

# Isolation and Characterization of Beneficial Microorganisms in Organic, Semi-Organic and Conventional Fertilizer Treated Agricultural Field Soil and Comparison of Bacterial Richness

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**How to cite this paper:** Rahman, M.M., Lisa, L.A., Ahmed, S., Uddin, M.N., Hasan, T. and Zaman, S. (2020) Isolation and Characterization of Beneficial Microorganisms in Organic, Semi-Organic and Conventional Fertilizer Treated Agricultural Field Soil and Comparison of Bacterial Richness. *Journal of Agricultural Chemistry and Environment*, 9, 223-239.

<https://doi.org/10.4236/jacen.2020.94018>

**Received:** September 24, 2020

**Accepted:** October 27, 2020

**Published:** October 30, 2020

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## Abstract

The effect of different farming systems on microbial communities in agricultural environment was investigated in the present study. Depending on the present farming trend, the microbial distribution in agricultural soils treated with organic, semi-organic and conventional fertilizers was analyzed. A total of 20 soil samples were collected from different types of agricultural fields of Bangladesh Agricultural Research Institute (BARI, Gazipur). Microorganisms playing beneficial roles in soil such as nitrogen fixation (e.g. *Rhizobium sp.*, *Azotobacter sp.*), phosphate solubilization (e.g. *Bacillus sp.*, *Pseudomonas sp.*, *Phosphobacteria*) and auxin production (e.g. *Pseudomonas sp.*, *Serratia sp.* and *Bacillus sp.*) were evaluated from each of the samples. The results revealed that agricultural fields treated with chemical fertilizers showed lower microbial count than that of organic fertilizer treated agricultural fields' soil samples. In addition, organic fertilizers amended field soils have higher phytohormone (Auxin) activities, phosphate solubilization bacteria and other bacterial richness compared to chemical fertilizer applied field soil.

## Keywords

Chemical Fertilizer, Organic Fertilizers, Bacterial Richness and Bacterial Diversity

## 1. Introduction

Soil microorganisms play a very important role in soil fertility not only because of their ability to carry out biochemical transformation but also due to their importance as a source and sink of mineral nutrients [1]. Soil microbes, the living part of soil organic matter, function as a transient nutrient sink and are responsible for releasing nutrients from organic matter for use by plants (e.g., N, P and K). An understanding of microbial processes is important for the management of farming systems, particularly those that rely on organic inputs of nutrients [2].

Chemical fertilizers have been extensively applied to sustain global agricultural production since the first Green Revolution [3]. However, on one hand, the production and use of these chemicals impart various negative effects on the agricultural ecosystem [4] [5]. On the other hand, application of organic compost enhances soil microbial activities, increase organic matter the levels and improve soil porosity, structural stability, moisture, and nutrient availability, as well as biological activity that consequently increase the product yield [6].

In Bangladesh, where agriculture is the main source of livelihood of two-thirds of the rural population, a serious concern has arisen about the sustainability of agriculture in the face of deterioration of land quality, declining yield, and increased population [7]. The increased use of inorganic fertilizers, insecticides and pesticides in limited amount of lands has led to deteriorate soil fertility, nutrient depletion, and contamination of water bodies and the spread of diseases, which have adversely affected aquatic life, livestock and people [8] [9]. More than two-third of the total agricultural area is suffering from declining soil fertility, and about 85 percent of the net cultivable land has less organic matter than the minimum requirement for maintaining soil productivity [7]. This has brought some major changes in cropping patterns, uses of agricultural inputs, and nature of soil fertility. Therefore, sustainable agriculture is an emphasis in response to the adverse environmental and economic impacts of conventional agriculture. In contrast, sustainable agriculture is seen as low-input and regenerative, which makes better use of a field's internal resources through incorporation of natural processes into agricultural production and more prominent utilization of improved knowledge and practices [10]. Therefore, the present study aims to compare the microbial quality of organic, semi-organic and inorganic agricultural fields' soil, its richness in beneficial microorganisms; effect of pesticides on beneficial microorganisms and finally isolation and characterization of plant growth hormone releasing bacteria.

## 2. Materials and Methods

### 2.1. Sample Collection

Total 20 soil samples from different controlled agricultural plots harvesting different vegetables at Bangladesh Agricultural Research Institute (BARI), Gazipur were collected. The plots were categorized into three types of farming activities

such as organic farming, semi-organic farming and chemical farming. Organic farming means those farms which are applying only organic fertilizers like pile or heap compost, vermi compost, quick compost, tricho compost, cow dung etc. in their crop production. Similarly, those farmers are applying both organic fertilizers and chemical fertilizers are treated as semi-organic farming and those who are applying only chemical fertilizer in their crop fields are treated as chemical farming. A total of 24 soil samples were collected for organic, semi-organic and chemical farming from 8 vegetables producing farms (3 samples from each vegetable field). The soil samples were collected from 15 cm depth from each plot with a stainless-steel soil probe. The soil cores from the same plot were placed in a clean plastic bucket and mixed thoroughly to form a composite sample. Composite samples were transferred immediately into sterilized polyethylene bags and kept in cool boxes for maximum 2 hours until transported to the laboratory. Once in the laboratory, all visible roots and plant fragments were removed manually from the soil samples. The field-moist soil samples were stored at 4 °C until analyzed.

## 2.2. Sample Preparation for Microbiological Analysis

About 25 gm of each sample was dissolved into 225 ml normal saline (0.85% W/V) and homogenized separately by a Stomacher<sup>®</sup> machine (Seward Stomacher 400 Circulator, United Kingdom) at 230 rpm for 60 seconds. The serial dilution was done in test tubes containing 9 ml of sterile normal saline each. Spread plate method was performed to isolate and enumerate the organism of interest present in different samples.

## 2.3. Detection Methods

Total aerobic bacteria, total *Coliform* bacteria and *E. coli* were analyzed in surface plate method using TSA (Tryptic Soy Agar) and Sorbitol MacConkey Agar medium (Oxoid, UK) followed by biochemical tests as per described by Gowsalya *et al.*, 2014 [11]. Other bacteria including *Bacillus sp.*, *Pseudomonas sp.*, *Azotobacter sp.*, *Rhizobium sp.*, *Klebsiella sp.* were identified using selective agar medium followed by API immunoassay analysis as per described by Jakaria Al-Mujahidy *et al.*, 2013; Ashish *et al.*, 2011; and Ridvan 2009, respectively [12] [13] [14]. Phosphate solubilizing fungi and Phosphobacteria was identified using surface plate method on Pikovskaya's agar (PVK) and yeast malt agar plate, respectively as described by Emilce *et al.*, 2011 [15]. In addition, total fungal count, and Nitrogen fixing fungi, was determined Sabouraud Dextrose Agar (SDA) in surface plate method followed by microscopy as described by Rohilla and Salar 2012 [16]. Furthermore, *Salmonella sp.*, and *Shigella sp.*, was detected using surface plate methods on *Salmonella-Shigella* (SS) agar (Oxoid, UK) followed by biochemical test as described by Romain *et al.*, 2013 [17]. The qualitative detection of *E. coli* O157, *Salmonella sp.* was performed by GLISA (Gold Labelled Immuno Sorbent Assay).

## 2.4. Detection of Phosphate Utilization Microorganisms

For determination of phosphate utilization, *Aspergillus sp.* was isolated from the collected samples using Sabouraud Dextrose Agar (SDA) and incubated with NBRIP (National Botanical Research Institute's phosphate growth medium) agar medium. Colony Forming Unit (CFU) was used to estimate the number of viable bacterial or fungal cells using the formula  $CFU/ml = (\text{number of colonies} \times \text{Dilution factor}) / \text{Volume of culture plate}$ . The measurement was then converted into common logarithmic value. Analytical Profile Index system (API 20E, API 20NE & API 50 CH) manufactured by bioMérieux® was used to identify microorganisms.

## 2.5. Detection of IAA Producing Microorganisms

IAA production by agricultural microbes was determined using Salkowski's method [18] and optical density (OD) of the test solution was measured at 530 nm by a UV spectrophotometer (Shimadzu, CPS-240A, Japan) [19] [20].

## 2.6. Effect of Pesticides on Beneficial Microorganisms

The effect of pesticides (*i.e.* Chlorpyrifos-1.5 ppm; Carbofuran-250 ppm and Carbaryl-80 ppm) on agricultural microorganisms was measured in vitro by Agar well diffusion method [21].

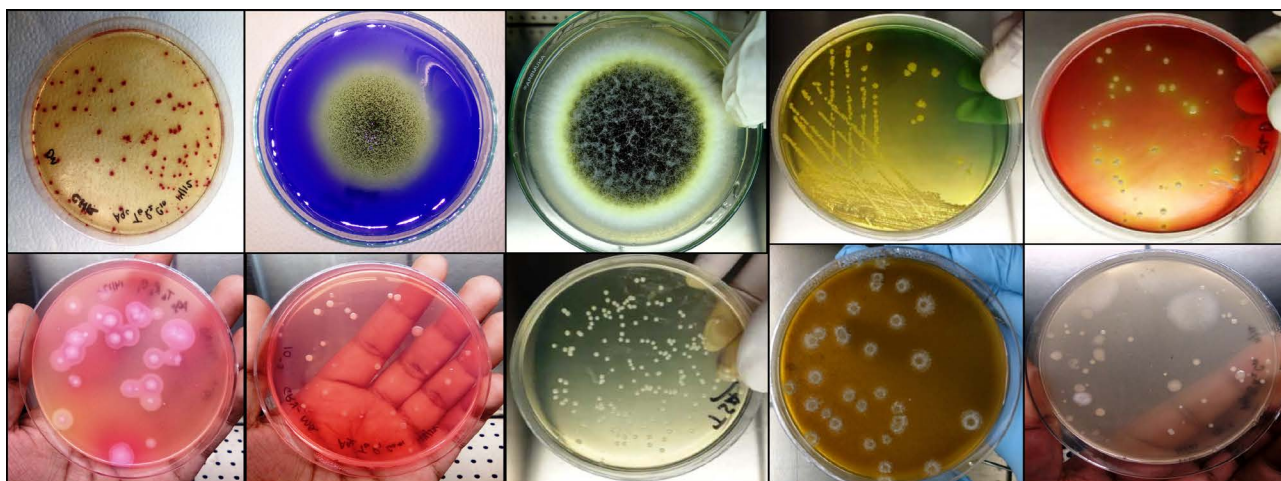
## 3. Statistical Analysis

All the trials were replicated three times. Reported data represented the mean values obtained from five individual trials, with each of these values obtained from duplicated samples. Data were subjected to analysis using the Microsoft Excel program (Redmond, Washington, DC, USA). Significant differences in plate count data were established by the least significant difference at the 5% level of significance.

## 4. Results and Discussions

### 4.1. Microbiological Quality, Safety and Richness

A total of 12 media preparations including Tryptic Soy Agar (TSA), Chromocult, Flurocult, Cetrimide, Xylose lysine deoxycholate (XLD), Thiosulfate-citrate-bile salts-sucrose (TCBS), Mannitol Salt Agar (MSA), NaCl-Glycine-Kim-Goepflect (NGKG) agar media, Congo Red Yeast (CRY), Nitrogen Free Agar (NFA), Sabouraud Dextrose Agar (SDA) and National Botanical Research Institute's phosphate growth medium (NBRIP) were used for the inoculation of samples and to isolate 12 types of organism for this study. The media preparations were used accordingly to isolate and determine total aerobic bacterial count, total coliform count, *E. coli*, *Salmonella sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Shigella sp.*, *Bacillus sp.*, *Rhizobium sp.*, *Azotobacter sp.*, Total fungal count and Phosphate solubilizing microorganisms. The colony characteristics of the isolates upon growth are shown as follows (Figure 1). Microbial counts (CFU/ml) on



**Figure 1.** Plates showing growth of microorganisms on different agar media.

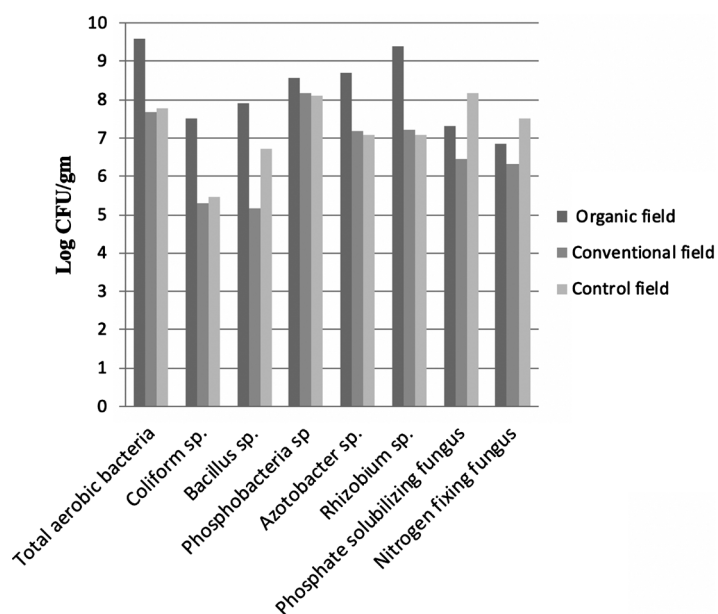
different types of selective agar plates and were converted into common logarithmic value. Then mean value and their corresponding standard deviation (SD) of each sample were calculated and were presented in **Table 1** and **Figure 2** & **Figure 3**. Further identification of 6 isolates at the level of species was conducted by using 3 types of biochemical testing apparatus such as API 20E, API 20NE and API 50CH (**Table 2**). The results were obtained from API web analysis software.

The average aerobic bacterial count in organic, semi-organic and conventional soil was recorded as was  $9.05 \pm 0.54$  CFU/g,  $8.96 \pm 0.28$  CFU/g and 8.76 CFU/g, respectively. Total coliform counts in these samples were recorded as  $7.54 \pm 0.01$  CFU/g, 6.51 CFU/g and 6.25 CFU/g. Presence of higher number of coliform bacteria was visible in all the soil samples indicating the lack of environmental hygienic practices. Irrespective of soil type and conditions, non-detectable level of pathogenic microorganisms (*E. coli*, *Salmonella sp.* and *Shigella sp.*) was observed in any of the field soil sample tested (**Table 1**). On the other hand higher number of essential microorganisms including *Bacillus sp.*, ( $6.18 \pm 0.28$ ) CFU/g, Phosphobacteria ( $9.41 \pm 0.42$ ) CFU/g, *Azotobacter* ( $9.36 \pm 0.27$  CFU/g), *Rhizobium* ( $9.76 \pm 0.19$  CFU/g), phosphate solubilizing fungi ( $9.28 \pm 0.14$  CFU/g), and Nitrogen fixing fungi ( $9.32 \pm 0.15$  CFU/g) was present in organic compared to control and conventional soil. Presence of higher number of *Rhizobium sp.* increases the plant health and soil fertility. Although higher number of *Bacillus sp.* ( $6.73 \pm 0.03$  log CFU/g) was recorded in all the field samples, lower number of *Pseudomonas sp.* ( $1.89 \pm 2.67$  log CFU/g) was observed in the field soil. Presence of higher number of *Pseudomonas sp.* is required for inhibiting some plant pathogens, and plant growth factor. However, all the soil samples tested contain significantly lower number of *Pseudomonas sp.* and hence supplementation of these bacteria is necessary in these fields to ensure that the bacteria reduce pathogens around the seed and root of the crop. It has been recommended that presence of 8.0 log CFU/g of phosphate utilizing bacteria (Phosphate solubilizing fungi, phosphobacteria etc.) and nitrogen fixing microorganisms (*Azotobacter*,

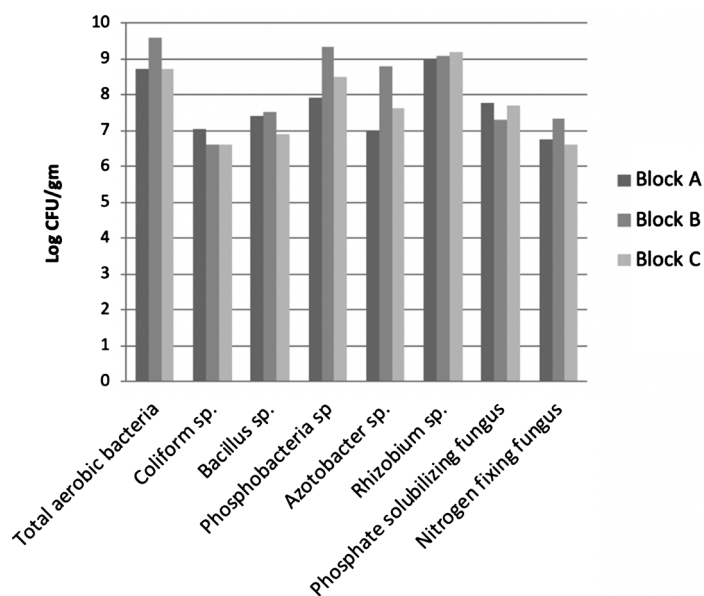
Table 1. Microbial counts from conventional fields, organic fields and control fields with semi-organic fertilizer with corresponding P<sup>H</sup> value.

	Mean log value of CFU ± SD																								
	Cauliflower			Tomato			Pumpkin			Eggplant			Capsicum			Bottle gourd			Cabbage			Broccoli			
	CF*	OF*	SOFF*	CF	OF	SOFF	CF	OF	SOFF	CF	OF	SOFF	CF	OF	SOFF	CF	OF	SOFF	CF	OF	SOFF	CF	OF	SOFF	
P <sup>H</sup>	7.81	6.69	6.43	6.85	7.17	6.73	7.06	6.92	6.85	6.82	7.17	6.92	6.99	6.95	8.81	7.46	6.54								
Total aerobic bacteria	8.27 ± 0.43	8.73 ± 0.64	8.27 ± 0.85	7.69 ± 0.85	9.59 ± 0.07	7.79 ± 0.45	8.50 ± 0.27	9.57 ± 1.17	8.20 ± 0.32	8.70 ± 0.40	8.34 ± 0.52	8.18 ± 0.20	8.25 ± 0.04	8.86 ± 0.04	7.85 ± 0.30	8.83 ± 0.16	8.38 ± 0.05								
<i>Coliform sp.</i>	6 ± 0.88	7.04 ± 0.85	6.07 ± 0.47	5.30 ± 0.23	7.52 ± 0.32	5.47 ± 0.38	5.50 ± 0.11	6.62 ± 0.40	5.18 ± 0.32	6.59 ± 0.34	6.28 ± 1.15	6.00 ± 0.00	6.10 ± 0.09	6.61 ± 0.10	5.90 ± 0.07	6.52 ± 0.08	0.30								
<i>E. coli</i>	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	3.40 ± 0.10	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00								
<i>Bacillus sp.</i>	6.47 ± 0.48	7.41 ± 0.47	6.77 ± 0.40	5.18 ± 0.16	7.92 ± 0.29	6.72 ± 0.07	5.57 ± 0.08	7.51 ± 0.64	6.47 ± 1.15	6.88 ± 0.12	6.84 ± 0.01	6.84 ± 0.08	6.78 ± 0.13	6.47 ± 0.10	6.76 ± 0.08	6.69 ± 0.12	0.13								
<i>Pseudomonas sp.</i>	4.04 ± 0.47	4.62 ± 1.15	4.17 ± 0.35	<1.00	6.79 ± 0.04	<1.00	4.34 ± 0.85	4.08 ± 0.12	<1.00	<1.00	<1.00	3.97 ± 0.12	<1.00	<1.00	3.73 ± 0.89	<1.00	4.31 ± 0.18								
<i>Phosphobacteria sp.</i>	7.54 ± 0.51	7.90 ± 0.51	7.44 ± 0.29	8.17 ± 2.62	8.57 ± 0.04	8.11 ± 0.85	7.61 ± 0.25	9.34 ± 0.45	8.32 ± 0.04	8.51 ± 1.73	7.69 ± 0.51	7.84 ± 0.08	8.20 ± 0.32	8.82 ± 0.07	7.85 ± 0.71	8.51 ± 0.16	0.20								
<i>Azotobacter sp.</i>	6.07 ± 0.01	7.00 ± 0.40	7.17 ± 0.40	7.17 ± 1.03	8.70 ± 0.51	7.09 ± 0.68	7.32 ± 0.32	8.77 ± 0.28	7.24 ± 0.25	7.63 ± 0.51	7.11 ± 0.52	6.78 ± 0.11	7.42 ± 0.73	7.30 ± 0.20	7.31 ± 0.18	7.97 ± 0.65	0.16								
<i>Rhizobium sp.</i>	7.11 ± 0.29	8.98 ± 0.64	8.23 ± 0.51	7.20 ± 0.07	9.38 ± 0.52	7.07 ± 0.27	6.91 ± 1.15	9.08 ± 0.40	8.41 ± 0.64	9.20 ± 0.45	7.69 ± 0.20	8.31 ± 0.04	8.22 ± 0.12	8.01 ± 1.05	8.17 ± 0.23	8.76 ± 0.14	0.06								
<i>Klebsiella sp.</i>	4 ± 0.40	5.48 ± 0.45	4 ± 0.43	3.90 ± 0.68	5.36 ± 0.52	3 ± 0.48	4.32 ± 0.64	5.34 ± 0.12	<1.00	4.85 ± 0.10	4.70 ± 0.17	ND	3.52 ± 0.66	3.20 ± 0.17	3.93 ± 0.43	2.10 ± 1.82	<1.00								
<i>Salmonella sp.</i>	<1.00	3.30 ± 0.28	<1.00	<1.00	<1.00	<1.00	ND	<1.00	<1.00	<1.00	3 ± 0.12	ND	<1.00	<1.00	ND	<1.00	<1.00								
<i>Shigella sp.</i>	5.32 ± 0.17	6.57 ± 0.12	5.43 ± 0.34	4.85 ± 0.23	6.33 ± 0.07	5.36	4.70 ± 0.16	5.48 ± 1.73	5.61 ± 1.15	5.15 ± 0.40	5 ± 0.12	4.32 ± 0.52	5.29 ± 0.15	5.16 ± 0.73	4.75 ± 0.68	3.80 ± 0.70	±.95								
Total fungal count	8.04 ± 0.34	8.92 ± 1.17	8.04	7.47 ± 0.74	9.72 ± 0.07	7.84	7.23 ± 0.13	9.85 ± 0.64	8	9.08 ± 1.17	8.23 ± 1.03	ND	8.36 ± 0.11	8.84 ± 0.03	8.25 ± 0.69	8.62 ± 0.33	0.07								
Phosphate solubilizing fungus	6.84 ± 0.51	7.78 ± 0.10	7.90 ± 0.13	6.47 ± 0.45	7.30 ± 0.25	8.17 ± 0.13	6.82 ± 0.06	7.30 ± 0.51	7 ± 0.74	7.70 ± 0.48	ND	<1.00	7.02 ± 0.66	6.53 ± 0.61	7.92 ± 0.21	ND									
Nitrogen fixing fungus	5.69 ± 0.35	6.76 ± 0.36	6.69 ± 0.17	6.32 ± 0.17	6.85 ± 0.01	7.51 ± 0.88	5.34 ± 0.32	7.32 ± 0.20	6.30 ± 0.06	6.60 ± 0.88	7.69 ± 0.52	6.45 ± 0.21	7.29 ± 0.15	7.02 ± 0.60	ND	ND									

\*CF: Conventional Field, OF: Organic Filed, SOFF: Semi-Organic Fertilizer Field.



**Figure 2.** Microbiological analysis of different tomato producing agricultural fields.



**Figure 3.** Microbiological analysis of different organic fields. (Block A = Soil treated with poultry compost; Block B = Soil treated with cow dung compost; Block C = Soil treated with both poultry and cow dung compost).

**Table 2.** Microorganisms identified through API.

API used	Microorganisms identified
API 20E	<i>Klebsiella pneumonia</i> <i>E. coli</i> <i>Vibrio sp.</i> <i>Serratia plymuthica</i>
API 20NE	<i>Pseudomonas sp.</i>
API 50 CH	<i>Bacillus cereus</i>

*Rhizobium*, Nitrogen fixing fungi) is required for better production, however, irrespective of field condition, one or two important beneficial bacterial population was found lower than the required number in the field soil [22]. Therefore, irrespective of field practices, supplementation of these beneficial microorganisms is necessary to improve the soil fertility and increased yield.

#### 4.2. Determination of IAA Production

Total 5 isolates were selected from the growth culture on NBRIP media for the determination of IAA (Indole 3-acetic acid) production assay (Figure 4 and Table 3). The isolates were coded as W14 (b), W14 (bc), W12, S5 and S18. The species of the isolates haven't been identified yet. Following incubation with Salkowski reagent, development of a pink color indicated IAA production and the amount of IAA were measured by spectrophotometric method at 530 nm [23]. On every sixth day, OD (optical density) of the incubated samples was measured at 530 nm. Total 4 readings were taken. The standard IAA calibration curve was set up by determining the prepared different concentrations of authentic IAA at 530 nm with UV spectrophotometer. IAA concentration values were obtained from the standard curve and the amount of IAA produced was

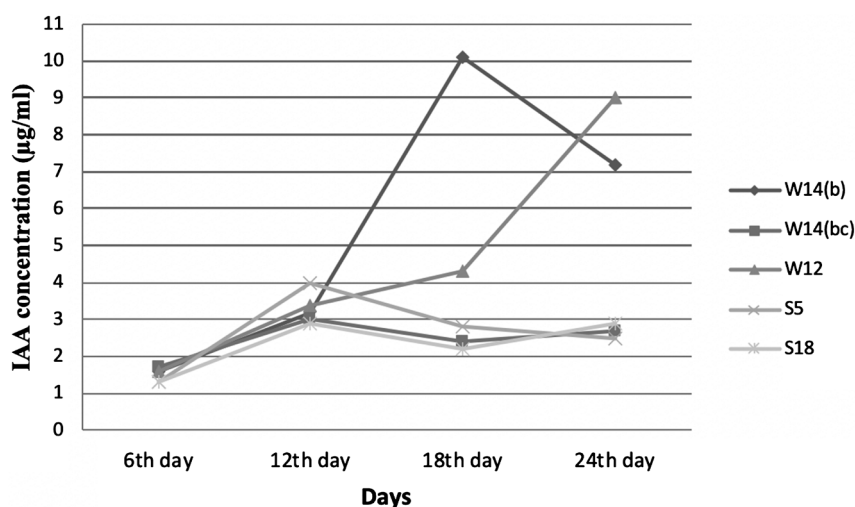


Figure 4. IAA Concentration ( $\mu\text{g/ml}$ ) on different days.

Table 3. Optical density and IAA concentration.

	6 <sup>th</sup> day	12 <sup>th</sup> day	18 <sup>th</sup> day	24 <sup>th</sup> day	6 <sup>th</sup> day	12 <sup>th</sup> day	18 <sup>th</sup> day	24 <sup>th</sup> day
	Optical density				IAA concentration ( $\mu\text{g/ml}$ )			
Blank	0.092	0.192	0.192	0.162	1.2	2.5	2.5	2
W14 (b)	0.134	0.236	0.678	0.477	1.6	3.2	10.1	7.2
W14 (bc)	0.141	0.228	0.190	0.204	1.7	3	2.4	2.7
W12	0.130	0.256	0.296	0.586	1.6	3.4	4.3	9
S5	0.102	0.280	0.210	0.178	1.3	4	2.8	2.5
S18	0.102	0.218	0.161	0.216	1.3	2.9	2.2	2.9



expressed as  $\mu\text{g}$  IAA secreted per unit of optical density.

### 4.3. Characterization of IAA Production Potential

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. IAA production was checked with use of Salkowski reagent. Color development was first visible at the highest IAA concentration within 30 minutes and continued to increase in intensity if kept for longer time. Hence optical density was measured after overnight incubation. If color development was not observed after 30 min, it was not kept for further incubation. All five isolates are positive for IAA production but among those five isolates W14 (b) and W12 were selected as potential IAA producers. It has been reported that IAA production by bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability. Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil [24]. Some bacteria need longer period for optimum IAA production [25]. As shown in **Table 3**, the optimum IAA productions of W14 (b) were found at 18th day incubation while the W12 were at 24<sup>th</sup> day incubation. The use of the technique for the detection of IAA using the Van Urk Salkowski reagent is an important option for qualitative and semi-qualitative determination that assure the presence of the hormone in the supernatant of bacterial cultures or liquid formulations of biological inoculants. The amount of IAA produced by the bacteria was within the detection limits of Salkowski reagent [18]. The reagent gives reaction with IAA and does not interact with L-tryptophan used [26]. Among the isolates W14 (b) and W12 were found to be the best producer of IAA. On the other hand, W14 (bc), S18 and S5 didn't produce significant amount of IAA as shown in **Table 3**.

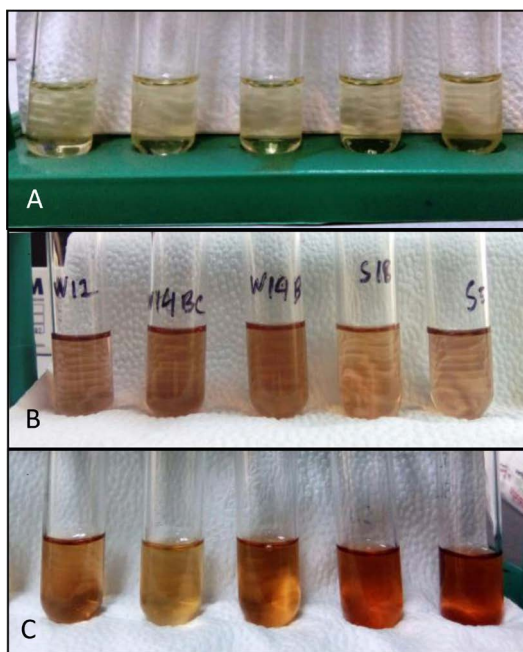
Auxin production by all isolates increased when culture medium supplemented with an IAA precursor; tryptophan which confirm the results of other scholar. Some microorganisms produce auxins in the presence of a suitable precursor such as L-tryptophan. The tryptophan increases the production of IAA in *Bacillus amyloliquefaciens* FZB42. Tien *et al.* (1979) showed that *Azospirillum* is able to produce auxins when exposed to tryptophan. Plants inoculated with the *Rhizobia* together with Ag<sup>+</sup> ion and L-tryptophan (Trp), give the highest root dry weight, and significantly increase the uptake of N, P and K compared to non-inoculated control plants [27]. Karnwal (2009) tested Fluorescent *Pseudomonas* isolates for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan and found that for both strains, indole production enhanced with increases in tryptophan concentration [28]. The significance of the study could be stated as the potential of these IAA producing isolates and optimization study for IAA production will flourish the growth and ultimately IAA production in the field and prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields.

#### 4.4. Phosphate Solubilization by *Aspergillus sp.*

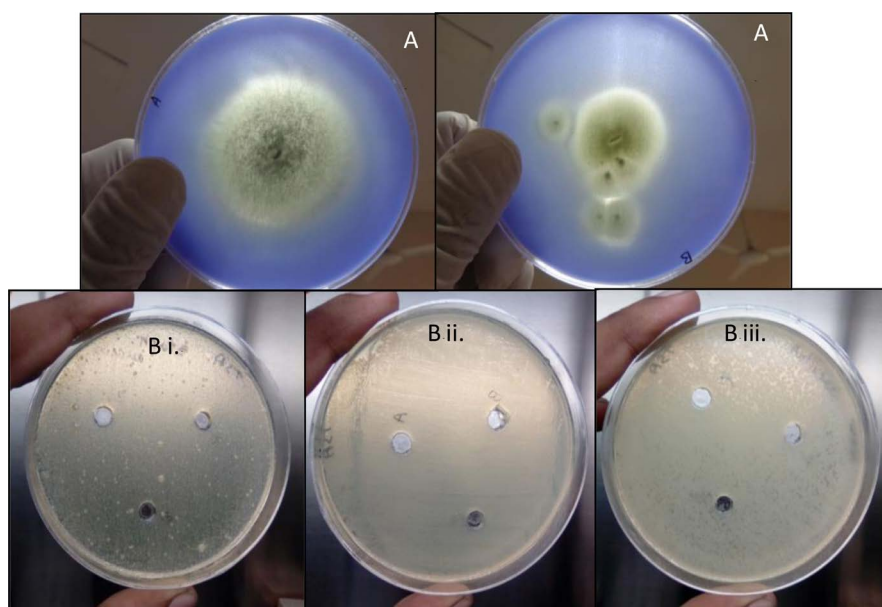
Microorganisms play critical role in natural P cycle, and the use of phosphate-solubilizing microorganisms (PSMs) has been proposed as a low-cost input to increase the agronomic effectiveness of insoluble phosphates. Several scientific reports showed that important genera of P-solubilizing bacteria include *Rhizobium*, *Bacillus*, and *Pseudomonas* [29]. Among fungi *Aspergillus sp.* is the dominant P-solubilizing filamentous fungi found in rhizosphere [30]. In the agar media phosphate utilization capability of *Aspergillus sp.* was determined through the appearance of halo zones or the discoloration of BPB dye around the fungal colonies. The plates were inspected every day (Figure 5 and Figure 6). Four out of 5 *Aspergillus* isolates that were spotted on NBRIP agar media showed halo zones around the colonies after 4 - 7 days of incubation at 28°C which was referred to as a phosphate solubilizing trait by Jyoti *et al.* (2013) [31]. The phosphate solubilizing efficiency of the isolates in NBRIP-BPB broth was also studied later in terms of decrease in intensity of the colour of bromophenol blue present in the media. The decolorization of bromophenol blue by all the selected *Aspergillus* isolates was observed after 4 days of incubation. This is an indicative trait of phosphate utilization in NBRIP broth media as referred by Bikash *et al.* (2016) [32].

#### 4.5. Effects of Pesticides on Bacteria

Pesticides are used in a number of human activities to be able to maintain high production efficiency. Pesticides have been linked to a wide range of human health hazards, ranging from short-term impacts such as headaches and nausea



**Figure 5.** Color changes due to production of IAA by the isolates. [(A) Control (uninoculated broth), (B) 6th day sample, (C) 24th day sample].



**Figure 6.** (A) Fourth day agar plates showing phosphate utilization by *Aspergillus sp.* in NBRIP media. (B) Bacterial plates showing no zone of inhibition by the pesticides. (i). *Staphylococcus aureus*; (ii). *Salmonella sp.*; (iii). *Bacillus sp.*.

to chronic impacts like cancer, reproductive harm, and endocrine disruption [33]. Chronic health effects may occur years after even minimal exposure to pesticides in the environment or result from the pesticide residues which we ingest through our food and water. A July 2007 study conducted by researchers at the Public Health Institute, the California Department of Health Services, found a six-fold increase in risk factor for autism spectrum disorders (ASD) for children of women who were exposed to organochlorine pesticides. Pesticides are toxic to living organisms. Some can accumulate in water systems, pollute the air, and in some cases have other dramatic environmental effects [34]. Scientists are discovering new threats to the environment that are equally disturbing. Pesticide use can damage agricultural land by harming beneficial insect species and soil microorganisms e.g. inhibit the transformation of ammonia into nitrates by soil bacteria [35]. Considering all these facts pesticide sensitivity of three bacteria including *Bacillus sp.*, *Staphylococcus aureus*, and *Salmonella sp.* was assessed by Agar well diffusion method, however, no visible zone of inhibition was found by the three selected pesticides on the growth of microbes. Further studies with higher concentration of pesticides may reflect the adverse effect on bacterial growth.

Microbiological analysis of experimental fields shows that, almost all types of plant beneficial microbial populations were present in low number in inorganic fields in comparison with plots having organic even with the control (Table 1). This finding focuses the possible lethal effects of chemical fertilizers on microorganisms when the fertilizer chemical components form  $H_2SO_4$ , anhydrous ammonia, chlorine gas etc. after conversion reactions [36]. But plants are extremely dependent on microbial activities like biological  $N_2$  fixation initiated by

*Rhizobium sp.*, *Azotobacter sp.* etc. contributing to about 70% of all nitrogen fixed on the earth per year [37]. Microorganisms like *Aspergillus sp.* and *Trichoderma sp.* have adopted special strategy to secrete low molecular weight organic acids which is the principle means of converting insoluble P to plant accessible form from both of organic and inorganic sources [38] [39] [40]. Except these, microorganisms in organic fertilizers have higher potential to aid the agriculture by secreting plant growth modulating enzymes [41], effective antibiotics against soil borne plant pathogens, numerous phytohormones and cyanogenic compounds as well [42]. Strength of soil health and crop production also depends on efficient decomposers of lands, namely earthworms, which are being harmed by the fatal effects of chemical agricultural practices [43]. Earlier researches had found the higher biological activity and organic matters content associated with organic fertilizers is potential to establish and increase soil fertility and productivity 22000 [44] [45] [46] [47] [48]. Avoidance of chemical pollutants in organic fertilizers amendment significantly reduces soil nutrient combustion thus improving organic matter status reported in previous studies [49].

In recent years, several studies have been conducted on fertilizer issues by different organizations in national and international levels in all over Bangladesh. All the studies were based on good quality food production, saving money, rehabilitation of problem soils and avoiding environmental pollution. Future impacts scenarios due to excessive use and dependency on chemical fertilizer is also well established for many sectors in different regional scale, but still our farmers are ignorant about it due to lack of knowledge. They only prefer to depend on chemical fertilizers during crop cultivation in their fields; as a result, soil is losing its natural productivity and fertility characteristics. Additionally, the integrated environment (soil-air-water continuum) is being imbalanced, polluted and deteriorating rapidly. On the other hand, for higher price of chemical fertilizer the farmers are suffering from financial crisis and return on investment during cultivation of crops. The cost which is required to cultivate the crops is generally higher than the cost which the farmers get from selling the crop in the market. But after facing all these drawbacks, they are yet not willing to use compost fertilizer in their field because they are ignorant about its beneficial impact and as they do not have opportunity and or timely availability of fertilizers from organic sources.

## 5. Conclusion

Intensive farming practices with chemical fertilizer to increase crop yields and poor management practices particularly of pests and diseases, excessive use of fertilizer have largely contributed to significant decrease in crop productivity. Experimental research findings on chemical fertilizer based conventional agriculture have revealed that this type of agriculture has enabled farmers to fulfill their quick needs at the expense of long-term environmental degradation and

nutrient depletion/nutrient loss in the soil ecosystem [50]. Therefore, the existing production practices of Bangladesh are no longer safe for soil health as well as for human health. Dependency on organic or compost fertilizer can reduce or compromise chemical fertilizers requirement. Thus, from the present study, it can be concluded that the application of organic manures and compost stuffed with better auxin producing and other beneficial microorganisms in crop fields will enrich the agricultural fields for sustainable productivity. Ultimately it would play significant role for increasing plant nutrients and favour long-term soil fertility. Therefore, cost-effective microbial biofertilizers can be suggested as safe and effective agricultural practice for selected vegetables production.

### Acknowledgements

The authors express their sincere gratitude to Dr. Md. Latiful Bari, Center for Advanced Research in Sciences, University of Dhaka for allowing them to conduct this study. Sincere help and valuable suggestions of Md. Kamruzzaman, Junior Faculty Member, Dept. of Applied Nutrition and Food Technology, Islamic University, Kushtia is very much acknowledged.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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