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Simulated crude oil pollution induced oxidative stress and modulates phytochemical production in *Rhizophora mangle* L.

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Abstract

In this study, pot experiment was carried out to determine the effects of crude oil pollution on the phytochemical production in *Rhizophora mangle.* Seedlings raised from propagules (viviparous seeds) were exposed to 1%, 3% and 5% concentrations of crude oil for 3 weeks. Physiological parameters as well as quantitative analysis of the phytochemical status of the seedlings were evaluated after the treatment period. The results showed that *R. mangle* seedlings were sensitive to crude oil toxicity and the effects were concentration dependent. It was observed that the treatment consistently reduced the phytochemical content as well as the growth index evaluated. However, there was a significant increase in the malondialdehyde level when plants were exposed to crude oil indicating oxy-radical production. The results may imply that crude oil treatment modulates phytochemical production and induced oxidative damage in *R. mangle*.

Keywords Pollution, growth, oxidative stress, secondary metabolites, *Rhizophora mangle*

Introduction

Mangroves form unique communities in tropical coastal regions and tidal lowlands. Nigeria has the largest mangrove forest in Africa occupying the lower stretches of the southern limit of the Niger Delta (NDES, 2000). Mangroves are mostly tropical trees or shrubs that grow between near mean sea level and the high spring tide mark in stable shores where they form distinct communities known as mangrove forest (Field, 1995). Mangrove vegetation provides shoreline protection to coastline communities and provide critical habitat for diverse marine and terrestrial flora and fauna. However, the healthy existence of mangrove forests in Nigeria is being threatened by various pollutants of anthropogenic sources which include: thermal pollution, oil spills, mining, and industrial as well as agricultural wastes (Odjegba, 2014).

Pollution caused by oil spills on plants has been shown to be directly proportional to the volume of spill (Baker, 1970). Amakiri and Onofeghara (1983) also reported that oil tends to act as a physical barrier that prevents absorption of nutrients and water required for plant growth and development. Whenever oil spill occurs, there is destruction of biodiversity, pollution of land, and damage to aquatic ecosystem (Suleiman, 1987). In the coastal areas of

Nigeria, spillages arising from accidents or sabotage occur frequently and plant species along these coastlines are affected. Disruption of biochemical processes, as well as reduction in water and nutrient uptake are among the common responses exhibited by these plants (Eriyamremu and Asagba, 2007).

Plant secondary metabolites are organic compounds synthesized by plants as part of defense system against herbivores and pathogens. They have also been implicated in conferring protection against environmental stresses in plants (Seigler, 1998). Plant secondary metabolites serve as unique sources for food additives, flavours, fragrances, dyes, insecticides and pharmaceuticals (Ravishankar and Rao, 2000).
The plant kingdom has proven to be the most useful hub in the treatment of diseases, and such a fit was attainable due to their contents of the secondary metabolites. The most important of these bioactive constituents are alkaloids, tannins, steroids, terpenoids, flavonoids, glycosides, saponins, and carotenoids (Ajayi *et al*., 2011). Accumulation of metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. The production of these compounds is often low (less than 1% of dry weight) and depends greatly on the physiological and developmental stage of the plant. These phytochemicals have been reported to exhibit haemolytic, antifungal, antibacterial, anti-inflammatory properties (Ramakrishna and Ravishankar, 2011).

The concentration of various secondary plant products is strongly dependent on the growing conditions and has impact on the metabolic pathways responsible for the accumulation of the related natural products. Expression levels of certain genes have been shown to increase in response to reactive oxygen species, cold treatment, high temperature, and osmotic stress (Tuteja, 2007). When plants are stressed, an exchange occurs between carbon to biomass production or formation of defensive secondary compounds (Bryant *et al*., 1983). A stress response is induced when plants recognize stress at cellular level. Secondary metabolites are involved in protective functions in response to both biotic and abiotic stress conditions (Ramakrishna and Ravishankar, 2011).

Formation of phenyl amides and accumulation of polyamines in bean and tobacco under the influence of abiotic stresses have been reported, suggesting antioxidative role of these secondary metabolites (Edreva *et al*., 2000). In the same vein, it was reported that *Rhizophora mangle* seedlings exposed to crude oil had increased levels of malondialdehyde, catalase and ascorbate peroxidise activities. Whether or not crude oil treatment also results in significant increase in the production of phytochemicals remain a subject of debate as this aspect has not been examined by past researchers. Therefore, in this paper, we present the effects of crude oil pollution on the growth and phytochemical production in *Rhizophora mangle*, a common mangrove species found in the coastal areas of Nigeria.

Materials and methods

Plant growth and treatments

Mature and healthy propagules (viviparous seeds) of *R. mangle* were collected in a single batch from Majidun estuary (06^{o} 36´ 20.2” N, 03^{o} 26´ 46.3” E) in Ikorodu area of Lagos State. For acclimation purpose, propagules were planted and grown for 4 weeks in a large plastic

bucket containing sediment collected from the estuary before oil treatment was effected. At the end of the acclimation period, seedlings of relatively equal height (12 cm) were selected and used for the study.

Bonny light crude oil was collected from Warri Refinery and Petrochemical Company in Delta State, Nigeria. The physicochemical properties of the oil were determined according to AOAC (2005). Soil treatment was done by manual mixing of weighed sediment with known volume of crude oil to achieve the required concentrations of 1%, 3% and 5% v/w oil/soil (Odjegba and Sadiq, 2002). Sediment without oil application served as control. One seedling was transplanted into each nursery bag representing each treatment, and replicated 12 times. The experimental set up was Completely Randomized Design (CRD) and stationed under natural light and photoperiod in the Botanical garden of the University of Lagos, Akoka. Samples were kept moist by adding water when necessary. The seedlings were allowed to grow in the treated soil and were harvested for analyses after 3 and 10 weeks of treatment to assess the short term and long term exposure effects.

Whole plant biomass determination

At the end of the treatment period, plants were carefully uprooted and rinsed with tap water. The shoot and the roots of each seedling were carefully severed from the propagule and were placed in labelled paper bags and oven dried at 65 ^{o}C for 72 hr. The dried samples were weighed on a digital top loading weighing balance (Mettler PM 34-K Delta) to determine the biomass accumulation.

Relative water contents of leaves

The second leaf on each plant was harvested for the determination of Relative Water Content (RWC) according to the method of Turner (1981) by using the formula:

RWC (%) = (fresh weight- dry weight) / (turgid weight- dry weight) x 100.

Lipid peroxidation evaluation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) content in the sample following the procedure of Wang and Jin (2005). Fresh root samples weighing 0.5 g were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 rpm for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid (TBA) solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450 nm, 532 nm and 600 nm. The concentration of MDA was calculated as $6.45(A_{532} - A_{600}) - 0.56\ A_{450}$.

Phytochemical analyses

The leaves of freshly harvested plants were collected from both the control and the treated plants for phytochemical analyses. Samples were rinsed in a running tap water and air dried at room temperature in the laboratory. The qualitative and quantitative analyses of alkaloid, flavonoid, glycoside, phenol, saponin, steroid and tannin present in the plant were conducted

using standard procedures as described by Harborne (1973); Sofowora (1980); Trease and Evans (1989).

Statistical analysis

Each analysis was conducted in triplicate. Numerical data were analysed using one way analysis of variance (ANOVA) and the results presented as mean ± standard error of mean (Zar, 1999).

Results

The physicochemical analyses of crude oil and soil showed that both have trace amount of cadmium, sulphur, nickel and lead. The density of the oil was 0.82 cm^3, while its percentage ash content was 0.072 % (Table 1).

Table 1 Physicochemical properties of the crude oil and soil.

Parameters	Levels detected	
	Soil	Oil
Density	N/A	0.82 cm^3
THC (%)	N/A	84.57
Ash (%)	N/A	0.072
pH	7.45	N/A
E.C.	36.4	ND
TOM (%)	42.5	ND
Cd (mg/kg)	0.19	0.08
Ni (mg/kg)	0.07	0.02
Pb (mg/kg)	0.32	0.13
S (mg/kg)	0.01	0.04

ND denotes Not Detected, TOM (Total organic matter), THC (Total hydrocarbon).

On the effect of crude oil treatment on the whole plant biomass, it was observed that crude oil consistently reduced the biomass production; the severity of the effect however increased with the concentration. The control plants had a mean value of 7.09 ± 0.05 g, while plants treated with 5 % crude oil had a mean value of 5.21 ± 0.02 g (Fig 1).

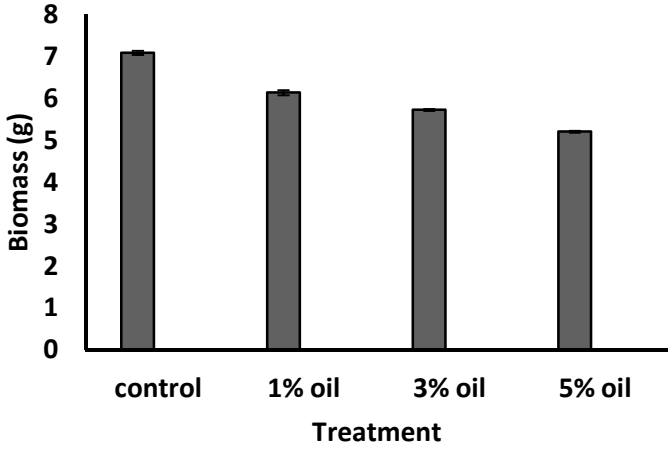

Fig. 1. Whole plant biomass of *R. mangle* seedlings treated with different concentrations of crude oil for 3 weeks. Data are means ± SE (n = 3).

The effect of crude oil treatment on the relative water content of *R. mangle* showed that exposure to crude oil caused a significant ($p<0.05$) reduction in the relative water content. After 3 weeks of exposure, the relative water content of the control had a mean value of 32.59 ± 0.06 % while plants treated with 1%, 3% and 5 % crude oil for the same period had 30.82 ± 0.40, 27.7 ± 0.28 and 19.81 ± 0.37 % respectively (Fig 2).

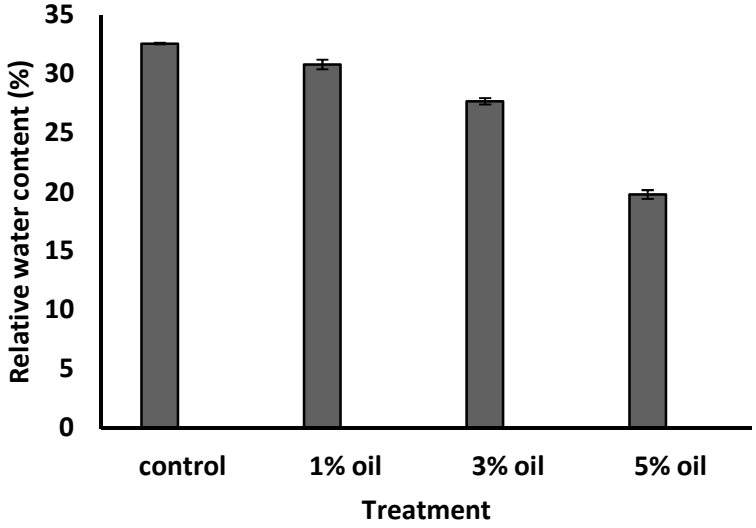

Fig. 2. Relative water content of *R. mangle* leaves as affected by crude oil treatment. Data represent means ± SE (n = 3).

It was observed in this study that crude oil treatment led to a significant increase in lipid peroxidation. After 3 weeks of exposure, the malondialdehyde content of the control was 4.15 ± 0.00 mg/g fresh weight as against 6.63 ± 0.09 mg/g fresh weight observed for plants treated with 5 % crude oil (Fig. 3).

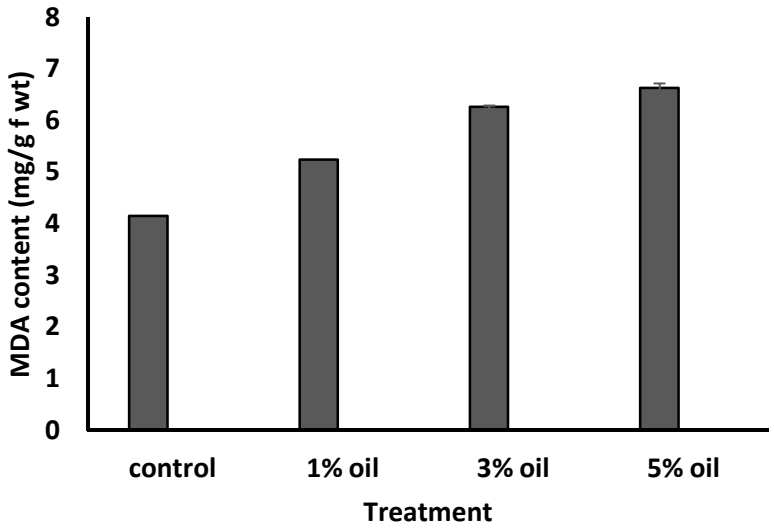

Fig. 3. Lipid peroxidation in root tissue of *R. mangle* after exposure to crude oil. Data are means ± SE (n = 3).

Oil pollution generally had significant effect on phytochemical production in *R. mangle* leaves. It was observed that after 3 weeks of treatment, the control plants had a mean alkaloid content of 46.41 ± 1.56 mg g^{-1} dry weight, while seedling grown in soil amended with 1%, 3% and 5% crude oil had 46.34 ± 4.46, 36.45 ± 0.83, and 21.72 ± 2.84 mg g^{-1} dry weight respectively (Fig. 4).

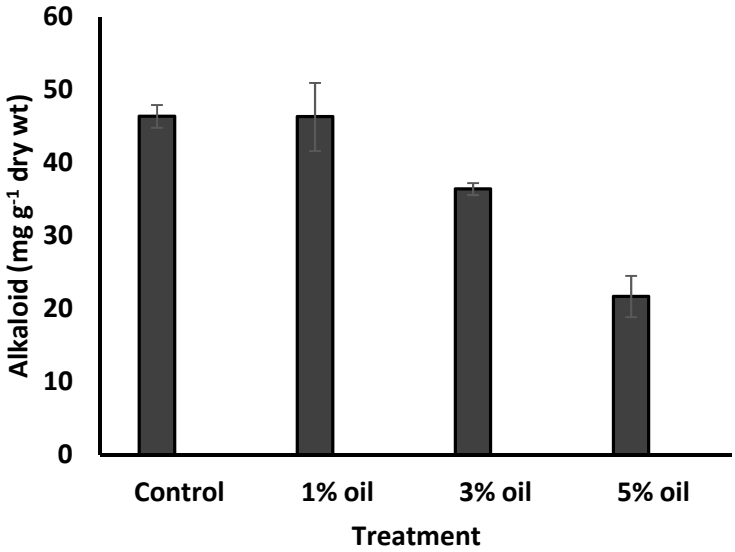

Fig. 4. Total alkaloid content in *R. mangle* leaves after exposure to crude oil. Data represent means ± SE (n = 3).

On the effect of crude oil treatment on cardiac glycoside production in *R. mangle,* it was observed that exposure of the plant to crude oil significantly reduced the cardiac glycoside concentration in individual seedling. The cardiac glycoside concentration in the control plants

was 26.47 ± 0.44 mg g^{-1} dry weight, this value was significantly higher than 24.66 ± 0.32, and 19.00 ± 0.55 mg g^{-1} dry weight that were observed in plants treated with 3 %, and 5 % crude oil respectively (Fig. 5).

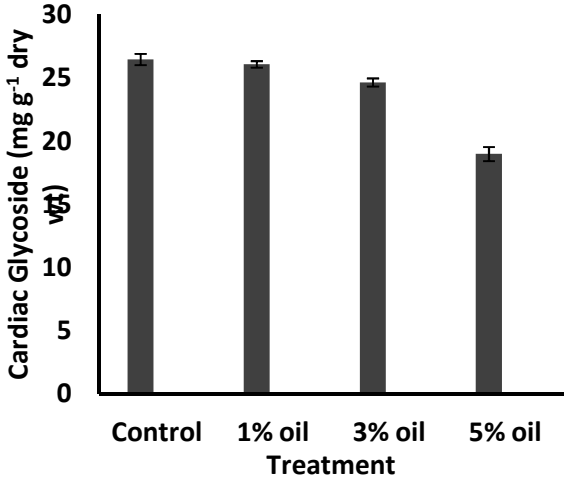

Fig. 5. Cardiac glycoside content in *R. mangle* leaves as affected by crude oil. Data are means ± SE (n = 3).

It was also observed that exposure of *R. mangle* seedlings to crude oil significantly lowered the level of flavonoid concentration. Seedlings that were treated with 5 % crude had a mean value of 18.51 ± 0.46 mg g^{-1} dry weight as against 25.49 ± 1.01 mg g^{-1} dry weight observed for the control (Fig 6).

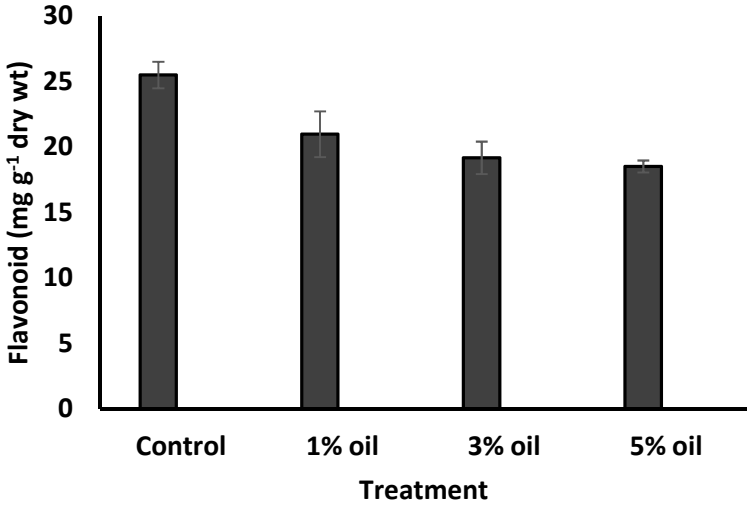

Fig. 6. Effect of crude oil treatment on flavonoid production in *R. mangle*. Data represent means ± SE (n = 3).

The total phenol content in *R. mangle* leaves after exposure to crude oil also shows the same trend. Crude oil treatment significantly reduced the concentration of phenol in the plant. It was observed that after 3 weeks of treatment, the control had a mean value of 3.32 ± 0.02 mg g^{-1} dry weight, while plants that were exposed to 1%, 3%, and 5% oil had 2.63 ± 0.08, 2.39 ± 0.07, and 2.29 ± 0.15 mg g^{-1} dry weight respectively (Fig. 7).

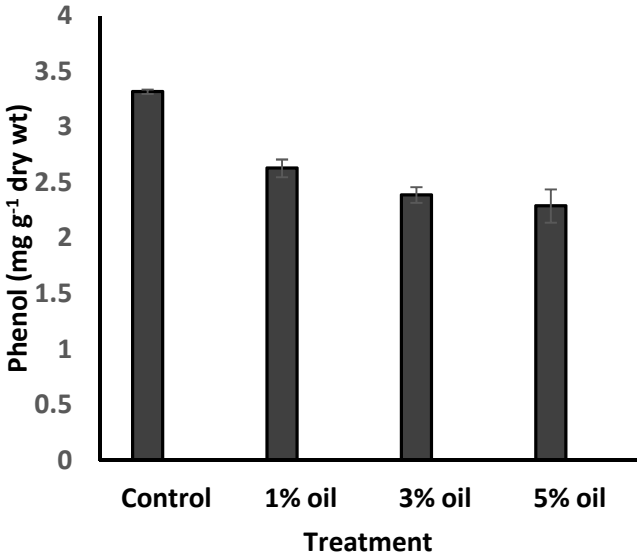

Fig. 7. Total phenol content in the leaves of *R. mangle* after a short term exposure to crude oil. Data are means ± SE (n = 3).

The effect of crude oil treatment on *R. mangle* seedlings as regard saponin production showed that crude oil treatment consistently reduced saponin concentration in the leaves of *R. mangle*. Significant difference ($p < 0.05$) was observed between the saponin concentration in the control and those of the treated plants. After 3 weeks of treatment, the control plants had a mean value of 20.11 ± 0.20 mg g^{-1} dry weight, while plants treated with 1 %, 3 %, and 5 % crude oil respectively had mean values of 15.94 ± 0.39, 13.96 ± 0.26, and 13.92 ± 0.25 mg g^{-1} dry weight (Fig. 8).

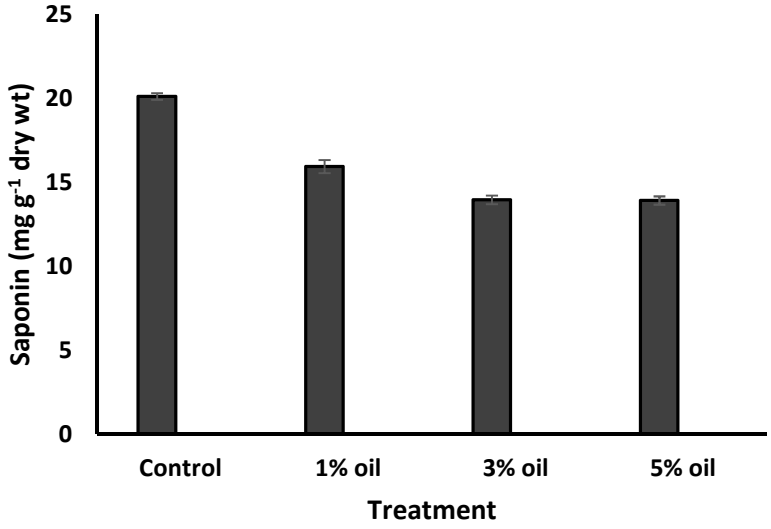

Fig. 8. Effect of crude oil treatment on saponin production in *R. mangle* leaves. Data are means ± SE (n = 3).

There was a significant reduction in the concentration of steroid present in the *R. mangle* leaves under simulated crude oil pollution (Fig. 9). The control plants had the highest steroid concentration in their leaves. The concentration of the phytochemical diminished as the oil concentration increased.

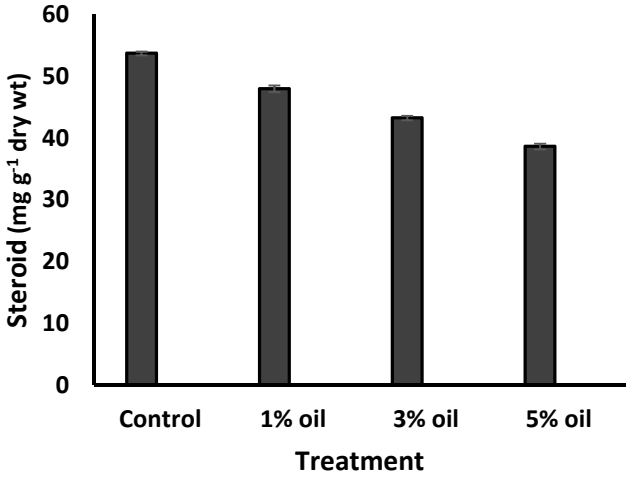

Fig. 9. Steroid concentration in the leaves of *R. mangle* after exposure to crude oil. Data are means ± SE (n = 3).

The tannin content in *R. mangle* leaves after a short term exposure to crude oil is presented in Fig. 10. Short term treatment with 3 % and 5 % crude oil for 3 weeks induced significant reduction in tannin concentration. It was observed that the control plants had a mean value of 41.72 ± 2.03 mg g^{-1} dry weight while 41.50 ± 2.27, 36.28 ± 1.26 and 24.37 ± 1.83 mg g^{-1} dry weight were observed for plants treated with 1 %. 3 % and 5 % oil respectively.

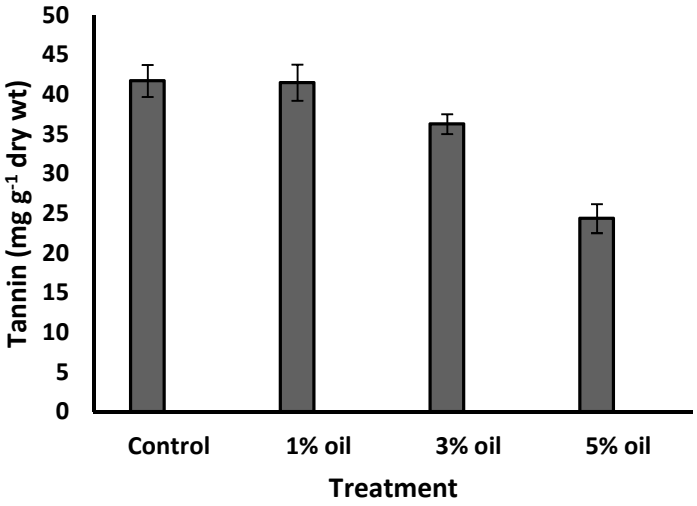

Fig. 10. Tannin concentration in the leaves of *R. mangle* as affected by crude oil treatment. Data represent means ± SE (n = 3).

Discussion

This study examined the crude oil effects on *R. mangle* seedlings when the propagules were anchored vertically in the sediment, a situation that mimicked the natural environment. Mangroves are susceptible to oil pollution as they are periodically inundated by tides carrying oily and water soluble fractions of the crude oil and also the refined products washed into the water body from the shores.
The growth index represented by whole plant biomass was significantly reduced in oil-treated seedlings. Zhang *et al*. (2007) reported that oiling reduced biomass of *Bruguiera gymnorrhiza*. The growth inhibition by oil could be partly due to the disturbances and imbalances of accumulation of nutrients.

The low relative water content observed in the leaves of oil-treated seedlings of *R. mangle* could be attributed to the disruption of the normal plant-water relations caused by oil application. Oily sediment repels water and this will consequently affect water absorption by plant roots. Similar observation was reported by Badejo and Odjegba (2014) that crude oil application significantly reduced the relative water content in *Capsicum annum*.

It has been reported that free radicals are produced in excess under environmental stresses such as heavy metals and oil pollution, and this caused lipid peroxidation and cellular membrane damage (Acworth and Bailey, 1997). The malondiadehyde (MDA) assay is a reliable diagnostic test for free radicals formation and its consequent cellular damage in a biological system (Halliwell and Chirico, 1993). It was observed that MDA content increased with crude oil treatment concentrations, an indication that damage caused by crude oil to plants could partly due to oxidative stress. Similar result was reported by Eriyamremu and Asagba (2007) that crude oil treatment increased the MDA content in *Phaseolus vulgaris* and *Zea mays*.

The phytochemical screening as well as the quantitative analysis of the untreated plants revealed high concentration of alkaloids, steroids and tannins; a moderate level of cardiac glycosides, flavonoids as well as saponins; while low concentration of phenol was observed. Previous works also showed similar findings (Fernandez *et al*., 2002; Kandil *et al*., 2004). However, a decrease in the quantitative values of the phytochemicals due to crude oil treatment concentrations was observed. Zobayed *et al*. (2007) stated that the synthesis of the basic skeletons for active secondary metabolites is dependent on the carbon assimilated during photosynthesis. The net photosynthetic rates decreased when plants are under stress such as drought, high temperature, high salinity, heavy metals, and oil pollution (Murch *et al*., 2003). The low concentrations of phytochemicals observed in oil-treated seedlings could be related to limited carbon assimilation that may consequently affect carbon allocation for secondary metabolite production.

The presence of these phytochemicals in high concentrations suggests that *R. mangle* is a potential source of useful pharmaceutics in the future. It has been reported that various parts of this plant are used in folk remedies to treat different ailments such as asthma, convulsion, diarrhoea, dysentery, stomach ache, dyspepsia, elephantiasis, and haemorrhage (Duke and Wain, 1981).

The data generated in this study provide the evidence of detrimental effects of crude oil pollution on the phytochemical production in *R. mangle*. However, the cellular mechanisms involved in the regulation of synthesis and accumulation of the secondary metabolites evaluated in this study, by crude oil remain to be explored.

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