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RESEARCH PAPER

IMPACT OF PALM OIL MILL EFFLUENT ON THE ACTIVITIES OF SOME ANTIOXIDATIVE PARAMETERS IN MAIZE PLANT (*Zea mays* L.)

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ABSTRACT
This study was carried out to evaluate the effect of palm oil mill effluent (POME) on some anti-oxidative parameters in maize plant. Fresh POME was used to pollute the soil at concentrations of 10%, 20% and 30% (w/w). Analysis of the maize plants after two, three and four weeks of planting showed alterations in the activities of the antioxidative enzymes measured. There was significant increase (p< 0.05) in catalase and peroxidase activities for plants grown in 20% and 30% POME-treated soils for 2, 3 and 4 weeks after planting. Significant increase in malondial-dehyde (MDA) levels was observed in the fourth week for maize grown in 20% and 30% POME-treated soil. Increase in the level of these enzymes and in MDA levels suggests that, palm oil mill effluent induces oxidative stress in the maize plant and may be deleterious to the growth of the maize plant.

Keywords: *palm oil mill effluent, maize, catalase, peroxidase, malondialdehyde*

INTRODUCTION

The oil palm – *Elaeis guineensis,* is indigenous to the tropical rain forest region of West Africa (Wattanapenpaiboon and Wahlqvist 2003; Olagunju, 2008). In Nigeria, palm oil production takes place mainly in the southern part where palm trees are found both in the wild, and in plantations (Nwaugo *et al.,* 2008).

The processes leading to the production of palm oil generate a number of waste products. These include empty fruit bunches, palm oil mill effluent (POME) and palm oil mill sludge (POMS). These wastes are produced in very huge quantities. Out of these wastes, POME is of great significance because it is considered harmful when it is discharged into the environment untreated (Rupani *et al.,* 2010).

It has been estimated that for every tonne of crude palm oil produced, 5-7.5 tonnes of water is used and that about half of this water ends up as palm oil mill effluent (POME) (Ahmed *et al.,* 2008;Oviasogie and Uzoekwe, 2011). The palm oil mill industry thus produces huge quantities of waste which if not properly managed, can cause significant environmental problems.

Excessive generation of reactive oxygen species (ROS) is an integral part of many stress situations. ROS include hydrogen peroxide, hydroxyl radical and superoxide. The levels of these ROS can be controlled by the activities of antioxidant scavenging enzymes such as superoxide dismutase, catalase and peroxidase (Blokhina *et al.,* 2003). The rate of expression of plant intracellular antioxidants is closely related to the plant's metabolic state and in response to changes or fluctuations in its environment. The levels of these antioxidant enzymes therefore provide information about the stress level of the plant.

This study therefore seeks to investigate the effect of POME on the levels of antioxidative enzymes in roots of maize grown in POME-polluted soil.

MATERIALS AND METHODS

Experimental design

Maize seeds (*Zea mays*) were purchased from Uselu Market in Benin-City, Edo State, Nigeria. The soil sample used for planting was obtained from a farmland in Oluku Junction in Benin-City. The POME was obtained from the Nigerian Institute for Oil Palm Research (NIFOR), along the Benin-Akure Road, Benin-City, Edo State, Nigeria.

Three kilograms of soil was weighed into each polythene bag. Six bags were labelled as control, with nothing added to them. Three other groups consisting of six bags each were treated with 10%, 20% and 30% (w/w) POME soil. Five viable maize seeds, selected by simple flotation method were planted, evenly spaced, in each bag. The bags were transferred to a greenhouse to simulate an almost natural environment. Each bag was moistened daily with 50ml of water. Biochemical analyses were carried out two, three and four weeks after planting.

Measurement of enzymatic activities and lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) were assayed by the method of Beuge and Aust (1978). Superoxide dismutase was assayed according to the method of Misra and Fridovich (1972). Catalase was assayed according to the method of Cohen *et al* (1970) and peroxidase was assayed according to the method of Chance and Maehly (1955).

Statistical analysis

Data was expressed as mean ± standard error of mean (SEM). The results were computed statistically utilizing statistical package for the social sciences (SPSS) software package, version 17. Using a one-way analysis of variance (ANOVA), post-hoc test was performed for inter group comparison using least significant difference. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Catalase activity between the control and 10% POME-polluted soil did not show significant difference ($p > 0.05$) (Table 1). However, there was significant increase ($p < 0.05$) in the activity of this enzyme between the control and 20% to 30% POME polluted soils.

Superoxide dismutase activity did not show significant difference ($p > 0.05$) between the control and the various treatments (Table 2). However, the activity of superoxide dismutase was observed to increase as POME concentration increased.

Significant increase ($p < 0.05$) in peroxidise activity between the control and 20% POME-treated soil was observed during this study. Peroxidase activity in the control and 10% POME-treated soil generally increased from week 2 to 4. The 20% and 30% POME-polluted soils showed reduction in peroxidise activity from week 3 to 4 (Table 3).

Table 4 shows malondialdehyde (MDA) levels in the maize plant post planting. No significant

Table 1: Catalase activity of maize roots grown in different concentrations of POME polluted soil

	Week 2	Week 3	Week 4
Control	0.08 ± 0.005[a]	0.22 ± 0.040[a]	0.31 ± 0.012[a]
10% POME pollution	0.10 ± 0.014[a]	0.32 ± 0.030[a]	0.37 ± 0.011[a]
20% POME pollution	0.11 ± 0.004[a]	0.41 ± 0.060[b]	0.44 ± 0.014[b]
30% POME pollution	0.13 ± 0.024[b]	0.44 ± 0.087[c]	0.51 ± 0.050[b]

All values represent mean ± standard error of mean (n = 5).
All units are expressed as units/mg protein.
Significant differences (p < 0.05) between groups are indicated by different alphabets

Table 2: Superoxide dismutase activity of maize roots grown in different concentrations of POME polluted soil

	Week 2	Week 3	Week 4
Control	0.67 ± 0.17[a]	2.67 ± 0.33[a]	2.67 ± 0.44[a]
10% POME pollution	0.83 ± 0.17[a]	3.13 ± 0.94[a]	3.50 ± 0.87[a]
20% POME pollution	1.00 ± 0.01[a]	3.83 ± 0.33[a]	4.33 ± 0.83[a]
30% POME pollution	2.67 ± 1.01[b]	7.33 ± 3.83[a]	3.67 ± 0.60[a]

All values represent mean ± standard error of mean (n = 5).
All units are expressed as units/mg protein/minute.
Significant differences (p < 0.05) between groups are indicated by different alphabets

difference (p> 0.05) in MDA levels between the groups occurred at weeks 2 and 3. Significant increase (p< 0.05) in MDA levels between the groups was however observed at week 4.

DISCUSSIONS

The problem of palm oil mill effluent pollution is unique to Nigeria for a number of reasons. No established control methods are possible because local palm oil mills located in the nooks and crannies are unlicensed and without quality control. Palm oil effluent treatment technology is non-existent and the prescribed environmental regulations on the allowable pollution loads are unenforceable. Minimization of effluent quantity is not pursued in the traditional palm oil mill and effluent utilization is not practicable. Sound water quality objectives to reduce the level of pollutants in the effluents are not pursued because of lack of

Table 3: Peroxidase activity in roots of maize grown in different concentrations of POME polluted soil

	Week 2	**Week 3**	**Week 4**
Control	0.43 ± 0.069[a]	1.39 ± 0.090[a]	1.46 ± 0.015[a]
10% POME pollution	0.49 ± 0.045[a]	1.28 ± 0.050[a]	1.20 ± 0.077[b]
20% POME pollution	0.36 ± 0.018[b]	1.63 ± 0.063[b]	1.21 ± 0.076[b]
30% POME pollution	0.46 ± 0.032[a]	1.51 ± 0.030[b]	1.30 ± 0.104[a]

All values represent mean ± standard error of mean (n = 5).
All units are expressed as units/mg protein/minute.
Significant differences (p< 0.05) between groups are indicated by different alphabets

Table 4: Malondialdehyde levels in roots of maize grown in different concentrations of POME polluted soil

	Week 2	Week 3	Week 4
Control	0.013 ± 0.0006[a]	0.017 ± 0.0013[a]	0.035 ± 0.0029[a]
10% POME pollution	0.013 ± 0.0008[a]	0.017 ± 0.0011[a]	0.035 ± 0.0008[a]
20% POME pollution	0.014 ± 0.0001[a]	0.019 ± 0.0017[a]	0.043 ±0.0018[b]
30% POME pollution	0.016 ± 0.0002[a]	0.022 ± 0.0040[a]	0.054 ± 0.0012[c]

All values represent mean ± standard error of mean (n = 5).
All units are expressed as mMolMDA/gm fresh root.
Significant differences (p < 0.05) between groups are indicated by different alphabets

awareness of the aquatic pollution problem being created (Olaleye and Adedeji, 2005).

The prevalent disregard for the environment in Nigeria as with regard to indiscriminate effluent discharge is far from the desired as most industries in developed countries strive towards quality and environmental conservation through sustainable development and cleaner technology approach (Awotoye *et al.,* 2011).

Plant cells contain antioxidants that prevent damage due to the action of reactive oxygen species (ROS). These highly reactive oxygen species when left uncontrolled are capable of causing cell death (Eze, 1991). Measurement of activities of antioxidative enzymes provides information about the extent to which tissues are exposed to reactive oxygen species. Available evidence suggests that the production of ROS may be a general alarm signal that serves to alert metabolism and gene expression about possible modification (Foyer *et al.*, 1994). Measurement of the level of thiobarbituric acid reactive substances (TBARS) in tissues is widely used as an index of lipid peroxidation (Lin and Kao, 2000). Extensive lipid peroxidation in biological membranes causes loss of fluidity, falls in membrane potential, increased permeability to H^+ and other ions and eventual rupture leading to release of cell and organelle content (Gutteride, 1995).

Some variations in antioxidant enzyme activities were observed in this study. Catalase activity significantly increased ($p< 0.05$) in the 20% and 30% POME-treated soil compared to the control. Superoxide dismutase activity did not show significant difference ($P > 0.05$) between the control and the various treatments. However, increase in the activities was observed as POME pollution level increased. Significant increase ($P < 0.05$) in peroxidase activity between the control and 20% POME soil was observed during this study. Significant difference in malondialdehyde levels at week 4 was also observed.

Yu and Rengel (1999) reported that, the activities of antioxidative enzymes usually increase in the initial stage of stress in plants, providing a certain degree of protection from oxidative damage and then decline with the duration of stress due to reduced synthesis, enhanced degradation or inactivation of the enzymes or all these factors. This trend was observed in the activity of peroxidise and superoxide dismutase at 30% POME application. Lipid peroxidation is a biochemical marker for the free radical mediated injury. The results also showed increase in the levels of TBARS as POME application increased suggesting that POME induces oxidative stress in *Zea mays*.

CONCLUSIONS

Increases observed in activities of the antioxidant enzymes and TBARS indicate some degree of oxidative stress in the maize plants. It can be concluded that, palm oil mill effluent induces oxidative stress in maize plant by altering the activities of antioxidant-marker enzymes and the levels of TBARS. The presence of palm oil mill effluent in the soil may therefore be detrimental to plant growth at the concentrations studied.

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