EFFECTS OF DUAL PURPOSE KEROSENE (DPK) ON THE GROWTH AND LEAF EPIDERMAL FEATURES OF *IPOMOEA AQUATICA* (ORDER – SOLANALES, FAMILY – CONVOLVULACEAE) IN AN EXPERIMENTALLY CONTAMINATED SOIL

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Abstract
The effects of Dual Purpose Kerosene (DPK) on the growth, survival and leaf epidermal features of Ipomoea aquatica Forsssk. (water spinach) were investigated. Seedlings were transplanted into plastic pots and grown for six weeks in soils irrigated twice a week with five different concentrations of DPK (0%, 25%, 50%, 75 % and 100%). Percentage survival of the plants declined from 100% in the Control to 75% in 25% DPK and to 0% in the 75% and 100% DPK concentrations. Plant height was significantly (p<0.05) reduced as the concentration of the pollutant increased compared with the Control. Leaf epidermal features in terms of stomata density, index and size were significantly greater in the Control plants than those obtained from plants in polluted soils. Other effects of DPK on the plant included stunted growth, chlorosis and death of the test plant as concentration of the pollutant increased. These results, therefore suggest that DPK has the potential of impairing plant growth and reducing some leaf epidermal features of the test plant.

Keywords: kerosene, growth, leaf epidermis, *Ipomoea aquatica*

Introduction
Pollution is the alteration of physical, chemical and biological characteristics of the biosphere, resulting from the release of unwanted materials notably wastes in amounts capable of becoming deleterious to the well-being and survival of biotic communities. Pollutants are generated by industries, agriculture, businesses, schools, vehicles, and even our homes, and when poorly managed contaminate the ecosystem and could render our environment unfit for habitation by life forms. Kerosene, sometimes spelled kerosine in scientific and industrial usage, also known as paraffin or paraffin oil in the United Kingdom, Hong Kong, Ireland and South Africa, is a combustible hydrocarbon liquid. Kerosene is one of the products obtained during fractional distillation of petroleum. This product is widely used in aviation industries as fuel to power jet-engined aircraft (jet fuel) and some rockets, but is also commonly used as a heating fuel and for fire toys such as poi as well as in homes for cooking in stoves and lightening in lanterns. In parts of Asia, where the price of kerosene is subsidized, it fuels outboard motors rigged on small fishing craft. The exploration, transportation and utilization of this petroleum product may pose serious dangers to the lives of both flora and fauna in the environment. For instance, Mckendrick (2002) reported that petroleum products have adverse effects on plants irrespective of their habits or habitats. Such effects are usually manifested as reduced and retarded growth, cellular and stomata abnormalities, morphological defects and irregular physiological processes such as alteration of normal plant water relation, nutrient availability, chlorosis and in extreme cases death.

Crude oil and its products such as diesel oil, lubricating oil and used engine oil have been variously reported to cause growth retardation in many plant species. Ogbo (2009) observed that 2% level of diesel fuel reduced radicle length in *Sorghum bicolor* and *Zea mays* than in *Arachis hypogaea* and *Vigna unguiculata.* Growth and yield of maize in terms of plant height, root length, root number, leaf area, ear length and grain yield, with the exception of stem girth, were observed to be significantly ($p< 0.05$) greater in the control plants (unpolluted soil) than those polluted with oil (Okonokhua *et al.,* 2007). Anoliefo and Vwioko (1995) reported that the contamination of soil with spent engine oil caused growth retardation in plants, with the effect more adverse on tomato (*Lycopersicom esculentum*) than pepper (*Capsicum annum* L.). Similarly, Odjegba and Idowu (2002) reported that germination of *Amaranthus hybridus* seeds was adversely affected when grown in spent engine oil –polluted soil.

Considering the adverse effects of petroleum products on the growth of crops, there is need to ascertain and monitor the pending risk or potential damage level of these pollutants from time to time. The dearth of information of the effect of these pollutants on the anatomical features of plants equally calls for investigation in this regards. In this work, we are focusing on an aquatic plant, *Ipomoea aquatica* Forssk. (water spinach, swamp morning glory) of family Convolvulaceae which belong to the order Solanales*. I. aquatica* is an aquatic leafy vegetable. The plant is a noxious weed of rice. In Southeast Asia, the vegetable is a common ingredient in dishes. In Singapore, Indonesia and Malaysia, the leaves are usually stir-fried with chile pepper, garlic, ginger, dried shrimp paste and other spices. In Penang and Ipoh, it is cooked with cuttlefish and a sweet and spicy sauce. In Laos, it is frequently stir-fried with oyster sauce or yellow soybean paste, and garlic and chillies. In Vietnam, *I. aquatica* once served as a staple vegetable of the poor. In the south, the stems are julienned into thin strips and eaten with many kinds of noodles. It is used as a garnish, as well. It has become a common ingredient of Vietnamese cuisine. In the Philippines, is usually sautéed in cooking oil, onions, garlic, vinegar, and soy sauce. This dish is called *adobong kangkong*. It is also a common leaf vegetable in fish and meat stews, such as *sinigang*. An appetizer in the Philippines, called crispy *kangkong*, uses the leaves coated with batter and fried until crisp and golden brown. In Bangladesh and West Bengal, it is stir-fried preparation of the leaves is a very popular dish. Of concern, the plant when eaten raw may transmit *Fasciolopsis buski*, an intestinal fluke parasite of humans and pigs, causing fasciolopsiasis.

Therefore, the objectives of this study were to examine the effects of dual purpose kerosene (DPK) on growth and leaf epidermal features of *I. aquatica.*

Materials and Methods

Source and Identification of Plant Material

I. aquatica seedlings were collected from their natural habitat at Zamfara Hostel Area, beside Kabeezy Resturant, University of Ilorin, Ilorin, Nigeria. Sixty seedlings of *Ipomoea aquatica* were collected. All seedlings of about 2 weeks old were healthy, growing well without any trace of malformation of their parts. The experiments were conducted in January to February, 2011. The seedlings were identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Transplanting and Grouping of Seedlings

The seedlings were transplanted into plastic pots. Two seedlings of about the same height were in each plastic pot of 30. The plants were grouped into two namely, groups A and B. Group A served as the experimental plants while group B were the Control plants.

Preparation of Kerosene Concentrations

DPK was diluted with distilled water to have 5 different concentrations namely 0% (Control), 25%, 50%, 75% and 100%. These concentrations were prepared by measuring appropriate amount into a measuring cylinder and made up to 100ml with distilled water. One hundred percent (100%) kerosene was not diluted with distilled water, it was absolute.

Irrigation of Plants

Distilled water mixed with respective concentrations of kerosene was used to irrigate the soils of group A plants while water was used for group B. The plants were irrigated twice a week (Mondays and Thursdays) for six weeks. The kerosene concentrations and water were poured on the soil from where the plant roots absorbed them. The DPK concentrations especially those of 75% and 100% coagulate the soil particles thus making soil impregnable solid block

Measurement of Plant Height
The plant height was taken with a ruler on weekly basis, and the readings were recorded. The measurement of plant height was taken as the part of stem above the soil level to the tip of the stem.

Isolation of Leaf Epidermal Layers
Leaf segments of an area of 1 cm square from each specimen were cut and immersed in concentrated solution of nitric acid for maceration. The upper (adaxial) and lower (abaxial) are separated with dissecting needle and forceps. These samples were picked from acid and rinsed with clean water. They were mounted on microscope slides in dilute glycerine for microscopic examination.

Determination of Frequency of Stomatal Complex Types
Prepared slides of macerated cuticles from the leaves of the plants were observed, using 35 fields of view at X40 objectives in an Olympus microscope as quadrats. The number of subsidiary cells per stoma were noted, and recorded to determine the frequency of the different stomatal complex types present in each specimen. Frequency of each complex type was expressed as percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001). The terminologies for naming stomatal complex types followed those of Dilcher (1974).

Determination of Stomatal Density and Stomatal Index
The stomatal density was determined as number of stomata per square millimeter (Stace, 1965).
The stomatal index was determined as follows: $SI = S/E + S \times 100$
Where S = numbers of stomatal per square millimeter
E = number of ordinary epidermal cells per square millimeter

Determination of Stomatal Size
The mean stomatal size of a seedling was determined as the product of length and breadth of guard cells.

Determination of Epidermal Cell Size
Epidermal cell size was determined as product of length and breadth of a cell based on a sample size of 35.

Data Analysis
One-way ANOVA and Duncan's Multiple Range Test were conducted at three different levels on the data obtained from the two groups of the seedlings (Bailey, 1995).

Results
Effects of DPK on Percentage Survival and Qualitative Growth of *I. aquatic*
Table 1 shows the effect of DPK on the percentage survival of *Ipomoea aquatica.* The percentage survival decreased from 100% in the Control group to 75% in 25% DPK, 50% in 50% DPK and 0% in 75% and 100% DPK. The results showed that the survival of *I. aquatica* was concentration dependents. On qualitative growth of the test plant as presented in Table 1, it was observed that *I. aquatica* grown in contaminated soil with various levels of DPK concentration (25%-100%) showed leaf discoloration, stunted growth, complete necrosis and death of plants as the level of the contaminant increases.

Effect of DPK on plant height of *I. aquatic*
The average stem heights of *I. aquatica* as affected by different levels of DPK for six weeks are shown in Table 2. The results were significantly different ($p<0.05$) starting from the 2^{nd} week of

growth till week 6. Plant height of *I. aquatica* decreased significantly ($p<0.05$) in the 25%, 50%, 75% and 100% of DPK concentration plants compared with the Control group. From the data at 6th week of growth, plant heights of the test plants were attenuated from 35.35cm in the control to 5.88cm, 5.33cm, 4.58cm and 3.60cm in 25%, 50%, 75% and 100% DPK, respectively.

Effect of DPK on Stomatal Features of *I. aquatic*

There exist some anatomical variations in the stomatal features of *I. aquatica* grown in soils polluted with different concentrations of DPK and those grown in unpolluted soil. In spite of these anatomical differences with respect to stomatal density, index and size, only paracytic type of stomatal complex occurred in both abaxial and adaxial leaf surfaces in both the Control and the treated plants (Table 3; Plate I). Significant differences at $p<0.05$ were observed in the stomata density and stomatal index of *I. aquatica* in both adaxial and abaxial surfaces of leaf between the control and the treated plants. The control plants had the maximum stomatal density and index in both adaxial and abaxial leaf surfaces with values of 131.5mm and 153.2mm, 16.6% and 18.9% respectively. Whereas minimum values of these parameters were observed in both adaxial and abaxial leaf surfaces in the *I. aquatica* plants grown in soil polluted with 25% kerosene with values of 30.9mm and 37.5mm, 9.8% and 10.2%, respectively. Stomatal size in the control and treated plants was only significantly different at $p<0.05$ on abaxial leaf surface. No significant difference was observed in stomatal size with respect to adaxial surface between the control and the treated. Although the stomatal size value was higher in the Control plants than those obtained in *I. aquatica* grown in soil polluted with 25% -100% of DPK concentrations.

Table 1: Effects of varying concentrations of DPK on survival and morphological appearance of *Ipomoea aquatica*

Treatments (DPK concentration in %)	Percentage survive (%)	Observable effects
Control plants (0.00)	100	-All plants survived and grow normally
25	75	-Gradual decrease in the plants growth rate -Resultant brown colouration and streak on leaves (necrosis)
50	50	-Reduction in the growth rate of plants (dwarfing) -Leaf Necrosis -Wilting of leaves
75	0	-Stunted growth -Leaves turned black (complete necrosis)
100	0	-Stunted and quick termination of growth -Leaf Necrosis -Stem turned pale

Table 2: Mean height (cm) of *Ipomoea aquatic* plants grown in soils with different concentrations of DPK

Week	25% DPK	50% DPK	75% DPK	100% DPK	Control
1	7.50^{a}	7.50^{a}	7.50^{a}	7.50^{a}	7.50^{a}
2	8.52^{a}	6.95^{b}	6.25^{b}	5.26^{b}	8.86^{a}
3	7.87^{b}	6.65^{bc}	5.68^{c}	5.00^{c}	13.10^{a}
4	7.25^{b}	6.57^{bc}	5.30^{c}	4.26^{cd}	19.79^{a}
5	6.50^{b}	6.00^{b}	4.93^{c}	3.90^{c}	28.30^{a}
6	5.88^{b}	5.38^{b}	$4.58b^{c}$	3.60^{c}	35.50^{a}

Means with same letters along rows are not significantly different at $p < 0.05$

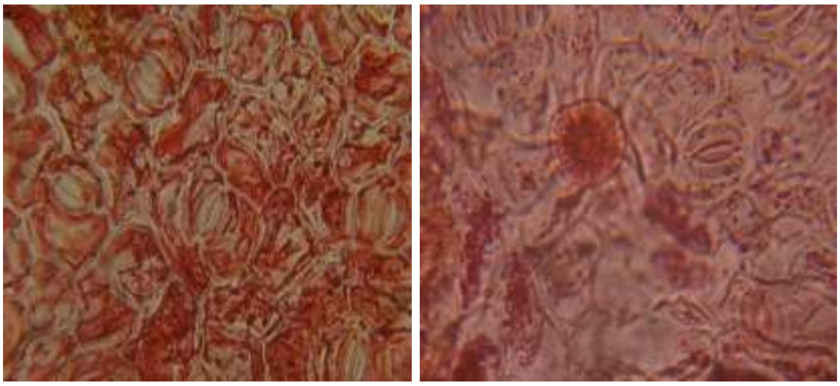
Table 3: Stomatal features of both control and experimental plants of *Ipomoea aquatica* plants grown in soils treated with concentrations of DPK

Treatments (% DPK concentration)	Leaf surface	Stomatal complex type	Frequency (%)	Stomatal Density (mm^2)	Stomatal index (%)	Stomatal size (µm)
25	Adaxial	Paracytic	100	30.90^d	$9.8o^c$	76.10^b
	Abaxial	Paracytic	100	37.50^d	10.20^c	79.70^b
50	Adaxial	Paracytic	100	78.90^c	15.30^{ab}	72.5^b
	Abaxial	Paracytic	100	90.10^b	17.80^a	76.10^b
75	Adaxial	Paracytic	100	80.90^b	13.30^b	72.30^b
	Abaxial	Paracytic	100	67.10^c	11.30^c	76.10^b
100	Adaxial	Paracytic	100	98.60^b	14.00^b	75.20^b
	Abaxial	Paracytic	100	90.10^b	13.00^b	72.20^b
Control (0.00)	Adaxial	Paracytic	100	131.50^a	16.60^a	83.30^{ab}
	Abaxial	Paracytic	100	153.20^a	18.90^a	90.60^a

Means with same letters along columns are not significantly different at $p<0.05$

Table 4: Qualitative features of leaf epidermal cells of *Ipomoea aquatica* plants grown in soils treated with concentrations of DPK

DPK concentration (%)	Leaf surface	Anticlinal wall pattern	Shape of cell
25	Adaxial	Straight	Pentagonal
	Abaxial	Straight	Pentagonal
50	Adaxial	Straight	Pentagonal
	Abaxial	Straight	Pentagonal
75	Adaxial	Straight	Pentagonal
	Abaxial	Straight	Pentagonal
100	Adaxial	Straight	Pentagonal
	Abaxial	Straight	Pentagonal
Control	Adaxial	Straight	Pentagonal
	Abaxial	Straight	Pentagonal



a b

Plate I: Leaf epidermal surfaces of *Ipomoea aquatica* showing paracytic stomata with oil films in experimental (a) and without oil films in control (b)

Discussion

Pollution in various forms has been shown to have adverse effects on plant growth which may range from morphological aberration, reduction in biomass to stomatal abnormalities (Sharma *et al.*, 1980). The findings of this study revealed that *I. aquatica* grown in soils polluted with various levels of DPK concentrations (i.e. 25%, 50%, 75% and 100%) showed decreased percentage survival from 100% in the control to 0% in soils irrigated with 75% and 100% DPK concentrations. The decrease in the rate of survival of *I. aquatica* in the polluted soil could be attributed to the physical properties of DPK which imposed stressful condition to the plant in terms of interfering with water uptake and gaseous exchange (Amakiri and Onofeghara, 1984). Atuanya (1987) reported that waste oil cause a breakdown of soil texture followed by soil dispersion. In this study, it was observed that DPK coagulate the soil particles thus making soil impregnable solid block. This adversely impairs water drainage and poor aeration within the soil and it could be the reason for the death recorded in 75% and 100% concentrations of DPK. The observable effects of DPK on *I. aquatica* in terms of stunted growth, complete necrosis, and leaf chlorosis are in line with the findings of Okonokhua *et al.* (2007) who observed that maize plants grown in oil polluted soil experienced chlorosis, necrosis, and upward curling of leaves from their tips.

Significant decreases in plant height were recorded from week 2 till the 6th week, between the Control and those polluted with 25% -100% DPK. Plant heights of the Control were significantly greater ($p<0.05$) than those in the polluted soil. This is congruent with the findings of Anoliefor and Vwioko (1995) who reported that spent oil in soil create unsatisfactory condition for plant growth ranging from heavy metal toxicity to insufficient soil aeration.

The results of this study showed that stomata were also affected by the pollutant. The effect was manifested as reduction in stomatal density, index, and size when compared to the Control plants. A strong relationship between the degree of distribution and concentration of DPK in the soil was noticed as the stomata in the leaves of the experimental plants shrunk (Plate I). Earlier Sharma *et al.* (1980) reported that since uptake of water and salt (ions) is carried out by the roots, the unpolluted plants with roots undisturbed grew normally while the polluted plants suffered morphological and anatomical aberrations, cell disruption in roots and other organs. In the entire concentrations studied, epidermal cell shape was pentagonal with straight anticlinal cell wall pattern. This translates to mean that the effect of DPK concentrations was not felt on stomatal complex type and other epidermal features except on the stomatal density, index and size. Furthermore, anatomical studies of the treated plants revealed the presence of oil films. Traces of oil were seen on the leaf epidermal surfaces of polluted plants, and thus making the structures unclear while the leaf epidermal surfaces of unpolluted plants were clear (Plate I).

Conclusion

The present study clearly showed that DPK has the potential of retarding plant growth. Also, the reduction in some stomatal features of *I. aquatica* could in turn have direct effects on carbon fixing capacity of the plant. Therefore, there is urgent need to focus on ways to minimize spills and leaks of this pollutant into the environment.

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