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Growth and Anatomy of *Amaranthus Hybridus* as Affected by Diferrent Crude Oil Concentrations

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Abstract: The response of the growth and anatomy of *Amaranthus hybridus* in provoked crude oil contaminated soil was investigated using 1-4% v/w crude oil and a control (0%). For growth parameters like plant height, number of leaves, leaf area, plant fresh weight and plant dry weight, the mean values obtained were higher for control (0%) and progressively decreased from 1-4%. The 4% concentration was lethal to the *A. hybridus* grown on it as they all died within 10 days after planting. *A. hybridus* grown in higher concentrations (2-3%) exhibited greater sinuosity in their epidermal cell walls than those grown in 0 and 1%. Stomatal index of leaves of *A. hybridus* progressively decreased as the crude oil concentration of soil increased. Also the thickness of walls of the cortical cells was more prominent in *A. hybridus* grown in crude oil polluted soil than in the control. The cortical cells of the roots and stems of *A. hybridus* in 2-3% polluted soil were flattened tangentially and smaller in size compared to that grown in control which had round polygonal cells that appeared larger. These changes in anatomy of *A. hybridus* due to contamination by crude oil were discussed as possible pollution indicators.

Key words: *Amaranthus hybridus* · Anatomy · Concentration · Crude Oil · Epidermis · Growth

INTRODUCTION

Crude oil in soil makes the soil condition unsatisfactory for plant growth [1], due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron and zinc [2]. Plants are highly susceptible to oil exposure and this may kill them within a few weeks to several months. There are several vegetal species that are capable of growing in soils polluted with hydrocarbons and they participate in their degradation through the rhizosphere, part of the root, which favours the growth of several microorganisms [3] and increases biomass and microbial activity, accelerating degradation processes [4].

Apart from using anatomy in the systematic of plants, some other workers have also used anatomy of plant to monitor environmental pollution. Sharma *et al.* [6] have reported morphological and stomatal abnormalities as an effect of environmental pollution on plants. Also Gill *et al.* [6] reported that stomata in *Chromolaena odorata* were grossly affected by crude oil which manifested as distortion and reduction in the number of stomata per unit area of the leaf. Other workers have mentioned the effects of crude oil on the growth and physiology of different plants [3, 6-8].

The plant *Amaranthus hybridus* Linn., a nutritious and leafy vegetable is a member of the Amaranthaceae family. It has a variety of uses, of which the common use is as a vegetable. *A. hybridus* is also used in the treatment of intestinal bleeding, diarrhea and excessive menstruation [9, 10]. A tea made from the leaves is astringent [11].

Edeoga and Otoide [12] reviewed the characteristics of leaf epidermal morphology of some *Amaranthus* species in Nigeria and they reported that the architecture of the epidermal cells was relevant to the taxonomy of *Amaranthus* species investigated. Odjegba and Sadiq [13] have investigated the effects of spent engine oil on some growth parameters of A. hybridus. Some other workers have also the effect of crude oil on growth and anatomy of different plants [2, 6, 14, 15].

The objectives of this study therefore, is to investigate the changes in growth and anatomy of *A. hybridus* grown in a crude oil contaminated soil and possible use of these changes in anatomy as a phytomonitoring technique of crude oil pollution.

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MATERIALS AND METHODS

Collection of samples: The soil used in study was obtained from the top soil (0-20cm) collected within the campus of Michael Okpara University of Agriculture, Umudike. Dried seeds of *A. hybridus* were bought from the local market. The crude oil was got from Nigeria National Petroleum Cooperation (NNPC).

Soil treatment: The soil was sterilized in the autoclave at 1.3 Kg cm^{-3} for six hours. The contamination with the crude oil was done by thoroughly mixing with the soil in their respective plastic buckets. Soil of 4000g was treated with 40ml, 80ml, 120ml and 160ml of crude oil to obtain 1, 2, 3 and 4% v/w crude oil contamination. Each treatment including the control (0%) was replicated five times.

Germination: Seedlings were reduced to five seedlings per pot five days after germination. Germination was observed as the emergence of a seed leaf the soil surface. Treatments were watered daily.

Design of the experiment: The design of the experiment was a completely randomized one. One way analysis of variance (ANOVA) was carried out and the Least Significance Difference (LSD) test was used to compare the means.

Recording of growth parameters: Growth parameters recorded were plant height, number of leaves, leaf area, plant fresh weight and plant dry weight. Plant height was measured from the soil level to the terminal leaf using a metre rule. Leaf area was determined by tracing the number of squares covered by the leaf on a graph sheet. Each square equals $1cm^2$. Fresh and dry weights were determined at 9 weeks after planting. Dry weight was determined by oven drying at 80°C for five days.

Anatomical studies: Some mature and fresh parts of the leaves, stems and roots of *Amaranthus hybridus* were collected. These plant parts were fixed in FAA (Formalin, acetic acid and alcohol in ratio of 1:1:18 respectively) for 48 hours. These plant parts were washed in water and sectioned with a Reichert rotary microtome. The sections were first stained with two drops of alcian blue for three minutes. The alcian blue stain is washed off and the sections are counter stained with safranin solution for about two minutes and then dehydrated with pure xylene at intervals for few seconds. The sections are finally mounted on slides using Canada balsam. A hot plate of 40°C was used to dry the slides.

Epidermal peels: Epidermal peels were obtained by soaking parts of the fresh leaves in concentrated nitric acid in a water bath for about 30-40 minutes. The plant samples were then carefully washed in water and the lower and upper epidermis teased from the mesophyll using dissecting needles and forceps. The epidermal peels were stained with safranin solution for about three minutes and washed off with water before mounting in glycerine. The slides of the anatomical sections and the epidermis were observed under a light microscope.

RESULTS

The results are presented in Table 1-6. The control (0%) had the highest mean plant height at 9 WAP (weeks after planting), while the lowest (9.00cm) was observed in the 3% treatment (9WAP). The difference in height was found to be very significant (P = 0.05). However there was no significant difference in plant height at 3 WAP between 2 and 3% crude oil treated *A. hybridus.*

The mean number of leaves obtained is shown in Table 2. The highest value the highest values were obtained for plants grown in 0% crude oil contaminated soil. The difference in treatment was also observed to be significant (P = 0.05. Table 3 shows the mean leaf area of *A. hybridus* plants grown in crude oil contaminated soil at 3, 5, 7, 9WAP. The highest values were obtained in 1 and 0% (control) crude oil concentration. Least values were obtained in 3% crude oil concentration. The difference in treatment and control were found to be significant (P = 0.05). No difference was found between 3 and 4%. Data obtained for plant fresh weight and plant dry weight showed significant differences between the control (0%), 1, 2 and 3% crude oil treatment (Table 4).

Epidermal characteristics: The epidermal cells of the *Amaranthus hybridus* control were mostly isodiametric in shape, while that of the 1% crude oil treatment had epidermal cells that were isodiametric-polygonal in shape. From 2-3% treatment, the irregularity of their shape increased. The anticlinal cell walls of the upper and lower epidermis of 0% treatment *A. hybridus* leaves were mostly straight, that of 1% were curved. The lower epidermis of 2% treated *A. hybridus* was also curved but the upper epidermis of 3% treated. *A. hybridus* and both the lower epidermis and upper epidermis of 3% treated *A. hybridus*

Table 1: Mean plant height of *A. hybridus* treated with different crude oil concentrations

Crude oil concentration	Plant height (cm) Weeks after Planting (wap) 3	5	7	9
0%	9.36[a]	16.24[a]	23.96[a]	44.64[a]
1%	5.42[b]	131.10[b]	16.88[b]	31.54[b]
2%	3.94[c]	9.34[c]	15.12[b]	21.20[c]
3%	2.98[c]	3.88[d]	5.44[c]	9.00[d]
4%	0.00[d]	0.00[e]	0.00[d]	0.00[e]

a, b, c,…………within each column, means with the same letter are not significantly different

Table 2: Mean number of leaves of *A. hybridus* treated with different crude oil concentrations

Crude oil concentration	No. Of leaves weeks after planting (wap) 3	5	7	9
0%	20.00[a]	30. 20[a]	42.20[a]	51.40[a]
1%	9.40[b]	15.00[b]	18.60[b]	23.80[b]
2%	5.20[c]	9.60[c]	13.80[c]	16.00[c]
3%	3.80[c]	6.80[d]	8.60[d]	10.00
4%	0.00[d]	0.00[e]	0.00[e]	0.00[d]

a, b, c,…………within each column, means with the same letter are not significantly different

Table 3: Mean leaf area of *A. hybridus* treated with different crude oil concentrations

Crude oil concentration	Leaf area (cm2) Weeks after Planting (wap) 3	5	7	9
0%	19.47[a]	28.86[a]	44.41[a]	83.85[a]
25%	11.60[b]	19.55[b]	25.33[b]	30.81[b]
50%	5.16[c]	8.88[c]	11.99[c]	16.94[c]
75%	0.65[d]	1.02[d]	2.31[d]	3.31[d]
100%	0.00[d]	0.00[d]	0.00[d]	0.00[d]

a, b, c,…………within each column, means with the same letter are not significantly different

were sinuous in shape. The stomatal index was highest (18.45%) (Table 5) in the lower epidermis of control experiment of *A. hybridus* and lowest (11. 63%) in 3% crude oil treatment *A. hybridus.* This trend was the same in the upper epidermis. The highest (12.90%) was observed in 0% crude oil treated *A. hybridus* and the lowest stomatal index was in the 3% crude oil treated *A hybridus.* The epidermis of all the *A. hybridus* investigated, had the anisocytic stomata type.

Table 4: Mean plant fresh weight and dry weight of *A. hybridus* treated with different crude oil concentrations at 9 weeks after planting

Crude oil concentration	Plant fresh weight (g) (9WAP)	Plant dry weight (g) (9WAP)
0%	131.30[a]	29.31[a]
1%	44.07[b]	10.25[b]
2%	24.95[c]	6.28[c]
3%	5.20[d]	1.18[d]
4%	0.00[d]	0.00[d]

a, b, c,…………within each column, means with the same letter are not significantly different

Table 5: Stomatal index of upper and lower epidermis of *A. hybridus* grown in different crude oil polluted soils

A. hybridus grown on different concentrations of crude oil polluted soil	*Stomatal index (Upper Epidermis)	(Lower Epidermis)
0%	12.90±2.34	18.45±3.55
1%	9.37±5.23	15.00±4.67
2%	9.09±5.43	12.77±3.53
3%	3.03±0.97	11.63±2.33
4%	-	-

*=mean±S.D.

Leaf anatomy: The epidermis in all the crude oil treated *A. hybridus* were one cell thick and conspicuous. There is a layer of cuticle over the epidermis in all the treated *A. hybridus.* These layers in the entire leaf cuticle were very thick and prominent over the epidermis of 2 and 3% crude oil treated *A. hybridus*. The palisade mesophyll is two layered. The spongy mesophyll cell are elongated lying parallel to the surface of lamina and occupies about half the entire mesophyll. Multicellular epidermal hairs were observed on both the lower and upper epidermal layers of all treatment.

Stem anatomy: The stem epidermis is made up of a single row of cells flattened tangentially and fitting closely along their walls. A well-defined cuticle extends over the epidermal cells. This cuticular layer is much thicker in the 2 and 3% treated. *A. hybridus.* In all the *A. hybridus* there were presence of multicellular epidermal hairs. The cortex of all *A. hybridus* consists of parenchyma cells. These are large polygonal thin walled cells with intercellular spaces. From the 1-3% crude oil treated *A. hybridus* the size of these parenchyma cells decreased. The outer parenchyma cells were most affected. The intercellular air spaces also reduced. Vascular bundles are collateral and open and arranged in a ring. Phloem lies externally and the xylem lies internally.

Table 6: Significant anatomical features of *A. hybridus* grown in different concentration of crude oil polluted soil

	Concentration of crude oil in soil			
Anatomical feature	0%	1%	2%	3%
Epidermal cell shape	isodiametric	isodiametric-irregular	irregular	irregular
Anticlinal cell walls UE	straight	curved	sinuous	sinuous
LE	straight	curved	curved	sinuous
Curticular layer of epidermis	thin	thin	thick	Thick
Shape of stem cortical cells	round	round-flattened tangentially	flattened tangentially	flattened tangentially
Shape of root Cortical cells	round	flattened tangentially	flattened tangentially	flattened tangentially
Intercellular air space of stem and root cortex	large	medium	reduced	reduced
Parenchyma cells	large	large	small	small

Root anatomy: The epidermal cells of all the *A. hybridus* are single layered. Flattened tangentially below the epidermis are cortical parenchyma cells visible in the *A. hybridus* grown in the 1-3% crude oil treated soil. The cortical parenchyma of 0% treated *A. hybridus* were round polygonal cells just like that observed in the stem cortex. A few layers of radially elongated cells lying in between the vascular bundles constitute the medullary rays. The pith of all the *A. hybridus* were very elaborate and extend from the vascular bundles to the center.

DISCUSSION

Few seeds of *A. hybridus* planted in the 4% crude oil treated soil germinated but very slowly and all eventually died at about 10 days after planting. Oil contaminated soils generally causes delayed seed emergence [16]. This is due to poor wettability and aeration of the soil [15] and loss of seed viability [17]. The progressive depression in height, numbers of leaves and leaf area of *A. hybridus* treated with different crude oil concentration is as a result of changes in soil condition, which imposed stressful conditions. These stress condition interfered with water uptake and gaseous exchange. This may have created a condition of physiological drought.

The disruption of soil physical properties by crude oil with anaerobic and hydrophobic condition was found largely responsible for reduction in plant growth [18]. Smith *et al.* [19] also reported stomatal closure and decrease in leaf, stem and root dry weight in poorly aerated soils. This can be attributed to the decrease in plant dry weight and plant fresh weight as the crude oil concentrations increased.

The high survival rate of the 1% crude oil treatment as shown by the comparatively high growth parameters recorded is due to low level of crude oil contamination. Anoliefo and Edegbai [20] reported that low level of oil pollution could be easily be degraded by natural rehabilitation in soils, increase organic matter in soil and improve the fertility, physical and chemical properties of the soil.

The values of the growth parameters decreased as oil concentration increased. This effect can be attributed to the fact that the crude oil formed a hydrophobic lager over the root, which limited absorption of water and nutrients [3]. Pezeshki and Delaune [7] stated that effect of crude oil on plants could be short term under field conditions, since plants would likely recover once residual oil is removed by rainfall or tidal action.

The irregular epidermal cell shape and the increasing level of sinuosity observed in the 1-3% treated *A. hybridus* were due to morphological aberrations. Sharma *et al*. [5] has reported the adverse effect of environmental pollution on plant growth to range from morphological aberrations, reduction in biomass to stomatal abnormalities. Esau [21] stated that the degree of sinuosity of epidermal cells is dependent on the degree of force exerted on the stomata in their course of development. This is evident in anatomy of *A. hybridus* grown in the provoked oil contaminated soilbecause of the stress condition caused by the physiological drought. Gill *et al.* [6] reported that stomata in C*hromolaena odorata* were grossly affected by crude oil, which manifested as distortion and reduction in the number of stomata per unit area of the leaf. This same condition was observed in this present study. The stomata index of the control of *A. hybridus* was higher than that of the treated *A. hybridus* in the 1-3% treated *A. hybridus.*

The reduction in parenchyma cell and thickness of their cell wall of the roots and stems in treated *A. hybridus* could be attributed to the earlier notion of a condition of physiological drought. This caused the cell to be smaller and the cell walls thickness as a way of reducing water loss.

In the present study, crude oil caused a reduction in the growth parameters considered viz plant height, numbers of leaves, leaf area, plant dry weight and plant fresh weight under laboratory conditions. From the range of 1-3% contamination, there were no lethal effects but at 4% treatment, the plants all died about 6-10 days after planting. There was a similar trend of effect in the anatomy of the treated *A. hybridus.* Under field conditions the plants would likely recover once residual oil is removed by rainfall or tidal actions. However these effects are likely to be short-term. These results are supported by previous studies in Nigeria where the effect of crude oil has been used in different plants [6, 8, 13, 15, 20, 22].

These visible significant anatomical changes seen in all the A. hybridus grown in the crude oil contaminated soil could be employed as an index of monitoring environmental pollution. This investigation can also be done in other plant species to ascertain their possible use in phytomonitoring.

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