Improve Quality of Ultisols (*Pokok Sena* Soil Series) Through Phototropic Bacteria Application

Aidee Kamal Khamis, Umi Aisah Asli, Siti Nazrah Zailani, and Firdausi Razali

Abstract**—**Healthy soils are the foundation of sustainable agriculture. One of the factors affecting the quality of soils is its acidity. Naturally, the soils became acidic because of the organic matters and minerals that are broken down in a soil over time or via leaching process due to heavy rainfall or irrigation. However, soils can be strongly acidic with the excessive use of high-nitrogen synthetic based fertilizers. The common effects of this acidic soil to the plants are stunting and yellowing leaves which related to a decrease in growth and yield of crops. In addition, plants grow in adverse pH conditions could be more prone to disease and fungal attack as the availability of essential plant nutrients (calcium, potassium, magnesium and sodium) are likely diminished in acidic soils. Hence, this study is aimed to decrease the soil acidity by natural way, where phototropic bacteria have been applied on the soils. In this study, *Pokok Sena* soil series from Ultisols and *Rhodopseudomonas palustris* have been chosen. The *R. palustris* was used as it has an ability to fix carbon dioxide to form biomass, convert nitrogen to ammonia, and produce hydrogen (as a by-product of nitrogen fixation) to decrease the soil acidity. The experiments were carried out by introducing a different amount of *R. palustris (*1.0, 2.5 and 5.0 % v/w) onto a controlled soils condition of the light exposure, temperature and moisture. The quality of soils were measured by the elementary analysis in order to quantify the C/N ratio, soils pH, cation exchange capacity (C.E.C) and soil electrical conductivity (EC). The analysis of CO_2 for O_2 uptake rate (OUR) was also recorded to monitor the *R. palustris* respiration in soil. Experimental results from this study have proven that the use of *R. palustris* had positively improved the soil acidity which affected other elements of nutrient content uptake capability.

Keywords**—** Ultisols, *R. palustris*, pH, Pokok Sena, Malaysia.

I. Introduction

HEALTHY soils are the foundation of sustainable agriculture [1] [2]. One of the factors affecting the quality of soils is its acidity. Naturally, the soils became acidic because of the organic matters and minerals that are broken down in a soil over time or via leaching process due to heavy rainfall or irrigation [3] [4]. However, soils can be strongly acidic with the excessive use of high-nitrogen synthetic based fertilizers. The common effects of this acidic soil to the plants are stunting and yellowing leaves which related to a decrease in growth and yield of crops [5]. In addition, plants grow in adverse pH conditions could be more prone to disease and fungal attack as the availability of essential plant nutrients (calcium, potassium, magnesium and sodium) are likely diminished in acidic soils. Hence, this study is aimed to decrease the soil acidity by natural way, where phototropic bacteria have been applied on the soils. Table I and Fig. 1 shows the soils characteristics and the soils textures by using FESEM.

TABLE 1
CHARACTERISTIC OF POKOK SENA SOIL SERIES

Parameters	Amount
pH	4.10
C/N ratio	9.42
Cation exchange capacity (C.E.C), meq/100g	10.14
Electrical conductivity (EC), μS	100.20
Clay, %	47.00
Silt, %	9.00
Coarse sand, %	6.00
Fine sand, %	38.00

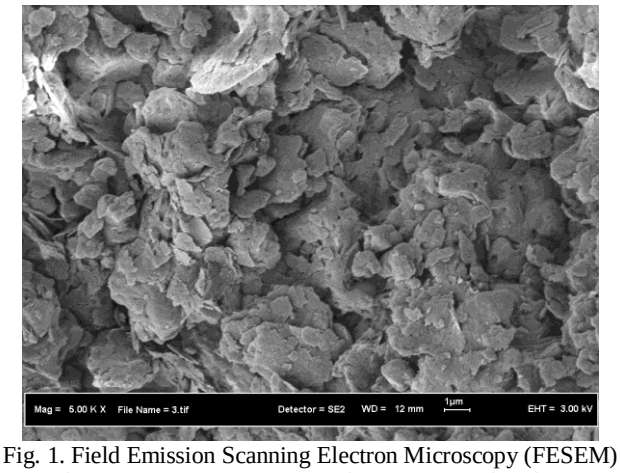

Fig. 1. Field Emission Scanning Electron Microscopy (FESEM) image of *Pokok Sena* soil with magnification view of surface 5.00K X.

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II. Methodology

250 g *Pokok Sena* soil (soils based on the Malaysian soil classification) was placed in a 1*L* conical flask. The number of colony forming unit (CFU) of *R. palustris* used in this experiment was 6 X 10^7 which was freshly prepared before being used. A composition of *R. palustris* with 1.0, 2.5 and 5.0% (v/w) was added to the soil in different conical flasks having the same aerobic condition. 100 ml of deionized water was added to each conical flask, to achieve a soil humidity of 30.0-40.0%. At the top of the conical flask, a set of respirometer was set up. This experiment was carried out for 30 days, with and without the presence of light. The light exposure is referred to a 60W light exposure in the laboratory. The soil pH, C/N ratio, C.E.C, and EC were analyzed after 30 days. During the experiments, OUR was consistently monitored and the results were collected daily.

A. Bacterium

The bacterium, *R. palustris* DSM 131 was purchased through DSM. The culture was stored in a container at temperature -80.0^{O}C. A typical length for this bacterium measures at 0.6-0.9 µm with optimum growth pH of between 6.5 and 7.5. The culture can reproduce itself through a asymmetrical polar cell division in a budding mode. This culture is grown in an incubator at 30°C with white neon light for 2 days and shaken at 150 RPM. In this process, Nutrient broth was used as a growth media.

B. Determination of Soil pH

A pH meter (Model : Hanna HI8424) was used to measure the pH of soil suspension with soil to water ratio of 1:2.5 (w/v) [6] [7].

C. Determination of Soil C/N ratio

Soil C/N ratio was measured by using a CHN analyzer (Model: Thermo Finnigan, Flash EA 1112). 2.5-3.0 mg of samples were used for each measurement including the standard [8].

D. Determination of Soil Cation Exchange Capacity (C.E.C)

The soil CEC was determined using cations displacement by 1 M NH_4OAc buffered solution at pH 7. Air-dry soil (0.5 g) was shaken with 20 ml of NH_4OAc and centrifuged at 5000 rpm for 15 min in a 50 ml centrifuge tube. The supernatant was discarded, and the extraction was repeated. The sample was then washed twice with 10 ml methanol to remove the remaining ammonium. The samples were then mixed with 20 ml of 0.5 M $CaCl_2$ to return the ammonium held on the exchange sites. Following that, the solution was centrifuged at 5000 rpm for 10 min and the supernatant was poured into a 100 ml volumetric flask. This extraction was repeated and the volume of supernatant was increased to 100 ml by adding deionised water. The ammonium concentration was finally measured with an ammonium electrode (Jenway 3045 Ion Analyser, Jenway Ltd, England). In this study, it is assumed that the amount of NH_4^+ corresponds to the amount of negative charge occupied previously by K^+, Na^+, Mg^{2+}, Ca^{2+} and H^+ [9] [10].

E. Determination of Soil Oxygen Uptake Rate (OUR)

A study of microbial activity of the soil was carried out using the respirometry method. A simple customized respirometer was designed to measure the O_2 uptake rate (OUR) or CO_2 released during respiration by bacterium. The respirometer consists of a sealed container with a linked specimen of the soil. The container is plugged with soda lime pellet which is connected to potassium hydroxide (KOH). The container is connected to a simple U-tube/manometer. The purpose of soda lime pellet is to adsorb the CO_2 released during respiration, while the U-tube/manometer measures the changes of the volume of gas produced during the activity. When the organism takes in O_2, it gives off an equal amount of CO_2. As CO_2 is absorbed by the soda lime, air is sucked from the U-tube/manometer to keep the pressure constant, hence displacing the liquid [11].

The relation of pressure and height of displacement of fluid in U-tube/manometer can be written as,

$$P = \rho g h \tag{1}$$

By assuming the ideal gas behavior for O_2, the ideal gas equation to determine the number of moles of O_2 consumed by microbes can be employed.

$$PV = nRT \tag{2}$$

where P is pressure (N/m^2), V is volume occupied by O_2 (m^3), n is number of moles of O_2 (gmol), R is gas constant, and T is temperature during the respiration. The fluid volume in the U-tube/manometer, V can be calculated by the displaced volume in the U-tube/manometer

$$V = \pi r^2 h \tag{3}$$

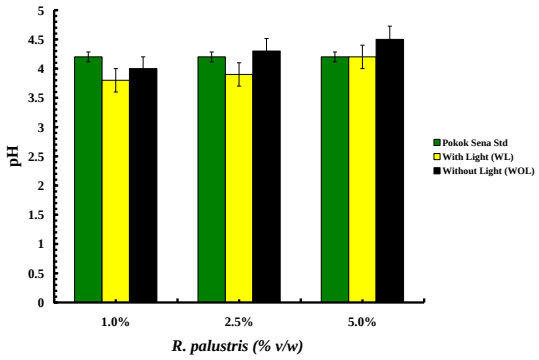
where r^2, is the square internal radius, and h is the height of the fluid level. By rearranging equations (2) and (3), with assumption 1 mole O_2 uptake equals 1 mol CO_2 emit, OUR is equivalent to :

$$\text{Moles of } O_2/\text{mass of soil (kg)} \times \text{Time (h)} \tag{4}$$

III. Result And Discussion

A. Effect of R. palustris on Soil pH

Fig. 2 shows the soil pH with application of *R. palustris* after 30 days. The value of pH for the control soil (with and without exposing to light) was no pH different after being left for 30 days even with the amount of 1.0-5.0 % (v/w) *R. palustris*. However, a significant reduction of acidity (from pH 3.8 to 4.2) was observed for the soil sample with 2.5 % (v/w) *R. palustris* after having been exposed to light. The best condition to increase the soil pH towards neutralization which is pH 4.5 with an addition of 5.0 % (v/w) *R. palustris* and without exposed to light.

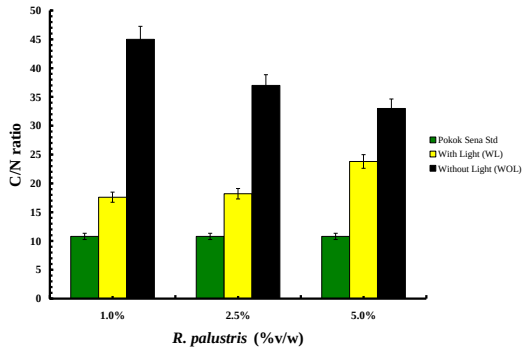


#Note : Results are the averages of three independent experiments ± SD. P<0.05 compared to (+) *Bungor* Std soil.
Fig. 2. Effect of *R. palustris* on soil pH.

The results in Fig. 2 show that without the presence of light, *R. palustris* is effective for converting H+ to hydrogen gas as a result of H^+ from *Pokok Sena* soil. As the H+ is reduced, the pH value is increased, resulting in the reduction of acidity [12] [13].

B. Effect of R. palustris on Soil C/N Ratio

The C/N ratio values of fermented soil are shown in Fig. 3. The value of C/N ratio for the control soil (with and without exposing to light) was no different after being left for 30 days even with the increasing amount of 1.0-5.0% (v/w) *R. palustris.* The value of C/N ratio with 2.5% (v/w) *R. palustris* added to the soil, had slightly increased from 18:1 to 25:1 after the exposure to light (WL). The highest amount of C/N ratio can be reached up to 45:1 by applying 1.0% (v/w) *R. palustris* into the soil without light exposure (WOL). By increasing the amount of *R. palustris* from 1.0-5.0% (v/w) without light exposure, the numbers of C/N ratio are gradually decreased.

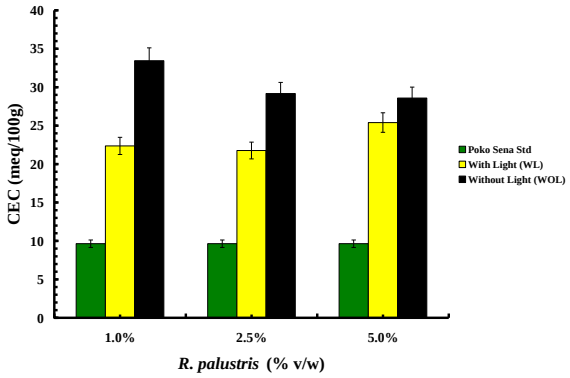


#Note : Results are the average of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.
Fig. 3. Effect of *R. palustris* on soil C/N ratio.

Analysis of the results from the C/N ratio has led to a conclusion that *R. palustris* could adjust the soil acquisition ratios according to the composition of resources consistently without the presence of light. This soil acquisition ratio is represented as the general soil nutrient cycling of Carbon (C), Nitrogen (N), and Phosphorus (P) in the soil. With the amount of C/N ratio 10.5:1 existing in *Pokok sena* soil, the *R. palustris* is capable of using the carbon and nitrogen as a food sources to survive [14] [15].

C. Effect of R. palustis on Soil Cation Exchange Capacity (C.E.C)

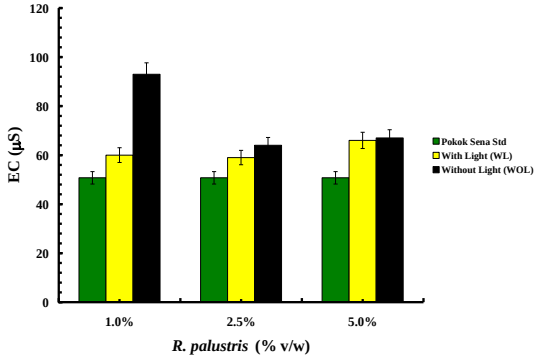
The C.E.C of *Pokok Sena* soil increased tremendously from 10.14 meq/100g to 22.50 meq/100g with the exposure to light (WL) and increased from 10.14 meq/100g to 33.50 meq/100g for soils without exposure to light (WOL) after the addition of 1% (v/w) *R. palustris* (Fig. 4). The C.E.C rise was related with the reaction of *R. palustris* with a variety of charges mineral such as Ca^+, Mg^{2+}, K^+ and Na^+. However, there were no significant or different increments of C.E.C values in this experiment, even though the amount of *R. palustris* was increased from 2.5% to 5% (v/w), and placed in environments with or without the presence of light [16].



#Note : Results are the average of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.
Fig. 4. Effect of *R. palustris* on soil C.E.C.

D. Effect of R. palustris on Soil Electrical Conductivity (EC)

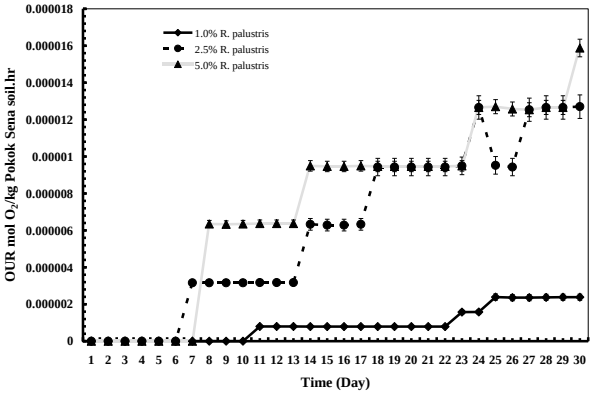
Fig. 5 shows that the applications of 1.0, 2.5 and 5.0% of *R. palustris* gave significantly different results of EC values as compared to the EC values of the standard soils for experiments conducted without the presence of light. The application of *R. palustris*, even at low percentage, was able to ameliorate the *Pokok Sena* soil electrical conductivity under the condition where there is an absence of light. The highest conductivity, 90.50 µS was achieved on day 30 after the application of 1.0 % (v/w) of *R. palustris*. These conductivity results show that increasing amounts of organic acid were secreted by *R. palustris* [17].



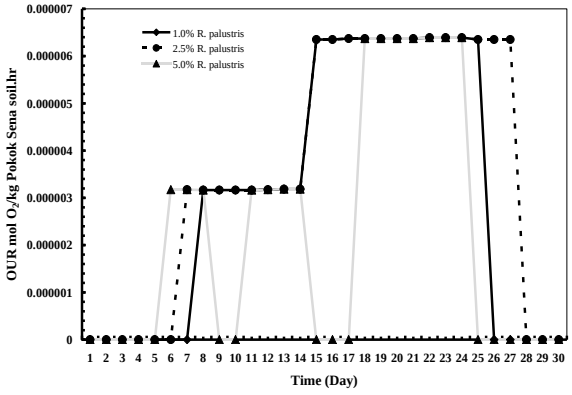
#Note : Results are the average of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.Fig. 5. Effect of *R. palustris* on soil electrical conductivity (EC).

E. Effect of R. palutris on Soil Oxygen Uptake Rate (OUR)

The results of the respiration of the bacterium in *Pokok Sena* soils in relation to time and bacteria treatment are presented cumulatively over a 30 day period as shown in Fig. 6. Fig. 6 (a) shows that by increasing the amount of *R. palustris* from 1.0 to 5.0% (v/w), with the presence of light, the OUR was approximately 300% higher after the 30 day duration. Fig. 6 (b) shows that without the absence of light, the highest OUR was achieved when 5% (v/w) *R. palustris* was used, and the respiration activity appeared to have been completed 3 times compared to 1.0 and 2.0% (v/w) within 25 days. From this result, it can be concluded that the best condition used for *R. palustris* to have a complete respiration process on the field is by using an amount of 5% (v/w) with the absence of light condition.



(a)



(b)

#Note : Results are the averages of three independent experiments ± SD. P<0.01 compared to (+) *Pokok Sena* Std soil.

Fig 6. Effect of *R. palustris* on soil oxygen uptake rate (OUR). (a) With exposure to light, WL (b) Without exposure to light, WOL.

The OUR results show that *R. palustris* without the presence of light, was able to provide better and higher respiration activities as compared to the experiment that had the presence of light. This respiration activity was parallel and in accordance with the results of C/N ratio in the soil (see Fig. 3). The bacterium grew until carbon and/or nutrients limited further growth and reached a maximum peak as reported elsewhere [18].

IV. Conclusion

All the experimental results have demonstrated that *R. palustris* has the capability to alter the chemical fertility of *Pokok Sena* soil. The effect on pH of the soil condition class was an upgrade from 3.8 (extremely acidic) to 4.5 (very strongly acidic). The C.E.C value of the soils was elevated from a low (6-12 meq/100g) to high range (25-40 meq/100g). The higher values of C/N ratio, EC and OUR obtained signified that the nutrient contents have been improved as well as nutrient uptake capability of the soils. The effect of the light exposure studied in this work may indicate as to whether the situation of day or night will affect the function of *R. palustris* which would alter the specific soil parameters. The research has shown that the soil treatment using this bacterium has a great potential as it could be used to utilize and improve fertility of Ultisols.

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