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Comparative Assessment of the Effect of RENA and Mycoremediation Techniques in Crude Oil Impacted Soil on Growth Performance of *Telfairiaoccidentalis* Hook F.

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Abstract: A field assessment was conducted using the growth performance of *Telfairiaoccidentalis*Hook F. to comparatively evaluate the bioremedial abilities of RENA and Mycoremediation on crude oil impacted soil. Triplicate plots were spiked with 7.5 litres of Bonny light and treated with RENA and Mycoremediation techniques. At the end of the five months remedial treatment period, *Telfairiaoccidentalis*Hook F. was planted on the treated plots and its growth response was investigated within an 8 week study period. Crop growth parameters investigated include rate of germination, number of leaves, plant height, leaf surface area and yield biomass weight. The growth performance results obtained showed significant difference $p<0.05$ between the plots treated with RENA, Mycoremediation, combination of RENA/Mycoremediation and the untreated crude oil impacted control plots. *Telfairiaoccidentalis* Hook F. planted in plots treated with a combination of RENA/Mycoremediation showed the highest growth performance and biomass yield in all the growth parameters studied while the crop planted in the untreated control plots showed the least growth performance in all the parameters investigated. Hence the study has shown that the use of combination of RENA/Mycoremediation technique will achieve site restoration of crude oil impacted soil and is beneficial as it creates a more crop friendly environment for improved agricultural yield.
Keywords: Crude Oil,Mycoremediation, *Pleurotusostreatus,* RENA, and *Telfairiaoccidentalis* Hook F.

I. INTRODUCTION

Since the exploration and processing of crude oil in Niger Delta area of Nigeria, the environment has been inundated with oil spill emanating from unplanned activities of multinational companies and vandals involved in crude oil bunkering activities [1]. Crude oil is a complex mixture of mainly hydrocarbon constituents. Petroleum hydrocarbon derivatives have deleterious consequences when oil is spilled in an environment. The Niger Delta soil has suffered both physiological and biological changes as a result of numerous oilspill incidences recorded within the area over the decades of oil exploration and production activities. Crude oil impacts the soil adversely, making the soil unfavorable to support life for microorganisms, plants and invariably animals that depend on plants for sustenance [2]. Crude oil impacted soil can remain unusable for agricultural activities because of its general effect on plant growth and development, some of these adverse effect is evident in seed germination, leaf chlorosis, delayed flowering/fruiting,bleaching, stunted growth, necrosis, crop biomass reduction, impaired fecundity or outright demise of crop [3].The re-vegetation or plant growth is seen as an evidence or indicator of success of soil remedial measures [4].*Telfairiaoccidentalis*Hook F. is a tropical vine crop that is grown for its leaves and edible seed [5]. It is also a commercial crop grown in both rural and urban areas of Nigeria and across West Africa [6].[7]reportedsome medicinal benefits of *Telfairiaoccidentalis*Hook F. which locals derive include its use in the treatment of anemia, liver problems, high blood pressure and convulsion.

There has been both a moral and legal burden on the operators of oil facilities causing the oil spillages to adopt measures to return the crude oil impacted environment to the status that would support growth of plant communities prior to contamination by toxic crude oil derivatives [8]. Over the years several methods have been tried for the rehabilitation of crude oil impacted soil. Bioremediation which is the reliance on living organism for the purpose of degradation of pollutants from impacted soil is one of such methods that have been used. Land farming or Remediation by Enhanced Natural Attenuation (RENA) is a form of bioremediation that is being used in the recovery of impacted Niger Delta soil. Overtime this technology has come under increased criticism by international agencies and local communities over its failure in the rehabilitation of crude oil impacted soil. This led to heightened tension between the oil operators and the communities thereby putting at risk the License To Operate of most oil companies.On the other hand Mycoremediation which is the use of fungi or mushroom has received world acknowledgment in its use in achieving remedial action for most recalcitrant chemicals or pollutants. Several researchers have attested to this bioremedial capabilities of mushroom[9-14]. Hence the aim of this present study is to use as indicator the growth and development response of

*Telferiaoccidentalis*Hook F. to comparatively evaluate the bioremedial abilities of these two techniques RENA and Mycoremdiaion using an indigenous mushroom *Pleurotusostreatus* in the rehabilitation of crude oil impacted soil.

II. MATERIALS AND METHOD

2.1 Study area description: The field study was set up within University of Port Harcourt, Rivers State of Nigeria. The study area soil type is clayey loamy. The area experiences a bimodal distribution pattern during the rainy season with peaks in July and September [15].

2.2Source of Research materials: Shell Petroleum Development Company of Nigeria provided the Bonny light crude. Spawn of *Pleurotusostreatus*was gotten from Federal Institute for Industrial Research, Oshodi (FIIRO), Lagos.Songhia Farm inTai Local Government Area, Rivers State provided the seed of *Telfairiaoccidentalis* Hook F.

2.3Research Design: The study was carried out in a Randomised Complete Block Design. Triplicate plots of one meter by one meter dimension was provided for each of the treatment options which included RENA, Mycoremediation, combination of RENA/Mycoremediation and Control. To achieve a desirable impact on the soil, 7.5 litres of Bonny light crude was used to spike each of the plots. The impacted plots were made to undergo remediation treatment. The plots for RENA treatment were continuously tilled with shovel and ridges were constructed to provide enhanced aeration for the impacted soil. The mycoremediation plots were inoculated with 400g of *Pleurotusostreatus* and allowed to stand for a five month period. Plots for combination of RENA/Mycoremediation treatment technique were inoculated with 400g of *Pleurotusostreatus* and intermittent tilling and ridges were also constructed. The control plots were spiked with crude oil and left without any form of treatment.

At the end of the five months remedial treatment period, five viable seeds of *Telfairiaoccidentalis* Hook F. were planted 75 x 90 cm spacing in each of the plot and growth parameters were investigated during an eight week study period. After two weeks the germinated crops were thinned down to one per plot till the end of the study period. During the eight week study period some plant growth parameters were studied non-destructively while some growth parameters were harvested at the end of the study period and investigated. Cultural practices applied during thestudyperiod include manual weeding and watering of plots when necessary.

Crop growth parameters studied include:

2.3.1.1 Seed Emergence Percentage (SEP): Enumeration of germinated seeds against seeds planted was done and this was expressed in percentage (%).

2.3.1.2 Plant Height (cm): Meter rule was used to measure in (cm) from the base of the stem to the tip of the terminal bud.

2.3.1.3 Number of leaves: The number of leaves was visually enumerated.

2.3.1.4 Leaf Area (cm^2):[16] method was applied in measuring the leaf surface area. The width and length of longest leaf was measured using 0.75 as correction factor.

2.3.1.5 Wet/Dry Shoot/Root biomass Weight: By the eight week the root and shoot of the crop was harvested and separated from one another. The yield biomass weight for wet shoot and root were obtained using a weighing balance. This exercise was repeated after air drying each crop component in a brown envelope. This gave the dry yield weight biomass.

2.4Statistical analysis: Analyses of variance (ANOVA) and least significant difference was used in statistical analyzing the data that was obtained.

III. RESULTS AND DISCUSSION

3.1Effect of bioremediation treatment techniques on *Telfairiaoccidentalis* Hook F.

3.1.1 Percentage emergence (%): After two Weeks of Planting (2 WAP) *Telfairiaoccidentalis*Hook F. had an emergence percentage for the different treatment technique as follows RENA (67 %), Mycoremediation (53 %), RENA/Mycoremediation (80 %) and Control (26 %). See details in (Figure 1). There was significance difference in the percentage emergence of all the treatment options. The combination treatment had the highest emergence percentage while the untreated control plots had the least percentage emergence. The reason for the result obtained in the combination RENA/Mycoremediation treatment may have been because of the enhanced productive soil environment which may have been triggered by the tilling and construction of windrows that enabled the microbes to degrade faster the toxic hydrocarbon components in the soil. This result is in line with the works of [17] that RENA or land farming of crude oil polluted soil lead to reduction of TPH concentration of the soil and an increase in the oxygen, moisture content, total organic matter in the soil for better crop productivity and yield. Also the activities of the *Pleurotusostreatus* may have contributed in the enhancement of soil environment for increased agricultural productivity as *Pleurotusostreatus* through researches[18-19]have shown its ability to degrade crude oil pollutants in the soil. On the otherhand the lowest percentage emergence recorded in this study in the untreated control plots could be as a result of the toxic effect of high petroleum hydrocarbon concentration in the soil which coated the seeds and adversely affecting the gaseous exchange and increased the soil physical water-repellant property. This study finding is consistent with the reports of [20-22]thatcrude oil by reducing the metabolic functions in the seed endangers the life of seed embryo thereby inhibiting germination of the seed.

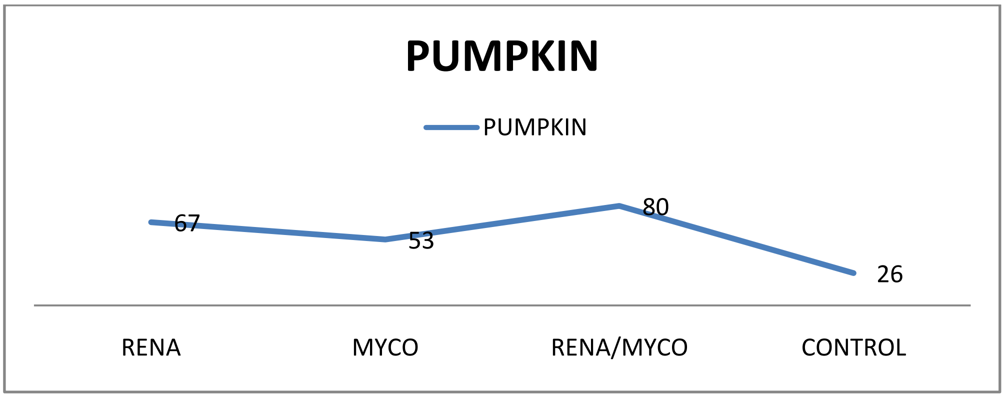

Figure 1: Trend of percentage emergence (%)

3.1.2 Number of leaves: The study investigatednumber of leaves of *Telfairiaoccidentalis* Hook F. in treated plots. RENA, Mycoremediation, combination of RENA/Mycoremediation and untreated control at 8th week recorded 43.33±1.53, 34.67±1.528, 60.67±2.52 and 29.67±1.16 respectively (See Figure 2). The plots treated with combination of RENA/Mycoremediation technique recorded the highest number of leaves 60.67±2.52 while the least was untreated polluted control with mean values recorded 29.67±1.16. The study showed significant differences in number of leaves from Weeks 2, 3, 4, 5, 6, 7 and 8 at p-values < 0.05. This study finding is in line with the works of [23]that low concentration of hydrocarbon plant growth is enhanced. As hydrocarbon concentration increases, it becomes toxic to plant growth leading to reduction in number of plant leaves [24]. Improved soil conditions in the combination RENA/mycoremediation plots may have induced greater number of leaves. This corroborates the findings of [25] on cow pea plant.

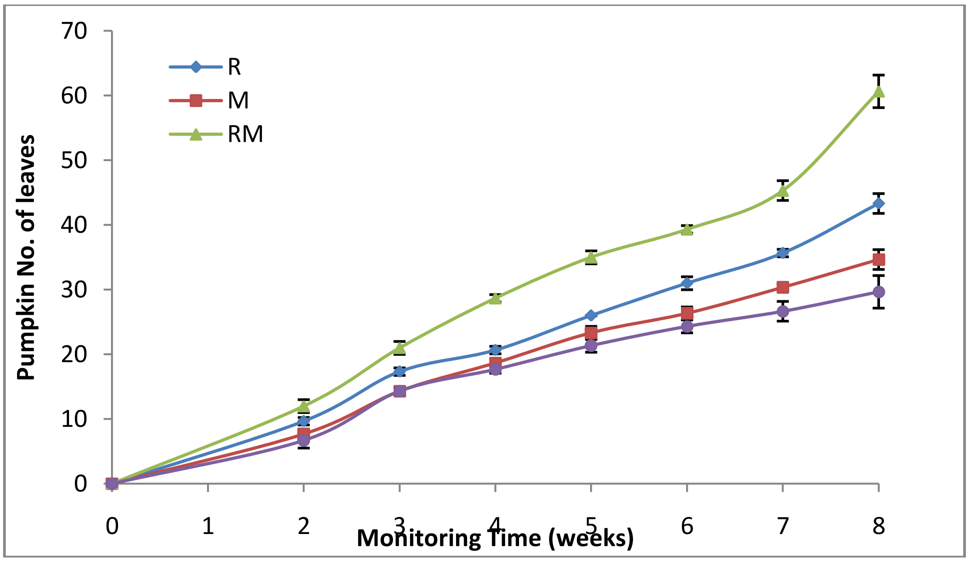

Figure 2: Changes in number of Telfairiaoccidentalis Hook F. leaves during the study

3.1.3 Plant Height: The study on parameter observedsignificant differences in the plants heights for all the treatment options from Weeks 1, 2, 3, 4, 5, 6, 7 and 8 at p-values < 0.05. The plants height recorded at the eight week were 144.47cm±8.54, 98.8cm±13.86, 180.53cm±15.497 and 70.5cm±1.1 representing RENA, Mycoremediation, Combination of RENA/Mycoremediation and Control respectively (Figure 3). The lowest height as evident in the untreated control plots is in line with the findings of [26-27] that unsuitable conditions in the soil because of the displacement of air pores by crude oil lead to poor aeration, decreased microbial metabolism [28], high levels of hydrocarbon leading to low water-plant interaction [29]. Also the high toxic components in the untreated control plots may have resulted in reduced nutrient availability in the soil as corroborated by [30].

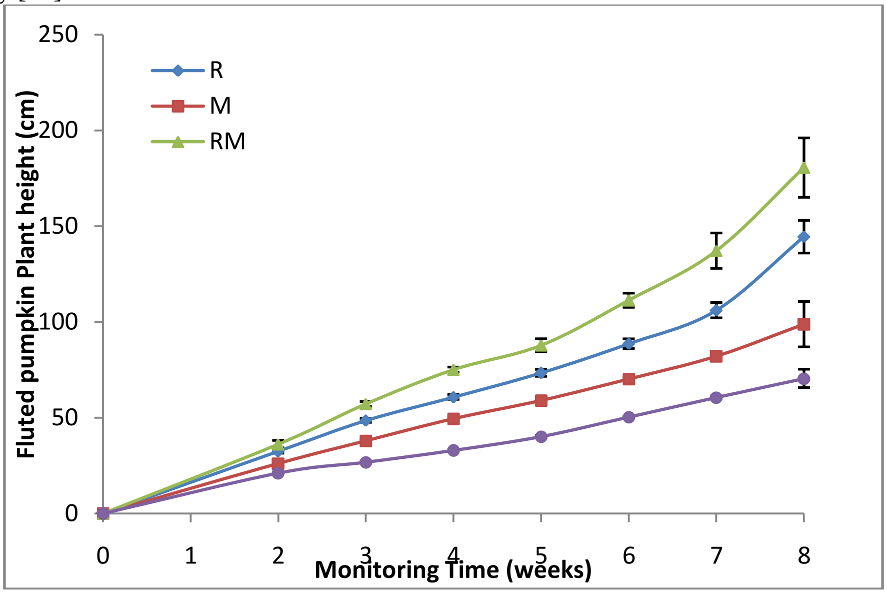

Figure 3: Changes in the height of Telfairiaoccidentalis Hook F. during the study period

3.1.4 Fresh/Dry Shoot Biomass: Fresh crop shoot biomass weight was investigated. Significance difference at ($p<0.05$) was observed from Weeks 2, 3, 4, 5, 6, 7 and 8. See Figure 4. Plots that were treated with combination technique of RENA/Mycoremediationrecorded the highest fresh shoot weight biomass measurement. Shoot biomass weights wereRENA 20.76g±1.4, Mycoremediation13.62g±1.3, RENA/Mycoremediation33.31g±2.1 and 10.5±0.9 untreated control. The ascending order of gain in shoot fresh biomass weight is as follows: Control<Mycoremediation<RENA<RENA/Mycoremediation. Dry shoot biomass weight was also measured. Results followed similar trend as in fresh biomass weight with the combination RENA/Mycoremediationtechnique gaining the most weight in dry biomass weight of 7.24g±0.76. The untreated control plots recorded the least gain in dry root biomass weight 1.24g±0.1. The study findings is in line with the works of [31]that high concentrations of petroleum hydrocarbon retarded the yield of pumpkin and Okra plants because of adverse alteration of the photosynthetic processes of crops. [32]reported that improved soil conditions will lead to increased number of leaves and higher photosynthetic surfaces consequently giving higher crop shoot biomass weight.

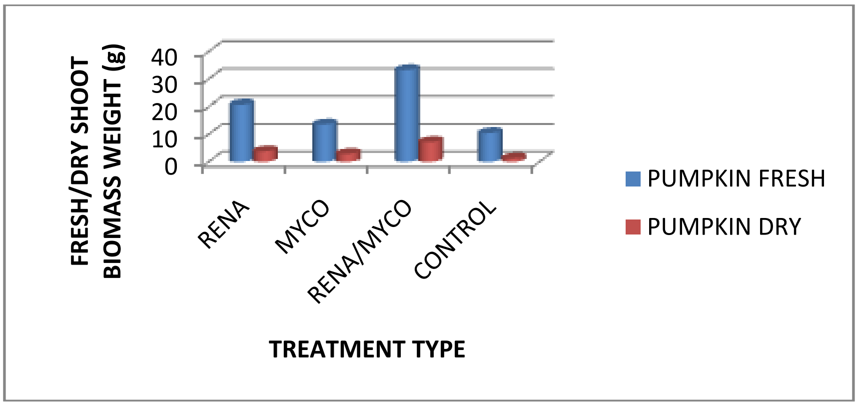

Figure 4: Changes in fresh and dry Shoot weight biomass during the study period

3.1.5Fresh/Dry Root Biomass: Root biomass weight was also investigated after eight (8) weeks of planting. The combination of RENA/Mycoremediation techniques recorded the highest measurement of 4.48g±0.5 while the untreated control plots had the lowest biomass weight measurement of 1.82g±0.04. See figure 5. Similar trend was observed as we observed significant difference $p<0.05$ in the roots biomass measurement after the roots were dried. The combination technique of RENA/Mycoremediationrecorded the most weight of 0.83g±0.16 while the least biomass weight was recorded in untreated control plots (0.24g±0.035). This outcome corroborates the finding of [33] that high concentration of crude oil in soil affects the absorption of vital nutrient and water which is needed for the metabolic processes in the plant thereby leading to reduced plant yield. [34]reported similar outcome as this was attributed to the adverse effect of immobilization and impermeability of soil nutrients due to the high concentrations of petroleum hydrocarbon componentsin the soil thereby retarding the yield of the *Vignaunguiculata* plant.

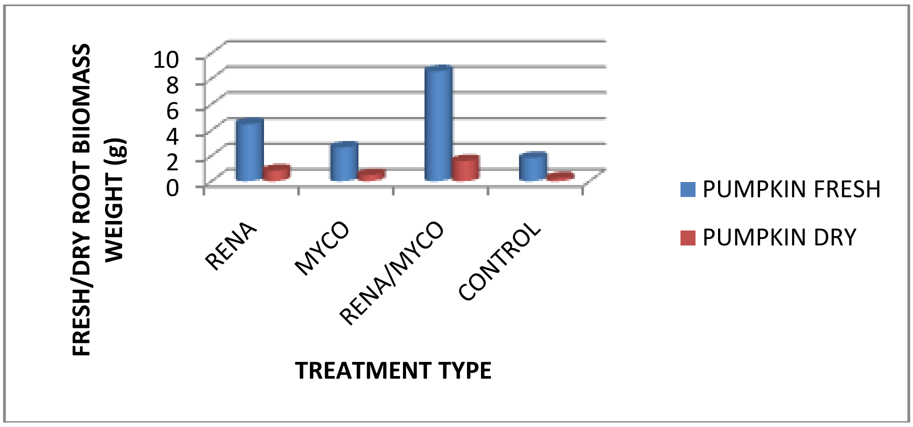

Figure 5: Changes in fresh and dry Root weight biomass during the study period

3.1.6 Leaf Surface Area: Leaf Surface Area was also investigated. The result showed significant difference at $p<0.05$ in the leaf surface area measurement from Weeks 2, 3, 4, 5, 6, 7 and 8. See details in Figures 6. At the eighth week the plots treated with combination of RENA/Mycoremediation technique recorded the largest leaf surface area of 153.26 cm^2±4.98 while the untreated control plots had the least measurement of 56.03 cm^2±1.26. RENA technique recorded leaf surface area measurement of 101.25cm^2±39 while Mycoremediationtechnique recorded 76.28cm^2±4.69. This result is consistent with the reports of [35-36] that high concentration of crude oil in the soil adversely affected the absorption of water and essential nutrient necessary for increased leaf surface area thereby leading to reduced leaf surface area.

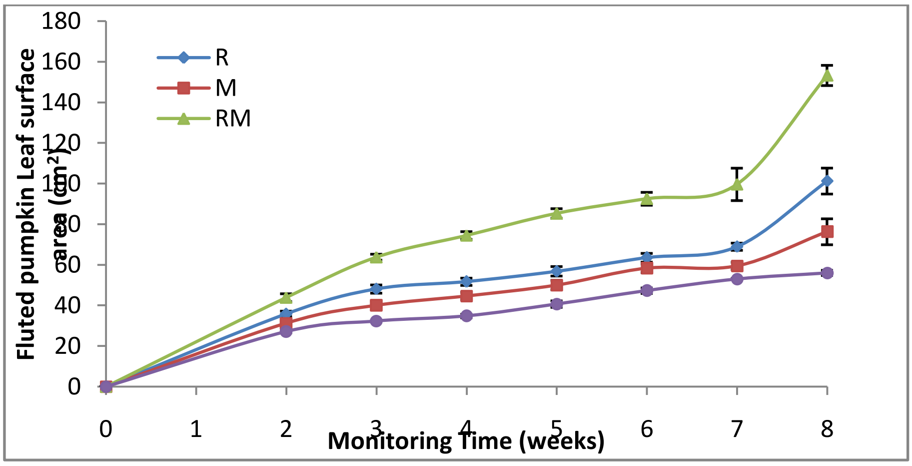

Figure 6: Changes in Telfairiaoccidentalis Hook F. leaf Surface area during the study period.

IV. CONCLUSION

The Study has demonstrated that crude oil impacted soil do not support the growth and development *Telfairiaoccidentalis* Hook F. It has also shown that agricultural yield in remediated soil is better achieved when *Pleurotusostreatus* is employed with RENA technique in the clean up of crude oil impacted soil.

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