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Research Article
Field Evidence of *Chlorella vulgaris* Potentials as a Biofertilizer for *Hibiscus esculentus*

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Abstract

Background and Objectives: The use of bio-fertilizer is a safe and economical method for increasing soil agricultural fertility and productivity rather than the conventional use of chemical fertilizer. Hence, this study was conducted to investigate the biofertilizer potential of *Chlorella vulgaris*, nitrogen, phosphorus and potassium (NPK) fertilizer and poultry manure on the microbiological and physico-chemical characteristics of the rhizospheres soil of *Hibiscus esculentus* at 2nd, 4th and 6th week of growth. The effect on germination, biometric and biochemical constituent of the plant at maturity was investigated and the best method of application of *Chlorella vulgaris* (bio-fertilizer) was determined. **Materials and Methods:** Disease free okra seeds were obtained from a market within Port Harcourt metropolis. They were inoculated with *Chlorella vulgaris*, NPK fertilizer, poultry manure and monitored for 6 weeks. Plant height, leaf count and fresh weight of the plants were determined alongside with microbiological and soil physico-chemical analyses. Obtained data were statistically analysed by one-way analysis of variance ANOVA using SPSS. **Results:** The results showed significant difference in the microbiological and physico-chemical constituent of the rhizospheres soil for all the treatment given and the bulk soil, bacteria count ranged from 3×10^{8}-30×10^{9} CFU g^{-1} while fungal count ranged from 5×10^{4}-90×10^{6} CFU g^{-1}, nitrogen content ranged from 0.15-0.99%, organic matter ranged from 2.95-8.67% potassium content ranged from 9-16.75 mg/100 mL and phosphorus ranged from 5.09-15.9 mg/100 mL. The combined seed and soil inoculation of *Chlorella vulgaris* speed up germination of the *Hibiscus esculentus* and its maturity at 3 days and 8 weeks, respectively. Highest pod yield and plant height were obtained in combined seed and soil inoculation with *Chlorella vulgaris*. **Conclusion:** C*hlorella vulgaris* (bio-fertilizer) is efficient and economical in improving soil nutrients for greater productivity of *Hibiscus esculentus*.

Key words: Biofertilizer, *Chlorella vulgaris*, *Hibiscus esculentus*, NPK fertilizer, poultry manure

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The continuous cultivation of soil results in the decrease of soil fertility based on many factors such as loss of nutrients, pile up of salts and other harmful elements, logs of water and soil erosion[1]. The use of fertilizer is therefore a very important factor necessary for efficient increase in agricultural yield[2]. Fertilizer enhances root growth, nutrient availability, insusceptibility to frost, tolerance to drought and plants resistance to pest and disease attacks. The conventional practice of adding chemical fertilizers deteriorates soil and causes air and ground water pollution[3]. It has also resulted in decrease in yield, increased invasion of pests and diseases and the pollution of the environment[4]. It is therefore, important to find safe solutions for increasing soil fertility and productivity[5-7].

Biofertilizer could be defined as any substance that is made up of living microorganisms, which can colonise the vicinity of the root or the plant's tissues and promote plant's growth by improving the state of their nutrients[3,8-10]. The rhizosphere soil is that space around plant roots where root exudates are released and constitute a source of useful nutrients to so many ranges of microorganisms which is therefore responsible for the types of microbes and the type of association or role they carry out in the root of various plants[11,12]. Rhizobacteria which are bacteria present in the rhizosphere can have neutral, detrimental or positive impact on a plant's growth[13-17]. The plant growth promoting rhizobacteria which include symbiotic nitrogen fixing bacteria, free nitrogen fixing bacteria, phosphorus solubilizing bacteria, cyanobacteria[18-20], green algae and seaweeds, all could be used as biofertilizers[1,12,21,22]. Biofertilizer is an alternative source of supply of nutrients for plant growth and provides an alternative to chemical fertilizers. It helps in waste recycling, increases in soil and plant nutrient level, improvement of animal and human life and also ensures an environmental friendly and low cost method of improving soil fertility and structure[23-25].

Algae are photoautotrophic organisms that can produce unlimited biomass by utilizing light energy and CO_2[26,27]. They can fix CO_2 to produce oxygen, organic matter and extra cellular metabolites in their vicinity[28-30]. Microalgae are employed in agricultural systems as biofertilizers[9,31-33]. Recently, a consortium containing *Anabaena variabilis*, *Chlorella vulgaris* and *Azotobacter* sp., was found to improve germination and growth of rice plants and it was recommended as a biostimulator and a biofertilizer for crops also the growth of *Zea may* was improved with two strains of *Chlorella* sp.[1,23,34].

Okra belongs to the mallow family *Malvaceae*, an important vegetable whose pod is used for soup to garnish salad and can be eaten when boiled or fried. Okra provides carbohydrates, protein, fat, minerals and vitamins which are essential for human health[35,36]. It is medicinal, it helps to lubricate the large intestines because of its laxative qualities, balances blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract and carries toxins dumped in cholesterol and bile acid by binding them via its mucilage. Optimum soil pH for cultivation of Okra is 6.0-7.5 and it reaches maturity after germination in 60 days[6,12,36].

Studies have shown previously that there were significant differences ($p<0.05$) in potassium, phosphorus, nitrogen, percentage organic carbon, organic matter, moisture and sulphur in rhizosphere soil of *Hibiscus esculentus*, when compared with the non-rhizosphere soil with *Bacillus*, *Micrococcus*, *Streptococcus*, *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium*, *Fusarium*, *Actinomyces*, *Streptomyces* and *Norcardia* spp. isolated from the studied rhizosphere soil[37].

Kumar *et al.*[28] had reported that *Bacillus* and *Pseudomonas* sp., (6 isolates) through molecular analysis were identified as rhizobacteria inhabiting the okra root which significantly increased germination and other growth parameters. In that study, *Pseudomonas flourescens* was most effective for okra seedlings length.

Thus, this study is aimed at investigating the potential of *Chlorella vulgaris* as a biofertilizer for *Hibiscus esculentus* and its role in enhancing soil fertility.

MATERIALS AND METHODS

Algal material: *Chlorella vulgaris* was obtained from a fresh water pond at the African Regional Aquaculture Center, Rivers State, Nigeria. It was bloomed using poultry manure digestate following the technique of Agwa and Abu[38]. The culture was streaked onto an agar plate containing a synthetic medium according to Agwa and Abu[39], viewed under the microscope using 10x objective lens and then bloomed using sterile poultry manure in transparent buckets for light penetration. The buckets were shaken intermittently twice daily to free trap gases, ensuring continuous mixing and prevention of scum accumulation at the top. Cells accumulated were harvested by centrifugation after 7 days. *Chlorella vulgaris* (2 g) was resuspended in 10 mL sterile distilled water and thereafter, inoculated on seeds and soil as described by Taher and Mohammed[1].

Plant materials: Okra seeds were obtained from oil mill market, Aba Road, Port-Harcourt, Nigeria. The experiments were carried out with non-diseased seeds carefully selected from the stock between March and July, 2016.

Pot experiment: The experiments were conducted in a completely randomized design with triplicates of each treatment following this sampling protocol:

OA: No fertilizer application in soil
OB1: Only Okra seed inoculation with *Chlorella vulgaris*
OB2: Only soil inoculated with *Chlorella vulgaris*
OB3: Soil and Okra seed inoculation with *Chlorella vulgaris*
OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed
OC: Soil amended with NPK fertilizer
OD: Soil amended with poultry manure

Determination of growth parameters of plant: Plant height, leaf count and fresh weights of the Okra plant were determined following the methods described by Kavitha *et al.*[4]. Biochemical analyses (chlorophyll, crude fibre, protein, carbohydrate, ash and moisture content) of the fresh vegetable plants were determined using methods adopted from AOAC[40].

Microbiological analyses: Total culturable heterotrophic bacterial and fungal counts were ascertained using nutrient agar and sabauroud dextrose agar, respectively. Bacterial isolates were characterized based on their colonial morphology, gram staining reactions and biochemical characteristics (production of catalase, coagulase, indole, utilization of citrate, fermentation of sugars, urease production, starch hydrolysis and growth on mannitol salt agar, etc.) as described by Oyewole *et al.*[37]. The isolates were identified by comparing their features with those of known taxa[41,42]. Fungal isolates were viewed under microscope and identified by comparing their characteristics with those of known taxa according to the schemes of Domsch and Gams[43].

Soil physicochemical analyses: Soil pH, potassium, nitrogen, phosphorus, organic carbon and matter were carried out following methods of IITA[44].

Statistical analysis: The obtained data in triplicate were statistically analysed by employing the one-way analysis of variance ANOVA using SPSS program Version 20.0, treatments means were compared by Duncan's multiple range tests at 5% level of probability[45].

RESULTS

Table 1-3 show the physico-chemical characteristics of the rhizosphere soil of okra for all the treatments and the bulk soil at weeks 2, 4 and 6. The mean totals of nitrogen, organic carbon, organic matter, phosphorus and potassium content of the soil were significantly increased ($p<0.05$) in all the treatments when compared to the control (OA) and the bulk soil (BS). The control was significantly higher than the bulk soil in all physicochemical parameters except pH and moisture. The highest pH was obtained in the bulk soil (BS).

Soil inoculated with *Chlorella vulgaris* (OB2) had the highest moisture value at week 2. The highest obtained nitrogen was in OD, organic carbon in OB4, organic matter in OB4, phosphorus in OB4 and potassium in OD were all obtained at week 2. Table 4 and 5 show the bacterial and fungal counts, respectively of the rhizosphere soil of fluted pumpkin for all the treatments and the bulk soil at week 2, 4 and 6. The bacterial and fungal counts for all the treatments were significantly higher ($p<0.05$) than the control (OA) which in turn is significantly higher than that of the bulk soil (BS). The highest bacterial count was obtained at week 6 in rhizosphere soil treated with poultry manure (OD) and the highest fungal count at week 4 in OB4 while the bulk soil (BS) had a the lowest bacterial count and a fungal count. Table 6 shows the

Table 1: Physico-chemical characteristics of okra rhizosphere soil at week 2

Treatments	pH	Nitrogen (%)	Organic carbon (%)	Organic matter (%)	Phosphorus mg/100	Potassium mg/100	Moisture (%L)
OA	7.800±0.06^{a}	0.200±0.05^{a}	2.580±0.04^{a}	4.460±0.06^{a}	5.600±0.06^{a}	12.950±0.06^{a}	1.200±0.06^{a}
OBI	7.440±0.06^{b}	0.380±0.06^{b}	3.590±0.05^{b}	6.200±0.06^{b}	9.350±0.08^{b}	14.160±0.06^{b}	2.350±0.06^{b}
OB2	6.810±0.06^{c}	0.480±0.05^{c}	3.930±0.06^{c}	6.800±0.060^{c}	11.600±0.060^{c}	14.100±0.060^{c}	2.590±0.06^{c}
OB3	7.010±0.06^{d}	0.520±0.060^{d}	3.690±0.06^{d}	6.380±0.02^{d}	10.500±0.06^{d}	15.650±0.05^{d}	2.250±0.06^{c}
OB4	7.010±0.170^{d}	0.490±0.060^{e}	5.060±0.060^{e}	8.740±0.06^{e}	15.900±0.06^{e}	15.550±0.06^{e}	1.900±0.06^{d}
OC	7.270±0.05^{e}	0.940±0.05^{f}	2.660±0.04^{f}	4.600±0.06^{f}	15.120±0.05^{e}	15.590±0.04^{f}	2.050±0.06^{e}
OD	6.630±0.06^{f}	0.990±0.06^{g}	3.060±0.060^{g}	5.290±0.06^{g}	11.980±0.06^{f}	16.750±0.06^{g}	2.150±0.05^{f}
BS	7.490±0.060^{g}	0.150±0.06^{h}	1.700±0.06^{h}	2.950±0.06^{h}	5.090±0.06^{g}	9.000±0.06^{h}	1.450±0.06^{g}

*Values are Mean±Standard deviation for 3 replicates (n = 3), *Values with no common superscripts were significantly different from each other at $p<0.05$, OA: No fertilizer application in soil, OB1: Only Okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and Okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer and OD: Soil amended with poultry manure

Table 2: Physico-chemical characteristics of okra rhizosphere soil at week 4

Treatments	pH	Nitrogen (%)	Organic carbon (%)	Organic matter (%)	Phosphorus (mg/100)	Potassium (mg/100)	Moisture (%L)
OA	7.830±0.06^{a}	0.200±0.06^{a}	2.370±0.06^{a}	4.100±0.05^{a}	10.400±0.05^{a}	12.000±0.06^{a}	1.150±0.06^{a}
OBI	7.310±0.06^{b}	0.350±0.06^{a}	3.490±0.06^{b}	6.070±0.06^{b}	13.500±0.06^{b}	14.06±0.06^{b}	1.650±0.06^{b}
OB2	6.490±0.06^{c}	0.430±0.06^{b}	2.970±0.05^{c}	5.130±0.06^{c}	14.520±0.06^{c}	14.000±0.06^{c}	1.890±0.06^{c}
OB3	7.000±0.06^{d}	0.460±0.06^{c}	2.940±0.06^{d}	5.080±0.06^{d}	14.930±0.06^{d}	14.950±0.06^{d}	1.700±0.06^{d}
OB4	6.720±0.06^{e}	0.440±0.06^{d}	3.370±0.06^{e}	5.830±0.05^{e}	14.500±0.06^{c}	14.860±0.06^{e}	1.790±0.06^{e}
OC	6.530±0.06^{f}	0.840±0.06^{e}	2.370±0.06^{f}	4.100±0.06^{f}	15.690±0.06^{e}	14.900±0.06^{f}	1.150±0.06^{a}
OD	7.220±0.06^{g}	0.900±0.06^{f}	2.980±0.05^{g}	5.160±0.060^{g}	13.740±0.06^{f}	14.980±0.06^{g}	1.450±0.06^{f}
BS	7.490±0.06^{h}	0.150±0.06^{g}	1.700±0.06^{a}	2.950±0.06^{h}	5.090±0.06^{g}	9.000±0.06^{h}	1.450±0.06^{f}

*Values are Mean±Standard deviation for 3 replicates (n = 3), *Values with no common superscripts were significantly different from each other at p<0.05, OA: No fertilizer application in soil, OB1: Only Okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and Okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer and OD: Soil amended with poultry manure

Table 3: Physico-chemical characteristics of okra rhizosphere soil at week 6

Treatments	pH	Nitrogen (%)	Organic carbon (%)	Organic matter (%)	Phosphorus (mg/100)	Potassium (mg/100)	Moisture (%L)
OA	7.520±0.06^{a}	0.220±0.06^{a}	2.540±0.05^{a}	4.400±0.06^{a}	10.150±0.06^{a}	12.200±0.06^{a}	1.400±0.06^{a}
OBI	6.890±0.06^{b}	0.350±0.06^{b}	3.450±0.04^{b}	5.970±0.05^{b}	13.650±0.06^{b}	13.900±0.06^{b}	1.590±0.06^{b}
OB2	7.010±0.06^{c}	0.400±0.06^{c}	3.410±0.06^{c}	5.890±0.060^{c}	14.500±0.06^{c}	14.400±0.06^{c}	1.400±0.06^{a}
OB3	7.450±0.06^{d}	0.430±0.06^{d}	3.240±0.06^{d}	5.600±0.06^{d}	14.550±0.06^{d}	14.740±0.06^{d}	1.550±0.06^{d}
OB4	6.580±0.06^{e}	0.400±0.06^{c}	3.390±0.06^{e}	5.860±0.06^{e}	14.800±0.06^{e}	14.700±0.06^{e}	1.540±0.06^{d}
OC	6.690±0.06^{f}	0.660±0.06^{e}	2.640±0.06^{f}	4.560±0.06^{f}	15.280±0.05^{f}	14.900±0.06^{f}	1.050±0.05^{e}
OD	6.900±0.06^{g}	0.740±0.06^{f}	3.440±0.06^{g}	5.950±0.06^{g}	13.600±0.05^{g}	14.750±0.06^{g}	1.250±0.06^{f}
BS	7.490±0.06^{h}	0.150±0.06^{g}	1.700±0.06^{h}	2.950±0.06^{h}	5.090±0.06^{h}	9.000±0.06^{h}	1.450±0.06^{g}

*Values are Mean±Standard deviation for 3 replicates (n = 3), *Values with no common superscripts were significantly different from each other at p<0.05, OA: No fertilizer application in soil, OB1: Only okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer and OD: Soil amended with poultry manure

Table 4: Total culturable heterotrophic bacterial counts (CFU g^{-1}) of rhizosphere soil of okra and the bulk soil during the study period

	Total culturable heterotrophic bacterial counts (CFU g^{-1})		
Treatments	Week 2	Week 4	Week 6
OA	8.0±0.05×10^{8a}	9.0±0.05×10^{8a}	1.0±0.04×10^{9a}
OBI	2.9±0.05×10^{9b}	1.2±0.06×10^{9b}	1.6±0.06×10^{10b}
OB2	2.8±0.06×10^{9c}	1.2±0.13×10^{9b}	1.4±0.06×10^{10c}
OB3	3.5±0.04×10^{9d}	1.1±0.05×10^{9c}	1.5±0.11×10^{10d}
OB4	2.5±0.06×10^{9e}	1.1±0.06×10^{9c}	1.9±0.06×10^{10e}
OC	2.3±0.06×10^{9f}	1.0±0.04×10^{9c}	1.3±0.05×10^{10f}
OD	5.6±0.06×10^{9g}	1.8±0.06×10^{9d}	3.0±0.04×10^{10g}
BS	3.0±0.06×10^{8h}	3.0±0.06×10^{8e}	3.0±0.06×10^{8h}

*Values are Mean±Standard deviation for 3 replicates (n = 3), *Values with no common superscripts were significantly different from each other at p<0.05, OA: No fertilizer application in soil, OB1: Only okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer and OD: Soil amended with poultry manure

Table 5: Fungal counts (CFU g^{-1}) of okra rhizosphere soil and bulk soil during the study period

	Fungal counts (CFU g^{-1})		
Treatments	Week 2	Week 4	Week 6
OA	8.0±0.06×10^{6a}	1.4±0.05×10^{5a}	1.0±0.12×10^{5a}
OBI	1.2±0.06×10^{7b}	2.8±0.11×10^{5b}	1.4 ±0.06×10^{5b}
OB2	1.3±0.05×10^{7c}	2.6±0.05×10^{5be}	2.3±0.06×10^{5c}
OB3	1.8±0.06×10^{7d}	4.8±0.04×10^{5c}	2.2±0.05×10^{5c}
OB4	1.5±0.05×10^{7e}	9.0±0.06×10^{5d}	1.8±0.06×10^{5d}
OC	1.0±0.12×10^{7f}	2.3±0.06×10^{5e}	1.4±0.05×10^{5b}
OD	1.5±0.06×10^{5}	2.4±0.06×10^{5e}	1.6 ±0.06×10^{5e}
BS	5.0±0.06×10^{4g}	5.0±0.06×10^{4f}	5.0±0.06×10^{4f}

*Values are Mean±Standard deviation for 3 replicates (n = 3), *Values with no common superscripts were significantly different from each other at p<0.05 OA: No fertilizer application in soil, OB1: Only Okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and Okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer and OD: Soil amended with poultry manure

biochemical characteristics of the okra pods at maturity. The mean totals of crude protein, crude lipid, crude fibre, chlorophyll a and total chlorophyll of all *Chlorella vulgaris* treated okra were significantly higher (p>0.05) than the control (OA). The highest moisture content was obtained in OA, the highest crude protein in OB2, OB4 and OC, crude lipid in OB3 and the highest crude fibre in OB4. Highest total chlorophyll was obtained in OB3.

Figure 1-4 show the weight of the pods, number of fruits, height of plant and the germination time for the various treatments. The lowest germination time (3 days) was obtained in OB3, whereas, the highest plant height (58 cm) and the highest number of fruits (14) were obtained in OB3 after 8 weeks of planting. In other treatments, similar plant heights and number of fruits were obtained in 9-10 weeks with the highest weight (39 g) obtained in OD. Table 7 shows

Table 6: Biochemical characteristics of okra pods at maturity

Parameters	OA	OB1	OB2	OB3	OB4	OC	OD	p-value
Moisture (%)	87.93^{a}±0.006	86.69^{b}±0.006	85.24^{c}±0.006	86.73^{b}±0.006	85.14^{d}±0.006	84.74^{e}±0.006	85.28^{f}±0.006	0.000
Crude protein (%)	3.00^{a}±0.006	3.01^{a}±0.006	3.20^{b}±0.012	3.10^{c}±0.006	3.20^{b}±0.012	3.20^{b}±0.058	3.10^{c}±0.006	0.000
Crude lipid (%)	0.80^{a}±0.006	0.91^{b}±0.120	1.00^{c}±0.120	1.30^{d}±0.120	1.01^{c}±0.120	0.93^{e}±0.006	1.00^{f}±0.006	0.000
Crude fibre (%)	1.39^{a}±0.006	1.45^{b}±0.058	1.42^{c}±0.006	1.48^{d}±0.006	1.51^{e}±0.006	1.3l^{f}±0.110	0.96^{g}±0.006	0.000
Total ash	2.00^{a}±0.006	2.50^{b}±0.006	2.81^{c}±0.006	3.60^{d}±0.017	4.05^{e}±0.006	2.90^{f}±0.006	3.50^{g}±0.006	0.000
Carbohydrate (%)	4.87^{a} 0.006	5.46^{b} ±0.006	6.30^{c}±0.006	3.34^{d}±0.006	5.46^{e}±0.006	6.33^{f}±0.058	6.76^{g}±0.006	0.000
Chlorophyll a	0.09^{a}±0.006	0.11^{bc}±0.006	1.12^{cd}±0.006	1.33^{d}±0.006	1.12^{cd}±0.006	0.10^{a}±0.006	0.11^{bc}±0.006	0.000
Chlorophyll b	0.09^{a}±0.006	0.10^{ab}±0.006	0.11^{bc}±0.006	0.10^{ab}±0.006	0.10^{ab}±0.006	0.10^{ab}±0.006	0.09^{a} ±0.006	0.000
Total chlorophyll	0.18^{a} ±0.006	0.19^{b} ±0.006	0.20^{bc}±0.006	0.23^{c}±0.006	0.20^{bc}±0.006	0.16^{d}±0.006	0.19^{bc}±0.006	0.000

*Values are Mean±Standard deviation for 3 replicates (n = 3) *Values with no common superscripts were significantly different from each other at p<0.05, OA: No fertilizer application in soil, OB1: Only Okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer, OD: Soil amended with poultry manure

Table 7: Fungal and bacterial isolates from the rhizosphere soil of the different treatments

	Treatments				
Isolates	A	B	C	D	Non rhizosphere
Bacteria	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus megaterium*
	B. mycoides	*B. subtilis*	*Micrococcus* sp.	*B. mycoides*	*B. mycoides*
	Citrobacter freundi	*Citrobacter freundi*	*Staphylococcus* sp.	*Citrobacter freundi*	*B. subtilis*
		Micrococcus sp.	*Citrobacter freundi*	*Escherichia coli*	*Streptococcus* sp.
		Pseudomonas sp.		*Micrococcus* sp.	
		Staphylococcus sp.		*Staphylococcus aureus*	
Fungi	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus fumigatus*	*Aspergillus niger*
	A. niger	*A. niger*	*Fusarium* sp.	*A. niger*	*Penicillium* sp.
	Fusarium sp.	*Fusarium* sp.	*Mucor* sp.	*Fusarium* sp.	
	Mucor sp.	*Penicillium* sp.	*Penicillium* sp.		

A: No fertilizer, B: Inoculations with *Chlorella vulgaris*, C: Soil amended with NPK fertilizer and D: Soil amended with poultry manure

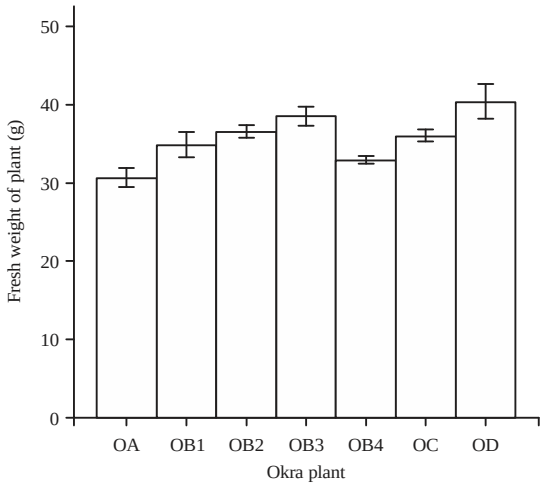

Fig. 1: Effect of various treatments on the fresh weight of okra plant. OA: No fertilizer application in soil, OB1: Only okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and Okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer, OD: Soil amended with poultry manure
Values are Mean±Standard deviation

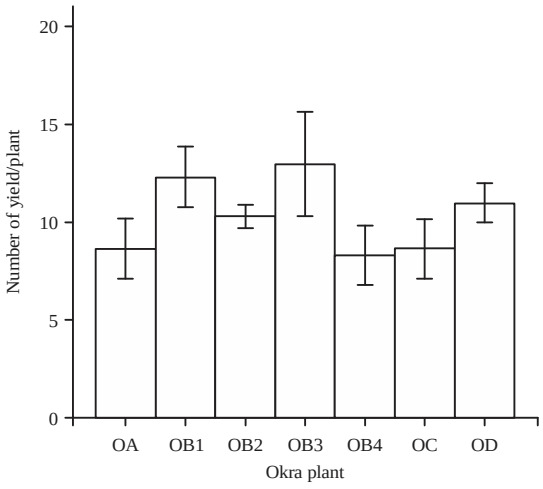

Fig. 2: Effect of various treatments on the number of yield of okra at maturity. OA: No fertilizer application in soil, OB1: Only okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer, OD: Soil amended with poultry manure
Values are Mean±Standard deviation

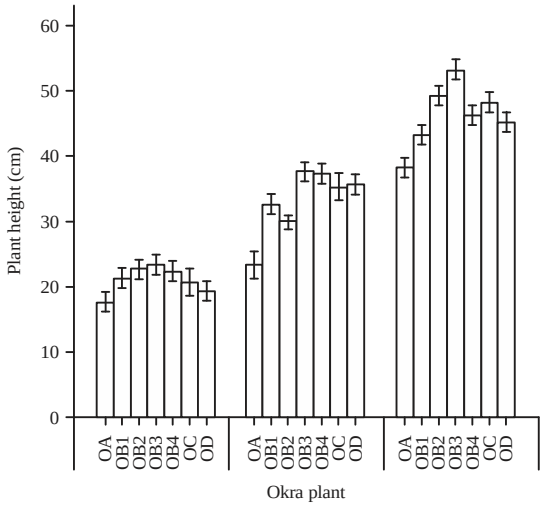

Fig. 3: Effect of various treatments on the height of okra during the study period. OA: No fertilizer application in soil, OB1: Only okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and Okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer, OD: Soil amended with poultry manure
Values are Mean±Standard deviation

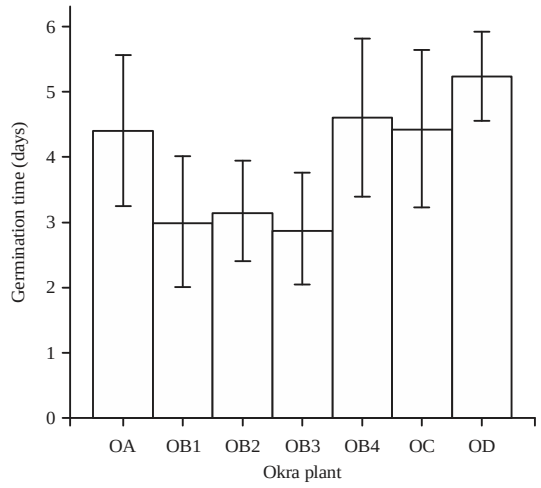

Fig. 4: Effect of various treatments on the germination time of okra seeds. OA: No fertilizer application in soil, OB1: Only okra seed inoculation with *Chlorella vulgaris*, OB2: Only soil inoculation with *Chlorella vulgaris*, OB3: Soil and okra seed inoculation with *Chlorella vulgaris*, OB4: Soil inoculation with *Chlorella vulgaris* after germination of seed, OC: Soil amended with NPK fertilizer, OD: Soil amended with poultry manure
Values are Mean±Standard deviation

the fungal and bacterial isolates from the rhizosphere soil of the different treatments and the control. *Bacillus* sp. and *Aspergillus* sp., occurred in the rhizosphere soil of all the different treatments and control. The rhizosphere soil treatment with *Chlorella vulgaris* (B) and that with poultry manure, showed more varieties of isolates than that of the NPK (C) and the control (A).

DISCUSSION

The nitrogen, organic carbon, organic matter, potassium and phosphorus contents in rhizosphere of soil of *Hibiscus esculentus* in every treatment administered (poultry manure, NPK and *Chlorella vulgaris* applications) were significantly increased over their control. A similar trend was earlier reported by Hatim[46], who stated that organic manure alone or inoculation of groundnut seeds with *Rhizobium* significantly increased soil physico-chemical characteristics when compared to control. The increase in soil physico-chemical characteristics may be attributable to the fertilizer application and the increased mineralization accomplished by higher populations of microorganisms. *Chlorella vulgaris* enriches the soil by fixing atmospheric nitrogen freely, production of growth promoting substances and reducing pest, increasing microbial population and organic matter of the soil[26].

The pH of soil in all treatments (poultry manure, NPK and all inoculations of *Chlorella vulgaris*) were significantly ($p>0.05$) lower than that of the bulk soil (7.49), this is in consonance with the data obtained by Baba *et al*. [47] and this trend may have been necessitated by microbial activities in the rhizosphere. However, elsewhere, no effect on pH was obtained after soil amendments as reported by Hatim[46].

The bacterial and fungal counts in soil were significantly higher ($p<0.05$) in all the treatments (NPK, poultry manure and all inoculations with *Chlorella vulgaris*) than in control and bulk soil. Likewise, the bacterial and fungal counts in rhizosphere soil (both the treated and control rhizosphere soil) were significantly higher ($p<0.05$) than in bulk soil. The relatively higher counts obtained in amended soil may be attributed to higher amounts of nutrients inherent in such soils due to fertilization and inoculation with *Chlorella vulgaris*. Soil amended with poultry manure and *Chlorella vulgaris* greatly increase microbial population than the NPK amended soil which had the lowest bacterial and fungal counts among all the fertilizers this is in consonance with report of Kavitha and Rajini[4]. Similar trends have been reported by Sule and Oyeyiola[12]. Such nutrient shore ups in soil usually enhance soil fertility and productivity [1].

The germination time was shortened by 3 days as a result of the seed and soil application of *Chlorella vulgaris*. *Hibiscus esculentus* had increased mean pod yield per plant of 14 ready for harvest 8 weeks after seed germination, whereas, for other treatments, the pods were ready for harvest only after 9-10 weeks following seed germination. Application of NPK fertilizer before planting inhibited germination of okra and this phenomenon may have been due to the toxicity high concentrations of chemical fertilizers elicited on the tender seeds. Consequently, seeds were left to germinate and grow for a week before addition of NPK. Highest fresh pod weight was produced by *Hibiscus esculentus* cultivated in soil amended with poultry manure (D). The highest moisture, crude protein, crude lipid and chlorophyll contents were associated with the plants cultivated in soil amended with *Chlorella vulgaris* inoculant or in plants that emanated from seeds inoculated with *Chlorella vulgaris.* Kavitha and Rajini[4] had reported a similar trend where maximum increase in chlorophyll of vegetable (*Amaranthus tristis*) was obtained in soil treated with mixed vermicompost and *Azospirillum*.

Species of *Bacillus*, *Citrobacter*, *Micrococcus*, *Pseudomonas*, *Staphylococcus*, *Aspergillus*, *Fusarium* and *Penicillium* were found in rhizosphere soil inoculated with *Chlorella vulgaris*. Species of *Bacillus, Pseudomonas* and *Penicillium* improve soil fertility by solubilizing phosphate and could also serve as bio control agents. *Aspergillus* also solubilizes phosphate while *Citrobacter* plays a very vital role in cycling nitrogen, responsible for reducing nitrate to nitrite. Kumar *et al.*[33] had isolated *Bacillus* and *Pseudomonas* in a previous study and were shown to be efficient plant growth promoting rhizobacteria that effectively enhance root and shoot length of okra seedlings and other vegetables.

CONCLUSION

This study has shown that *Chlorella vulgaris* application (especially, combined seed and soil inoculation) is efficient and economical in improving soil nutrients for greater productivity of *Hibiscus esculentus*. Thus, it was recommend that novel biofertilizer formulation to farmers seeking for an alternative to the use of NPK fertilizers which have posed a huge challenge to post harvest stability, product quality and environmental sustainability.

SIGNIFICANCE STATEMENTS

This study suggested that *Chlorella vulgaris* (bio-fertilizer) is efficient safe and economical for productive cultivation of *Hibiscus esculentus.* The plants are exposed to the micronutrients inherent in the biofertilizer which are beneficial for plant growth and have no detrimental effect on the plants hence, providing an economical and environmental friendly method of enhancing crop production. The finding of this study has provided a veritable alternative to the use of chemical fertilizers for enhanced cultivation of *Hibiscus esculentus* by farmers in Sub-Saharan Africa.

REFERENCES

1. Taher, M.T. and Y. Mohammed, 2015. Improvement of growth parameters of *Zea mays* and properties of soil inoculated with two *Chlorella* species. Rep. Opin., 7: 22-27.
2. Huang, X.F., J.M. Chaparro, K.F. Reardon, R. Zhang, Q. Shen and J.M. Vivanco, 2014. Rhizosphere interactions: Root exudates, microbes and microbial communities. Botany, 92: 267-275.
3. Youssef, M.M.A. and M.F.M. Eissa, 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. E3 J. Biotechnol. Pharm. Res., 5: 1-6.
4. Kavitha, S. Sivagami and Ranjini, 2013. Individual and combined effect of biofertilizer, chemical fertilizer and vermicompost on *Amaranthus tristis*. Int. J. Pharm. Sci. Rev. Res., 20: 190-195.
5. Doolotkeldieva, T., S. Bobusheva and M. Konurbaeva, 2015. Effects of *Streptomyces* biofertilizer to soil fertility and rhizosphere's functional biodiversity of agricultural plants. Adv. Microbiol., 5: 555-571.
6. Shiyab, S., B. Al-Qarallah, M. Akash, M. Statieh, J. Ayad, A. Jordan and K. Al Sane, 2014. Response of okra (*Abelmoschus esculentus* (L.) to different levels of hoaglands solution. Life Sci. J., 11: 1080-1086.
7. Sule, I.O. and G.P. Oyeyiola, 2012. Fungal population in the root region of cassava cultivar TMS 30572. World J. Agric. Sci., 8: 73-79.
8. Kandil, A.A., M.H. El-Hindi, M.A. Badawi, S.A. El-Morarsy and F.A.H. Kalboush, 2011. Response of wheat to rates of nitrogen, biofertilizers and land leveling. Crop Environ., 2: 46-51.
9. Mazid, M. and T.A Khan, 2014. Future of bio-fertilizers in Indian agriculture: An overview. Int. J. Agric. Food Res., 3: 10-23.
10. Okhchelar, R.A. and R. Amirnia, 2012. Survey simple correlation, yield and yield components of (*Cucurbita pepo* var. Styrica) influenced by treatments different bio-fertilizer. Life Sci. J., 9: 1501-1509.
11. Derkowska, E., L.S. Paszt, P. Trzcinski, M. Przybyl and K. Weszczak, 2015. Influence of biofertilizers on plant growth and rhizosphere microbiology of greenhouse-grown strawberry cultivars. Acta Sci. Pol. Hortorum Cultus, 14: 83-96.
12. Rajasekaran, S., P. Sundaramoorthy and G.K. Sankar, 2015. Effect of FYM, N, P fertilizers and biofertilizers on germination and growth of paddy (*Oryza sativa* L.). Int. Lett. Nat. Sci., 35: 59-65.

13. Alia, A.A., N.K. Shahida, J. Bushra and A.A. Saeed, 2013. Phosphate solubilizing bacteria associated with vegetables roots in different ecologies. Sudan Pak. J. Bot., 45: 535-544.
14. Eze, C.S. and J.E. Amadi, 2014. Studies on rhizosphere and rhizoplane microflora of tomato (*Lycopersicon esculentum* Mill) seedlings. Int. J. Eng. Sci. Res. Technol., 3: 666-672.
15. Rao, P.H., R.R. Kumar, V. Subramanian and V. Sivasubramanian, 2010. Environmental impact assessment of *Chlorella vulgaris* employed in phycoremediation of effluent from a leather-processing chemical industry. J. Algal Biomass Utiln., 1: 42-50.
16. Raposo, M.F.D.J. and R.M.S.C. de Morais, 2011. *Chlorella vulgaris* as soil amendment: Influence of encapsulation and enrichment with rhizobacteria. Int. J. Agric. Biol., 13: 719-724.
17. Farfour, S.A., M.A. Al-Saman and A. Hamouda, 2015. Potential activity of some biofertilizer agents on antioxidant and phytochemical constituents of faba bean plant. Global Adv. Res. J. Agric. Sci., 4: 26-32.
18. Wafaa, M.T., F.M. Eletr, A.A.M. Ghazal and H.Y. Gehan, 2013. Responses of wheat-rice cropping system to cyanobacteria inoculation and different soil conditioners sources under saline soil. Nat. Sci., 11: 118-129.
19. Grzesik, M. and Z. Romanowska-Duda, 2014. Improvements in germination, growth and metabolic activity of corn seedlings by grain conditioning and root application with cyanobacteria and microalgae. Polish J. Environ. Stud., 23: 1147-1153.
20. Toppo, S.R. and P. Tiwari, 2015. Phosphate solubilizing rhizospheric bacterial communities of different crops of Korea District of Chhattisgarh, India. Afr. J. Microbiol. Res., 9: 1629-1636.
21. Bashan, Y., L.E. de-Bashan, S.R. Prabhu and J.P. Hernandez, 2014. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). Plant Soil, 378: 1-33.
22. Lopez, B.R., Y. Bashan, A. Trejo and L.E. de-Bashan, 2013. Amendment of degraded desert soil with wastewater debris containing immobilized *Chlorella sorokiniana* and *Azospirillum brasilense* significantly modifies soil bacterial community structure, diversity and richness. Biol. Fertil. Soils, 49: 1053-1063.
23. Al-Shakankery, F.M., R.A. Hamouda and M.M. Ammar, 2014. The promotive effect of different concentrations of marine algae as biofertilizers on growth and yield of maize (*Zea mays* L.) plants. J. Chem. Biol. Phys. Sci., 4: 43201-43211.
24. Muthulakshmi, R. and K. Meenatchisundaram, 2015. Biodiesel energy production from microalgae-*Spirogyra* sp., *Oscillatoria foreani* and *Chlorella pyrenoidosa*. Life Sci. Arch., 1: 84-89.
25. Aggani, S.L., 2013. Development of bio-fertilizers and its future perspective. Sch. Acad. J. Pharm., 2: 327-332.
26. Bileva, T., 2013. Influence of green algae *Chlorella vulgaris* on infested with *Xiphinema index* grape seedlings. J. Earth Sci. Climate Change, Vol. 4. 10.4172/2157-7617.1000136.
27. Ponnuswamy, I., S. Madhavan and S. Shabudeen, 2013. Isolation and characterization of green microalgae for carbon sequestration, waste water treatment and bio-fuel production. Int. J. Bio-Sci. Bio-Technol., 5: 17-26.
28. Kumar, K., K. Madhuri, V. Murugan, K. Sakthivel and A. Anantharaj *et al*., 2014. Growth enhancement in vegetable crops by multifunctional resident plant growth promoting rhizobacteria under tropical Island ecosystem. Afr. J. Microbiol. Res., 8: 2436-2448.
29. Kitada, K., S. Machmudah, M. Sasaki, M. Goto, Y. Nakashima, S. Kumamoto and T. Hasegawa, 2009. Supercritical CO_2 extraction of pigment components with pharmaceutical importance from *Chlorella vulgaris*. J. Chem. Technol. Biotechnol., 84: 657-661.
30. Kumar, N., H.K. Singh and P.K. Mishra, 2015. Impact of organic manures and biofertilizers on growth and quality parameters of strawberry cv. chandler. Indian J. Sci. Technol., 8: 1-6.
31. Faheed, F.A. and Z. Abd-El Fattah, 2008. Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. J. Agric. Soc. Sci., 4: 165-169.
32. Haque, M.A. and M.K. Khan, 2012. Effects of phosphatic biofertilizer with inorganic and organic sources of phosphorus on growth and yield of lentil. J. Environ. Sci. Nat. Resour., 5: 225-230.
33. Kumar, R., A. Sinha, S. Srivastava and M. Srivastava, 2013. Effect of green manuring of *Sesbania aculeata* L. on rhizosphere microflora of okra (*Abelmoschus esculentus* L.). Crop Res., 46: 200-204.
34. Zayadan, B.K., D.N. Matorin, G.B. Baimakhanova, K. Bolathan, G.D. Oraz and A.K. Sadanov, 2014. Promising microbial consortia for producing biofertilizers for rice fields. Microbiology, 83: 391-397.
35. Olanrewaju, O.S., E.B. Akinro and O.A. Oladipupo, 2014. Fungi colonization of the rhizoplane of okra (*Hibiscus esculentus*) plant. IOSR J. Environ. Sci. Toxicol. Food Technol., 8: 18-22.
36. Oyeyiola, G.P., M.O. Arekemase, I.O. Sule and T.O. Agbabiaka, 2013. Rhizosphere bacterial flora of okro (*Hibiscus esculentus*). Sci. Int. (Lahore), 25: 273-276.
37. Oyewole, O.A., I.N. Okoliegbe and E.E. Akwu, 2012. Comparative rhizosphere microbiological and physicochemical properties of *Arachis hypogeae* (Groundnut) and *Hibiscus esculentus* (Okro). Nat. Prod.: Indian J., 8: 352-360.
38. Agwa, O.K. and G.O. Abu, 2014. Utilization of poultry waste for the cultivation of *Chlorella* sp. for biomass and lipid production. Int. J. Curr. Microbiol. Applied Sci., 3: 1036-1047.
39. Agwa, O.K. and G.O. Abu, 2016. Influence of various nitrogen sources on biomass and lipid production by *Chlorella vulgaris*. Br. Biotechnol. J., 15: 1-13.

40. AOAC., 1990. Official Methods of Analysis. 14th Edn., Association of Official Analytical Chemist, Arlington, VA., USA., pp: 503-515.
41. Buchanan, R.E. and N.E. Gibbons, 1974. Bergey's Manual of Determinative Bacteriology. 8th Edn., Williams and Wilkins Co., Baltimore.
42. Cheesbrough, M., 2000. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edn., Cambridge University Press, UK., ISBN-13: 9780521665452, pp: 42-51.
43. Domsch, K.H. and W. Gams, 1970. Fungi in Agricultural Soils. 1st Edn., Longman Group Limited, London, UK., pp: 20-152.
44. IITA., 1979. Selected Methods for Soil and Plant Analysis. 2nd Edn., International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, pp: 3, 6-7, 11-12, 41-42.
45. SPSS., 2011. IBM SPSS Statistical for Windows. IBM Corporation, Armonk, New York.
46. Hatim, A.S., 2013. Effect of bio-organic fertilizers on soil fertility and yield of groundnut (*Arachis hypogaea* L.) in Malakal area, Republic of South Sudan. J. Nat. Resour. Environ. Stud., 1: 14-19.
47. Baba, Z.A., M.Y. Zargar and S.A. Mir, 2010. Effect of inorganic and biofertilizers on soil physico chemical properties and micronutrient availibility in strawberry (*Fragaria×ananassa* Duch). Asian J. Soil Sci., 5: 90-93.