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The Role of Ethylene Gas in Local Rice Cultivars (Oryzae *sativa*) in Bali Local Government Area, Bal. Bali Town as the Case Study.

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Abstract
Rice (Oryza sativa) is a staple food that provides the staple food requirements for more than 50% of the global population. As the human population increases fast, there a need to increase its production to satisfy their needs. However, rice production is associated with low nitrogen use efficiency as a result of leaching and denitrification. Ethylene plays important roles in root elongation, partial disease resistance, and inhibition of nitrification, grain filling, regulating root structure, increasing water use efficiency and triggering growth in rice. Three experiments were set up in the Research Farm of the Department of Agricultural Technology, Federal Polytechnic, Bali Taraba State Nigeria. The rice cultivars used were Yar Ai, Maikano, Chikwa and CP Jamila and all of them were grown under flooded, aerobic and alternate wetting and drying (AWD) soil management The objective of the study was to find out whether rice could produce ethylene gas and study it, to find out if the production of ethylene gas varied with local rice cultivars and to determine whether or not the produced ethylene could inhibit biological nitrification. On the first experiment of weather local varieties of rice produce ethylene gas or not, result showed that local rice varieties could produce ethylene gas ($P < 0.01$). On the second experiment of weather the production of ethylene gas varied with the local rice cultivar or not, the result showed the production of ethylene gas varied with local rice cultivars ($P< 0.05$).On the third experiment to determine if the produced ethylene gas could inhibit biological nitrification, the result showed that the concentration of ethylene gas could no inhibit the oxidation of ammonium when the obtained value was compared with the ammonium oxidation limit.

***Key Words**: Rice, ethylene gas, ammonium, oxidation, global warming*

INTRODUCTION

Rice is an essential food being consumed by more than half of the world populace. Its production exceeds 423. 6 million tons (Faggeria, 2018). It also provides B-vitamin (Manful *et al*., 2010). Globally, human population gets its more than 20% of calorie and more than15% of protein from rice (Redman, *et al*., 2012). It is also estimated that rice provides 40% of energy out of the 100% of the total energy provided by all the food crops (De Datta, 2016). Rice farming is a source of employment to many people that would have been redundant in the rural areas (De Datta, S.K, 2016). The cultivation and consumption of rice is always in increasing rate and it is regarded as one of the most consumed cereals (Foo and Hameed, 2015). Rice husk is now used to generate energy more especially to those countries that import oil to provide energy (Wang *et al*., 2015). Ganvir (2011), reported that, health problems may result when the fluoride concentration in drinking water exceeds 1.5 mg/L. This may include failure of the human body to metabolize calcium and phosphorus. To remove this, aluminium hydroxide is coated with rice husk ash (Gavril *et al*., 2011). Industrial activities are origin of heavy metal that contaminate soil and they can be injected into human body through food consumption (Pain et al., 2013). For the purpose of reducing the cost of their remediation, rice straw can be used to remove them. The lignin content of rice straw will react with the heavy metals to form chelate and detoxify them (Richard *et al*., 2014).

Rice brand contains high amount of oil that can be related to palm kernel and cotton seed oil (Levinch, 2017). But the growing of rice since it is hydrophyte crop, it requires flooded areas and it is known as another way of increasing the concentration of greenhouse gasses in the atmosphere. Rice is mostly grown in flooded lands or semi flooded lands, only some varieties of rice are cultivated in a well-drained land. Upon all the advantages of rice farming, it leads to the generation of greenhouse gases, such as nitrogen oxides and their derivatives, methane and carbon dioxide (de Miranda *et al*., 2015) Ethylene also known as ethane is a simple colourless and combustible gas made up of two carbons linked together by dual bonds. The gas has a molecular formula as C_2H_4.According to Bleeker (2014), it is plant hormone that plays major roles in plant life cycle stating from germination to the final ripening. The biological role of this gas was known before its discovery; it is known to control the growth and development of plants and that is why it is called a plant hormone. It is considered to play an important role in seed germination, fruit ripening, response to pathogenic infection and environmental stress and petal and leave abscission. But upon all these roles, nothing was known about ethylene until in the late nineteen century, a young researcher Dimitri Nikolayevich was able to identify the gas. Later in twentieth century, a gas chromatograph was used to detect the gas and classify it as a plant hormone. (Gowda *et, al*, 2015).

Nitrogen use efficiency (NUE) in rice

Nitrogen is considered to be one of the growth limiting factors for many years, an agricultural researchers have been paying their attention on how to supply enough nitrogen to crops in order to get best yield .The efficacy of this can only be achieved by maximizing the amount of nitrogen retuning to the soil and minimising its amount leaching out of the plant root zone(De Willigen, P., 1991) Rice is one of the crops that can consume high amount of nitrogen, but its utilization efficiency is small, which is not more than 35% (Dobermann *et al*., 2002).According to Subba Rao (1999), apart from denitrification, leaching is another way by which nitrogen is lost ranging to loss of 20 to 50% of the applied nitrogen. It is considered as the most prominent way by which nitrogen is lost in the rice farm in which more than 50% of the applied nitrogenous fertilizer is lost by leaching. It is estimated that more than 80 million tons of nitrogen fertilizer is applied to the soil world-wide (Frink *et al*., 1999), but plant can efficiently utilize only 30% of it, the larger percent is either leached down the soil which contaminates the ground water or denitrified and into the atmosphere to cause global warming (Glass, 2003).The loss of nitrogen through leaching and denitrification when fertilizer is applied to the soil can be mitigated if ammonia oxidation is inhibited. This will make rice to have efficient fertilizer utilization (Hauck, 1983). Rice is a plant that can tolerate high concentration of ammonia (Wang *et al*., 1993a, b). Research has shown that the ability of the rice to withstand the high degree of ammonia concentration is related to the enzyme called glutamine synthetase it has and lack of more build-up of free ammonia in its tissues (Magahals and Hubber, 1991; Balkos *et al*., 2010).

Impact of ethylene gas on soil microbes and impact of soil microbe on ethylene in the rice rhizosphere.

Rhizosphere is the portion of the soil within the influence of root. This area has numerous microorganisms due to root exudates. But the presence of these microorganisms depends on the physical and chemical properties of the soil (Peiffer, *et al*., 2013). The amount of organic matter and percentage of soil fungi and bacteria are higher in the rhizosphere, but still the concentration of ethylene is lower than non-rhizosphere soil due to the decomposition and consumption of ethylene by microbes, transfer of ethylene to the leaves and adsorption process (Otani, T and Ade, N., 1993). Microbes play a role in determining the concentration of ethylene in the rhizosphere by the decomposition of organic matter to produce ethylene and then consume it (Abeles, 1992). Microbes are the key basis of ethylene in the soil both in aerobic and flooded condition, but it is oxidized in aerobic condition (Zeccmeister-Boltenstern & Smith, 1998). The microbial activities are said to be affected when there is a small change in ethylene concentration (Abeles *et al*., 1992). Increase in organic matter increases the ethylene concentration. (Arshad &Franken Berger, 1990). This organic matter is decomposed by microbes.

Biological nitrification inhibition (BNI) and its importance

The ability of plant root to release substances that can inhibit the conversion of ammonia to nitrate is what is called biological nitrification inhibition (BNI) (Subbarao *et at.*, 2013). A biological nitrification is an aerobic process that developed many years ago (Berner, 2006). The process is the function of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archea (AOA). The key examples of ammonia-oxidizing bacteria are *Nitrobacter spp* and *Nitrosomonas spp* (Lininger et al., 2006; Taylor *et al*., 2010). These oxidizers are mostly accountable for ammonia oxidation large agricultural soil, however, bacterial spp like *Nitrosospira* and *Nitrosocystus* and fungus *Aspergillus flavus* also have a significant impact on ammonia oxidation more especially in forested ecosystem (Summer et al., 1976). The nitrification has an agricultural and environmental implication. Agriculturally, it encourages leaching, causes low nitrogen use efficiency and increases the cost of production. Environmentally, the leached nitrate contaminates the ground water and nitrous and nitric oxide are greenhouse gases that cause global warming (IPCC, 1996).

Biological nitrification inhibition will maximize nitrogen use efficiency (NUE) through improvement of nitrogen that will be lost by leaching and denitrification (Subbarao *et al*., 2012a).Ethylene produce by rice inhibits nitrification (Hommes *et al.*, 1998).According to Tanaka (2010) conducted an experiment to investigate the biological nitrification inhibition using two cultivars of rice which were upland rice cultivar (IAC25) and lowland rice cultivar (IR64or Nippon bare).The result showed that the upland rice cultivar had higher biological nitrogen inhibition in the rhizosphere than the lowland cultivar (IR64 or Nippon bare).The higher inhibition was as a result of higher release of the exudates. This was endorsed by noticing higher concentration of nitrate in the lowland cultivar IR64 than the upland cultivar IAC25. A part from ethylene inhibition of ammonium oxidation, compounds like dicyandiamide (DCD) and (3, 4-Dimethylpyrazole phosphate (DMPP) inhibit it by deactivating ammonia monooxygenase (AMO) which is an enzyme of ammonium oxidation bacteria. For instance, *Nitrosospira and nitrobacteria* (Di and Cameron, 2011).

Statement of the Problem

The population of the globe is seriously increasing and so food production has to be increased, too. But in this food production, rice cannot be left at the back but included. The cultivation of rice includes the huge use of nitrogen fertilizer which can produce greenhouse gases like nitrogen oxide and dioxide. Research has shown that certain crops can produce ethylene gas that can inhibit the oxidation of ammonia and rice is among them. If ammonia oxidation is inhibited, the ammonia will remain in the soil in its positive form and rice will make use of nitrogen efficiently without being lost to the atmosphere and caused global warming.

METHODOLOGY

The seeds of rice cultivars (*Oryza sativa*), Yat Ai, Maikano, Chikwa and CP Jamila and were bred by the Depatment of Agricultural Technology, Federal Polytechnic, Bali, Taraba State, Nigeria. A pot experiment was set up in the Department of Agricultural Technology, Federal Polytechnic Bali Research Farm. Four (4) different cultivars of rice (Yar Ai,Maikano, Chikwa and CP Jamila) were planted in white and black pots and were irrigated every day until they were ready for transplanting.

Transplanting

After the filling of all the pots with the soil, twelve seedlings of the planted rice were gently and randomly uprooted and transplanted in to the prepared pots. This transplanting was carried out in the evening to avoid any stress to the plants due to transpiration. After the plants were fully recovered from the transplanting stress, the prepared metal tubes were inserted in to the pot and very close to the rice root. If the tubes were to be far away from the root, this would have a negative effect on the accumulated concentration of the ethylene gas. The closer the tube is to the root, the more the accumulation of the ethylene gas that may be predicted (Yamauchi et al., 1995). The distance between the soil in the pot and the head of the tube was 2 cm. This had given space for regular watering and the application of nutrient solution.

Figure 1: Rice cultivars under flooded Soil Management

Figure 2: Rice cultivars under Alternate Wetting and Drying

Method of measuring the concentration of ethylene gas

According to Bulens (2011), ethylene can be measured by a gas chromatograph after the samples have been collected directly from the head space inserted in to the samples. But the concentration of the ethylene is quantified by a gas chromatograph when a straight lined relation between the peak and the computed concentration is known. The calibration graph should be at least three to cover the whole range of the detector range. For the accuracy of measurement, the calibration curve has to be repeated before separate measurement is done.

Method of sampling and analysis of ethylene gas

According to Yamauchi (1995), the ethylene sample was collected by placing the rice in a test tube which was plugged with a head stopper. Then 1 ml of ethylene gas was withdrawn and injected into a gas chromatograph for the determination of its concentration. The chromatograph will only show the area, but the calibration graph was used to determine the concentration. In this experiment, 5- ml samples were withdrawn by syringe and needle from the prepared metal probes or tubes that was inserted very close to the rice root of each pot and injected into the evacuated collection bottle. After the samples were collected, then, they were taken to the laboratory for analysis and the machine used for the analysis was gas chromatograph. After the analysis, the data collected were subjected to one way analysis of variance (ANOVA) together with a turkey test using a statistical package for social science (SPSS) as the soft wire used.

Figure 3.A metal tube for the accumulation and collection of ethylene gas

RESULTS AND DISCUSSIONS

Three experiments were conducted with four local varieties of rice (Chikwa, Maikan,CP Jamila and Yar Ai) in Federal Polytechnic Bali School of Agricultural Technology Department of Agricultural Technology. The first experiment is to investigate whether rice produces ethylene gas. The second research is to find out if the production of ethylene gas by the rice plant differs with rice variety. The third experiment is to see if the produced ethylene gas will inhibit the oxidation of ammonium. The rice varieties were cultivated under three management condition, which were flooded, aerobic and alternate flooding and drying (AWD). The results obtained were displayed and discussed below. On the experiment to investigate on the possibility of rice to produce ethylene gas, the original hypothesis was rice could not produce ethylene gas. But this result showed that there was a statistically production of ethylene gas by rice ($P < 0.01$).

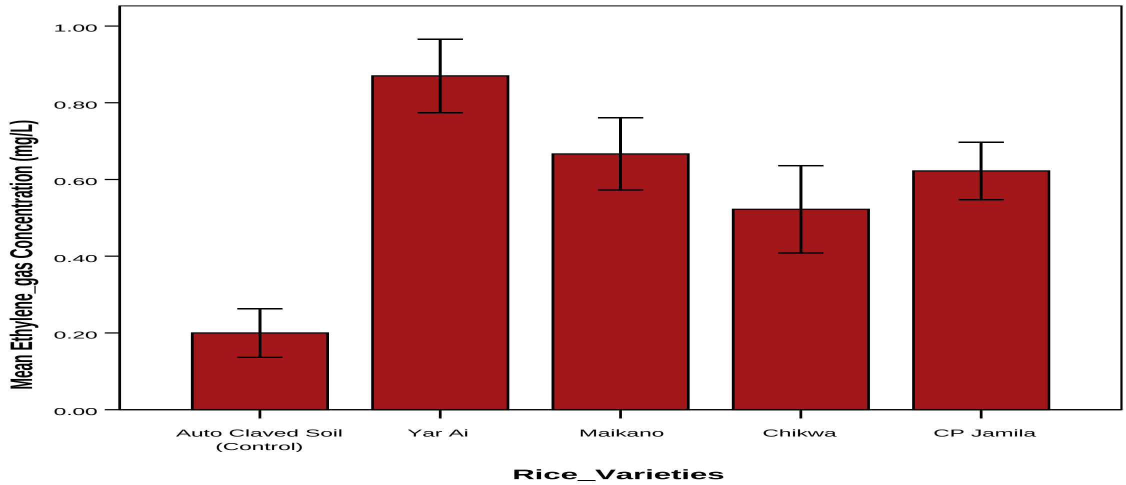

Figure 4: Mean of ethylene gas concentration

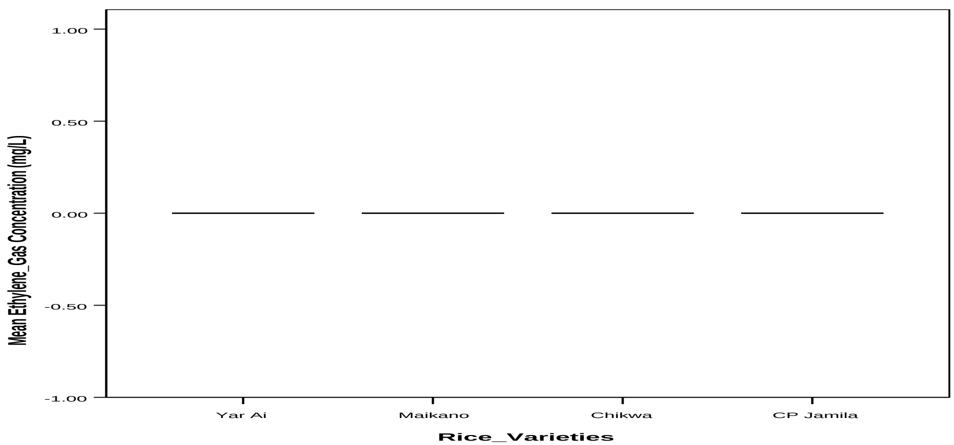

Figure 5: Mean concentration of Ethylene gas

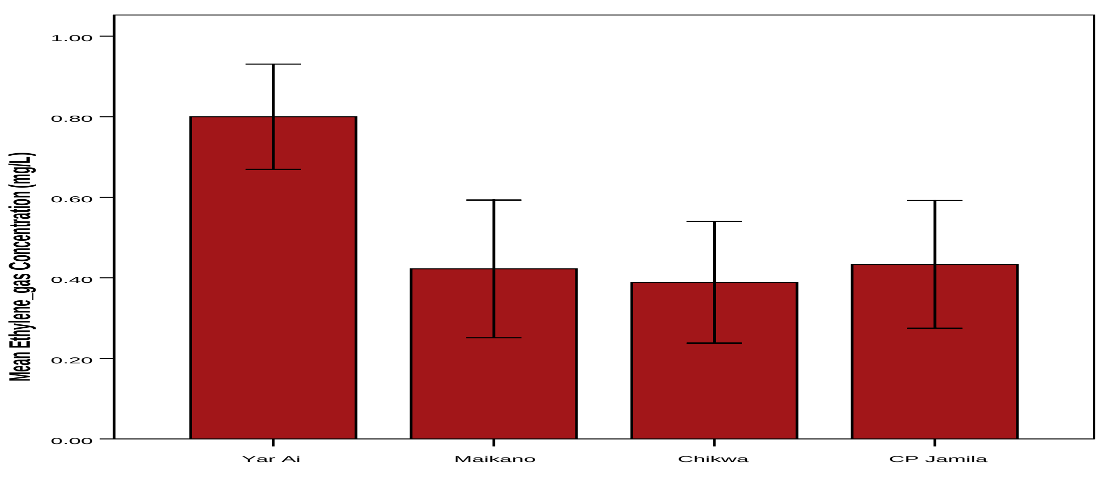

Figure 6: Mean Concentration of Ethylene gas (mg/L)

The results of figure 4, 5 and 6 showed the total mean of ethylene concentration produced by the local rice varieties under flooded, aerobic and alternate wetting and drying (AWD) soil management. This result indicated that there was a statistically significant production of ethylene gas under flooded soil management. This might be as a result of lack of ethylene oxidizing bacteria to oxidize the accumulated ethylene gas as shown in figure 4. The result is in the same opinion with the result of Levinsh et al., (1992), in which research was conducted to find out whether cereals could naturally produce ethylene (without being artificially induced to produce). At the end of the experiment, the pots with barley, wheat and rye seedlings produced ethylene. Even though the study was not on rice, the but result is the same with the result of rice in this study. Another study that supports this experiment is the result of Pana et al., (2019), who conducted experiment to investigate what causes poor spikelet filling. At the end of the experiment, ethylene was estimated in rice. This estimation supports the view that rice can produce ethylene. Another research which is in accordance with this study is the research of Lutts et al., (2018), they were able to investigate how salt stress affects ethylene production in rice leaves using 5 cultivars. They concluded that salt stress could increase ethylene production in salt tolerant cultivars and that the rice parts could produce ethylene. In figure 5, in which the local rice varieties were cultivated in aerobic condition, there was no statistically significant accumulation of ethylene gas in their rhizosphere. This might be as a result of ethylene gas oxidation by the ethylene oxidizing bacteria as was confirmed by Frederick at, al (2016). In figure 6, in which there was a significant accumulation of ethylene gas which was lower than the flooded and higher than the aerobic soil management, might be as a result of lack of ethylene oxidizers sometimes and the presence of ethylene gas oxidizer sometime as shown in figure 6

On the experiment to investigate on if the production of ethylene gas by the local varieties of rice varies with the rice cultivar, the original hypothesis was the production of ethylene gas does not vary with rice cultivars. But this result showed that there was a statistically significant different production of ethylene gas by the local varieties of rice. ($P < 0.05$) as shown in figure 4 and 6 below.

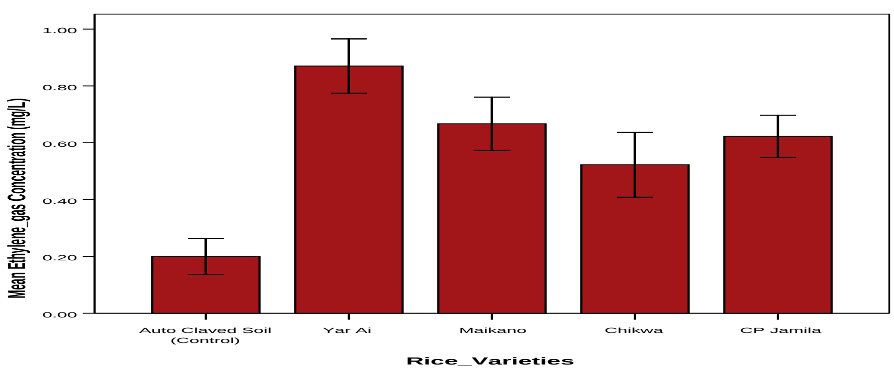

Figure 4: Mean Concentration of Ethylene gas

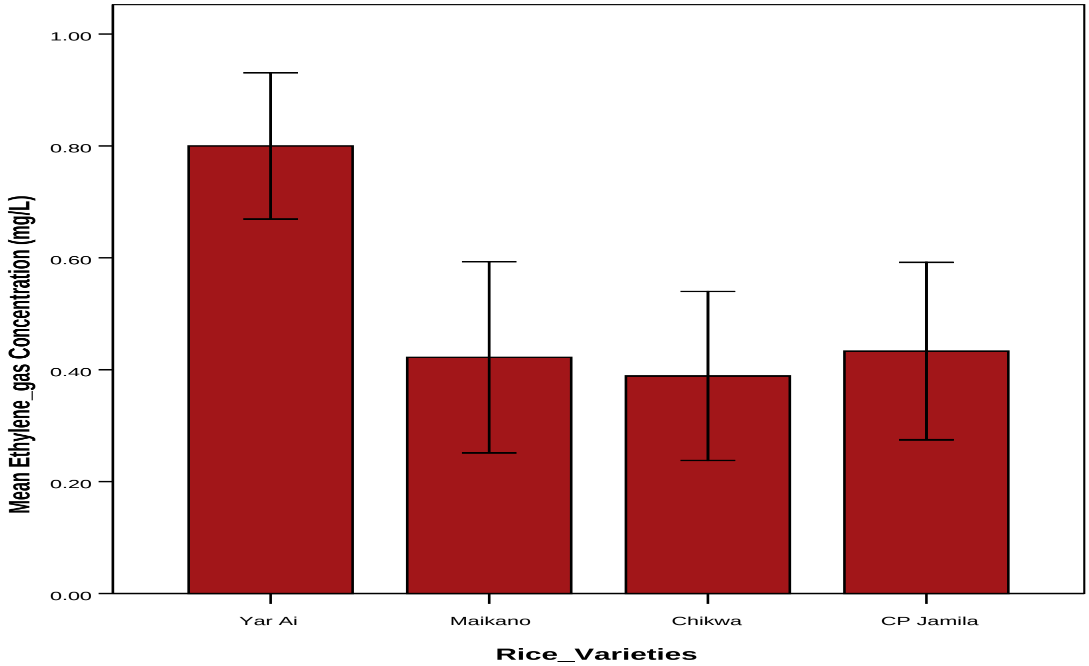

Figure 6: Mean Concentration of Ethylene gas

This difference in the production of ethylene gas might be related to different chemical reactions that might be taking place in different rice varieties. . The study of Imakawa et al., (2002) showed that the result of (Imakawa et al., 2002), where they conducted an experiment on 16 rice cultivars and 10 soya beans cultivars to find out if there is variation in the rate of ethylene production and growth between them. The result of their experiment reported that there was variation in the rate of ethylene production between them, but the difference existing in rice cultivars in relation to ethylene production was much lower than the soybeans. Larsen & Vangdal (2013), also conducted a research on 9 plum cultivars to investigate whether there is variation in the rate they produce ethylene and respire in relation to parking. At the end, they concluded that there was a difference in the rate of ethylene production among the 9 plum cultivars. The result of the two researchers is on the same vein with this research.as shown above.

On the experiment to find out if the concentration of the ethylene gas produced by the local rice varieties could inhibit the oxidation of ammonium, the original hypothesis was the produced ethylene gas could not inhibit the oxidation of ammonium. The result of this finding testified that the obtained concentration of ethylene gas could not inhibit the oxidation of ammonium as shown in figure 9 below.

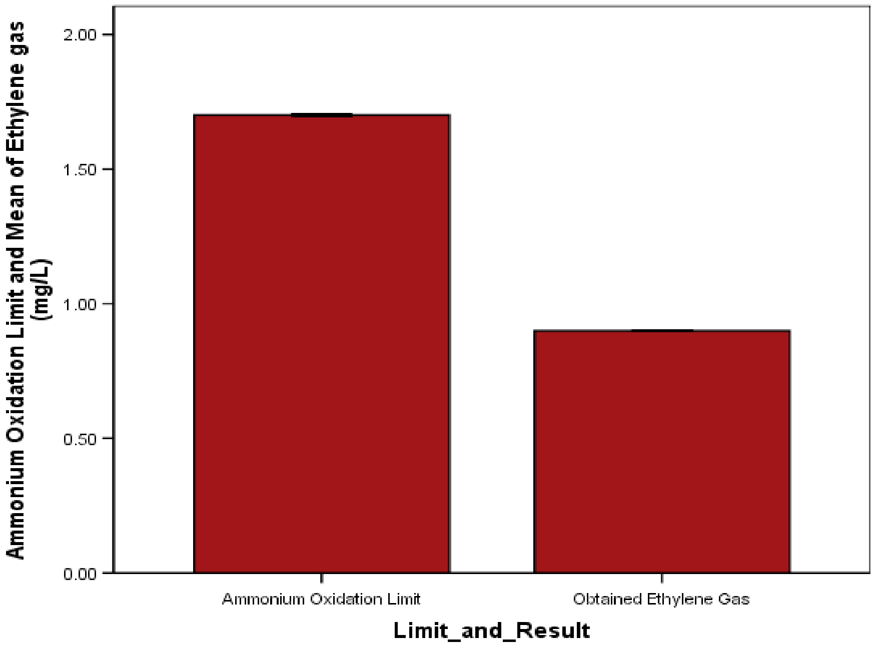

Figure 7: Ammonium Oxidation Limit and Mean Concentration of Ethylene gas obtained

The overall objective of this study was to investigate the possibility of rice plants to have an impact on nitrification via the inhibitory effect of ethylene on ammonia oxidase. The original hypothesis was the ethylene gas that would be obtained would inhibit the oxidation of ammonia. But the local rice varieties grown under different soil managements could not inhibit biological nitrification as shown in figure 7 above. According to McCarty & Bremner (2019), for ethylene to inhibit biological nitrification, the required ethylene gas concentration should be 100 ppm. The concentration of ethylene produced was far below the ammonium oxidation limit as shown in figure 7. But Porter (2013) conducted an experiment to see the effect of ethylene gas on nitrification. A clay loamy soil was treated with ammonium sulphates under different ethylene partial pressure and later analysed the soil to see the concentration of ammonia (NH_4), nitrate (NO_3) and nitrite (NO_2). It was found that at the ethylene concentration of 0.17 ppm, the nitrification was the same as untreated soil, at the ethylene concentration of 1.7 ppm, there was a partial inhibition of nitrification and at the ethylene concentration of 45.5 ppm, there was a complete inhibition of ammonia oxidation. This reveals that the amount of ethylene gas to inhibit the complete nitrification that would increase the nitrogen use efficiency and reduce the accumulation of greenhouse gases should be 100 mg/L.

Conclusion

The role of ethylene in rice cannot be over emphasised, it plays a function in all stages of rice growth and development. In this study, four local varieties of rice (Yar Ai, Maikano, Chikwa and CP Jamila) were able to produce a statistically significant concentration of ethylene gas ($P < 0.01$). This varieties produced a statistically significant concentration of ethylene gas ($P < 0.03$). But the concentration of the produced ethylene gas could not inhibit the oxidation of ammonia to reduce the concentration of nitrogen oxide and its derivatives being sent to the atmosphere to cause a global warming and in the other hand, to increase the nitrogen use by rice and other crops. There is a need to further a research on how to enhance more production of ethylene in rice and other crops so as to increase their nitrogen use efficiency and reduce the loss of nitrogen through leaching and denitrification as well as looking at its negative effect on rice if the concentration is high. The enhancement of ethylene production will maintain the quality of ground water by minimising NO_3 leaching and eutrophication and maintain the air quality by reducing N_2O emission from nitrification and denitrification, and this helps to convert climate change.

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