INVESTIGATION ON THE EFFECT OF PALM OIL EFFLUENT ON THE EARLY GROWTH AND OXIDATIVE STRESS PARAMETERS ON *TELFERIA OCCIDENTALIS* LEAVES FOLLOWING CHRONIC EXPOSURE.

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ABSTRACT

The effect of palm oil effluent on early growth and oxidative stress parameters on *Telferia occidentalis* leaves from soil exposed to the effluent over a long period of time was studied. *Telferia occidentalis* leaves from soil devoid of palm oil effluent served as the control. The concentrations of glutathione (GSH), protein thiol (PSH), total thiol (TSH), ascorbic acid (AA), malondialdehyde (MDA) and protein contents from the aqueous extract of *T. occidentalis* leaves from the two environments were determined. Four viable seeds of *T. occidentalis* were planted in each perforated polythene bag containing soil sample in triplicate. *Telferia occidentalis* seeds from soil samples devoid of palm oil effluent had 100% germination while those from palm oil effluent-polluted soil samples had 33% germination. Results revealed that there was a significant ($p<0.05$) difference in all the oxidative stress parameters investigated from the aqueous extract of *T. occidentalis* leaves from palm oil effluent-polluted soil sample compared to the control indicating that palm oil effluent induced stress on *T. occidentalis* leaves and delayed germination of the seeds.

Keywords: *Telferia. occidentalis*, palm oil effluent, oxidative stress, pollution.

INTRODUCTION

The impact of palm oil effluent on our agricultural soil has not been given any proper recognition it deserves. Its effects on terrestrial plants are poorly understood as research on palm oil effluent is scanty (Elendu, 1998).

Pollution from palm oil effluent is an environmental problem in the South-East and South-South region of Nigeria where the processing and extraction of palm oil is prominent (Henson, 1998).

Disposal of palm oil effluent into our agricultural land is a common practice especially by palm oil mill operators. Previous studies (Bossert and Bartha, 1984; Odjegba and Sodiq, 2002; Zakaria *et al*., 2002; Ma *et al.*, 1996 ; Ken, 2005) revealed that oil in whatever form (palm oil, crude oil, engine oil and diesel oil) penetrate the pore spaces of soil thereby affect terrestrial vegetation and subsequently impede photosynthesis and other plant physiological processes. Plants on such soil become suffocated due to exclusion of air by the oil (Udo *et al.,* 1999). The exclusion of air in soil interferes with the plant-soil-water relationship, effect the seeds and causes morphological and anatomical aberration in the leaves, stem and roots. (Gill *et al.,* 1994).

Palm oil seed (*Elaeis guineensis*) was discovered in the tropical rain forest region of west African countries. The oil has been in wide use as cooking oil among West African people (Enwere, 1998). Palm oil is rich in carotene, (although boiling destroys the carotenoids) from which it derives its deep red colour when ripe (Hartley, 1997). Palm fruit is a high yielding source of edible and manufacturing oil, the oil palm is now grown in plantations in most West African countries with high rainfall. The palm bears its fruits in bunches varying in weight from 10 to 40 kg, the weight of individual fruits ranges from 6 to 20 grams. They are made up of an outer skin (the exocarp), a pulp (the mesocarp) containing the palm oil in the fibrous matrix, a central unit consisting of a shell (the endocarp) and the kernel which itself contain palm kernel oil that is quite different from palm oil (Ma *et al*., 1996). The wild oil palm plantations of West African region consist mainly of a thick shelled variety with a thin mesocrap called the Dura. Cross breeding between the dura and a shell-less variety the pisifera have led to the development of a hybrid called the tenera (Elendu, 1998).

Natural palm oil obtained from fresh palm fruit is often accepted for local consumption without further treatment. Other simple treatment that palm oil is sometimes exposed to may be simple filtration and settling operation to remove any impurities. The satisfactory extraction of palm oil from its fresh fruit bunches require specially designed machinery either manually or mechanically operated and the provision of ancillary equipment for preparation of the fruit for subsequent extraction of the oil (Enwere, 1998). The general process of oil extraction include the sterilization process where the fruit is usually softened by heat treatment. The pulp is broken up to release the oil. Extraction releases the oil from mashed fruit while purification and clarification process separates the oil from water and debrics called effluent. It is this palm oil effluent that is discarded indiscriminately into the environment that affect the soil chemistry which in turn affect terrestrial plants (Zakaria *et al.,* 2002). The aim of this study was to investigate the effect of palm oil effluent pollution on germination, early growth and development and the stress parameters this effluent might have on *Telferia occidentalis* leaves which is the vegetable mostly consumed in the South-East and South-Southern parts of Nigeria.

MATERIALS AND METHODS

Soil preparation and Planting

Seeds of *T. occidentalis* used for the study were purchased from Ihiagwa market Owerri, Nigeria. The palm oil effluent-polluted soil was collected near a palm oil processing mill in Umuchima village in Ihiagwa, Owerri, Nigeria.

The garden soil was collected from Umuchima village in Ihiagwa Owerri, Nigeria. Two groups of nine perforated polythene bags containing 700grams of garden soil and palm oil effluent-polluted soil samples were measured and labeled. Each group had triplicate representation respectively. Four viable seeds of *Telferia occidentalis* were planted in each bag. Water was added to the soil samples when necessary to keep the soil samples moist. After germination/ emergence, and after two leaf stage, the seeds were thinned down to two plants in each bag. The germination/ emergence were recorded in all the groups. The experiment was allowed to stand for 50 days after which the leaves were harvested. The experiment was conducted from April to June, 2009.

Preparation of Extract:

Extract of the *Telferia occidentalis* leaves was obtained using sodium phosphate buffer (pH 7.4) according to the method as described by Levine *et al.* (1990). The aqueous extract from each group was used for the various analyses.

Reduced glutathione (GSH) concentration was determined by the method of Jollow *et al.* (1974). The method is based on the formation of a relatively stable chromophoric product on reacting with a sulphydryl compound with Ellman's reagent. The concentration of GSH in the extract was calculated using standard GSH. Protein thiol concentration was determined using the method of Levine *et al* . (1990). Total thiol concentration was determined by the summation of glutathione and protein thiol concentrations (Levine *et al.,* 1990) Ascorbic acid (AA) concentration was determined using the method of Roe and Kuether (1961). Lipid peroxidation was detected spectrophotometrically by assessing the level of thiobarbituric acid substances and expressed as malondialdehyde (MDA) equivalent. This was measured according to the method of Albro *et al* (1986). Protein content was determined using a protein test kit (Biosystem, USA) that utilizes the Biuret method as described by Gornall et. al. (1949) for protein determination.

Statistical Analysis:
Each determination was performed in triplicate. Data generated were expressed as means ± standard deviation and statistically analysed using one way Analysis Variance (ANOVA) with $p \leq 0.05$ taken to be significant.

RESULTS AND DISCUSSION

The indiscriminate discharge of palm oil effluent by palm oil mill operators and individuals who are involved in palm oil processing has become a common practice over the years, thereby polluting the surrounding environment where palm oil are processed. The palm oil effluent-pollution is commonly noticed in the South-South and South-Eastern regions of Nigeria.

The germination / emergence of *T. occidentalis* seeds in the two groups of soil samples (Table 1) revealed that the soil samples devoid of palm oil effluent (the control) had 100% germination/ emergence while those from palm oil effluent-polluted soil samples had 33% germination/ emergence respectively. The result revealed that palm oil effluent pollution significantly ($p<0.05$) delayed germination and inhibited emergence of some *T. occidentalis* seeds. The poor germination/ emergence from palm oil effluent-polluted soil samples was attributed to poor aeration. The inhibition of germination may also be due to absorption of palm oil from the effluent by the seeds, which caused some seeds to be slimmy, as observed when the seeds were excavated from the palm oil effluent-polluted oil. The palm oil in the effluent may also have reduced physical contact of the seeds to water and oxygen thereby inhibiting germination. There were marked differences in the result obtained from the germination of the seeds from the control compared to those from palm oil effluent-polluted soil.

Glutathione is an antioxidant located in the cytosol, nuclei and mitochondria and is a major soluble antioxidant in these cell compartments (Masella *et al.*, 2005). Glutathione function as antioxidant in many ways. It reacts chemically with singlet oxygen, superoxide and hydroxyl radicals thereby function as a free radical scavenger. It also stabilizes membrane structure by removing acyl peroxides formed by lipid peroxidation reaction (Price *et al*, 1998). The result obtained for glutathione from the aqueous leaf extract indicated that glutathione as an antioxidant was responsive to the effect of palm oil effluent on *Telferia occidentalis* leaves. This manifested in a significant ($p< 0.05$) reduction in the concentration of glutathione in the leaves of *T. occidentalis* from palm oil effluent-polluted soil sample (146.81 ± 0.01mg/ml) when compared to the control (387.23 ± 0.03 mg/ml) (Figure 1).

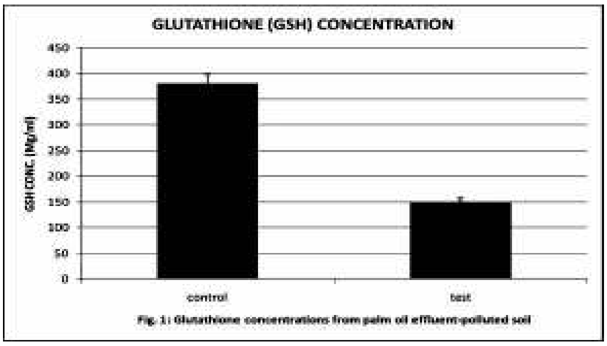

Fig. 1: *Glutathione concentrations from palm oil effluent-polluted soil*

Table 1:
Germination / emergence of *T. occidentalis* seeds in palm oil effluent-polluted soil and soil devoid of palm effluent

Groups	Soil Types	Germination/ emergence within 15 (days) 5 6 7 8 9 10 11 12 13 14 15	Total Germination	% Germination
1	Garden soil (Control)	2 0 1 1 1 1 0 0 0 0 0	6	100
2	Palm oil effluent-polluted soil	0 0 0 0 1 0 0 1 0 0 0	2	33

The significant reduction was expected as glutathione in the leaf extract of *T. occidentalis* from palm oil effluent-polluted soil might have been used to scavenge the reactive intermediates from palm oil effluent pollution or used in the transport of reduced sulphur from the leaves that sink into other plant tissues such as roots (Ruegsegger *et al.*, 1990).

Amino acids are building blocks of proteins. Amino acids and their denatures are used as markers of level of oxidative damage in plants (Leeawenbang *et al.,* 1990). The result obtained for protein thiol and total thiol are presented in (Figures 2 and 3). The results revealed that there were significant ($p<0.05$) reduction in the concentrations of these parameters in the aqueous leaf extract of *T. occidentalis* grown from palm oil effluent-polluted soil compared to those from the control. The reduction in the mean concentrations of protein thiol and total thiol are attributable to increased oxidative protein damage due to reactive intermediate from palm oil effluent pollution.

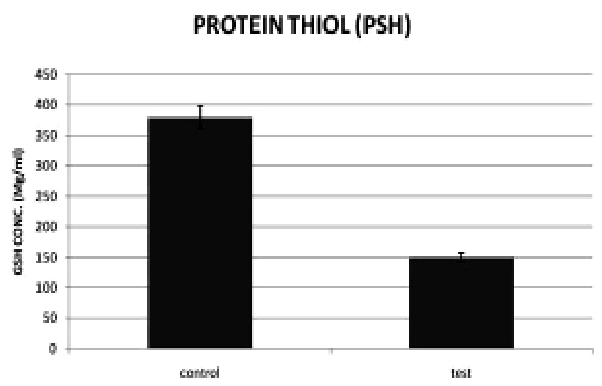

Fig 2: Protein Thiol concentrations from palm oil effluent-polluted soil

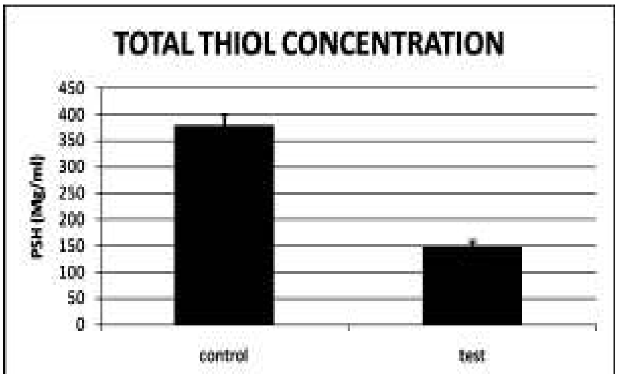

Fig. 3: Total thiol concentrations from palm oil effluent-polluted soil

Ascorbic acid is a water-soluble antioxidant molecule which scavenge reactive intermediates in the soluble cytoplasm. It readily donates electrons to reduce reactive intermediates in the cellular compartments. Palm oil effluent pollution significantly ($p < 0.05$) reduced the mean concentration of ascorbic acid (109.26 ± 0.05 mg/ml) in *Telferia occidentalis* leaves from palm oil effluent-polluted soil when compared to the value obtained for that from the control (284.72 ± 0.04mg/ml), this observed significant reduction in ascorbic concentration due to palm oil effluent pollution in the soil could be attributed to the involvement of ascorbic acid in reactions with reactive intermediate, thereby reducing its concentration in the *Teleferia occidentalis* leaves (Figure 4)

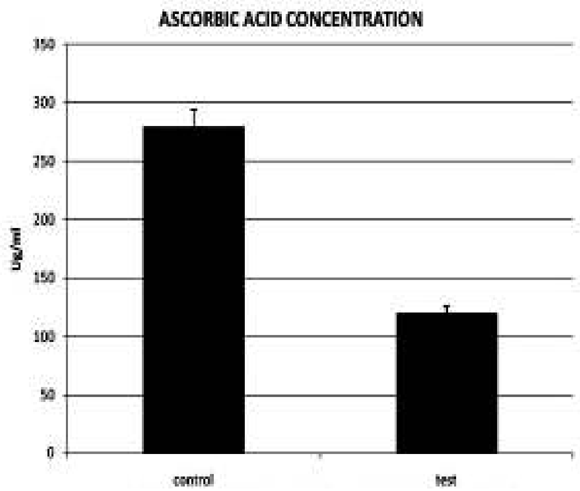

Fig. 4: *Ascorbic acid concentrations from palm oil effluent-polluted soil*

The result obtained for lipid peroxidation is presented in Figure 5. There was high concentration of malondialdehyde from the aqueous leaf extract of *T. occidentalis* from palm oil effluent-polluted soil when compared to that from the control. The observed significant ($p < 0.05$) difference is not surprising as the pollution caused increased peroxidation in membranes of living organisms (plants and animals). This finding agrees with the report of Eirewele (2001) who reported that petroleum hydrocarbons pollution induces oxidant stress in living organisms through lipid peroxidation. Reactive intermediates from palm oil effluent might have mediated the high lipid peroxidation observed in the aqueous leaf extract of *Telferia occidentalis* from palm oil effluent -polluted soil.

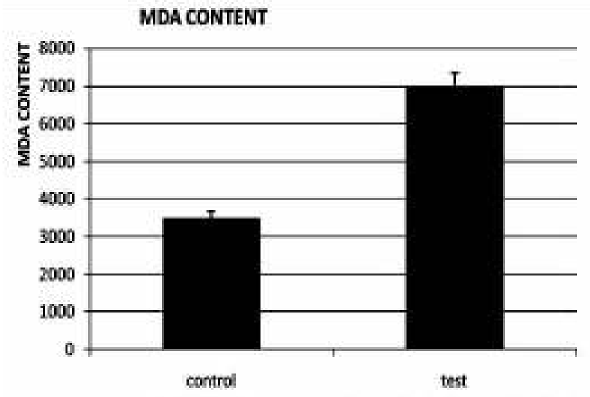

***Fig. 5:** Malondialdehyde concentration from palm oil effluent -polluted soil*

The result obtained for protein content from the aqueous leaf extract of *T. occidentalis* is presented in figure 6. The result revealed that *T. occidentalis* from palm oil effluent polluted soil significantly ($p < 0.05$) reduced protein content from 5.69 ± 0.08 to 17.52 ±0.01 mmol/L. The reduction is attributed to interference of pollution due to palm oil effluent in the soil physical and chemical properties which subsequently affected transpiration and photosynthesis in the plant's leaves, which might be related to the reduction in the total primary production of the plant thereby bringing about the reduction in the protein content.

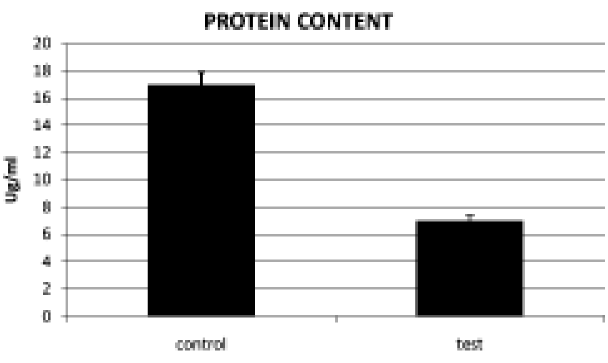

***Fig. 6:** Protein content from palm oil effluent-polluted soil*

Our results revealed that chronic exposure of *Telferia occidentalis* to palm oil effluent affected the germination of seeds and induced oxidative stress on the leaves. It is the opinion of the authors that greater environmental consciousness should be instilled into palm oil mill operators in order to discourage them from indiscriminate discharge of palm oil effluent through seminars and workshops as this unhealthy practice is not only an eye sore, it also affect the physical and chemical properties of the soil.

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