academicJournals

Vol. 12(15), pp. 345-353, 21 April, 2018
DOI: 10.5897/AJMR2017.8601
Article Number: FD1F09E56856
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

*Full Length Research Paper*

# Effect of indigenous and effective microorganism fertilizers on soil microorganisms and yield of Irish potato in Bambili, Cameroon

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Received 25 May, 2017; Accepted 13 February, 2018

**Irish potato (*Solanum tuberosum*) is one of the world's most consumed staple worldwide and an important crop in terms of food security in the face of population growth and increased hunger rates. Potato yields in Cameroon have often been low as a result of decrease in soil fertility. Soil fertility has often been regarded as the chemical and physical properties of soil, with the microbial aspect often being ignored. An experiment was carried out in Bambili, Cameroon to evaluate the effect of two organic fertilizers (indigenous microorganism fertilizer, IMO, and effective microorganism fertilizers, EM) on the yield of Irish potato and to identify some soil bacteria and fungi. A randomized complete block design with three treatments (EM, IMO and control), and four replications was used. Fertilizers were applied one week before planting and repeated four and eight weeks after planting. Soil samples were collected before the application of fertilizers, and then 1, 6 and 10 weeks after application of the fertilizers and used to find out microorganisms present in the different treatments at different periods of plant growth. Different culture media were used for the primary cell culture of the bacteria and fungi using the spread plate technique while isolation of pure bacteria cultures was done by streaking. The fresh weight of tubers under IMO fertilizer was higher than those with EM fertilizer and the control. Some microorganisms identified in the different treatments included: *Aspergillus*, *Rhizopus, Penicillium*, *Fusarium, Saccharomyces, Enterobacteria* and *Pseudomonas* which were present in all the treatments but at different growth stages of the plants. Both IMO and EM fertilizers had significant positive effects on the tuber yield and the soil microbial population in the different treatments.**

**Key words:** Bacteria, fungi, *Solanum tuberosum*, tuber yield.

## INTRODUCTION

Microorganisms play an important role in the improvement of soil quality, thereby favoring the growth of plants. In most soil fertility studies, attention is usually focused on soil physical and chemical properties while

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Indigenous microorganisms (IMO) are ‘‘naturally’’ occurring microbes that have adapted to the environmental conditions where they are found and are therefore capable of accelerating decomposition of organic materials found in the same area (Singh and Sharma, 2003).

Effective microorganisms (EM) consist of mixed cultures of beneficial and naturally occurring microorganisms which are applied to the soil in other to increase the microbial diversity of soils and the growth of plants (Muthaura et al., 2010). The concept of EM was first discovered by Higa (Suthamathy and Seran, 2013). EM is used by the crops as a means of improving the efficient utilization of organic matter.

Microorganisms are important attributes in agriculture because they promote the decomposition, cycling and circulation of plant nutrients and reduce the need for chemical fertilizers. Biofertilizers are organic products containing living cells of different types of microorganisms that have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally sustained nutrient supplies (Muthaura et al., 2010). The increased use of chemical fertilizers and some organic fertilizers in agriculture helps the country in achieving self-sufficient food grain production (Sumathi et al., 2012). The soil fertility of an area or location is very important and optimum productivity may turn to long-term economic benefits, which will reflect on the yield and yield components based on the perceived knowledge of soil fertility (Ibeawuchi et al., 2007). Application of organic matter positively affects the growth and development of plant roots and shoots (Ghosh et al., 2004)

EM and IMO are all products of natural farming and have beneficial effects both on the soil and the crops. Notwithstanding, there are differences between them. In terms of number and types of microorganisms found in them, EM has more microbes than IMO. EM has three main families of over 80 different species (Daly and Stewart, 1999). On the other hand, IMO has mainly Lactobacillus and sometimes *Rhizobium* with a few other species (Hiddink et al., 2005). It is easier for farmers to get adequate results while using EM since the microbes are available from a reliable source. In terms of cost, IMO is cost effective than EM since it is collected from the locality (Carandang, 2003). It is less expensive, but with EM, it must be bought from a reliable source. EM has well combined microbes which produce a symbiotic and mutualistic interaction among the constituent microbes (Daly and Stewart, 1999). These microbes therefore work synergistically thereby producing a very effective ecosystem which can ensure survival of most of the microbes. On the other hand, microbes in IMO do not have a mutualistic and synergetic effect like EM as they are collected by chance (Hiddink et al., 2005). In terms of adaptability, IMOs adapt more to the environment since they grow within the same climatic and environmental conditions. Contrary to this, EM are most probably collected from an area with a different climatic and environmental condition. Considering these differences, it is very evident that comparing the effects of EM and IMO on crop productivity will vary according to these differences and it will be difficult to say with precision which fertilizer will produce better results in the soil types.

There are many opinions on what an ideal agricultural system is. Many will agree that it should produce food on a long-term sustainable basis. Others will insist that it should maintain and improve human health, be economical and beneficial to both producers and consumers, actively preserve and protect the environment, be self-contained and regenerative, and above all produce enough food for an increasing world population (Higa, 1991). It will be therefore better for agriculture to be geared towards less chemically intensive to more biologically based practices so as to improve soil health and agricultural production and be less harmful to humans and the environment than conventional agricultural production methods.

A survey carried out in the national territory of 2000 and 2001 showed that potato yields in Cameroon vary according to the production zone, from 3.3 to 6.7 t $ha^{-1}$ with an overall mean of 6.0 t $ha^{-1}$ (Njualem, 2010). In the Western Highlands of Cameroon, it is estimated that over 200,000 small holder farmers, mostly women, are involved in the production of potato. Their production accounts for more than 80% of the national production, estimated at 142,000 t $yr^{-1}$ cultivated on 45,000 ha. In addition, between 1986 and 2009, these farmers were able to increase potato yields from 2.5 to 5 t $ha^{-1}$ (Fontem et al., 2004). The aim of the study was to investigate the effects of indigenous and effective microorganism based fertilizers on soil microbial activities and their effect on the yield of Irish potato.

## MATERIALS AND METHODS

### Study area

This research was carried out in the research farms of Higher Teachers Training College (HTTC) of the University of Bamenda at the Bambili campus. Bambili is located in Tubah sub-division, Mezam division of the Northwest region of Cameroon. The town has a total surface area of about 250.69 $km^2$. It is located between latitudes 5° 60’ 0 ’’ and 6° 05’ 0 ’’ north of the equator and between longitudes 10° 12’ 0’’ and 10° 22’ 0 ’’ east of Greenwich Meridian. It has a humid tropical climate with an average annual rainfall of about 2200 mm. The temperature is about 20.7°C (Focho et al., 2009). Bambili has an undulating topography with altitude varying between about 900 and 2270 m above sea level (Yerima and Van Ranst, 2005). The climate is characterized by two distinct seasons: a long wet season (March to October) with high winds followed by a short dry season (November to March) with high light intensity.

### Preparation of IMO fertilizer

IMO fertilizer was prepared according to the method of Park and Du


**Figure 1.** Procedure for collecting microorganisms, (a) Boxes ready to be buried, (b) A box of cooked rice buried, and (c) Rice covered with white mold 7 days after burying.


**Figure 2.** Culturing the IMO manure, (A) weighing of molded rice, (B) hand kneading of molded rice/sugar mixture, (C) clean clay pot 2/3 full of the rice/sugar mixture

Ponte (2008) using local materials, as elaborated below:

***Collection of microorganism from the environment***

Five wooden boxes were ¾ filled with steamed rice. The boxes were covered with white paper towel, rubber bands were then used round the top of the boxes to secure the paper towel in place (Figure 1a). The boxes were partially buried, such that their surfaces were left exposed to the atmosphere, and they were covered with fallen leaves (Figure 1b). After 7 days, the boxes were removed (Figure 1c).

The molded rice was transferred from the wooden boxes into a bowl and weighed (Figure 2a). Equal weight of granulated brown sugar was gradually added to the molded rice and the mixture was hand kneaded until it turned uniform, soft and sticky (Figure 2b). A clean clay pot was filled, 2/3 full with the rice/sugar mixture, and covered with paper towel (Figure 2c). The pot was then stored away from direct sunlight for 7 days to allow the mixture to ferment. After 7 days, the pot was removed and water in a ratio of 1:500 (v/g) was added to the fermented mixture. The mixture was then compressed in a 200 L drum, covered with a lid, and kept away from sunlight for another 7 days. After the 7 days, the IMO was ready for use.

**Preparation of EM fertilizer**

In order to prepare 100 kg of EM fertilizer, 1 kg of brown sugar, 1 L molasses and 1 L of EM inocula were mixed in a clean container using a wooden spoon until a homogeneous solution was obtained (Figure 3a and b). Twenty liters of chlorine-free water was added which served as a favorable medium for the survival of microorganisms. Fifty kilograms of rice and 50 kg of wheat bran were poured on a clean dry cemented floor and mixed thoroughly using a spade. The liquid mixture was then poured in a hole made in the middle of the dry ingredients and mixed with the hands and spade until the mixture was homogeneous (Figure 3c and d). The mixture was then put in a 300 L plastic tank and compressed very well to maintain anaerobic conditions. The tank was properly closed and left unopened for 7 days. At this point, the EM was ready for use.

**Land preparation and planting**

A piece of land of dimensions 20 m by 15 m was selected using a measuring tape. The land was cleared using a cutlass and tilled using a hoe. A randomized complete block design (RCBD) with three treatments (EM, IMO and the control) and four replications was used. Holes of about 10 cm deep and 30 cm apart were dug and 38 g of EM fertilizer and 38 g of IMO fertilizer was applied according to the treatments one week before planting. It was repeated at 4 and 8 weeks after planting (WAP) round the plant making sure it does not touch the stem. One potato seed (CIPIRA variety) was planted per hole on the same day one week after the first application of treatments.

**Harvesting**

Harvesting was done at 12 WAP, when the plants had already withered showing that tubers were matured. Tubers from each


**Figure 3.** Preparation of EM manure. (a): 1 L of molasses and EM, (b) 1 kg of brown sugar mixture, (c) Liquid mixture poured in the rice, (d) Mixing rice and wheat bran.

treatment were counted and weighed.

**Collection and preparation of soil samples for microbial analysis**

Before the application of the fertilizer, soil samples were randomly collected at a depth of 0 - 15 cm using a sampling auger and then bulked and labeled. This procedure was repeated for different treatments at 1, 6 and 10 weeks after planting on the spots where fertilizers were applied. All samples were stored in sterilized bottles, ready for microbial analysis.

**Preparation of culture media**

Primary cell culture of bacteria was done as described by Ahmed et al. (2013) in which ten-fold dilution ($10^{-4}$) was used for bacterial culture following the spread plate technique. One hundred microliters of diluted sample (from $10^{-4}$) was pipetted unto the surface of agar. A sterile spreader was used to spread the sample evenly on the entire agar surface. The plates were labelled and incubated at 37°C in a bacteriological incubator (BINDER, USA) for 72 h.

Nutrient agar medium which is a multipurpose medium for bacteria, Sabouraud Dextrose Agar, a multipurpose medium for fungi, Cled Agar medium also for *Salmonella* and *Pseudomonas*, and Mac Conkey Agar medium which is medium for *E. coli, Enterobacteria* and coliforms, were used.

**Primary cell culture of bacteria**

Primary cell culture of bacteria was done as described by Ahmed et al. (2013) in which, ten-fold dilutions ($10^{-4}$) was used for bacterial culture following the spread plate technique. One hundred microliter of diluted sample (from $10^{-4}$) was inoculated unto the surface of agar. A sterile spreader was used to spread the sample evenly on the entire agar surface. The plates were inoculated and incubated at 37°C in a bacteriological incubator (BINDER, USA) for 72 h.

**Isolation of pure bacterial cultures**

The streak plate technique described by Cheesbrough (2000) was used for the isolation of pure bacterial cultures. A sterile inoculation loop was used to pick out small amounts of bacteria from separate morphologically distinct colonies. This was used to inoculate sterile nutrient agar surfaces by streaking. The plates were inoculated and incubated at 37°C in a bacteriological incubator for 24 to 48 h.

**Extraction and culture of fungi**

Fungi species found in the treatments were identified using the method of Gautam et al. (2011). Chloramphenicol antibiotic (0.03 mg/L) was added to the media to avoid bacterial contamination.

**Identification of fungal isolates**

Fungal isolates were identified as described by Navi et al. (1999) on the bases of morphological and microscopic examinations.

**Morphological characterization of bacterial isolates**

Isolated bacteria were characterized based on colony and cellular morphology as described by Cheesbrough (2000).

**Characterization by colonial morphology**

Colonial morphology was described using parameters such as colony form, margin, colour, elevation and appearance. Freshly cultured bacterial isolates (24 to 48 h cultures) were characterized morphologically by observing and recording the above colonial parameters.

**Characterization by cellular morphology**

Characterization by cellular morphology was carried out following Gram's staining. This was done as described by Cheesbrough (2000). Freshly cultured bacteria isolates (48 h cultures) were used for these purposes.

**Gram stain**

***Preparation and fixing of smears***

With the use of a sterile inoculation loop, 2 to 3 loopful of sterile distilled water was placed on a clean dry labeled grease-free slide.

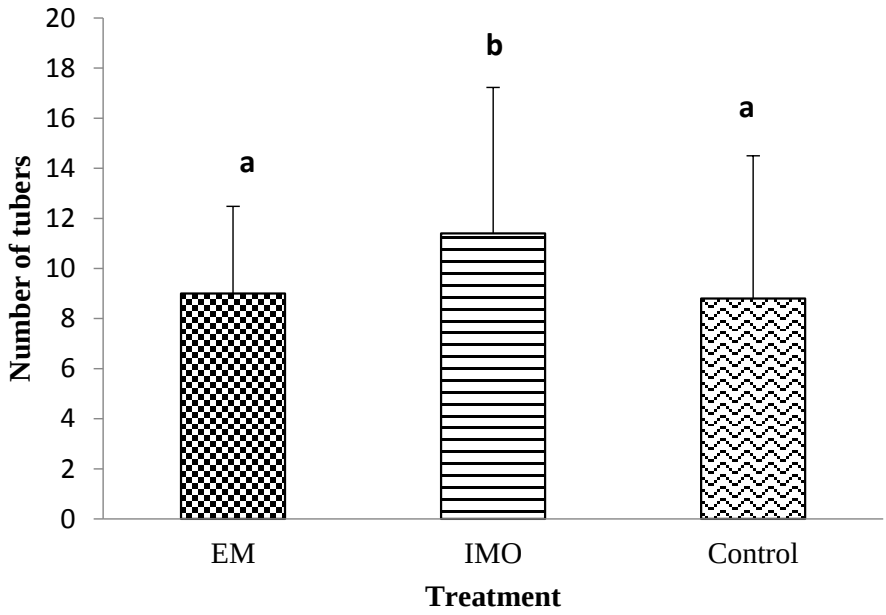


**Figure 4.** Average number of tubers per plant and per treatment. Histograms with same letter are not significantly different at P ≤ 0.05 (DMRT).

The loop was re-sterilized by heating in a Bunsen flame till it became red hot, and then cooled and used to pick up a small amount of bacteria from single colonies. The bacteria were then emulsified in saline water on the slide to form a thin smear. The slides were air-dried completely. Smears were fixed by rapidly passing the slide, smear uppermost, three times through the flame of a Bunsen burner. The smear was allowed to cool before staining.

***Gram staining procedure***

The fixed smear was flooded with crystal violet solution for 30 to 60 s. The dye was quickly drained and washed with clean running water. The smear was then covered with Lugol's iodine for 30 to 60 s. The iodine was drained and slide washed gently using clean running water. Rapid decolourization of the smear was done using acetone-alcohol for 5 s and slide washed gently using clean running water. The counterstain, carbolfuchsin was used to flood the slide for 30 s after which it was drained and washed with clean running water. The slides were then dried in a preheated oven for 5 min and then observed under oil immersion lens (100x objective). Bacteria cells were then characterized as either Gram-positive if they stained dark purple or Gram-negative if they stained pink.

**Identification of bacteria strains**

The different bacteria strains were identified using the identification technique of Cheesbroug (2000).

**Statistical analysis**

The data collected were analyzed using Microsoft Excel (2010 version). Data obtained were expressed as means ± SD and analyzed statistically using SPSS statistical software version 17.0 (SPSS Inc., Chicago). Significant differences between mean values were determined using analysis of variance (ANOVA). Duncan multiple range Test (DMRT) was used to compare treatment means at 0.05 level of significance.

## RESULTS

### Number of tubers per plant

Plants treated with IMO fertilizers produced the highest number of tubers per plant (11.40 ± 5.83), followed by those treated with EM manure (9.00 ± 3.48), and then the control plants, the least (8.80 ± 5.69). The number of tubers of plants treated with IMO was significantly different from the number of tubers of plants treated with EM manure and the number of tubers of control plants (Figure 4).

### Fresh weight of tubers

Plants treated with IMO fertilizers produced potato tubers with the heaviest weight (241.64 ± 32.94 g), followed by those treated with EM manure (227.62 ± 44.58 g) and the control which produced tubers with the least weight (125.66 ± 31.63 g). Statistical analysis revealed significant differences (p ≤ 0.05) between plants treated and control plants (Figure 5).

### Morphological identification of fungi

The fungi were colonially and microscopically identified and found to belong to the same phylum of Ascomycota,

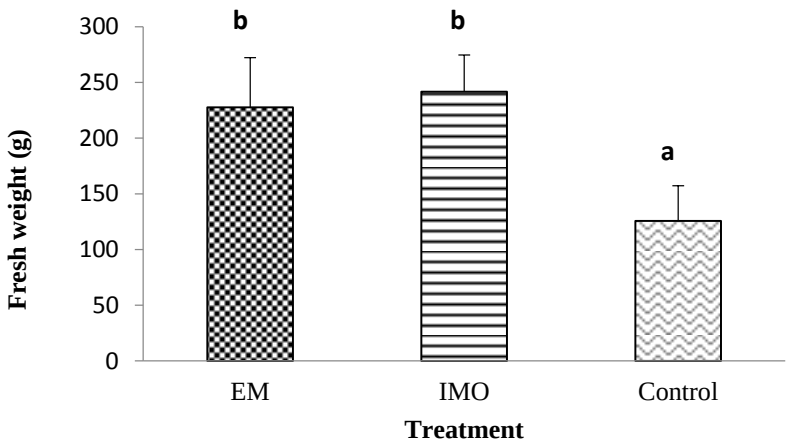


**Figure 5.** Average weight of tubers per treatment. Histograms with same letter are not significantly different at P ≤ 0.05 (DMRT).

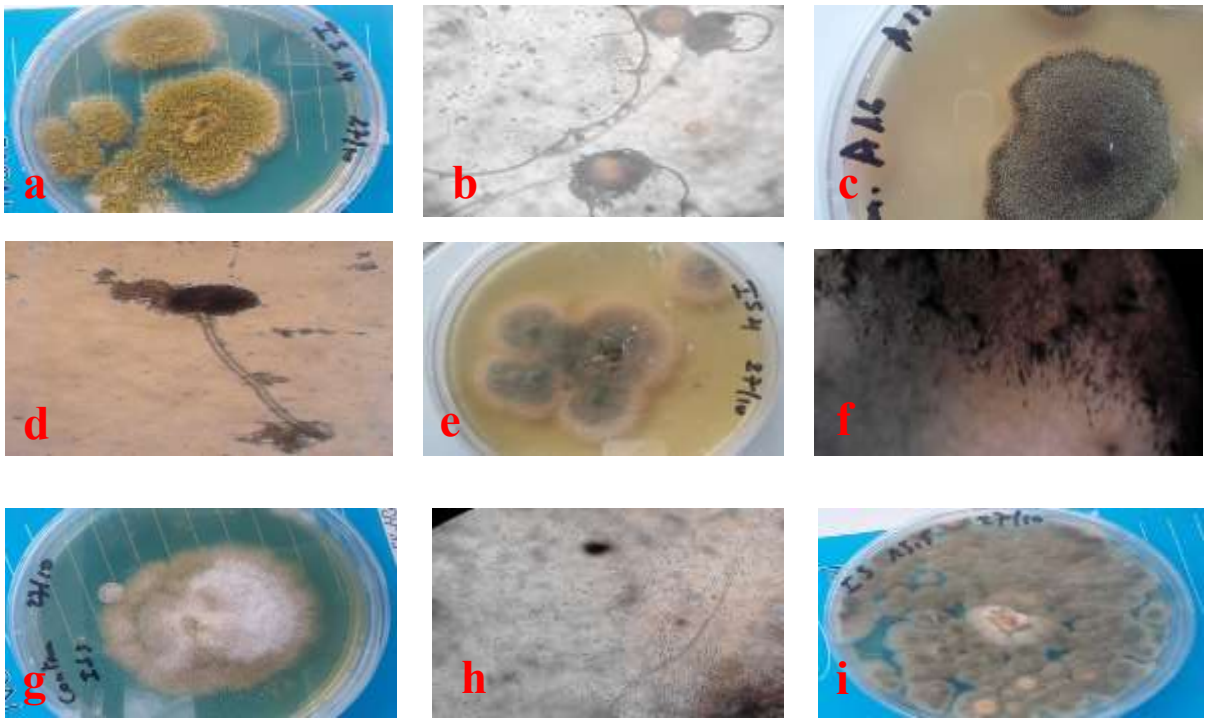


**Figure 6.** Surface and microscopic view of fungi in different treatments; (a, b) *Aspergillus*, (c, d): *Rhizopus*, (e, f): *Penicillium*, (g, h): *Fusarium*, (i):*Saccharomyces.*

but from five genera (*Aspergillus*, *Rhizopus, Penicillium*, *Fusarium* and *Saccharomyces*, respectively) (Figure 6).

## Characterization of bacterial isolates by colonial morphology

A total of 6 distinct bacterial isolates were obtained in the soil samples. These isolates were distinguished based on their colonial morphology observed on nutrient agar plates (Table 1).

## Characterization of bacterial isolates by cellular morphology

Of the six isolates, two ($I_5$ and $I_6$) were Gram positive rods with $I_6$ having central spores. The remaining

**Table 1.** Morphological characterization of bacteria isolates.

| Parameter | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | $I_6$ |
|---|---|---|---|---|---|---|
| Margin | Entire | Undulate | Undulate | Entire | Entire | Undulate |
| Form | Circular | Irregular | Irregular | Circular | Circular | Irregular |
| Elevation | Raised | Raised | Flat | Convex | Flat | Undulate |
| Colour | Pale orange | Dark green | Creamy | Creamy | Brick red | Creamy |
| Appearance | Mucoid | Mucoid | Dull | Mucoid | Mucoid | Dull |

I = isolates.

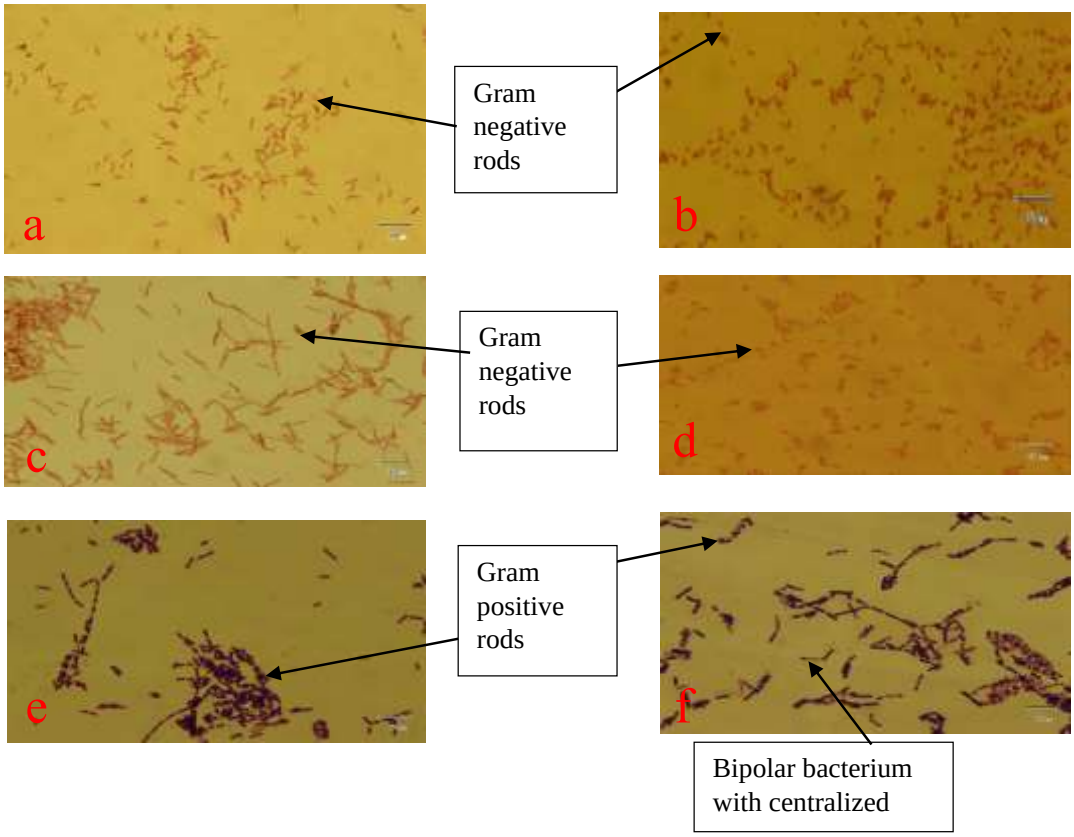


**Figure 7.** Gram stains of bacteria isolates in different treatments; (a) Gram stain of I1, (b) Gram stain of I2, (c) Gram stain of I3, (d) Gram stain of I4, (e) Gram stain of I5, (f) Gram stain of I6.

fourwere Gram negative rods (Figure 7).

**Fungi and bacteria found in the different soil treatments with time**

*Aspergillus* was found in both IMO and EM soil at 6 WAP but at 10 WAP, it was only present in the IMO soil. Before the application of the different treatments, it was not present and throughout the growth period of the plants, it was absent in the control. *Rhizopus, Penicillium and Fusarium* were absent before the application of treatments and *Rhizopus* was present only at 6 WAP for the control, while for IMO, it was present throughout and absent at the 10$^{th}$ WAP for EM. *Saccharomyces* were present in all the different treatments before and after application of the manures (Table 2).

From the Gram staining results, the bacteria were identified to belong to two main groups: the *Enterobacteriaceae* which were the Gram negative rods and the *Pseudomonas* which were Gram positive rods. *Enterobacterium* was present in all the different treatment before and after application of the manures. *Pseudomonas* was not in the soil before the application of fertilizers and at 1 WAP. It was present in the control

**Table 2.** Variation in some fungi and bacteria found in the different soil treatments with time.

| Microorganism | Before | 1 WAP | | | 6 WAP | | | 10 WAP | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | E | I | C | E | I | C | E | I | C |
| *Aspergillus* | - | - | - | - | + | + | - | - | + | - |
| *Rhizopus* | - | + | + | - | + | + | + | - | + | - |
| *Penicillium* | - | - | - | + | - | - | - | - | - | - |
| *oFusarium* | - | - | - | + | + | + | + | + | + | + |
| *Saccharomyces* | + | + | + | + | + | + | + | + | + | + |
| *Enterobacteriaceae* | + | + | + | + | + | + | + | + | + | + |
| *Pseudomonas* | - | - | - | - | + | + | - | + | + | + |

E = EM, I = IMO, C = control, + = present, - = absent.

only at 10 WAP, while for EM and IMO soil, it was present at 6 and 10 WAP (Table 2).

## DISCUSSION

The crop yield was greater in the treated soil than the control soil with IMO manure having a greater and more significant tuber yield than EM fertilizer. The number of tubers per plant and fresh weight of tubers were higher in IMO and EM treated soils. EM and IMO manures are organic fertilizers which play a significant role in maintaining and improving the chemical, physical and biological properties of soils and in sustaining crop yield. Beneficial microorganisms in IMO were indigenous to the soil and environmental conditions of the farm and could more easily adapt, unlike those in EM manure which were only imported from abroad (Prell, 2010). According to Koon-Hui et al. (2013), IMO treated plants did best because mycorrhizae contributed to the soil tilt in IMO plots. This result is confirmed by Woo et al. (2006) with the explanation that beneficial fungal species colonize plant root and stimulate increased nutrient uptake to improve yield (Muyang et al., 2014). However, this result was different from the observations made by Yamada and Xu (2000) where EM treated plants did better than IMO treated plants. Mbouobda et al. (2014) showed that carrots grown with EM manure did better than those grown with IMO manure and the control.

*Aspergillus, Penicillium, Enterobacteria* and the *Pseudomonaceae* are amongst the most powerful phosphate solubilizers (Whitelaw et al., 2000). *Aspergillus* was found in both IMO and EM soil at 6 WAP and at 10 WAP, it was only in the IMO soil. Before the application of the different treatments, it was not present and throughout the growth period of the plants, it was absent in the control. This was similar to studies carried out by Gaur and Sadasivam (1993) where the nutrient status of sorghum stalk and wheat straw compost was improved when inoculated with *Aspergillus niger* and *Penicillium* species. However, it was not the case with *Penicillium* which was present only in the control at 1 WAP. *Rhizopus* is a type of mold which grows on organic matter and could be the reason why it appeared more in the inoculated soils. *Fusarium* was present only in the control at 1 WAP and in all the treatments at both 6 and 10 WAP. *Fusarium* is a plant pathogenic microorganism which causes wilting in potatoes. When a soil has a high population of *Fusarium, Phytophthora* and *Pythium*, it is considered to be a disease inducing soil (Higa, 1994). *Saccharomyces* was present in all the different treatments before and after application of the fertilizer. When microbial amendments are applied to the soil, their fermentative activities can increase drastically and overwhelm the indigenous soil microflora for an indefinite period (Higa and Parr, 1994). *Enterobacterium* was present in all the different treatments before and after application of the manures. *Pseudomonas* was not present before the application of the fertilizer and at 1 WAP. It was present in the control only at 10 WAP while for EM and IMO soil, it was present at 6 and 10 WAP. *Enterobacteria* and *Pseudomonas* are phosphate solubilizers which play an important role in supplementing phosphorus to plants (Tamberkar et al., 2009). This also explains why the yield was higher in the two amended soils as compared to the control.

### Conclusion

The purpose of this study was to evaluate the effect of IMO and EM fertilizers on the yield of Irish potato (*Solanum tuberosum*) and on some of the microorganisms in the soil. From this study, the following conclusions were drawn:

(i) Both IMO and EM fertilizers had a better effect on the yield of Irish potato with IMO producing the best yields in terms of number and weight of tubers.
(ii) *Aspergillus, Penicillium, Enterobacteria* and *Pseudomonaceae* were the most common microorganisms and are amongst those that help in improving crop productivity.

According to the results obtained, the use of

microorganisms (indigenous and effective microorganism manure) was shown to significantly increase the yield of Irish potato and therefore could be recommended as organic amendment for improving yield of Irish potato.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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