Applied Biological Research 17(2): 190-194; (2015)
DOI: 10.5958/0974-4517.2015.00016.6 **SHORT COMMUNICATION**

EFFECTS OF PALM OIL MILL EFFLUENT ON SEED GERMINATION AND TOTAL CHLOROPHYLL, PHENOL AND CAROTENE CONTENTS OF MAIZE (*Zea mays* L.)

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ABSTRACT

Palm Oil Mill Effluent (POME) is produced in large quantities in Nigeria and is amenable to microbial degradation. Studies were conducted to assess the effect of various concentration of POME on germination, total chlorophyll, phenol and carotene contents of maize plant. Different concentrations of POME tested were 0, 25, 50, 75 and 100% (prepared in water). Each treatment was stored in separate container. Maize seeds were planted in 25 sterilized petri-plates and each treatment replicated five times. POME (10 mL) taken from a given concentration was used to irrigate the planted seeds as per treatment at 48 h interval. Results revealed that the use of 25% POME gave highest seed germination and total chlorophyll content of 68% and 17.56 mg mL^{-1}, respectively while 100% POME gave 26% germination and 9.18 mg chlorophyll mL^{-1}. The phenol content was highest in 75% POME and lowest in control. Carotenoid content in maize seedlings was highest carotene in 50% POME treatment. The study revealed that POME at proper dilution could serve as a bio-fertilizer for seedling growth.

Keywords: Chlorophyll, maize, palm oil mill effluent, phenol, germination

INTRODUCTION

Maize (*Zea mays* L.), also called corn, is a large grain plant domesticated by indigenous peoples in Mesoamerica in prehistoric times. The leafy stalk produces ears which contain grains, which are seeds called kernels. Maize kernels are used in cooking as a starch. Maize is most widely grown grain crop throughout America with 332 million metric tons grown annually in USA alone (Raouf, 2011). Approximately 40% of the crop i.e. 130 million tons is used for corn ethanol. Genetically modified maize made up 85% of the maize planted in USA (USDA, 2010). The crop has high nutrient requirement of crop and the use of low input/easily available substrates is one of the means to overcome compensate crop requirement in sustainable manner.

Palm oil mill effluent (POME) is a thick unpleasant-smelling brownish viscous liquid waste toxic to aquatic plants and contains soluble materials that may have a significant impact on the environment. Oil palm *(Elaies guineensis* Jacq.*)* belongs to the family *Palmae* and is an indigenous plant in West Africa. Oil palm is often regarded as the most productive and economic oil crop in the world (Ahmed *et al*., 2003). Nigeria is currently the world's 5th largest producer of palm oil (Nwaugo *et al*., 2008). It has been a major agro-based enterprise in Nigeria especially in its southern part where palm trees are found both in wild and plantations. Palm oil processing is carried out using large quantities of water in mills where oil is extracted from the palm fruits. During extraction process, about 50% water results in palm oil mill effluent often known as 'POME'. As per conservative estimates for every one ton of crude palm oil produced 5.0-7.5 tons of water ends up as POME (Wu *et al*., 2009). Raw POME, consisting of complex vegetative matter, is thick, brownish and colloidal slurry of water, oil and solids including about 2% suspended solids originating mainly from cellulose fruit debris (Bek-Nielsen *et al*., 1999).

The brownish and colloidal suspension of POME contains high organic matter content, high total solids (40,500 mg L^{-1}), oil and grease (4,000 Mg L^{-1}), chemical oxygen demand [COD] (50,000 mg L^{-1}) and biological oxygen demand [BOD] (25,000 Mg L^{-1}) (Ahmed *et al*., 2005). However, it also contains appreciable amounts of plant nutrients like nitrogen, potassium, magnesium and calcium and their concentrations are usually sub-lethal i.e. <17.5 mg g^{-1} (James *et al*., 1996, Habib *et al*., 1997). The raw or partially treated POME, therefore, may serve as mulch and biofertilzer in crops (Okwute and Isu, 2007). The present study was aimed to assess the impact of POME on maize seed germination and total chlorophyll, phenol and carotene contents of maize plant.

MATERIALS AND METHODS

The present study was conducted in Abakaliki, the capital of Ebonyi state, Nigeria. Abakaliki is located approximately 6°20'49''N and 8°06'11''E. It is at 118 m masl with relative humidity between 70-90% and average temperature of 31°C. The residents are predominantly of Igbo ethnic group. This study was carried out between January and August, 2013.

Maize seeds cv. "Oba super" were procured from the National Root Crop Research Institute, Umudike, Nigeria. The seeds were washed with distilled water to remove any contaminants present on seed coat. POME was obtained from a local palm oil mill plant at Afikpo in Ebonyi State (Nigeria) and transported in 5 L containers to Ebonyi State University Greenhouse Laboratory.

Laboratory analysis of POME

Raw POME was analyzed for COD, BOD, pH, potassium, phosphate and total nitrogen. COD determination was based on closed reflux dichromate oxidation calorimetric method and was read out in Dr/2000 spectrophotometer at 700 nm (Apha, 1992). BOD was estimated as per the modified standard method (Apha, 1992) and 2-chloro 6 trichloro-methyl pyridine was used to inhibit nitrification. Lovibond BOD DR Sensomat Measurement Device and BOD-sensomat and stirring system were used. pH was determined using Hach-one pH meter while potassium and phosphate determination were carried out by wet oxidation method (AOAC, 1965). POME temperature was measured using mercury in glass thermometer. POME was later stored at 4°C before use.

Experimental details

Five concentrations of POME were prepared by dilution with distilled water. For this, 0, 25, 50, 75 and 100 mL POME in different containers were diluted with 100, 75, 50, 25 and 0 mL water, respectively. Twenty five sterilized petri-dishes of 8 cm dia. were lined with moist filter and arranged in a completely randomized design. Four maize (*Zea mays*) seeds were planted in each petri-plate and incubated under a room temperature.

Seed germination and chemical analysis

For determination of seed germination 10 mL of various concentrations of POME was used to irrigate various treatment groups at 48 h interval, with exception of control where 10 mL distilled water was used. This was carefully done to avoid any water-logging of maize seed and causing it to imbibe so much water and rupture. Number of seeds that germinated in each Petri-plate were recorded at 24, 48, 72 and 96 h of experiment. The percentage germination was calculated using the formula below.

$$\text{Germination\%} = \frac{\text{Total number of seeds germinated in a particular treatment}}{\text{Total number of seeds germinated in control (distilled water)}} \times \frac{100}{1}$$

For total chlorophyll content, 4 g freshly cut leaf sample from maize plants from each treatment group was collected. The samples placed in a resalable plastic bag were frozen indry ice in a cooler. Samples were kept in a dark room to prevent chlorophyll degradation. Total chlorophyll was assessed as per the method of Arnon (1949). The calculations were made to ascertain chlorophyll concentrations in the samples and concentrations expressed on an area basis.

$$\text{Chlorophyll a [mg mL}^{-1}] = 12.7\ A_{663} - 2.69\ A_{645}$$

$$\text{Chlorophyll b [mg mL}^{-1}] = 22.9\ A_{645} - 4.68\ A_{663}$$

where A_{663} and A_{645} are the absorbance at a wavelength of 663 and 645 nm, respectively;

$$\text{Total chlorophyll [mg mL}^{-1}] = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Total chlorophyll [mg mL^{-1}] in tissue sample = Total chlorophyll [mg mL^{-1}] x final volume (mL).

To express on the basis of area, the amount of chlorophyll was divided by the area (mm^2) in the tissue samples analyzed.

The **c**arotene content of fresh leaves samples were analyzed as per the method of Duxbury and Yentsch (1956) and phenol content as per the method of Bray and Thorpe (1954).

Statistical analysis

The data was reviewed according to the quality assurance/quality control considerations. The data was subjected to ANOVA test for assessing the significance differences in various parameters studied. Mean value and standard deviation (SD) were also calculated.

RESULTS AND DISCUSSION

Physico-chemical analysis revealed that POME had high acidic content (3.79) and the biological oxygen demand (BOD), chemical oxygen demand (COD), potassium, phosphate, total suspended solid and oil and grease were 8.98 mg L^{-1}, 18.27 ppm, 19.34 mg L^{-1}, 7.00 mg L^{-1}, 14.15 mg L^{-1} and 69.92 mg L^{-1}, respectively (Table 1). POME was dark brown in colour due to the presence of high suspended particles and dissolved solids.

There was a significant increase in seed germination of maize with increase in dilution of POME, with highest germination of 68% in 25% POME treatment and least germination of 26% in 100% POME [Fig. 1]. The total chlorophyll content in maize was significantly high (17.56 mg mL^{-1}) in 25% POME treatment as compared to other treatment groups which depicted a considerable decrease in chlorophyll content (Fig. 2). A significant difference in phenol content was observed due to POME treatment with highest phenol contents in 75% POME treatment (1.77 mg m L^{-1}) and least in control (1.01 mg mL^{-1}) [Fig. 3]. Highest carotene content

Table 1: **Physio-chemical characteristics of POME**

Parameter	Rang	Mean
pH	3.2 - 4.4	3.79
B.O.D	2.5 - 15.4	8.98
Oil and Grease	41.6 - 98.2	69.92
C.O.D	12.3 - 24.2	18.27
Potassium	9.5 - 29.1	19.34
Phosphate	5.3 - 8.7	7.00
TSS	7.6 - 20.7	14.15

Note: All parameters are in Mg L^{-1}, except pH

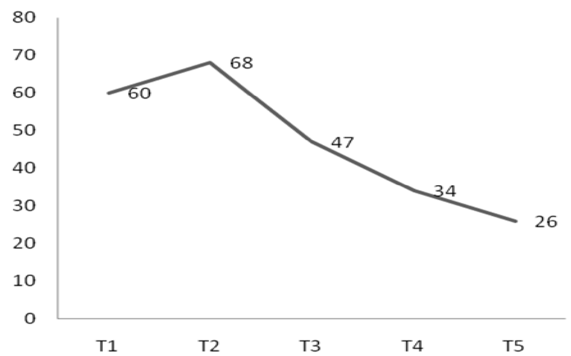

Fig. 1: Percent germeniation of maize [T_1 = control and T2, T3, T4 and T5 are POME: water in 25:75, 50:50, 75:25 and 100:0 ratio respectively]

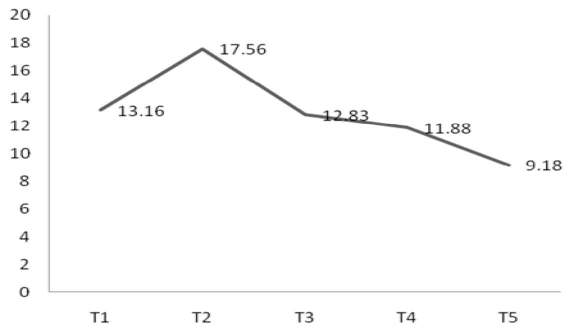

Fig. 2: Total chlorophyll content in maize seedlings [T1 = control and T2, T3, T4 and T5 are POME: water in 25:75, 50:50, 75:25 and 100:0 ratio, respectively]

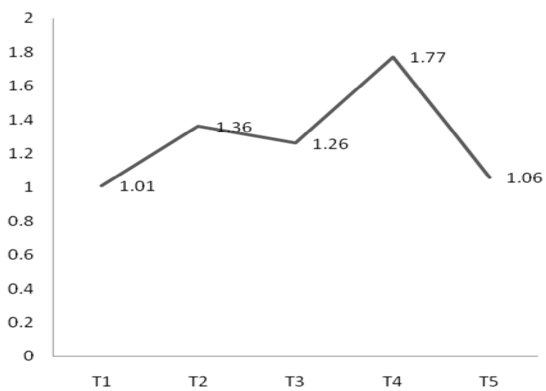

Fig. 3. Effects of POME on phenol content of maize seedlings [For notations see Fig. 1]]

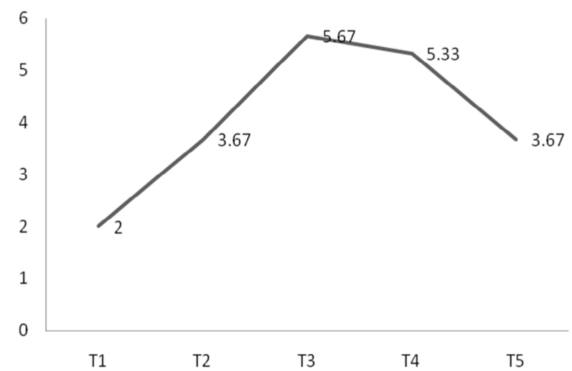

Fig. 4: Effect of POME on the carotene content of maize plant [For notations see Fig. 1]

was in 50% POME treatment (5.67 mg m L^{-1}) and least in control (2.00 mg mL^{-1}) [Fig. 4].

POME was dark brown in colour due to high level of suspended particles and dissolved solids. This could likely pose threat to aquatic life. Physico-chemical parameter analysis revealed the acidic nature of POME in terms of high BOD and COD. The low pH in POME could be attributed to the presence of phenolic acids and oxidation of other organic acid compounds in POME. The results are supported by Dhouib *et al.* (2006) who while using olive mill effluent (OME) suggested anaerobic digestion with white rot fungi decreases BOD/COD ratio and toxicity.

The presence of potassium and phosphate in POME suggested its possible use as organic amendment to improve soil fertility. This observation is consistent with the works of Onyia *et al.* (2001) and Kittikun *et al.* (2000). The study revealed that seed germination of maize was affected by variation in the concentrations of POME with highest germination in 25% POME treatment and lowest in control. This suggested that at proper dilutions POME favours seed germination but exhibits inhibitory effects at higher concentration. The study suggests that POME contains some nutrients that enrich soil and favour growth when properly administered. Lowest germination in 100% POME revealed that as undiluted POME has high oil and grease content it is capable of creating anaerobic environment when present in excess and this could be unfavourable to seed germination and plant growth.

The study revealed significant decrease in total chlorophyll content with increase in POME concentration. The increase in chlorophyll content seen in 25 and 50% POME treatments may be attributed to high nutrient uptake or translocation of nutrient probably facilitated by optimum availability of iron and magnesium in POME (Rajeev *et al.,* 2013). High phenol content in 75% POME treatment could be attributed to certain elements present in POME effluent. Similar observations were made by Orhue *et al.* (2005). The highest carotene content in 75% POME suggests that if POME is properly administered into the soil it could serve as potential source of carotene content for germinating plants. This has revealed that proper dilution reduces the toxic substances present in the POME while still retaining its rich carotene content.

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