The application of *Klebsiella* sp. and *Rhizobium radiobacter* as biofertilizer and Palm Oil Mills Effluent (POME) as organic fertilizer on growth of *Paraserianthes falcataria*

Suliasih* and S Widawati

Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences, Cibinong Science Center, Jl. Raya Jakarta-Bogor km 46, Cibinong 16911, Indonesia

Email: lishadari@yahoo.co.id

**Abstract.** The bio and organic fertilizers are cheap and environmentally friendly source of plant nutrients for agricultural yields and environmental quality improvement. The study aimed to evaluate the application of biofertilizer and Palm oil mills effluent (POME) either singly or in combination on growth of *Paraserianthes falcataria* under greenhouse condition. The study was laid out in factorial based Completely Randomized Design (CRD) design which comprised of biofertilizer treatments (Control without bacteria, *Klebsiella* sp, *Rhizobium radiobacter*, *Klebsiella* sp + *Rhizobium radiobacter*) and five concentrations of POME treatments (0%, 10%, 25%, 50%, 100%) with 3 replicates for each treatment. The results revealed that inoculation with biofertilizer treatments along with POME significantly enhanced plant growth parameters, soil available P, soil phosphatase activity and soil bacterial population. Combination of *Rhizobium radiobacter* and POME 50% induced the highest increase of shoot length (10.17 ± 0.83 cm), root length (28.67 ± 0.88 cm), shoot dry weight (0.48 ± 0.006 g), and root dry weight (0.25 ± 0.013 g). The highest soil phosphatase activity was obtained in the combination *Klebsiella* sp. and POME 25% treated soil. The application of *Rhizobium radiobacter* along with POME at 10% and 25% concentrations reached its highest level of soluble soil P (4.07 ppm) and soil bacterial population (48.33 10^7 cfu/g of soil) respectively.

1. **Introduction**

The increase of continuously intensive land exploitation leads to the decrease of soil organic. Consequently, it causes the decline of soil fertility and land productivity. The addition of organic matter at the time of planting as bio and organic fertilizer is one of the alternative methods to maintain and increase the organic matter level and soil fertility [1,2]. In sustainable agriculture, microbial inoculants and organic fertilizers are affordable and environmentally friendly nutrition sources and are important for the management of soil nutrient [3].

*Klebsiella* sp. and *Rhizobium* sp. which are used as biofertilizers, are root-associated bacteria that have an important role as plant growth promoting rhizobacteria (PGPR). These bacteria are able to solubilize both organic and inorganic phosphates, fix nitrogen, produce IAA growth hormone and ACC Deaminase activity [4,5,6,7,8,9].

In agricultural sector, waste utilization as nutrients enrichment to improve plant growth is often needed. One of the best agricultural waste that can be converted into organic fertilizer is palm oil
industry waste [10]. POME (Palm Oil Mill Effluent), one of the palm oil industry by-products, is oily brownish liquid obtained from the extraction of palm oil. POME can be used as fertilizer on agricultural land due to its macro nutrients content such as N, P and K. The application of POME to the soil is not only able to increase soil fertility but also improve soil structure resulting in better root health and plant growth [11]. Some researchers suggested that the use of organic and sole biofertilizer or a combination of both provided positive response in regard to plant growth, nutrient uptake and availability of plant nutrients [12,13,14].

The objective of this research was to investigate the effectiveness of bacterial inoculants as biofertilizers combined with POME in various concentrations as organic fertilizer on *Paraserianthes falcata* in greenhouse-scale.

2. Materials and Methods

2.1. Green house experiment

*Rhizobium radiobacter* (InaCC B834) and *Klebsiella* sp. (InaCC B833), used as biofertilizers, were obtained from the InaCC Collection, Microbiology Division of the Biology Research Center, LIPI. The abilities of these bacteria in fixing N, ACC deaminase activity, producing IAA, solubilizing inorganic and organic phosphates have been tested in vitro [15].

| Table 1. The abilities of bacteria as PGPR |
|-------------------------------------------|
| Isolates & N2 fixation & ACC Deaminase & IAA (ppm) & Inorganic P (ppm) & Phosphatase (Unit) |
|---------|-----------|----------------|---------------|-----------------|-----------------|
| R       & +        & +              & 88.69         & 0.83            | Acid 0.11       |
| B       & +        & +              & 104.96        & 1.57            | Alkaline 0.50   |

Note: R = *Rhizobium radiobacter*, K = *Klebsiella* sp.

For the inoculants preparation, 1 ml of each *Rhizobium radiobacter* and *Klebsiella* sp. was inoculated on 250 ml Erlenmeyer containing Yeast mannitol (YM) broth and liquid Pikovskaya respectively [16]. The erlenmeyer was incubated on a shaker at the speed of 120 rpm for 3 days at room temperature. Bacterial culture was centrifuged at the speed of 12000 rpm for 10 minutes and the pellets obtained were diluted into sterile aquadest until the cell density was $10^6$ cfu/ml. The bacterial solution was used as seed inoculant.

Palm Oil Mill Effluent (POME), used as organic fertilizer, was taken from ponds in PTP VIII Cikasungka, West Java. *Paraserianthes falcata* seeds were obtained from the collection of Bogor Botanical Garden. The seeds were sterilized in sodium hypochlorite then rinsed with sterile distilled water three times. The seeds were germinated in sterile Petri dish which had been coated with sterile filter paper beforehand. The sprouts of *P. falcata* were inoculated by being immersed in liquid culture of *Rhizobium radiobacter* and *Klebsiella* sp. for 60 minutes. Inoculated sprouts then were planted in pots filled with 300 grams of soil. Non-inoculated plants were used as controls.

The experiment used a Completely Randomized Design (CRD) with a factorial pattern. The first factor was biofertilizer consisting of K (non-inoculated plants as control); R (*Rhizobium radiobacter* inoculant); B (*Klebsiella* sp. inoculant); M (*Rhizobum radiobacter* + *Klebsiella* sp.). The second factor is the POME concentration consisting of P0 (control without POME); POME 10% (P1); POME 25% (P2), POME 50% (P3), POME 100% (P4) and each treatment had 3 replications.

Planting was carried out in a greenhouse in Microbiology - Research Center for Biology - LIPI Cibinong. As much as 300 grams of soil was loaded into the pot. Then each pot was given POME solution based on the treatment and incubated for 2 weeks. Three *Paraserianthes falcata* sprouts were transferred into each pot. Harvesting was carried out when the plants was 60 days old. The
observed variables included root and shoot length (cm), root and shoot dry weight (mg), soil available P (ppm), phosphatase activity (unit) and total bacterial population (cfu/g of soil).

2.2. Analysis of available P content, Phosphatase and Total bacterial population in soil

Samples of soil rhizosphere during the harvesting (2 months after planting) from each treatment were analyzed to measure the level of available P, phosphatase activity and total bacterial population.

2.2.1. The measurement of available P. Available P content were determined using the Olsen method, soil samples were extracted with the Olsen’s extracting solution (sodium bicarbonate, pH 8.5). Place on a mechanical shaker for 30 minutes at room temperature and centrifuge the suspension for 10 minutes, 12000 rpm. Measured the supernatant colorimetrically [17].

2.2.2. The measurement of phosphatase activity. A mix of 1 gram of soil sample, 4 ml of substrate p-NPP 0.115 M and 5 ml sterile aquadest was put on a shaker for 30 minutes at room temperature. The mixture was centrifuged at the speed of 12,000 rpm for 15 minutes. As much as 0.5 ml of acetate buffer was added into 0.1 ml of supernatant and incubated for 15 minutes. After incubation, 0.5 ml of 0.5M CaCl₂ and 2 ml of 0.5M NaOH were added. The absorbance of the sample was measured at the wavelength of 880 nm. The standard and blank were treated in the similar way as sample. A p-nitrophenol solution with a concentration of 1-6 ppm was used as standard, while distilled water was used as blank [18].

2.2.3. Total bacterial population in soil. The pour plate method was used to count the bacterial population [19]. As much as 1 gram of soil was diluted into 9 ml of sterile aquadest to obtain a 10⁴ dilutions. From the dilution a dilution series up to 10⁻⁷ was prepared. As much as 0.1 ml from 10⁻³,10⁻⁵ and 10⁻⁷ dilution was taken into a sterile Petri dish, then poured over with nutrient agar on top. Plates were incubated for 7 days at room temperature. The number of viable cells was measured by CFU (colony forming unit/g of soil).

2.3. Statistical analysis

The acquired data were analyzed using SPSS and values were given as means ± SD for triplicate samples. Duncan's Multiple Range Test (DMRT) was applied to test the significance of treatment means at P ≤ 0.05.

3. Results and Discussion

3.1. Greenhouse experiment

3.1.1. The Effect of biofertilizer and POME on shoot and root length. The application of the biofertilizer significantly increased the length of shoots. Plants that were inoculated with Rhizobium radiobacter (R), Klebsiella sp. (B) and mixture of both (M) showed an increase in shoot length ranging from 39.3-48.2% compared to control without biofertilizer. Similar result was reported by [20] that there was an increase in the length of chickpeas shoots and roots inoculated with phosphate solubilizing rhizobia which had the ability to solubilize P and produce IAA (Figure 1).
3.1.2. The effect of biofertilizer and POME on shoot and root dry weight. In figure 2, there was an escalation in shoot and root dry weight in plants treated with biofertilizer. The application of *Klebsiella* sp. and *Rhizobium radiobacter* as biofertilizers improved shoot and root dry weight compared to plant control without inoculants and POME. Similar result was reported by [21] found that inoculation with phosphate solubilizing *Rhizobium leguminosarum* biovar phaseoli increased the dry weight of lettuce and corn plants. Correspondingly, [9] study provided that the inoculation of *Vigna. radiate*, *V. tetragonoloba* and *V. unguiculata* with phosphate solubilizing and IAA producer *Klebsiella pneumonia* showed significant differences in the wet and dry weight of plants compared to controls.

According to [22,23,24], the application of PGPR which has various abilities in solubilizing phosphate, producing phosphatase, IAA and ACC deaminase activity, was more effective in increasing shoot and root length, as well as shoot and root dry weight. This is in accordance with the research results that *Klebsiella* sp and *Rhizobium radiobacter* which belong to Plant Growth Promoting Rhizobacteria (PGPR), have the ability to nitrogen fixation, ACC deaminase activity, produce IAA, solubilize inorganic P and phosphatase activity (Table 1). [25] reported that several strains of *Rhizobium* and *Klebsiella*, *Azospirillum*, *Azotobacter*, aside from possessing the ability to solubilize inorganic phosphate from P source, namely Ca₃(PO₄)₂ also had phosphatase activity. Some *Klebsiella* species such as *K. oxytoca* Rs-5 showed the ability to produce IAA and ACC deaminase activity [26], *K. varicola* AY13 produced IAA [27], *K. pneumoniae* solubilized P, while producing IAA [9]. Similarly, *Rhizobium* isolated from plant legumes nodules produced IAA, ACC deaminase and solubilized both inorganic and organic P [28,29].

The usage of POME as organic fertilizer demonstrated positive plant growth responses. Plants treated with POME concentrations of 10%, 20%, 50% and 100% showed significant increase in shoot length, dry weight of roots and shoots compared to plant control without POME. This result is in accordance with the research of [30], that reported an increase in the growth of corn plants treated with fermented POME compared to controls.

The combination of biofertilizer and POME stimulated and improved the growth of *P. falcataria*. In addition to the length of shoots and roots, dry weight of shoots and roots showed the highest increase in plants inoculated with *Rhizobium radiobacter* at 50% POME concentration.
improvement of growth was between 26.5-92% compared to plants treated with sole biofertilizer or POME only. [31] reported that the combination of bio-organic fertilizer would increase plant height and dry weight of ginger plants and also increased the population of bacteria and actinomycetes. [14] suggested that the application of organic fertilizers and biofertilizers can increase plant height and leaf number. It is predicted that the biofertilizer used in conjunction with organic fertilizer help the plants to develop better and absorb more nutrients.

![Figure 2. Effect of biofertilizer and POME on the dry weight of shoot and root]

Note: K=Control without Inoculatin, R=Rhizobium radiobacter, B=Klebsiella sp. M= R. radiobacter + Klebsiella sp, P0=Control without POME, P1=POME 10%, P2=25%, P3=50%, P4=100%

3.2. Analysis of available P content, phosphatase and total bacterial population in soil

3.2.1. The effect of biofertilizer and POME on soil available P. The effects of biofertilizer and organic POME fertilizer application on available P in soil at harvest (2 months after planting) are presented in Figure 3. All biofertilizer treatments resulted in positive responses of the increase in available P, compared to controls. The highest level of available P (1.84 ppm) was obtained on the soil treated with biofertilizer of mixed Klebsiella sp. and Rhizobium radiobacter inoculant. This result corresponds the observations of [32], reported that several bacteria such as Rhizobium, Klebsiella and Pseudomonas were able to solubilize insoluble phosphates thus become available to plants. The application of biofertilizers on selected strains increased available P in the soil, it is probably a result of the presence of larger microbial activity in solubilizing P bound or mineralizing organic material [33].

The application of POME of 10, 25, 50 and 100% concentrations as organic fertilizer significantly higher available P than control without POME. [34] found that available phosphorus, soil pH, organic matter, total nitrogen, exchangeable acidity and bulk density of the soil is affected by the use of POME.

The combination of biofertilizer and organic fertilizer treatment was more effective in increasing the amount of available P than control and treatment of sole biofertilizers or organic fertilizers only. The maximum available P (4.07 ppm) occurred in soil treated with Rhizobium radiobacter inoculant along with POME 10%.
3.2.2. The effect of biofertilizer and POME on the activity of phosphatase. The application of Klebsiella, sp. displayed significant effect on phosphatase activity with an increase of 28%, followed by Rhizobium radiobacter (11%) compared to controls without inoculants. [22] that reported an increase in phosphatase and organic P mineralization due to the treatment of phosphate solubilizing bacteria inoculant, and phosphatase activity was considered as the major contributor to the increase in phosphorus in the soil.
The application of low concentration POME without inoculants enhanced phosphatase activity and increased while the usage of high concentration POME caused a decrease on observed parameters. The soil applied with POME concentration of 10% showed a rise in phosphatase activity of around 5.4% compared to controls without POME. On the contrary, the application of POME of 25, 50 and 100% concentrations resulted in lower level of soil phosphatase than controls. This was also observed by [35] who reported that phosphatase and dehydrogenase activity in POME-contaminated soils decreased significantly compared to control soil samples (Figure 4).

The application of Klebsiella sp. inoculant along with 25% POME concentration showed the highest soil phosphatase activity (64.72 units).

3.2.3. The effect of biofertilizer and POME on soil bacterial population. The population of soil bacteria treated with biofertilizer showed significant difference when compared to the control without inoculants. Plants inoculated with *Rhizobium radiobacter* produced the highest number of soil bacterial population (38.33 x 10⁷ cfu/g soil).

The soil treated with POME 10% and 25% demonstrated an increase in bacterial population. Although in the higher concentration of POME treatment, 50 and 100%, the total count of bacterial population tended to be lower than the control. Likewise, [36,37,38] reported that heterotrophic bacterial population, phosphate solubilizing bacteria, nitrifying and lipolytic decreased significantly in soil contaminated with high concentrations of POME; contrariwise with low concentration.

![Figure 5](image-url)

**Figure 5.** Effect of biofertilizer and POME on soil bacterial population

*Note:* K=Control without Inoculation, R=*Rhizobium radiobacter*, B=*Klebsiella* sp. M= *R. radiobacter* + *Klebsiella* sp, P0=Control without POME, P1=POME 10%, P2=25%, P3=50%, P4=100%

The combination of biofertilizer and organic fertilizer treatment was more effective in increasing the bacterial population than control and treatment of sole biofertilizers or organic fertilizers only. *Rhizobium radiobacter* combined with 25% POME concentration resulted in the highest bacterial population (48.33 x 10⁷ cfu/g of soil). [39] stated that the application of biofertilizers in combination with organic fertilizers increased soil bacterial populations more effectively than using sole biofertilizers or organic fertilizers only. Similar observations have been reported by [40], which discovered a significant increase in soil microflora such as bacteria, fungi and actinomycetes and soil enzyme activities such as phosphatase and dehydrogenase in soil treated with phosphate solubilizing
bacterial biofertilizer combined with organic fertilizer in the form of compost. The activity of phosphatase, soil microbial populations and organic matter content can be increased by the application of organic fertilizers [41,42,43].

4. Conclusion
Plants inoculated with sole *Klebsiella* sp. and *Rhizobium* sp. or POME or a combination of both showed an increase of growth in *Paraserianthes falcataria*. All biofertilizer treatments and combinations with organic fertilizers presented positive response in regard to the increase of available P, phosphatase and total count of bacterial population in soil. The application of low concentration POME without inoculants increased phosphatase activity and soil bacterial population, whereas the use of high concentration POME resulted in a decrease of those parameters.

5. References
[1] Venkatashwarlu B 2008 Role of bio-fertilizers in organic farming: Organic farming in rain fed agriculture; Central institute for dry land agriculture *Hyderabad* 85-95
[2] Chiconato, DA, Simonli, Galbiatti, JA, ranco C, Caramelo AD 2013 Resposta da alfalfa aplicacao de biofertilizante sob dois níveis de irrigacao *Biosci. J.* **29**(2) 392-9
[3] Abdullahi R, Sheriff H H and Lihan S 2013 Combine effect of bio-fertilizer and poultry manure on growth, nutrients uptake and microbial population associated with sesame (*Sesamum indicum L*) North-eastern Nigeria *IOSR J. Environ. Sci. Toxicol. Food Technol.* (IOSR-JESTFT) **5**(5) 60-5
[4] Bhattacharjee R B, Jourand P, Chaintreuil C, Dreyfus B, Singh A, Mukhopadhyay S N 2012 Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. *trifoliisN10* promote rice growth, and in the process undergo colonization and chemotaxis *Bio. Fert. Soils* **48** 173–82
[5] Machado RG, Sá ELS, Bruxel M, Giongo A, Santos N S, Nunes A S 2013 Indole acetic acid pro-rhizobia promote growth of Tanzania grass (*Panicum maximum*) and Pencasola grass (*Paspalum saurae*) *Int. J. Agric. Biol.* **15** 827–34
[6] Ravi Kumar P and Raghu Ram M 2012 Production of indole acetic acid by *Rhizobium* isolates from *Vigna trilobata* (L) Verdc *Afr. J. Microbiol. Res.* **6**(27) 5536-41
[7] Buddhhi Charana Walpola, Arunakumara KKIU, Min Ho Yoon 2014 Isolation and characterization on phosphate solubilizing bacteria (*Klebsiella oxytoca*) with enhanced tolerant to environmental stress *Afr. J. Microbiol. Res.* **8**(31) 2970-8
[8] Rochel de Souza, Adriana Ambrosini, and Luciane, Passaglia MP 2015 Plant growth-promoting bacteria as inoculants in agricultural soils *Genet. Mol. Biol.* **38**(4) 401-19
[9] Gurvesh Bhardwaj, Rushabh Shah, Bhrugesh Joshi, Prittesh Patel 2017 *Klebsiella pneumoniae* VRE36 as a PGPR isolated from *Saccharum Officinarum* cultivar Co99004. *J. Appl. Biol. Biotechnol.* **5**(1) 47-52
[10] Aisueni NO, Omoti U 1999 *The making of compost from empty oil palm bunch refuses* In: Books of Abstracts, Soil Science Society of Nigeria Conference, Benin, 21–25 November 1999
[11] Chan KW, Watson I, Lim KC 1981 Use of oil palm waste material for increased production *Planter* **57** 14-37
[12] Maman N and Mason S 2013 Poultry manure and inorganic fertilizer to improve pearl millet yield in Niger *Afr. J. Plant Sci.* **7**(5) 162-9
[13] Barna chakraborty and Manab kundu 2016 Effect of biofertilizer in combination with organic manures on Growth and foliar constituents of mulberry under rainfed lateritic soil condition *Int. J. Eng. Sci. (IJES)* **4**(3) 16-20
[14] Negi Ekta, Punetha Shailaja, Pant, Kumar Sandeep SC, Bahuguna Pankaj, Mekap Bengia and Nautiyal BP 2017 Effect of organic manures and bio-fertilizers on growth, yield, quality
and economics of Broccoli (Brassica Oleracea L. var. italica PLENCK) cv. Green head under high-hill conditions of Uttarakhand. Int. J. adv. Biol. Res. 7(1) 96-100
[15] Widawati S, Suliasih, Susiloswati DN, Muramatsu Y and Sudiana IM 2018 Screening of growth promoting rhizobacteria isolated from Gunung Pancar and Bukit Bangkirai, Indonesia Unpublish
[16] Rao NSS 1995 Soil Microorganisms and Plant Growth 3th edition New Hampshire Science Publishers Inc.
[17] Olsen SR and Sommers LE 1982 Phosphorus In Page AL et al. (eds.) Methods of soil Analysis part 2 Agron. Monogr. 9 403-30 2nd ed. ASA and SSSA Madison WI
[18] Tabatabai MA and Bremner JM 1969 Use of p nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1 301-7
[19] Vincent JM 1982 Nitrogen Fixation in Legumes. London Academic Press
[20] Sachin Singh, Govind Gupta, Ekta Khare, Behal KK and Naveen Arora K 2014 Phosphate Solubilizing Rhizobia Promote the Growth of Chickpea under Buffering Conditions Int. J. Pure Appl. Biosci. 2(5) 97-106
[21] Chabot, R., H. Anton and M.P. Cescas. 1996. Growth promotion of maize and lettuce by phosphate solubilizing Rhizobium leguminosarum biovar. phaseoli. Plant and Soil 184 311-21
[22] Minaxi LN, Yadav RC and Saxena J 2012 Characterization of multifaceted Bacillus sp. RM-2 for its use as plant growth promoting bioinoculant for crops grown in semi arid deserts Appl. Soil Ecol. 59 124-35
[23] Muhammad Iqbal Hussain, Hafiz Naeem Asghar, Muhammad Javed Akhtar and Muhammad Arshad 2013 Impact of phosphate solubilizing bacteria on growth and yield of maize. Soil Environ. 32(1) 71-8
[24] Li Y, Liu X, Hao T and Chen S 2017 Colonization and Maize growth promotion induced by phosphate solubilizing bacteria isolates Int. J. Mol. Sci. 18(7) 1253
[25] Monica del Pilar Lopez-Ortega, Paola Jimena Criollo-Campos, Ruth Milena Gomez-Vargas, Mauricio Cameo-Rusineque, German Estrada-Bonilla, María Fernanda Garrido-Rubiano, Ruth Bonilla-Buitrago 2013 Characterization of diazotrophic phosphate solubilizing bacteria as growth promoters of maize plants Rev. colomb. biotecnol. 15 (2) 115-23
[26] Liu Y, Shi Z, Yao L, Yue Hi H, Li C 2013 Effect of IAA produced by Klebsiella oxytoca Rs-5 on cotton growth under salt stress J. Gen. Appl. Micobiol. 59(1) 59-65
[27] Ah-Young Kim, Raheem Shahzad, Sang-Mo Kang, Chang-Woo Seo, Yeon-Gyeong Park, Hyun-Jin Park, In-Jung Lee 2017 IAA-producing Klebsiella varicola AY13 reprograms soybean growth during flooding stress J. Crop Sci. Biotech. 20(4) 235-42
[28] Uretimicigdem Kucuk, Cenap Cevheri 2016 Isolated from Pea (Pisum sativum L. ssp. arvense) Indole Acetic Acid Production by Rhizobium Turk. J. Life. Sci. 1(1) 43-5
[29] Pankajkumar L, Dubey RC, Maheshwariandvivek Bajpai DK 2016 ACC Deaminase Producing Rhizobium Leguminosarum RPN5 Isolated from root nodules of Phaseolus vulgaris Bangladesh J. Bot. 45(3) 477-84
[30] Nwoko, Chris O; Ogungbemi, SolaNwoko, Chris O; Ogungbemi, Sola 2010 Evaluation of Palm Oil Mill Effluent to Maize (Zea Mays. L.) Crop: Yields, Tissue Nutrient Content and Residual Soil Chemical Properties Aust. J. Crop Sci. 4(1) 16-22
[31] Nan Zhang, Ruhao Pan, Yifei Shen, Jun Yuan, Lei Wang, Xing Luo, Waseem Raza, Ning Ling, Qiwei Huang, Qirong Shen 2017 Development of a novel bio-organic fertilizer for plant growth promotion and suppression of rhizome rot in ginger Biol.Control 114 97–105
[32] Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA 2010 Plant growth promotion by phosphate solubilizing fungi – current perspective Arch. Agro. Soil Sci. 56 73–98.
[33] Rihardson A E and Simpson RJ 2011 Soil microorganisms mediating phosphorus availability Plant Physiol. 156 989-96
[34] Chibuike Samuel Ubani, Chukwudi Onwuneme, Victor Eshu Okpashi, Akudo Chigoziri Osuji, Ugochukwu G.E.M. Nwadike 2017 Palm oil mill effluent effect on soil fertility: A longitudinal assessment of Zea mays plant Environ. Qual. 23 43-53

[35] Nwaugo VO, Chinyere GC, Inyang CU 2008 Effects Of Palm Oil Mill Effluents (Pome) On Soil Bacterial Flora And Enzyme Activities In Egbama Plant Prod. Res. J. 12 10-13

[36] Eneje Roseta C1, and Ifenkwe Innocent 2012 Effect of agricultural and industrial wastes on the physicochemical properties of a sandy clay loam soil Int. J. of Appl. Agric. Res. 7(3) 187-96

[37] Ibe IJ, Ogbulie JN, Orji JC, Nwanze PI, Ihejirika C and Okechi RN 2014 Effects of Palm Oil Mill effluent (Pome) on soil bacteria and enzymes at different seasons Int. J. Curr. Microbiol. Appl. Sci. 3(10) 928-34

[38] Iyakndue ML, Brooks A A, Unimke AA and Agbo BE 2017 Effects of Palm Oil Mill Effluent on Soil Microflora and Fertility in Calabar – Nigeria Asian J. Biol. 2(3) 1-11

[39] Muhammad Fakhar-u-Zaman Akhtar, Moazzam Jamil, Maqshoof Ahamd and Ghulam Hassan Abbasi 2017 Evaluation of biofertilizer in combination with organic amendments and rock phosphate enriched compost for improving productivity of chickpea and maize Soil Environ. 36(1) 59-69

[40] Balakrishnan V, Venkatesan K, Ravindran KC 2007 The influence of halophytic compost, farmyard manure and phosphobacteria on soil microflora and enzyme activities Plant Soil Environ. 53(4) 186-92

[41] Hao XH, Liu SL, Wu JS, Hu RG, Tong CL, Su YY 2007 Effect of long-term application of inorganic fertilizer and organic amendments on soil organic matter and microbial biomass in three subtropical paddy soils Nutr. Cycl. Agroecosyst. 81 (1) 17-24.

[42] Ed-Haun Chang, Ren-Shih Chung and Yuong-How Tsai 2007 Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. Soil Sci. Plant Nutr. 53 132–40

[43] Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q, Shen W 2010 The effects of mineral fertilizer and organic manure on soil microbial community and diversity Plant Soil 326 (1-2) 511-22

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