

*Full Length Research Paper*

# **Spikelet Numbers, Filled Grains and Spikelet Fertility Potential of NERICA Rice (Mecux, Tox and WitA.4) Grown in Hydrocarbon Polluted Soils**

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Rice plants with relatively high tolerance to organic pollution will be of great interest to crop producers. Therefore, ability of three NERICA rice varieties (Mecux, Tox and WitA.4) to grow and reproduce in petroleum polluted soils was investigated. The rice plants were grown for 120 days on soil contaminated with organic pollutants mixture (fresh diesel, gasoline and spent engine oil). Thereafter, each variety was analyzed based on its ability to produce panicles, spikelets and fertile spikelets. Numbers of filled spikelets were mostly affected, for instance; Mecux, Tox and WitA.4 numbers of filled spikelets were significantly reduced when compared with the control at  $P < 0.01$ ,  $P < 0.001$  and  $P < 0.0001$ . It seems that exposure of rice seedlings to organic xenobiotics has some relationships with agronomic characteristics, especially spikelet filling, although there were no significant reductions in percentage spikelet fertility of all the three varieties of the NERICA rice. The analysis in this study strongly reinforced the pertinence of enforcing environmental regulations and equally looking for food crops that can withstand organic xenobiotics thereby increasing yield potentials.

**Keywords:** Panicle number, spikelet fertility, NERICA rice, grain filling, petroleum, flag leaf area.

## **INTRODUCTION**

Rice like other crop plants passes through a succession of growth phases at rates that are controlled by both biological and environmental factors (Sheehy *et al.*, 2001). In rice plants, there are three main developmental stages. These are: vegetative growth, reproductive development and grain ripening stages. The reproductive stage begins with panicle initiation and flowering typically follows one day after heading (Moldenhauer and Gibbons, 2003; Falodun *et al.*, 2012). Blanking or spikelet sterility caused by poor anther dehiscence and low pollen production and hence low numbers of germinating pollen grains on the stigma is induced at this stage (Jagadish *et al.*, 2007).

Series of investigations have shown that spikelet sterility or blanking is induced by low temperatures during the reproductive growth phase, especially during the booting stage in areas with a cool climate (Alvarado, 1999; Shimono *et al.*, 2010). Furthermore, Farrel *et al.*, (2006) reported that low temperature during reproductive growth stage disrupts proper pollen development, leading to a shortage of sound pollen at the flowering stage.

Apart from water stress caused by variable dry and wet seasons, rice plants are equally exposed to adverse soil conditions such as excesses of heavy metals like aluminium, manganese, lead, zinc and/or deficiencies of macro-nutrients (Yoshida, 1981). In Nigeria, oil spillage and uncontrolled dumping of petroleum products such as diesel, gasoline and spent engine oil on agricultural farm lands are some of the adverse soil conditions crops are subjected to (Falodun *et al.*, 2012). Therefore, pollution of soil by petroleum and its products is of great concern to crop production in general.

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The purpose of this study therefore was to find out the sterility/fertility performances of three NERICA rice (Tox, Mecux and WitA.4) varieties in order to assess the variety – environmental pollution factors as they affect sterility. It is of interest to know if the age of plants could impact on efficient partitioning of synthesized carbohydrates to the spikelets (low blanking) during reproductive growth stage of the rice seedlings due to the abundance of petroleum degrading microorganisms that increase with plant age. Moreover, since rice belongs to the grass family (Poaceae), it has a monophyletic origin together with sorghum, maize, barley and wheat. Information derived from rice could be useful for studying adaptability of other cereal crops to hydrocarbon polluted environments.

## MATERIALS AND METHODS

NERICA rice seeds of the varieties Mecux, Tox and WitA.4 were obtained from College of Plant Science (COPLANTS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. These varieties were derived from the cross between the African varieties – *Oryza glaberrima* and the Asian *Oryza sativa*. Therefore, NERICA rice varieties combined the ability to withstand harsh environmental conditions of *O. glaberrima* and high yield traits of *O. sativa*. The soil was prepared by bagging 15kg of loamy soil in thoroughly washed polythene bags. Fifty-four bags were used in all. The fifty-four bags were divided into 3 equal parts, that is, eighteen bags for each of the three NERICA rice varieties. Fresh gasoline, diesel and spent engine oil were thoroughly mixed together in ratio 1:1:1. The amount of the mixtures added to the soil in different concentrations were 1%, 2%, 3%, 4%, and 5% weight per weight (w/w). The added petroleum product mixtures were mixed thoroughly with the soil in each bag and each treatment was replicated three times. The treatments and the controls were left for 5 days to allow for proper settling of the mixture of gasoline, diesel fuel and spent engine oil in the soil.

The NERICA rice seeds were then directly-seeded in the polluted soils at normal atmospheric temperature, daylight period and watered regularly in a screen house. Ten seeds were planted but later thinned to four per replicate. Panicles were harvested at physiological maturity and data on spikelet numbers, numbers of filled and unfilled spikelets per panicle, flag leaf area and numbers of grains per panicle were recorded. Spikelet fertility was estimated as the ratio of number of filled spikelets to total number of spikelets and expressed as percentage. Each floret was pressed between the forefinger and thumb to determine if the spikelet was filled or not.

Number of filled spikelets included both completely and partially filled spikelets.

These growth parameters were further analyzed based on  $\alpha = 0.05$  significance level using Pearson correlation coefficient. Relationships between agronomic characters and concentrations of the pollutant mixtures were analyzed using computer software package of Graphpad Prism version 5.04 for Windows.

## RESULTS

The observed petroleum product – induced changes in agronomic characteristics of the three NERICA rice (Mecux, Tox and WitA.4) are given in Table 1. Based on the analysis of variance (ANOVA), the interaction between different concentrations of petroleum pollutants mixture and panicle number was not significant in Tox variety grown in 1 to 5% concentrations ( $P > 0.05$ ), meanwhile the data showed significant adverse effect on Mecux variety grown in 4% concentrations ( $P < 0.01$ ) and WitA.4 variety sown in 2 to 5% concentrations of the pollutants mixture ( $P < 0.05$ ,  $P < 0.01$ ) as shown in Table 1.

The mean spikelet number for the three varieties of NERICA rice varied between 300 and 1400 per replicate for each concentrations of pollutants mixture (Table 1). The correlation analysis showed that spikelets number in Mecux ( $r^2 = 0.7166$ ) and WitA.4 ( $r^2 = 0.9447$ ) varieties were significantly correlated with increase in pollutants concentrations, whereas, there was no significant correlation between spikelet number of Tox variety and different concentrations of the pollutant mixtures (Figure 1).

Significant reductions in number of filled spikelets were observed in the three NERICA rice varieties sown in 1 to 5% concentrations of pollutant mixtures ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  and  $P < 0.0001$ ) compared with the control plants (Table 1). Further correlation analysis showed high significant correlation between number of filled spikelets and pollutant mixture concentrations ( $r^2 = 0.8101$ ) in WitA.4 variety (Figure 2).

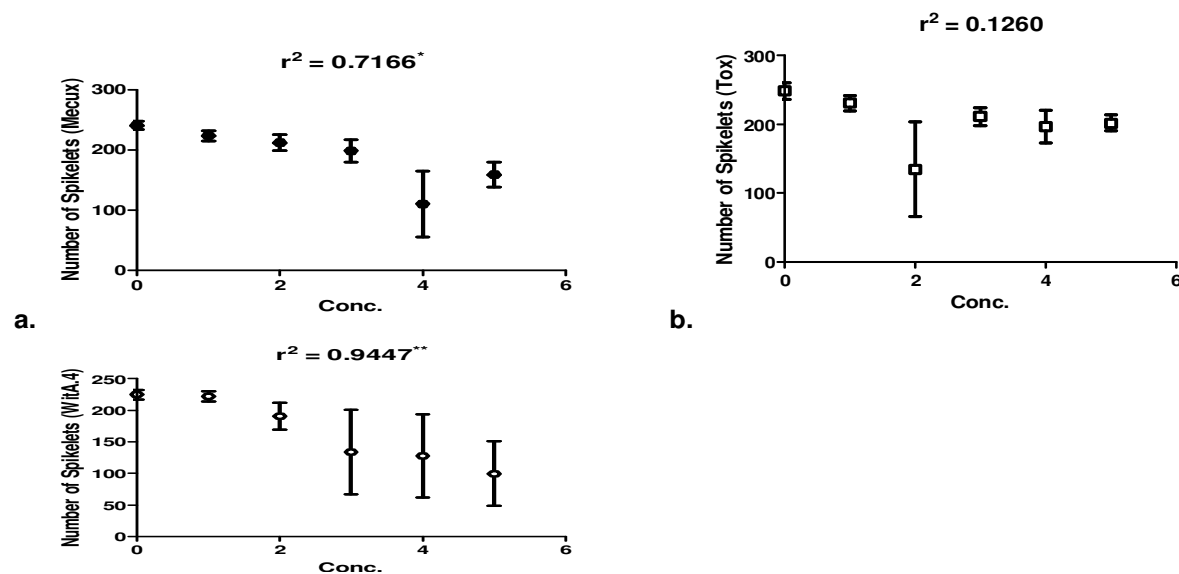
Table 1 shows the data obtained for the relationship between the different concentrations of the pollutants and percentage spikelet fertility of the three NERICA rice varieties. The interaction between the pollutant mixtures and spikelet fertility was not significant for all the crops, except 2% treatments in Tox ( $P < 0.01$ ), 4% in Mecux ( $P < 0.05$ ) and 3% in WitA.4 ( $P < 0.01$ ).

The correlation analysis results obtained showed no significant relationship between the concentrations of the pollutants and spikelet fertility in the three NERICA rice varieties (Figure 3). The same results were obtained for flag leaf area and number of filled

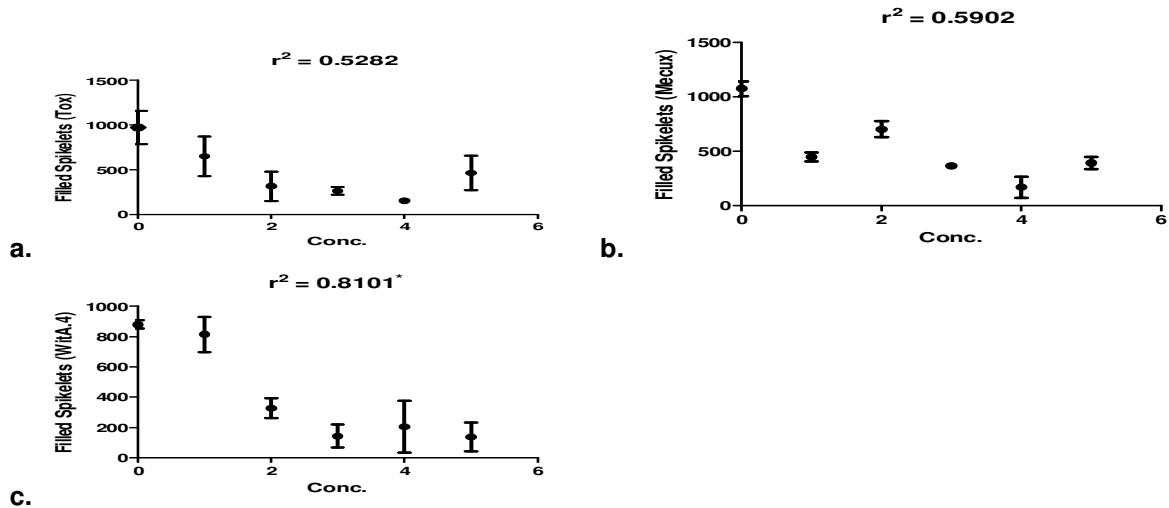
**Table 1.** Panicle number (PN), Spikelet number (SN), Filled spikelets (FS), Unfilled spikelets (US), Spikelet fertility (SF) and Flag leaf area (FLA) of NERICA rice (Tox, Mecux and WitA.4) grown in hydrocarbon pollutants

Var.	Mecux						Tox						WitA.4					
	Conc.	PN	SN	FS	US	SF (%)	FLA (cm <sup>2</sup> )	PN	SN	FS	US	SF (%)	FLA (cm <sup>2</sup> )	PN	SN	FS	US	SF (%)
0	11.33	1437.00	1075.3 3	361.67	74.87	79.10	8.00	1403. 67	975.33	428.33	69.57	111.30	11.67	1412.6 7	879.33	533.33	62.27	68.40
1	7.67	852.67	449.33 b	403.33	52.70	78.83	5.33	932.3 3	654.00	278.33	70.07	96.60	8.33	1307.6 7	815.00	492.67	62.33	90.87
2	6.33	803.33	703.33	100.00	87.53	67.51	4.67	850.3 3	321.00 b	529.33	25.17b	116.58	5.00a	777.67	328.00 a	449.67	42.20	89.60
3	5.67	669.67	367.00 b	302.67	54.83	65.17	4.33	476.3 3	268.67 b	207.67	56.43	88.50	3.33b	547.33	143.33 c	404.00	17.43b	92.09
4	3.00b	303.33b	170.67 d	132.67	37.53a	39.87a	2.00	278.6 7b	158.67 c	120.00	57.10	77.00	3.00b	384.00 a	205.33 b	178.67	35.53	74.16
5	7.00	653.33	393.33 b	260.00	60.30	81.89	5.00	663.6 7	468.33	195.33	70.50	82.50	3.00b	310.67 a	136.67 c	174.00	29.40	77.87

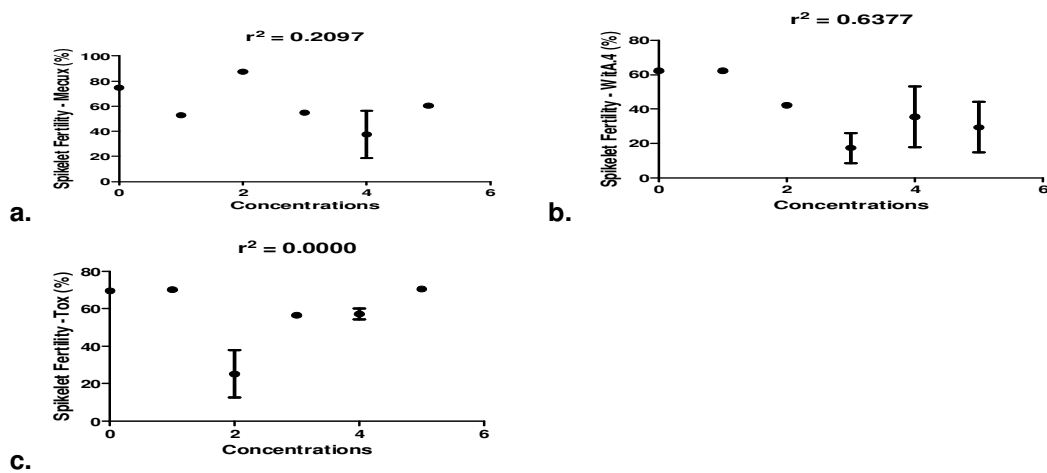
Differences are significant to the control crops at p values of: a = P < 0.05, b = P < 0.01, c = P < 0.001, d = P < 0.0001.



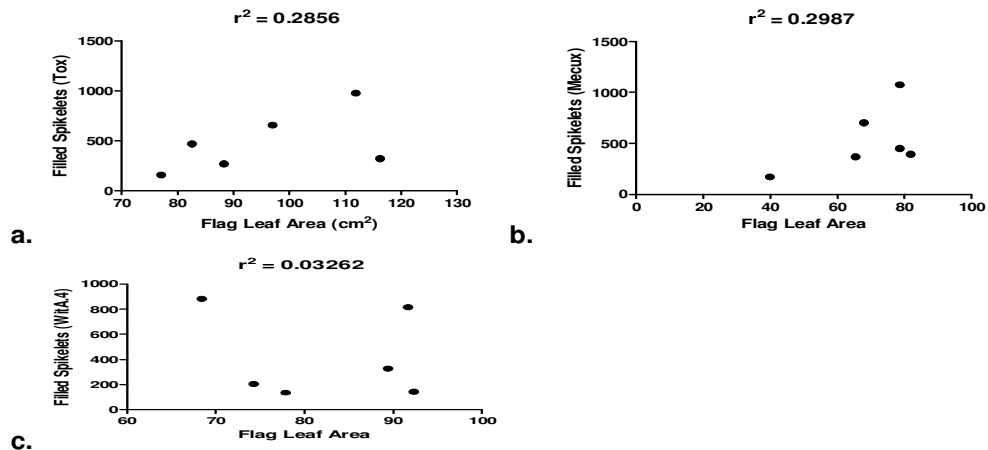
**Figure 1.** Correlations between number of spikelet and concentrations of pollutants mixture. P value: \* and \*\* = significantly correlated at P < 0.05 and P < 0.01, respectively.



**Figure 2.** Correlations between number of filled spikelet and concentrations of pollutants mixture. P value: \* = significantly correlated at  $P < 0.05$ .



**Figure 3.** Correlations between spikelet fertility and concentrations of pollutants mixture. P value: No significant correlations,  $P > 0.05$ .



**Figure 4.** Correlations between No of filled spikelet and Flag Leaf Area. P value: No significant correlations,  $P > 0.05$ .

spikelets of the three rice varieties grown in 1 to 5% pollutant concentrations (Figure 4).

## DISCUSSION

According to Bonner and Galston (1952), the growth and development of a plant are results of the interaction between the genetic constitution of the plant and the factors of the environment. The genetic constitution of the plant determines both the nature of the individual and how this individual will react to the influence of the environment. The above statement explains the interaction between the three NERICA rice varieties' panicle number and different concentrations of the pollutant mixtures. Although the three NERICA rice varieties are from the same parents (*O. Sativa* and *O. glaberrima*) and exposed to the same pollutant mixtures, Tox variety proved to be a better stress – tolerator than Mecux and WitA.4 varieties.

For example, it has been reported that changes induced by anthropogenic activities in the environment lead to variable phenotypic reactions between plant species and within species (Medina *et al.*, 2003). To buttress this point, Medina *et al.*, (2003) quoting Taylor *et al.*, (1991) indicated that one of the underlying causes of phenotypic variabilities in tolerance to xenobiotic chemical exposure is as a result of genotypic variation occurring in cultivated and natural populations.

Furthermore, the genetic differences in the ability of plants to acquire resources like water, nutrients, carbon dioxide and light under organic pollution stress have been found in diverse plants grown in organic xenobiotics. For instance, live oak (*Quercus virginiana*), saw palmetto (*Scennoa repens*), castor bean (*Ricinus communis*) grown in trichloroethylene polluted soils (Doucette *et al.*, 2003) and maize, soybean, wheat and rice grown in hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT) (Villa *et al.*, 2007).

In this present study, we found that Tox and Mecux NERICA rice varieties performed well compared with the control (Table 1). At 2 – 5% concentrations, WitA.4 panicle number was significantly reduced. Obviously the genotypic differences in the NERICA rice varieties' panicle numbers may be attributed to differences in tolerance, initiation and growth of panicles. Therefore, screening and breeding the cultivars with ability to tolerate organic xenobiotics with low or no reduction in yield is probably most feasible.

Although, the spikelet numbers of the three NERICA rice varieties grown in the pollutant mixture were significantly reduced at 4% concentrations ( $P < 0.05$  and  $P < 0.01$ ), Tox and Mecux varieties sown in 5% concentration showed no significant reduction. Similar results were reported by Lin and Mendelsohn (1996), who indicated that aboveground biomass of *Sagittaria lancifolia* (bulltongue grass), increased with increasing oil

dosage. Lin and Mendelsohn (1996) also stated that the mechanisms by which oil stimulates the growth of some plants are not clearly understood.

Furthermore, Lin and Mendelsohn suggested that, this occurrence may be due to: an indirect increase in microbial N – fixation; or directly by the addition to the soil of some plant growth stimulatory analogues in the petroleum or its metabolites and shifting competitive interactions. The present research exhibited that the effect of petroleum products on the growth and development of rice spikelets differed in rice varieties, therefore the sensitivity and tolerance of NERICA rice varieties to soil petroleum products pollution among others are cultivar – dependent.

Spikelet filling or ripening growth stage in rice extends from flowering to physiological maturity (Fageria, 2007). During this stage, stored carbohydrates, for instance, starch and other polysaccharides are translocated to the grains (spikelets) or else used up as a substrate for respiration (Yoshida, 1972). The data obtained from this work showed that spikelet filling in Mecux variety was significantly reduced in seedlings grown in 1, 3, 4 and 5% concentrations of the pollutant mixture ( $P < 0.01$  and  $P < 0.0001$ ), same for Tox variety sown in 2 to 4% concentrations ( $P < 0.01$  and  $P < 0.001$ ) and WitA.4 seedlings grown in 2 to 5% concentrations of the pollutants mixture. Therefore, it could be speculated from these results that the translocation of carbohydrates from leaves and stems (photosynthetic parts) to the grains or spikelets in these NERICA rice seedlings were inhibited, either due to partitioning of synthesized foods to stems and leaves that are still recovering from the stress occasioned by the petroleum products or reduced photosynthetic rate (Grime, 1979). Also, accumulation of soil organic acids or other toxic compounds due to unfavourable oxidation-reduction conditions may contribute to low productivity vis-a-vis spikelet filling (Grime, 1979).

The results also indicated that there were no significant reduction in percentage spikelet fertility of all the three varieties. The same observation was made using Pearson correlation coefficients where there were no significant correlations between the increase in concentrations of the pollutants mixture and spikelet fertility. Although high proliferation of hydrocarbon degrading microbes is expected, which can prove beneficial in that it acts as Nitrogen sink which will slowly remineralize microbial nitrogen that later become available to the plant (Riser-Roberts, 1998), thereby contributing positively to the health of the rice seedlings.

It can be concluded from our results that exposure of rice seedlings to organic xenobiotics has some relationships with agronomic characteristics, especially spikelet filling and other morphological characteristics (Falodun *et al.*, 2011). Therefore, more studies should be undertaken in order to completely understand the interactions between agronomic crops such as rice

and organic pollutants.

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