (Table 3).

Treatments effect on rice yield attributes

The effect of bacterial and fungal strains inoculation on rice plants varied although there were no significant interactions in all the tested parameters (Table 4). The main plot effect for the tested parameters (number of panicles, panicle length and 100 grain weight) were all significantly different at $p < 0.05$. However, for the number of panicles, concentration 2 (10^7cfu spores^{-1} ml^{-1}) performed better than concentrations 1 and 3 (10^9cfu spores^{-1} ml^{-1} and 10^5cfu spores^{-1} ml^{-1} respectively). Plants treated with concentration 2 had the highest number of panicles / plant (5.11) as opposed to those inoculated with treatment 1 and 3 (4.71 and 4.86) respectively.

The main plot effect for panicle length and 100 grain weight were better for plants inoculated with concentration 1 than those of concentration 2 and 3 (Table 4).

The sub-plot treatment effect for number of panicles and 100 grain weight were significantly different at $p < 0.05$ as opposed to panicle length. Results also revealed that all inoculated plants with the exception of those inoculated with B1, F1 and MBF (3.92, 4.33 and 3.50 respectively) had more panicles than the uninoculated control (C, 4.50). At the same time, plants inoculated with B2 (5.25), B7 (5.33) and MB (5.25) were not significantly different from those supplied with inorganic / chemical fertilizer (C2, 6.75) despite the latter having more panicles.

Table 4. Effect of inoculation on rice yield attributes.

	Yield components of rice plants			
	Treatment			
	Concentration	Number of panicles	Panicle length (cm)	100-grain weight (g)
Conc 1	10^9cfu spores^{-1} ml^{-1}	4.71a	18.14a	2.01a
Conc 2	10^7cfu spores^{-1} ml^{-1}	5.11b	17.64a	1.91b
Conc 3	10^5cfu spores^{-1} ml^{-1}	4.86a	16.84b	1.94c
	SE (+/-)	0.203	0.608	0.027
Isolate	Inoculant			
B1	*Micrococcus yunnanensis*	3.92ab	16.67a	1.93ab
B2	*Brevudimonas naejangsanensis*	5.25bc	17.67a	1.89ab
B3	*Acinetobacter pittii*	5.00ab	16.28a	1.97ab
B4	*Staphylococcus saprophyticus*	4.92ab	18.36a	1.94ab
B5	*Enterobacter aerogenes*	5.00ab	16.45a	1.99ab
B6	*Pseudomonas plecoglossicida*	5.08ab	17.43a	1.91ab
B7	*Bacillus tequilensis*	5.33bc	18.17a	1.93ab
B8	*Bacillus stratophericus*	4.92ab	17.73a	1.98ab
B9	*Enterobacter cancerogenus*	5.00ab	17.43a	2.02ab
F1	*Penicillium chrysogenum*	4.33ab	17.61a	1.96ab
F2	*Trichoderma harzianum*	4.67ab	18.31a	1.95ab
F3	*Penicillium pinophilum*	4.92ab	17.30a	1.81a
F4	*Aspergillus oryzae*	4.83ab	17.86a	2.06b
MB	MB	5.25bc	18.70a	1.90ab
MF	MF	4.92ab	17.91a	1.97ab
MBF	MBF	3.50a	17.75a	2.02ab
C	Cont 1	4.50ab	16.65a	1.97ab
C2	Cont 2	6.75c	17.46a	2.01ab
	SE (+/-)	0.717	1.329	0.093
Interaction		NS	NS	NS

Despite there being no significant differences for the sub-plot effect on panicle length, all inoculated plants had longer panicles in comparison to the uninoculated plants (16.65 cm) except those inoculated with B3 (16.28 cm) and B5 (16.45 cm). Plant inoculated with (B2, B4, B7, B8, F1, F2, F4, MB, MF and MBF) had longer panicles than the fertilizer treated plants (Table 4). The application of PGPMs significantly affected the 100 grain weight of the rice plants. Results reveal that B8, B9, F4 and MBF inoculated plants (1.98 g, 2.02 g, 2.06 g and 2.02 g respectively) had more 100 grain weight than the uninoculated control (1.97 g) and at the same time B9, F4 and MBF inoculated plants' 100 grain weight was more than that of the fertilizer applied plants (C2 – 2.01g).

Discussion

The present study revealed that the rice ecosystem harbors a diverse range of microorganisms (Table 1 and 2) with the dominant spps. being *Bacillus, Pseudomonas, Enterobacter, Penicillium, Aspergillus* and *Trichoderma*.

Rice fields have been reported by Prassana *et al* (2011) to represent a remarkable diversity of soil microbes comprising of bacteria and fungi. In the current study, the number of bacteria surpassed that of the fungi isolated from the same samples indicating that the bacterial population in the soil was more compared to that of the fungi. These results are in consistence with those of Saharan and Nehra (2011) who observed the abundance of bacteria in the rhizosphere in relation to other microorganisms.

Microbial inoculants have been known to play a major role in organic matter decomposition and availing plant nutrients including N and P to plants. Plants through their roots release metabolites from which the rhizospheric microbes obtain their energy (Lebuhn *et al*., 1997).

These metabolites determine the microbial conditions in the rhizosphere as they form compounds such as sugars, amino acids, organic acids, vitamins, enzymes amongst others. Plant growth is as a result of mutual relationship between the plant and the soil microorganisms where the organisms avail nutrients to the plants while they obtain their energy from the plants (Rodriguez and Fraga, 1999). The microorganisms also synthesize auxin which due to its role of cleavage and cell elongation results to plant growth promotion (Vessey, 2003). The use of bio-fertilizer containing microbes have been reported by Mezuan *et al.* (2002) to improve the physical, chemical and biological properties of soil hence increasing crop yields.

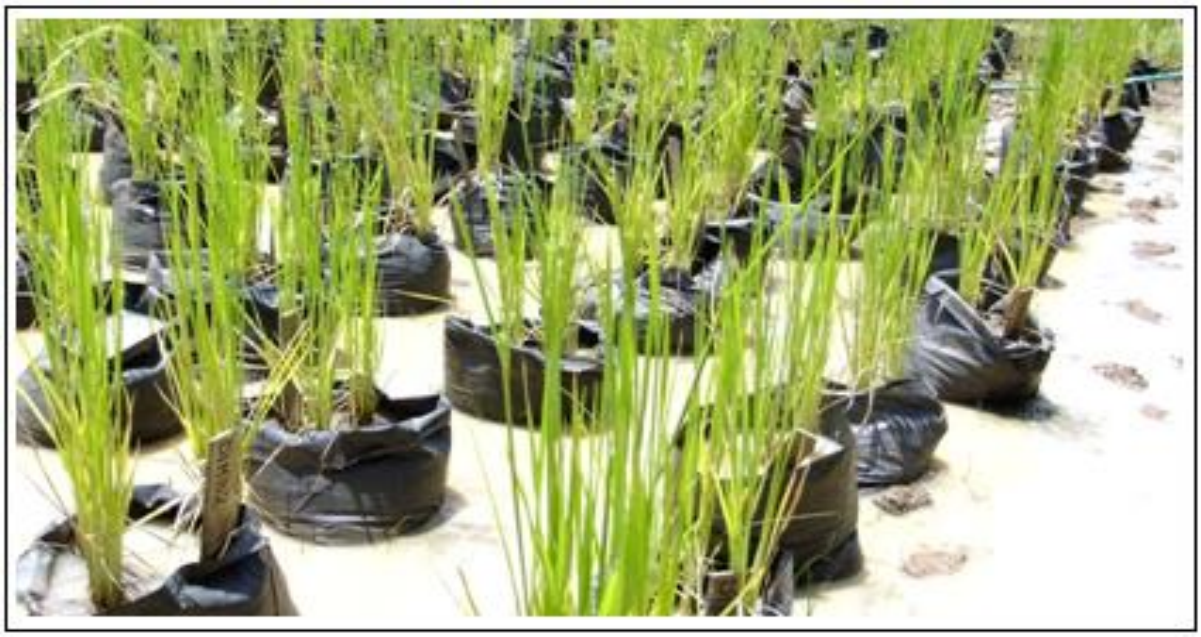

Fig. 1. Pots in the field maintained under paddy conditions.

The effectiveness of soil microorganisms in releasing P from inorganic complexes through solubilization is certain. According to Rodríguez and Fraga (1999) almost all P solubilizing microorganisms produce gluconic, glycolic and 2-ketogluconic acids amongst others when multiplied in media containing simple 'C' source. All the tested bacterial and 75 % fungal isolates in this study had the ability to solubilize P. As indicated by Verma *et al.* (2001) in their report, the rhizospheric bacteria have the ability to solubilize P. Also, Johri *et al.* (2003) in their study observed that several fungal spps. in addition to rhizobacteria can effectively solubilize P. All the studied bacterial isolates showed acetylene reduction activity (ARA) property. Nitrogen fixation associated with roots of grasses is an important component of N cycle. Most soil-borne microorganisms exist in the rhizosphere which is an active zone where biological, physical and chemical activities influence the soil.

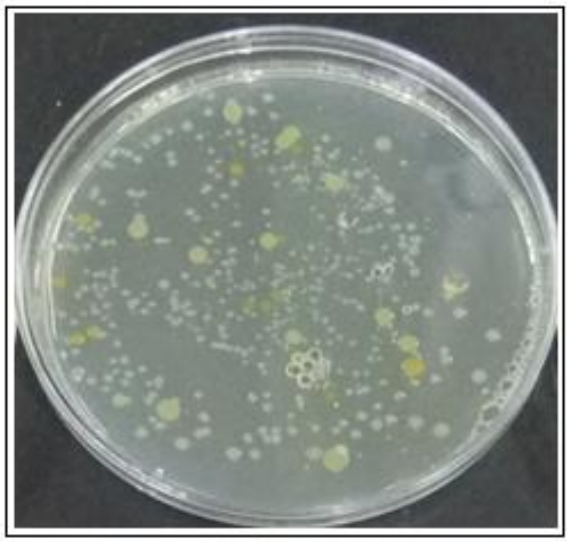

Fig 2a. NA cultures before purification.

The presence of N- fixing bacteria in the rhizosphere had been reported by Knief *et al.* (2011) whereas high ARA associated with the soil and roots of kallar grass was reported by Zafar *et al.* (1986). Almost all the tested bacterial isolates apart from *Acinetobacter* and a spps. of *Pseudomonas* and 50 % of the fungal isolates had the ability to induce IAA production.

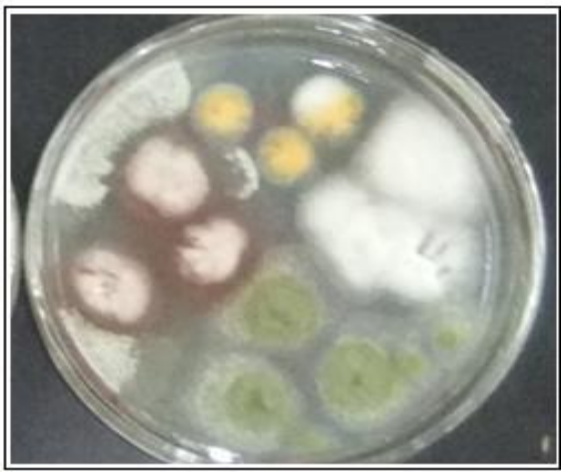

Fig. 2b. PDA cultures before purification.

There are many phytohormones but the native auxin, IAA is considered the most essential. Most bacteria are known to synthesize IAA and it is supposed that 80 % of those at the rhizosphere secrete IAA (Leinhos, 1994). Nevertheless, according to Joseph *et al.* (2007), the production of IAA in the rhizosphere by these bacteria is dependent on the accessibility of IAA precursors and the uptake of microbial IAA by the plant.

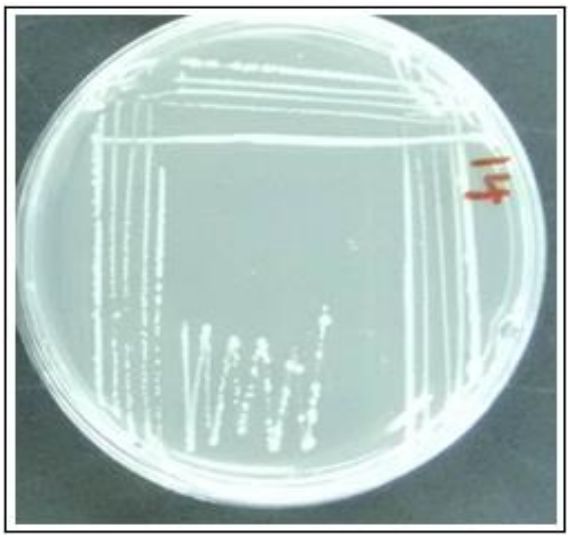

Fig. 3a. Pure bacterial culture.

The results of this study indicated that the effect of the different concentrations and microbial inoculants were significant in all the examined yield components apart from the panicle length for the microbial inoculants (Table 4). These results are similar to those of Alam *et al.* (2008) who reported that inoculation of rice plants with phosphate solubilizing microorganisms (PSM) in field experiments have been confirmed by researchers to significantly increase some yield attributes.

The panicle length and 100 grain weight of plants inoculated with concentration 1 outperformed those treated with concentrations 2 and 3 (Table 4). It has been reported that soaking seeds in the highest density of inocula increases the infection probability (Isaac, 1992; Fitri and Gofar, 2010). This is as a result of osmotic differences between the seed and the microbial inoculant suspension hence the seed via the pedicel imbibing the microorganisms.

Bio-fertilizers have been used worldwide for growing different types of crops with excellent results (Bashan *et al.*, 2004; Cakmakc *et al.*, 2006). In the present study, apart from the number of panicles where the C2 treatment had the highest (6.75), all the other inoculants performed better than the uninoculated plants and were either superior or at per with the C2 (fertilized plants) in the panicle length and 100 grain weight except *Micrococcus* spps.

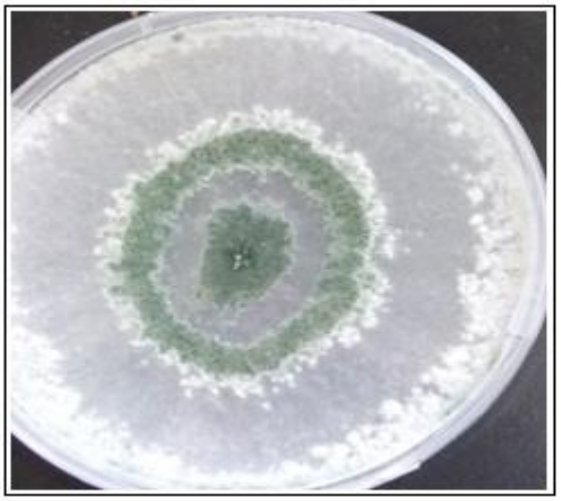

Fig. 3b. Pure fungal culture.

It is also evident from the results that the use of combined inoculants (MB, MF and MBF) had highest values for some of the evaluated parameters (Table 4). Bashan *et al.* (2004) reported significant increases in yield of agronomical important crops in relation to inoculation with PGPMs. Cakmakc *et al.* (2006) also proved that some microorganisms such as *Pseudomonas* had the potential for agricultural exploitation and thus can be utilized as natural fertilizers.

Since most of the tested microbial isolates in this study were able to solubilize P, fix N and induced the production of IAA (Table 3), these properties are supposed to be responsible for the promotion of yield (100 grain weight) and yield attributes (number of panicles and panicle length).

Solubilization of the insoluble P in the soil and the releasing of considerable amounts of PGPS including auxin are some of the activities of PGPMs (Kannapiran and Ramkumar, 2011). These activities coupled with the availability of balanced nutrients (N and P) might have led to the improvement of the yield and yield attributes in this study.

Conclusion

The PGPM inoculants used in this study which had the ability to solubilize P, fix N and to induce the production of IAA, improved the yield and yield attributes of the rice plants (with the best PGPM giving a yield increase of 5 % over the control).

Their usage as inoculant bio-fertilizers is an efficient approach to phase out chemical fertilizers for sustainable rice production in Kenya. As opposed to the utilization of chemical fertilizer which results to environmental hazards such as water pollution and soil degradation, the bio-fertilizer are non-pollutants to the environment and are ecofriendly. Therefore the present study suggest the utilization of PGPM isolates such as *Bacillus, Enterobacter and Aspergillus* as inoculant bio-fertilizer are beneficial for rice production.

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