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Mutagenicity of Oil Drilling Fluid (Potassium Chromate) on the Seedlings of *Vigna unguiculata* L. (Walp). in the Niger Delta, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors participated fully in conducting this research work. They also read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The morphological, leaf epidermal and anatomical distortions caused by the exposure of *Vigna unguiculata* L. to potassium chromate was investigated using 1-4%w/w of an oil field chemical Potassium chromate and Control (0). Results showed that 2-4%w/w concentrations elicited noticeable effects on the plant. Variations occurred in plant height, leaf area, number of leaves between the treated and the control plants. *V. unguiculata* treated with 1%w/w concentration had malformed and chlorotic leaves. The treated plant had reduced stomatal indices in both epidermal surfaces: adaxial (18.3%) and abaxial (66.3%) as against (26.3%) and (75.3%) observed in the control. The mid rib of the treated plants had thicker cuticle than the control plant. There were 6 layers of collenchymatous cells in the mid rib of the treated plant as against 5 layers of collenchymatous cells in the control. The petiole of the treated plant also had thick sclerenchyma cells around the vascular bundle while the sclerenchymatous cells around the vascular bundles in the control plant were thin. Oxalate crystals were seen in the parenchyma cells of both the treated and the control plants but more of the crystals were observed in the treated plants. Obviously some

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mutagenic effects were elicited on the test organism indicating that the oil field chemical should be excluded where this plant is grown so as to avoid losses or other adverse consequences of potassium chromate on *V. unguiculata.*

Keywords: Vigna unguiculata; potassium chromate; oxalate crystals; oil drilling fluid.

1. INTRODUCTION

Vigna unguiculata (L.) Walp (cowpea) is a dicotyledonea belonging to the order Fabales, family Fabaceae, subfamily Faboidea, tribe *Phaseoleae*, genus *Vigna*, and section *Catiang* [1]. The precise origin of *Vigna unguiculata* is not known but the centre of maximum diversity of the cultivated cowpea is found in West Africa, in an area encompassing the Savannah region of Nigeria, Southern Niger, part of Burkina Faso, Northern Benin, Togo and the North-western part of Cameroun [2]. *Vigna* is a pan tropical genus and is the most cultivated grain legume of commercial importance in Nigeria [3]. Cowpea seeds are a cheap source of protein [4], and are nutritious in both human and livestock diets [5].

The importance and the phenotypic plasticity of *V. unguiculata* have endeared the plant to so many researchers; hence Achuba [6] studied the effects of crude oil, Obute [7] studied the effects of colchicine, Agbogidi [8] studied the effects of spent engine oil, Ogbemudia [9] studied the effects of hydrocarbon on seeds and seedlings of cowpea. Other workers including Girija [10] studied the effects of gamma rays, Eze [11] studied the effects of Bonny light crude oil, Rajendiran [12] studied the effects of aqueous extracts of *Lantana camara*, Eisa and Ali [5] studied the effects of microelements, Iyagba and Offor [13] studied the effects of crude oil and biostimulant while Adu [14] studied the effects of spent engine oil and unused engine oil on *Vigna unguiculata*. The crop is grown in small amounts in the Niger Delta area of Nigeria where farmlands compete with oil explorative and exploitative activities. Some oil field chemicals such as Potassium chromate may be exposed to crop plants.

Potassium chromate is a corrosion inhibitor, an important oilfield chemical used during oil exploration and production activities. In the bid to harness this resource, a large extent of the environment has been negatively impacted because of poorly managed operational activities of the oil and gas industries. According to Kloff and Wicks [15], during drilling operation, one production platform may discharge about 60,000 m^3 of drilling fluids into the environment. As these fluids find their way through the environment, the physical, chemical and microbiological properties of the soil are affected [16] most times adversely. This leads to build up of essential (organic C, P, Ca, Mg) and non-essential (Mg, Pb, Zn, Fe, Co, Cu) elements in soil and their eventual translocation into plant tissues [17]. Hence, many researchers [18-21] have stressed the need for proper handling and disposal of oilfield drilling fluids and wastewater. Although the effects of this chemical on cocoyam has been documented, it is known that different plants react differently to pollutants [17]. Indigenous folks in the Niger Delta practise mixed farming in small holdings and there is need to ascertain the effects of drilling fluids on other crops grown in the area. The objective of this study is to investigate the mutagenic effects of Potassium chromate on some characteristics of *V. unguiculata*.

2. MATERIALS AND METHODS

The experiment was conducted at the Ecological Research and the Regional Centre for Biotechnology and Bio fuel Research Centres of the University of Port Harcourt. Loamy soil obtained from the Botanical garden of the University of Port Harcourt was bagged in polythene bags to a weight of 5 kg. Dry seeds of *Vigna unguiculata* were bought from ADP (Agricultural Development Programme) Rivers State, Port Harcourt. Viable seeds were determined using the floatation method and four bean seeds were planted in each bag. Industry grade Potassium chromate was obtained from the Scientific Chemical vendors in Ariaria International Market Aba, Abia State. Four different concentrations were used in this experiment: 1, 2, 3 and 4% w/v while 0% served as the control. The chemical concentrations were measured out and thoroughly mixed in appropriate quantities of distilled water to make 100 ml of solution and applied to the plants immediately after planting. The seedlings were monitored and parameters of interest were measured after four weeks of seedling growth up to the 10^{th} week. Parameters measured include germinability, gross morphology, leaf epidermal features and leaf and petiole anatomical structures of treated and control plants.

2.1 Germinability of Bean Seeds

Germination assessment was carried out from the first through the third week after planting. Percentage germination was calculated as:

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times \frac{100}{1}$$

2.2 Morphological Assessments

Morphological assessments were carried out from the 4th week after planting to the 10th week at 2- week intervals. Plant height was obtained by measuring the plant from the soil level to the collar of the uppermost leaf. The leaf area was determined by measuring the length and width (at the widest point) of each leaf. The product of this was multiplied by a correction factor of 0.75 to cater for leaf shape [17]. The number of leaves, number of leaves with chlorosis and number of malformed leaves were counted for control and treatment plants.

All data generated were exposed to analysis of variance (ANOVA) and the means were compared using the Duncan's multiple range test (DMRT) at 5%.

2.3 Leaf Epidermal Assessments

Epidermal sections were obtained by applying nail vanish on a smooth surface of the leaf, allowing the varnish to dry naturally, then a small transparent cello tape was pressed firmly on the leaf surface and removed. This piece of cello tape with the leaf epidermal impression was placed on a microscope slide with the part having the impression on the slide. This preparation was then observed under the microscope. The types of stomata, number of stomata, number of epidermal cells, nature of the anticlinal walls, and shape of the epidermal cells as well as trichome distribution were visually scored. The stomatal indices and frequencies were calculated using the formulae below while good plates were photographed to reveal foliar epidermal features of interest. Stomatal indices and frequencies were calculated thus:

$$\text{Stomatal index (\%)} = \frac{S}{S+E} \times \frac{100}{1}$$

$$\text{Stomatal frequency (\%)} = \frac{S}{E} \times \frac{100}{1}$$

where: S = means number of stomata; E = means number of epidermal cells.

2.4 Anatomical Features

Free hand anatomical sections of the midrib and petioles of the control and treated *V. unguiculata* were made. The sections were stained with methylene blue for 5 minutes, washed to remove excess stain and mounted in a drop of 100% glycerin on a clean slide. Cover slips were placed on the preparations and were examined under the microscope. Good plates were photographed to show the anatomical features of interest.

3. RESULTS AND DISCUSSION

3.1 Germinability

The effects of potassium chromate on the germination of *Vigna unguiculata* revealed that at one week after planting, there was 50% germination (Table 1) in 1% w/v but 25% germination in the control. This showed that the chemical at this concentration apparently acted as germination enhancer to the plant within the first week, however, one of the seedlings died off in the second week after planting. Although the chemical enhanced the germination of *V. unguiculata* at 1% w/v the chemical was lethal to the plant at 2, 3 and 4% w/v concentrations. This indicates that potassium chromate is toxic to *V. unguiculata* since the seeds could not survive at higher concentrations of the chemical. Consequently, all the results showed the effects of the chemical only at 1% w/w concentration.

Table 1. Percentage germination and seedling development of *V. unguiculata* treated with potassium chromate

Chemical conc. (%w/v)	Percentage germination weeks after planting		
	1 week	2 weeks	3 weeks
0	25	25	25
1	50	25	25
2	0	0	0
3	0	0	0
4	0	0	0

3.2 Plant Height

Effects of potassium chromate on plant height showed that at the fourth to sixth weeks after planting, the treated plant was taller than that of the control plant. This is in line with Anoliefo and Vwioko [22] findings in their studies on *Capsicum* *annuum* using spent lubricating oil, they reported enhanced growth at 1% concentration in relation to the control. From the eighth to the tenth weeks, the control plant became taller than the treated plants (Fig. 1). This indicates that the effect of this chemical on *V. unguiculata* was more adverse to plant height with time. However, statistical evidence showed that the difference in plant height between the control and the treated plants was not significant at ($p \leq .05$) but the difference in mean plant height 10 weeks after planting (Table 2) was significant at ($p \leq .05$). In other words, the drilling fluid probably elicited reduced growth in the plant as time increased.

3.3 Leaf Area

Effects of potassium chromate on the leaf area showed that at four week after planting, the leaf areas of the treated plants were higher than that of the control plant; but as from the sixth to the tenth week after planting, the control plant had higher leaf area than the treated plants (Fig. 2). Progressive decrease in leaf area with increased pollutant concentration has been attributed to interference of water uptake and gaseous exchange caused by the stress of the pollutant [23] on the plant under study. In addition, Adu [14] reported decreased leaf area in *V. unguiculata* with increased concentration of engine oil. They attributed the reduction in leaf area to limitation of nutrients uptake necessary for expansion of leaf area occasioned by high levels of pollutants. However, statistical evidence showed that there was no statistical difference between the control and the treated plant; also the difference in leaf areas weeks after planting were not significant at 5%. Therefore, it is possible to adduce other reasons to the findings

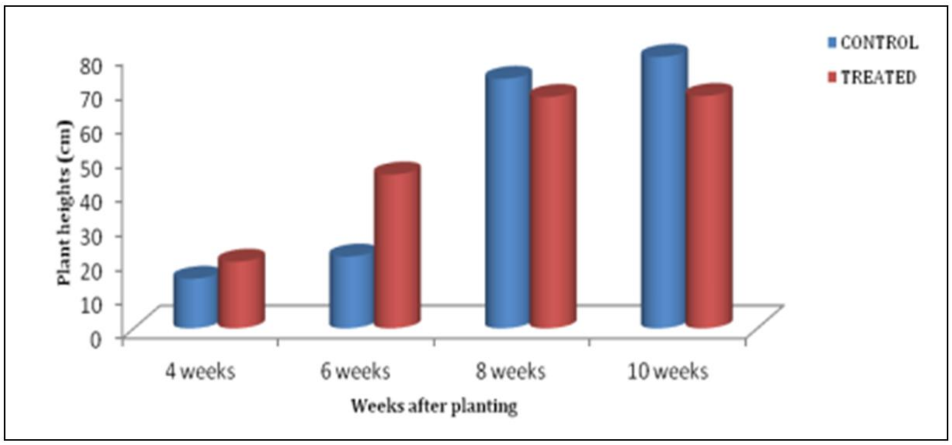

Fig. 1. Plant heights of control and treated *Vigna unguiculata* weeks after planting

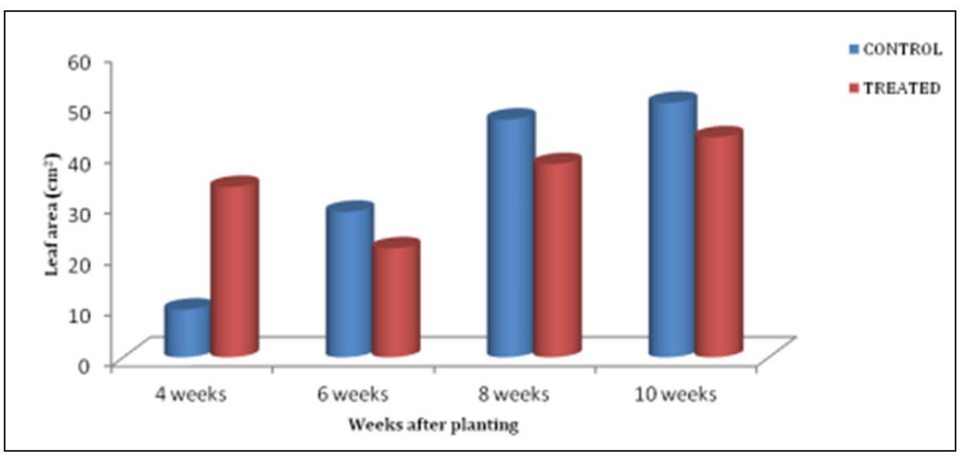

Fig. 2. Leaf area of control and treated *Vigna unguiculata* weeks after planting

that leaf area was reduced in treated material even if statistical evidence here shows non-significance difference. One of such may be the small sample size in this and previous similar cases. Increased sample size is needed to increase the sensitivity of this treatment in cowpeas in further studies.

Table 2. Plant heights of *V. unguiculata* treated with potassium chromate

Chemical conc. (%)	Plant heights (cm) weeks after planting (WAP)			
	4 weeks	6 weeks	8 weeks	10 weeks
Control	14.5[b]	21.0[b]	73.0[a]	79.4[a]
Treated	19.5[b]	45.0[b]	67.7[a]	68.0[a]

Different alphabets within rows are significantly different at ($P≤.05$)

3.4 Number of Leaves

From the fourth to the sixth weeks after planting, treated plants had higher number of leaves than the control plants (Fig. 3); this observation is in line with that of Fernandes and Henriques [24] who inferred that some heavy metals when present at low concentrations, act as essential micro-nutrients for plants, but at a high concentrations induce metabolic disorders and growth inhibition for most plant species. However, as from the eighth to the tenth week after planting, the control plants had higher number of leaves than the treated plants. Adu [14] reported similar results and they agreed that as the level of pollution increases, the number of leaves decreases.

3.5 Other Morphological Parameters

Potassium chromate-treated plants had two malformed leaves (Plate 1). Obute [7] also reported malformed leaves in colchicine treated *V. unguiculata*; the author deduced that chromosome breakage, reduction in the auxin level, change in enzyme activity and variation in ascorbic acid concentration are some factors, which lead to the development of abnormal leaves. There were also chlorotic leaves as at 8 weeks after planting in *V. unguiculata* treated with potassium chromate. Njoku [20] working with spent engine oil on *Zea mays* reported similar result; they attributed this phenomenon to interference of pollutant to mineral uptake thereby causing reduced rate of photosynthesis and food production. There was no observable difference between the treated and the control plants in the pattern of pigmentation of the stem and perianth; for both the treated and the control plants had trifoliate leaves. This result is also similar to the reports of Obute and Ugborogho [4] and Obute [7].

3.6 Foliar Stomatal Attributes

The paracytic type of stomata was expectedly prominent on the adaxial and abaxial epidermes for both the control and treated plants (Plates 2 and 3). Contiguous stomata and stomatal complexes were observed in the abaxial epidermes of both treated and control plants. Stomatal distribution showed that *V. unguiculata* was amphistomatic; but the number of stomata on the abaxial epidermis was higher than the

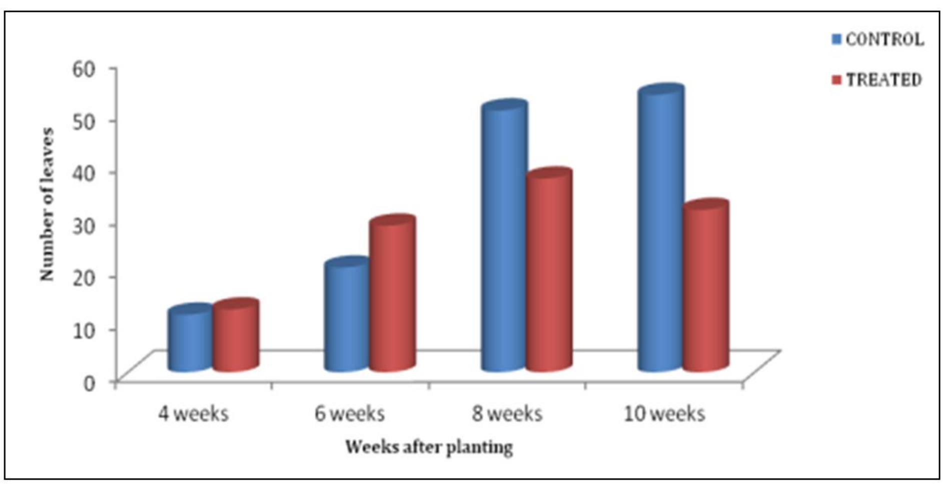

Fig. 3. Number of leaves of control and treated *Vigna unguiculata* weeks after planting

number of stomata on the adaxial epidermis for both the treated and the control plants. The effect of potassium chromate on stomatal distribution showed that the stomatal index of the control plants on both the adaxial (26.3%) and the abaxial (75.3%) epidermal surfaces were higher than that of the treated plants: (18.3%) and (66.3%) respectively. This result is in line with the findings of other researchers [4,23,25]. The decreased stomatal index explains why there was reduced plant height, leaf area, number of leaves and chlorosis in the potassium chromate treated plants. Reduced stomatal presence in the treated plant means reduced gaseous exchange hence, reduced photosynthetic activities. Statistical evidence showed that the difference between the mean number of stomata in the adaxial and the abaxial epidermes of the treated and the control plants were highly significant at 5% at (p≤.05).

Plate 1. Malformed leaves of treated *Vigna unguiculata*

3.7 Epidermal Cells

The shape of the epidermal cells on both epidermes of both control and treated plants were quadrilateral to octagonal in shape. The anticlinal walls of the epidermis were straight in both epidermis (Plates 2 and 3) for both control and treated plants. Esau [26] stated that the nature of the anticlinal wall is dependent on the force exerted on the stomata in the course of development. Since the anticlinal walls of the epidermal cells are straight, it means that potassium chromate did not exert pressure on the stomata. The number of subsidiary cells around the stoma on the adaxial epidermis was four to six on the control plant while they were four to five on the treated plant (Plate 3). The mean number of epidermal cells was more on the adaxial epidermis than at the abaxial epidermis of both the treated and control plants, in addition there were more epidermal cells in the control plants than in the treated plants (Fig. 4). However, the difference between the mean number of epidermal cells of the adaxial and the abaxial epidermis were not significant at 5%. In the same vein, the difference in the mean number of epidermal cells (Table 2) between the control and the treated plants was also not significant at (p≤.05).

3.8 Trichomes

The glandular type of trichome was observed on both epidermes for control and treated plants. The trichome consisted of a gland with 2-3 intercellular walls (Plates 2 and 4). The distribution was however, more on the abaxial epidermis than on the adaxial epidermis for both treated and control plants. However, there was reduction in the number of trichomes (Fig. 5) on the treated material compared with the control. It follows that the drilling fluid most probably adversely affected the number of trichomes on both leaf surfaces than the case of control. Trichome density is believed to be a strong taxonomic indicator for delimitation of species; Metcalfe and Chalk [27] hold that trichome frequency and size are environmentally controlled while Alege [28] indicated that trichome distribution greatly depends on the fertility status of the soil. Since both treated and control materials were exposed to the same environment it may be concluded that potassium chromate indeed reduced the number of trichomes in *V. unguiculata*.

Table 3. Mean values of trichomes, stomata and stomatal indices of treated and control *V. unguiculata*

Plants	Epidermal surface	Type of stomata	Anticlinal cell wall	Mean no of stomata± SD	Mean no of epidermal cells	Stomatal index (%)	Stomatal freq. (%)	Mean no of trichome±SD
Control	Adaxial	Paracytic	Straight	26.3 ± 2.1^{a}	68.3 ± 9.1^{e}	27.8	38.5	28.0 ± 2.8^{g}
	Abaxial	Paracytic	Straight	75.3 ± 15.6^{b}	39.7 ± 14.1^{e}	65.5	189.7	79.0 ± 18.4^{g}
	Adaxial	Paracytic	Straight	18.3 ± 1.5^{c}	60.7 ± 2.3^{e}	23.2	30.2	8.0 ± 4.2^{g}
Treated	Abaxial	Paracytic	Straight	66.3 ± 4.5^{d}	25.3 ± 10.1^{e}	72.4	262.1	21.5 ± 9.2^{g}

Mean values with different alphabet within column means they are significantly different at (P≤0.05)

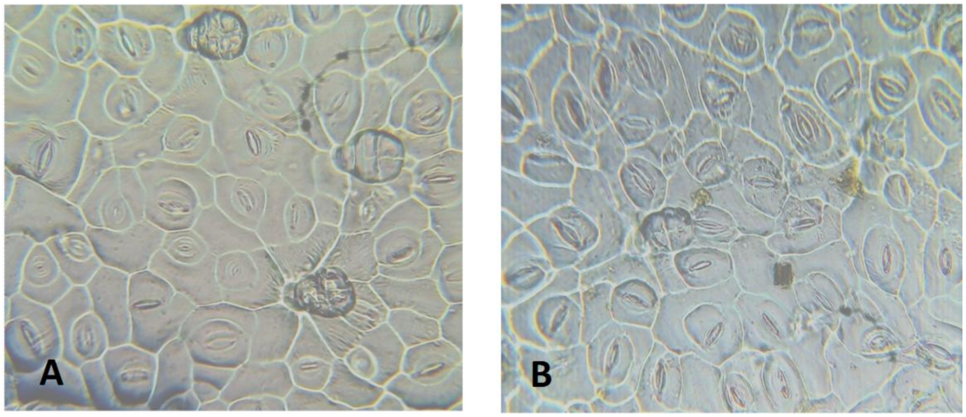

Plate 2. Abaxial epidermis of *V. unguiculata.* (A) Control. (B) Treated

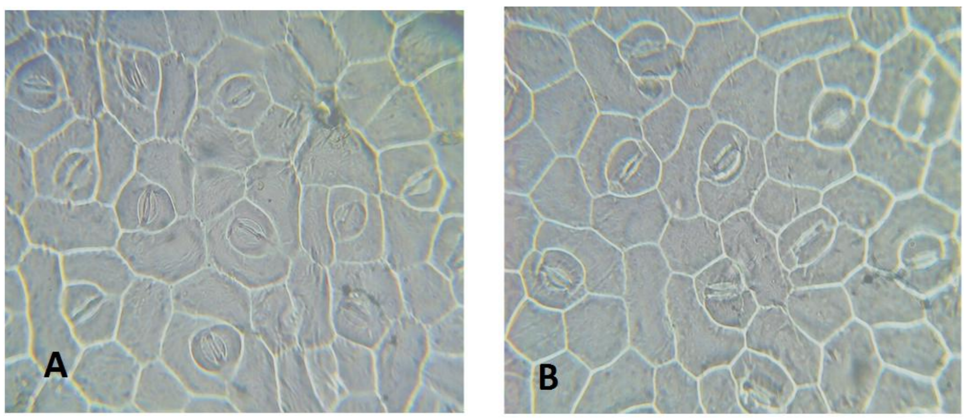

Plate 3. Adaxial epidermis of *Vigna. unguiculata*. (A) Control). (B) Treated

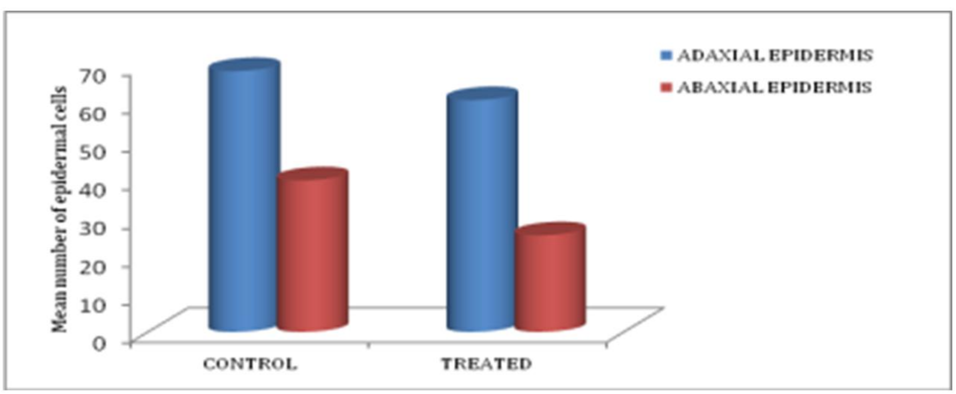

Fig. 4. Mean epidermal cells distribution on *Vigna unguiculata*

3.9 Midrib Anatomy

The midrib of both treated and control plants had sclerified perivascular sheath with round-shaped epidermal cells. The cuticle of potassium chromate treated plants was thicker than the cuticle of the control plants. The midrib of the control plants had 5 layers of collenchymatous cells while the treated plants had 6 layers of collenchymatous cells. Luis [29] opined that the thicker the collenchyma, the more resistant the plant is to pathogens. The control *V. unguiculata* had extension of palisade mesophyll towards a tapered dorsal end (Plate 5D) but the treated plant had bundle sheath sclerenchyma (Plate 6D) towards a concave-shaped dorsal end. Furthermore, the vascular bundle of the potassium chromate treated *V. unguiculata* was surrounded by a border parenchyma at both the dorsal and ventral (Plate 6B) ends while the vascular bundle of the control plants had border parenchyma only at the ventral part of the midrib (Plate 5B).

3.10 Petiole Anatomy

The petiole of control *V. unguiculata* revealed a round-shaped epidermal cells with thin cutinized walls (Plate 7B). The parenchymatous cells were round- to oval-shaped and contained rod-like structures that resembled oxalate crystals (styloids). The endodermal cells enclosed vascular bundle (Plate 7C) surrounded with thin sclerenchymatous cells. Meanwhile, the treated *V. unguiculata* had round-shaped epidermal cells, thick cuticles and multicellular epidermal hairs as reflected in (Plate 8B). Eisa and Ali [5], also reported thicker cells in treated *V. unguiculata*, they agreed that probably increased enzyme and metabolic activities stimulated auxin synthesis, cell division and expansion. The parenchymatous cells were round to oval in shape but with many rod-like structures suspected to be styloid type of oxalate crystal. Stace [30] described oxalate crystals as waste products and the occurrence of more of these structures in treated *V. unguiculata* indicates their probable accumulation in such plants. The endodermal cells were surrounded by these rod-like structures but the sclerenchymatous cells surounding the vascular bundles appeared thicker than the ones (Plate 8C) in the control plant.

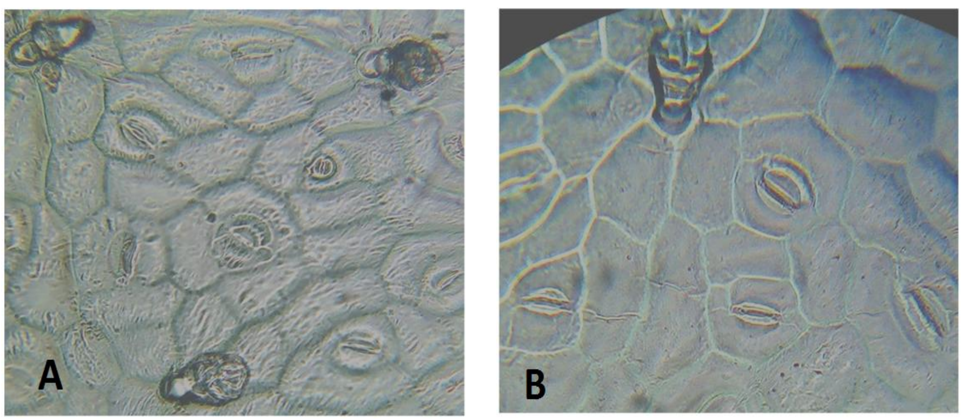

Plate 4. Adaxial epidermis of *Vigna unguiculata* with glandular trichome. (A) Control (B) Treated

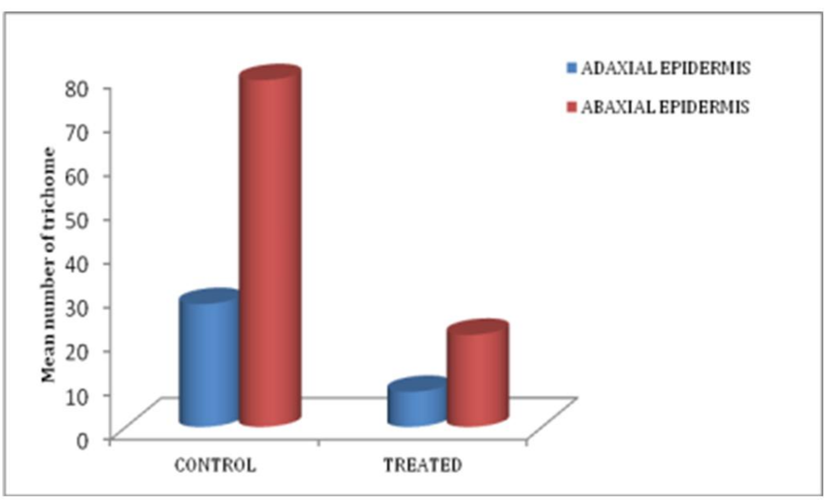

Fig. 5. Mean trichome distribution on *Vigna unguiculata*

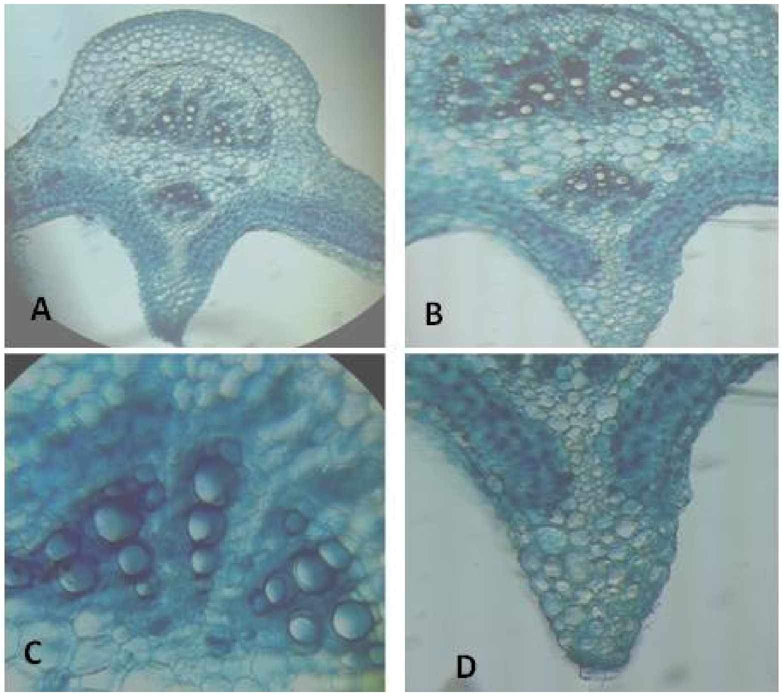

Plate 5. Midrib of *Vigna unguiculata* (Control). (A). General outline of the mid rib sheath. (B). The vascular bundle (C). The xylem (D). The tapered end of the mid rib

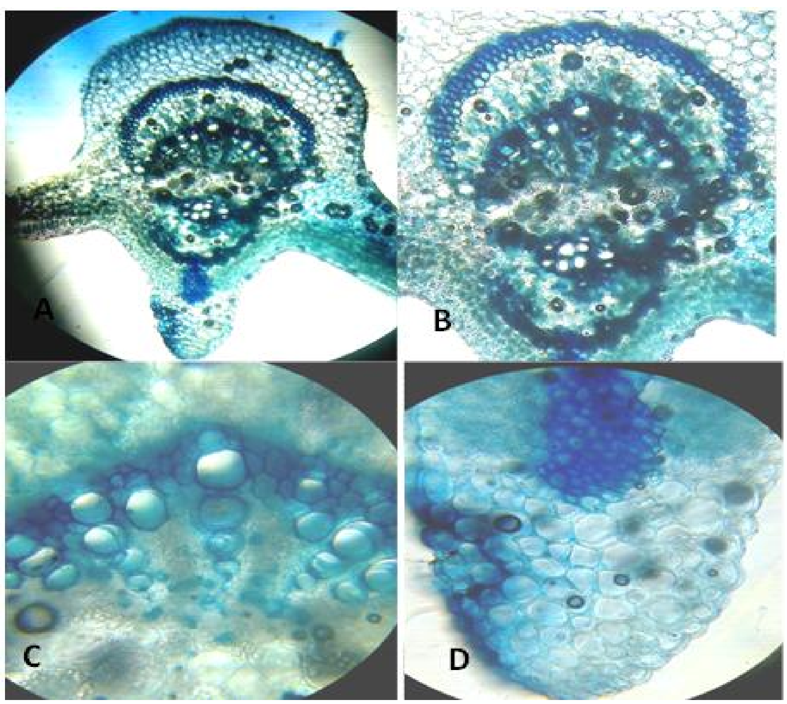

Plate 6. Midrib of *V. unguiculata* (treated) (A)-The general view of the mid rib sheath (B)-The vascular bundle (C)-The xylem (D)-The concave end of the mid rib

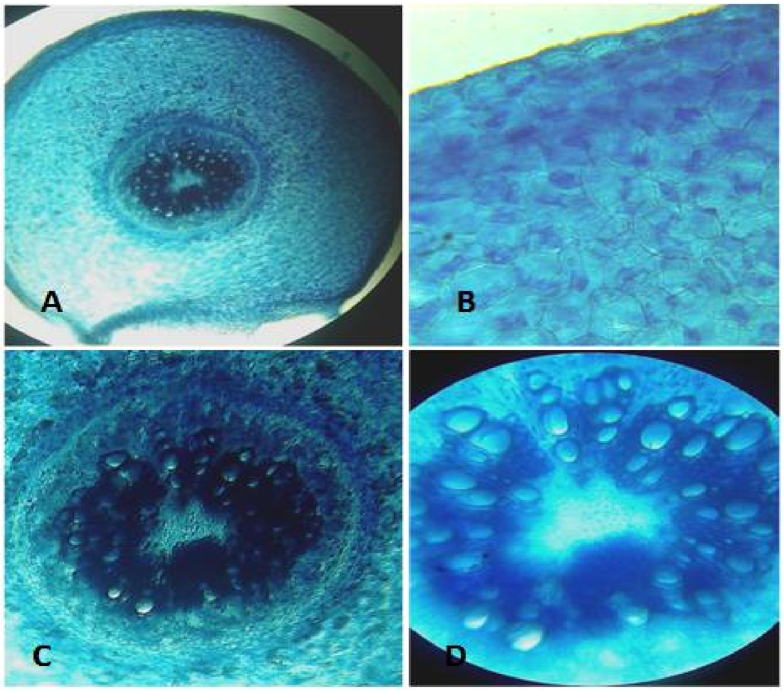

Plate 7. Petiole of *Vigna unguiculata* (control) (A)- The general view of the petiole (B)- Round shaped epidermis with thin cuticle and an oval to round shaped parenchyma cells (C)-The endodermis with thin sclerenchyma cells (D)- Vascular bundle and pith

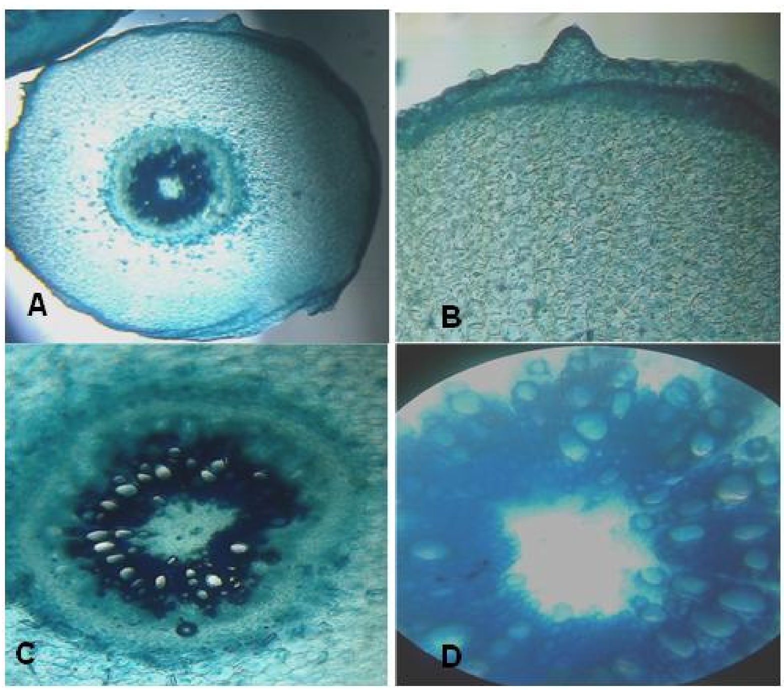

Plate 8. Petiole anatomy of *Vigna unguiculata* (Treated). (A). General outline of the petiole anatomy. (B). Round shaped epidermis with thick cuticle and an oval to round shaped parenchyma cells (C).The endodermis with thick sclerenchyma cells (D). Vascular bundle and pith

4. CONCLUSION

The present study has shown that potassium chromate may elicit mutagenic effects on *V. unguiculata.* As an oilfield chemical, special care must be taken when handling this chemical because it can be accumulated in plant tissues and reduce primary productivity as observed in reduced leaf area, stomatal features, anatomical features and accumulation of crystal oxalates.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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