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Epidermal Characterization of *Colocasia esculenta* (L.) Schott. and *Xanthosoma maffafa* (L.) Schott. Accessions As Affected by Different Concentrations of Sodium Azide and Potassium Chromate in Nigeria

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Authors' contributions

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

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ABSTRACT

Epidermal assessment of five accessions of *Colocasia esculenta* and three accessions of *Xanthosoma maffafa* exposed to different concentrations of sodium azide and potassium chromate treatments was carried out. Each accession was planted in four different concentrations (2.5, 5, 7.5 and 10 mg/kg) of each chemical, while the accessions planted with no chemical additive served as the control experiment. Results showed that both the treated and control accessions were amphistomatous, but with more stomata on the abaxial epidermis. Four types of stomata (brachyparacytic, amphibrachyparacytic, brachyparatetracytic and brachyparahexacytic-monopolar)

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were observed in these species. Contiguous stomata were observed on the abaxial epidermes of some treated samples of NXs 002 and NXs 003; control sample of NXs 003 however had contiguous stomata on both epidermes. The mean stomatal index survey showed that in both epidermes, sodium azide treatments induced higher stomatal index in the accessions than potassium chromate treatments. The anticlinal walls were straight in both control and treated accessions except for the abaxial epidermis of NXs 003 treated with 5 mg/kg of potassium chromate that had wavy anticlinal walls. The shapes of the epidermal cells were not affected by the treatments as they were observed to range from quadrilateral to nonagonal in all the accessions. However, some isodiametric and circular shaped epidermal cells were observed on the adaxial epidermis of NXs 002 treated with 7.5 mg/kg of potassium chromate and, on both epidermes of control NXs 003. Statistical evidence showed that the differences in stomatal indices and epidermal cell numbers between the adaxial and abaxial epidermes were significant at 5% likewise the differences between various accessions but the differences between treatments were not significant at *P*=.05. The study showed that both chemicals were capable of distorting epidermal architecture but sodium azide proved to be more potent than potassium chromate. Based on this, further studies were encouraged for a clearer understanding of the potentials of these chemicals in crop improvement programs.

Keywords: Stomata; oilfield chemicals; environment; abaxial; adaxial; stomatal index.

1. INTRODUCTION

Nigeria is a country endowed with many natural mineral resources; one of these resources is the crude oil. In the bid to harness this resource, a large extent of the environment has been negatively impacted due to poorly managed operational activities of the oil and gas industries. The consequences of this, is the introduction of environmental pollutants which are accumulated in living organisms; these accumulations are persistent because of their poor biodegradable qualities. The relationship between human activities and the polluted environment has been strongly recognized and the need to protect human health, recreation and fisheries production has led to strategic environmental monitoring [1].

When oilfield chemicals and its associated crude pollutants get in contact with the environment; they enhance the diminution of soil nutrients, undermine aquatic organisms, decrease oxygen diffusion and cause an alteration in the food chain [2]. Studies have shown that plants are structurally dynamic and can adapt to various environmental conditions which are likely to result in different morphological, epidermal, physiological and anatomical variations [3,4]. However, the degree of impact in plants depends on the concentration of the pollutants, mode of entrance into the plants and the nature of the species under consideration [5].

Morphological variations are one of the first signs observed in plants growing in polluted environment but, studies have shown that plants growing in polluted environment sometimes do not show any morphological defect and occasionally have more vegetative growth than plants in an unpolluted environment [6,7,8]. Because of this trend, many scholars resorted to using epidermal and anatomical characters of plants for environmental monitoring [9,10,11,12,13]. The reason for this is not far-fetched as these characters are considered bioindicator of environmental quality [4] and are believed to be much valuable because they are rarely affected by environmental factors [14].

Environmental bioindicator refers to the ability of an organism (plant) to exhibit sharp negative effects in the presence of an environmental chemical. Cocoyam is the third most important tuber crop in Nigeria; it serves as food, raw material for biotechnology and pharmaceutical industries as well as has therapeutic properties [15]. Cocoyam (*Colocasia esculenta* and *Xanthosoma maffafa*) a root tuber crop commonly cultivated in the Niger Delta area of Nigeria where majority of the oil exploration and drilling activities take place has been seen as a poor environmental bioindicator [16,17]. Udosen [17] reported the inability of cocoyam to absorb and accumulate most heavy metals from paint waste soil; in the same vein, Ajah et al. [15] on the effects of sodium azide and potassium chromate on this plant also reported more vegetative vigour in the treated plants when compared to the control.

Therefore, this study intends to investigate the distortions in the epidermal architecture of *Colocasia esculenta* and *Xanthosoma maffafa* accessions exposed to sodium azide and potassium chromate treatments.

2. MATERIALS AND METHODS

3 accessions of *Xanthosoma maffafa* (NXs 001, NXs 002 and NXs 003) and 5 accessions of *Colocasia esculenta* (NCe 001, NCe 002, NCe 003, NCe 004 and NCe 005) were identified and collected from the National Root Crops Research Institute (NRCRI), Umudike. The native names of these accessions are given as: NXs 001 (Ede ocha), NXs 002 (Ede uhie), NXs 003 (Okorokoro), NCe 001 (Coco indica), NCe 002 (Ede ofe), NCe 003 (Ede ofe, green petiole), NCe 004 (Ede ofe giant) and NCe 005 (Ukpong). Each accession was planted in two different oilfield chemicals (sodium azide and potassium chromate) with the following concentrations: 2.5, 5, 7.5 and 10 mg/kg. The unpolluted soil was used as the control experiment. Each of these accessions was planted in polythene bags containing 10 kg soil and the chemicals were applied by mixing each concentration with 400 ml of water. This mixture was used in watering the plants immediately after planting. The plants were watered with 200 ml of water when needed as this experiment was done during the rainy season. Weeding was done by hand picking. This experiment was set up in a Completely Randomized Design without replicates and kept at the Ecological Research Centre of the University of Port Harcourt.

Cuticular assessments of both the treated and the control plants were carried out five months after planting. Epidermal sections were obtained using two different methods; the hand teasing [18] and the leaf impression [19] methods. The leaf impression method involves applying nail vanish on a smooth surface of the leaf. This nail vanish was allowed 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 is placed on a microscope slide with the part having the impression on the slide. The preparation was then observed microscopically. The type of stomata, number of stomata, number of epidermal cells, nature of the anticlinal walls and the shape of the epidermal cells were all visually scored. Good plates were photographed to reveal foliar features of interest. Stomatal indices and frequencies were calculated as follows:

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

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

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

All data generated were exposed to Microsoft excel two way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Effects on Stomatal Distribution

The effects of sodium azide and potassium chromate on all the accessions were summarized in Tables 1a and 1b. Investigation showed that *Colocasia esculenta* and *Xanthosoma maffafa* accessions were amphistomatic (stomata occurred on both abaxial and adaxial epidermes) and generally had more stomata on the abaxial epidermis than at the adaxial epidermis. The occurrence of stomata on both epidermal surfaces but with more on the abaxial epidermal surface has also been reported by some authors in *Solanum macrocarpon* and *Solanum nigrum* [20], *Colocasia esculenta* and *Xanthosoma mafaffa* [18]. The effects of these two oilfield chemicals did not alter this design as there were more stomata on the abaxial than at the adaxial epidermis of all the treated accessions. Moreover, calculated stomatal index and stomatal frequency also showed that the stomatal indices and frequencies were lower at the adaxial epidermis than at the abaxial epidermis. Statistical evidence further showed that the differences in stomatal indices between adaxial and abaxial epidermes were significant at 5%.

Accessions treated with the chemicals generally showed decreased stomatal indices and frequencies when compared with their controls however, there were cases of decreased stomatal indices and stomatal frequencies due to the chemical treatments. Lower stomata frequency reduces gaseous exchange between the environment and the plants; it also signifies the ability of the plant to conserve moisture that would have been loss through transpiration. Reduced stomatal index and frequency due to pollution is not strange as many scholars have reported the same result in *Amaranthus hybridus* [21], *Datura alba* [22] and *Ipomea fistulosa* and *Calotropis gigantia* [5]. However, Kokilavani and

Rajendiran [23] reported higher stomatal index and stomata frequency in *Vigna unguiculata* exposed to ultraviolet-B radiation. Mean stomatal index calculation showed that in both epidermes, sodium azide treatments induced higher stomatal index in the accessions than potassium chromate treatments. Survey into the stomatal index also showed that accession NCe 002 (9.9% with sodium azide treatments) had the highest mean stomatal index [derived from all the treatments] at the adaxial epidermis (Fig. 1) while at the abaxial epidermis, accession NXs 003 (18.7% with potassium chromate treatments) had the highest mean stomatal index (Fig. 2). *Xanthosoma maffafa* accessions were observed to have more cases of increased stomatal index and frequency than the C*olocasia esculenta* accessions. This observation confirms the assertion by Osuji and Nwala [18], that variations within these two species (*C. esulenta* and *X. maffafa*) indicate their unique ecological adaptations to different environmental conditions. The only accession without a case of increased stomatal index and frequency when compared with the control was NCe 004, however this accession did not respond well to the treatments as there were lack of germination in three concentrations of the chemicals. Statistical analysis further showed that the differences in stomatal indices between treatments were not significant at *P*=.05 but the differences in stomatal indices between accessions were significant at *P*=.05 (Table 2).

Furthermore, stomatal complexes and contiguous stomata were observed in the abaxial epidermes of some treated accessions; these stomatal complexes were however observed on the adaxial epidermes of accessions NCe 003 and NCe 005. The accessions with stomatal complexes on their abaxial epidermes included: NCe 002 treated with 2.5 mg/kg of sodium azide; with 2.5 and 10 mg/kg of potassium chromate. NCe 004 treated with 5 and 7.5 mg/kg of sodium azide and with 2.5mg/kg of potassium chromate. NCe 005 treated with 7.5 and 10 mg/kg of sodium azide and with 5 and 7.5 mg/kg of potassium chromate. The accessions with stomatal complexes on their adaxial epidermes were NCe 003 treated with 2.5 mg/kg of sodium azide; and NCe 005 treated with 10 mg/kg of sodium azide and with 7.5 mg/kg of potassium chromate. It was generally observed that sodium azide treatments induced more cases of stomatal complexes on the accessions than potassium chromate treatments. Contiguous (Siamese) stomata were observed on the abaxial epidermes of NXs 002 and NXs 003 treated with 7.5 mg/kg of sodium azide; abaxial epidermis of NXs 003 treated with 10 mg/kg of potassium chromate also had contiguous stomata (Plate 1). Contiguous stomata were also observed on the both epidermes of NXs 003 control. The occurrence of contiguous stomata has also been reported by Essiett et al. [24] in Euphorbia species. They affirmed the occurrence of such stomatal abnormality as a result of environmental factor.

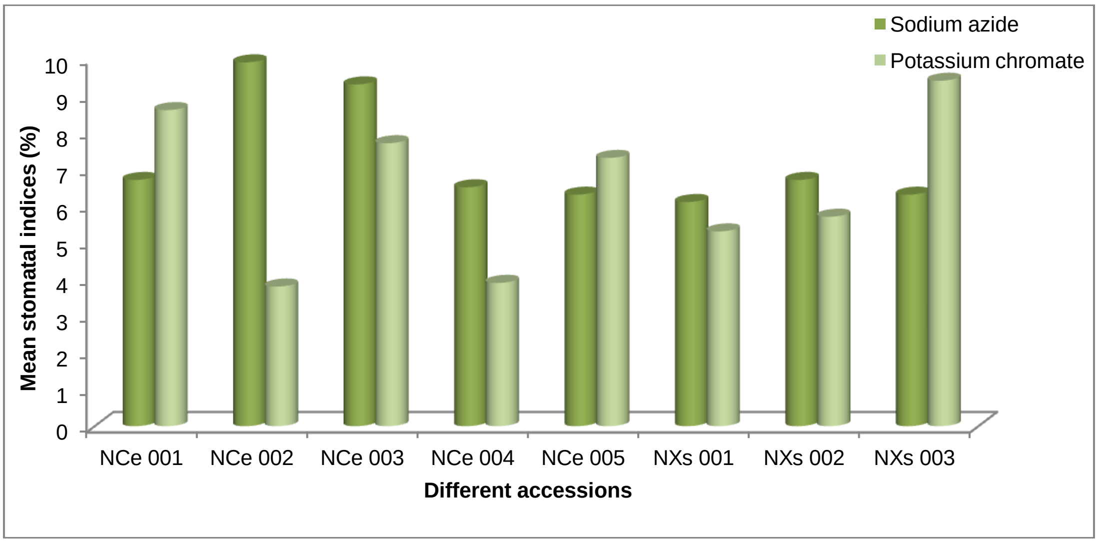

Fig. 1. Effects of sodium azide and potassium chromate on the mean stomatal indices of the adaxial epidermes of different accessions

Table 1a. Stomatal indices and frequencies in the epidermes of different Colocasia accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NCe 001				NCe 002				NCe 003				NCe 004				NCe 005			
			S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F
Sodium azide	2.5	Adaxial	12	185	6.1	6.5	23	150	13.3	15.3	21	152	12.1	13.8	_	_	_	_	10	190	5.0	5.3
		Abaxial	21	130	13.9	16.2	32	154	17.2	20.8	38	219	14.8	17.4	_	_	_	_	25	182	12.1	13.7
	5	Adaxial	8	123	6.1	6.5	14	164	7.9	8.5	9	232	3.7	3.9	20	175	10.3	11.4	12	124	8.8	9.7
		Abaxial	18	124	12.7	14.5	27	129	17.3	20.9	25	183	12.0	13.7	32	199	13.9	16.1	21	150	12.3	14.0
	7.5	Adaxial	6	105	5.4	5.7	18	213	7.8	8.5	22	221	9.1	10.1	24	253	8.7	9.5	4	112	3.4	3.6
		Abaxial	15	128	10.5	11.7	26	141	15.6	18.4	46	264	14.8	17.4	28	274	9.3	10.2	26	198	11.6	13.1
	10	Adaxial	15	148	9.2	10.1	25	212	10.6	11.8	19	136	12.3	14.1	17	224	7.1	7.6	15	170	8.1	8.8
		Abaxial	18	123	12.8	14.6	30	146	17.1	20.6	31	180	14.7	17.2	27	211	11.3	12.8	28	258	9.8	10.9
Potassium chromate	2.5	Adaxial	7	167	4.0	4.2	10	177	5.4	5.7	22	205	9.7	10.7	25	319	7.3	7.8	22	193	10.2	11.4
		Abaxial	10	168	5.6	6.1	31	180	14.7	17.2	35	203	14.7	17.2	37	316	10.5	11.7	39	251	13.5	15.5
	5	Adaxial	17	155	9.9	11.1	_	_	_	_	13	266	4.7	4.9	18	203	8.2	8.9	12	142	7.8	8.5
		Abaxial	31	144	17.7	21.5	_	_	_	_	35	206	14.5	17.1	24	187	11.4	12.8	27	159	14.5	17.1
	7.5	Adaxial	23	147	13.5	15.7	_	_	_	_	15	217	6.5	6.9	_	_	_	_	10	152	6.2	6.6
		Abaxial	40	172	18.9	23.3	_	_	_	_	27	210	11.4	12.9	_	_	_	_	26	144	15.3	18.1
	10	Adaxial	9	119	7.0	7.6	20	189	9.6	10.6	18	168	9.7	10.7	_	_	_	_	13	249	5.1	5.2
		Abaxial	16	92	14.8	17.4	31	182	14.6	17.0	26	172	13.1	15.1	_	_	_	_	28	196	12.5	14.3
Control		Adaxial	21	125	14.4	16.8	18	161	10.1	11.2	27	221	10.9	12.2	23	180	11.3	12.8	12	171	6.6	7.0
		Abaxial	22	99	18.2	22.2	29	119	19.6	24.4	47	169	21.8	27.8	31	182	14.6	17.0	26	173	13.1	15.0

Table 1b. Stomatal indices and frequencies in the epidermes of different Xanthosoma accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NXs 001				NXs 002				NXs 003			
			S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F
Sodium azide	2.5	Adaxial	8	137	5.5	5.8	9	188	4.6	4.8	5	100	4.8	5.0
		Abaxial	20	128	13.5	15.6	27	263	9.3	10.3	22	119	15.6	18.5
	5	Adaxial	13	173	7.1	7.5	13	211	5.8	6.2	11	121	8.3	9.1
		Abaxial	20	150	11.8	13.3	39	195	16.7	20.0	35	124	22.0	28.2
	7.5	Adaxial	7	123	5.4	5.7	14	193	6.8	7.3	9	109	7.6	8.3
		Abaxial	23	146	13.6	15.8	30	198	13.2	15.2	22	118	15.7	18.6
	10	Adaxial	10	144	6.5	6.9	20	190	9.5	10.5	4	89	4.3	4.5
		Abaxial	11	124	8.2	8.9	40	231	14.8	17.3	19	104	15.5	18.3
Potassium chromate	2.5	Adaxial	8	187	4.1	4.3	8	200	3.9	4.0	9	100	8.3	9.0
		Abaxial	40	201	16.6	19.9	27	144	15.8	18.8	20	91	18.0	22.1
	5	Adaxial	8	173	4.4	4.6	11	182	5.7	6.0	9	81	10	11.1
		Abaxial	26	146	15.1	17.8	31	218	12.5	14.2	18	90	16.7	20.0
	7.5	Adaxial	15	194	7.2	7.7	12	199	5.7	6.0	9	85	9.6	10.6
		Abaxial	26	201	11.5	12.9	30	218	12.1	13.8	22	90	19.6	24.4
	10	Adaxial	9	161	5.3	5.6	13	162	7.4	8.0	12	111	9.8	10.8
		Abaxial	19	122	13.5	15.6	27	144	15.8	18.8	24	93	20.5	25.8
Control		Adaxial	9	153	5.6	5.9	13	230	5.4	5.7	22	221	9.1	10.1
		Abaxial	16	149	9.7	10.7	51	238	17.7	21.4	29	212	12.0	13.7

(KEYS: S= Number of stomata, E= Number of epidermal cells, S.I= Stomatal index (%), S.F= Stomatal frequency (%)

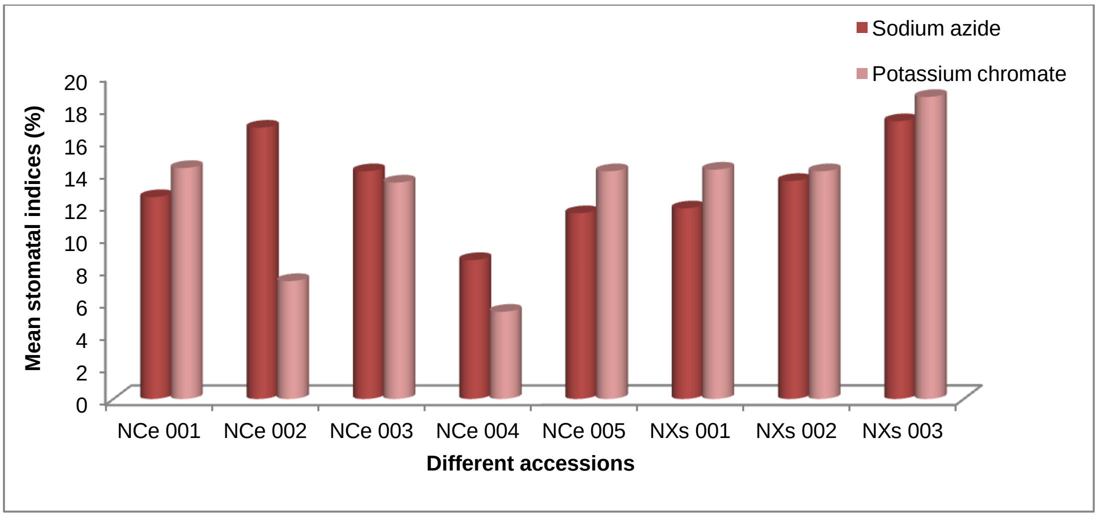

Fig. 2. Effects of sodium azide and potassium chromate on the mean stomatal indices of the abaxial epidermes of different accessions

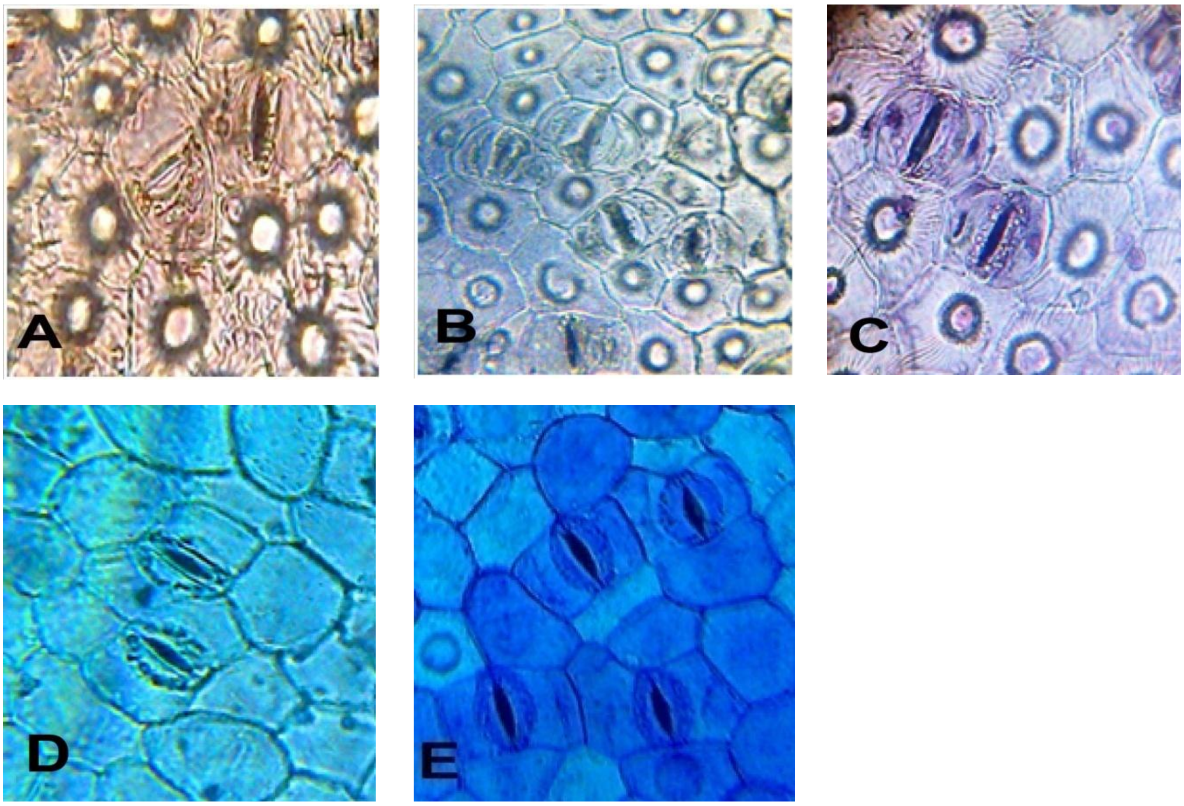

Plate 1. Contiguous stomata: (A.) NXs 002 treated with 7.5 mg/kg of sodium azide, (B.) NXs 003 treated with 7.5 mg/kg of sodium azide, (C.) Abaxial epidermis of NXs 003 treated with 10 mg/kg of potassium chromate, (D.) Adaxial epidermis of NXs 003 control, (E.) Abaxial epidermis of NXs 003 control

3.2 Effects on Stomatal Types

The brachyparacytic stomata was observed as the basic type of stomata in this study. This observation was at variance with Osuji and Nwala [18] who reported the paracytic stomata as the basic type of stomata but in agreement with Ajah et al. [25]. Other types of stomata also observed on the epidermes of these accessions included: amphibrachyparacytic, brachyparatetracytic and brachyparahexacytic-monopolar types of stomata (Plate 2). The occurrence of more than one type of stomata on an epidermis is not new as Essiett et al. [24] reported this phenomenon with *Euphorbia sp*.; they deduced that the occurrence of more than one type of stomata on the epidermis has taxonomic value and is useful in the delimitation of species. Rai [26] also reported similar result with *Jatropha sp*.

Table 2. Analysis of variance showing mean square of effects of the treatments on stomatal indices

Source of variation	df	MS	F	F crit
Accessions	15	126.99	9.36**	1.75
Treatments	8	20.78	1.53	2.01
Error	120	13.56		
Total	143			

***= indicate significance at P=.05*

Table 3 showed detailed types of stomata observed in both epidermes of the control and treated accessions. Observations showed that sodium azide and potassium chromate treatments induced different types of stomata on the epidermes apart from the types of stomata present on the control. Similar result was reported by Ajah et al. [25], they surmised that it is as a result of the rearrangement of the subsidiary cells by the environmental chemicals. Scrutinizing the results, it was noted that 12 occurrences of different stomata types were observed in NCe 004, 6 occurrences were observed in NCe 001 and NCe 003, 5 occurrences were observed in NXs 001, 2 occurrences were seen in NCe 005 but in NCe 002, NXs 002 and NXs 003 no new type of stomata was introduced by the chemicals. Generally, it was observed that the induction of different types of stomata on the epidermes was more with sodium azide treatments than with potassium chromate treatments.

3.3 Effects on Epidermal Cell Distribution

The epidermal cells were almost evenly distributed between the adaxial and abaxial epidermes; the epidermal surfaces were glabrous without trichome but the presence of papillae characterized both epidermal surfaces but their presence was more on the abaxial surfaces. Ogie-Odia [27] gave the description of papillae as the simplest of trichomes, characterized by wall projection followed by the protoplast of epidermal cells. However, the precise function of the papillae is not clear and the need to extensively investigate the function of papillae on plant epidermes was reiterated by De Oliveira and Miglioranza [28]. Furthermore, the anticlinal walls were straight in the control accessions and were straight in all the treated accessions except in one sample of NXs 003. It was observed that potassium chromate treatment induced wavy anticlinal walls (Plate 3) in the abaxial epidermis of NXs 003 treated with 5 mg/kg treatment (Table 4). Ajah and Obute [29] believed that the orientation of the anticlinal wall is influenced by the force exerted on the stomata in the course of development. It can therefore be affirmed that potassium chromate exerted pressure on the stomata of this accession thereby causing their anticlinal walls to become wavy.

The subsidiary cells that surrounded each stoma ranged from five to nine in all the control accessions but treatments with these oilfield chemicals caused a change in the number of the subsidiary cells that surrounded a stoma in all the accessions (Table 5). All the accessions were affected as the chemicals caused the number of subsidiary cells to change; the change ranged from four to eleven but the accessions mostly affected were NCe 005, NXs 002 and NXs 003. The shapes of the epidermal cells of the treated accessions did not show much variation when compared to the control as their shapes ranged between quadrilateral to nonagonal. However, some circular and isodiametric shaped epidermal cells were spotted on the adaxial epidermis of NXs 002 treated with 7.5 mg/kg of potassium chromate and, on both epidermes of control NXs 003 (Plate 4).

For the control accessions, the numbers of epidermal cells on the adaxial epidermes were more than the numbers of epidermal cells on the abaxial epidermes; but in accessions NCe 004, NCe 005 and NXs 002 the reverse was the case (Table 1a and 1b). However, the effects of the treatments on the epidermal cells showed that the number of epidermal cells on the abaxial epidermis was more than the number of epidermal cells on the adaxial epidermis. Statistical evidence also confirmed that the differences in the number of epidermal cells between the adaxial and abxial epidermes were significant at 5%. Additionally, the mean epidermal cell number showed that on both epidermes, sodium azide treatments induced more number of epidermal cells in the accessions than potassium chromate treatments. It was observed that NXs 002 (221.8 with sodium azide treatments) had the highest mean number of epidermal cells on the abaxial epidermis and NCe 003 (214.0 with potassium chromate treatments) had the highest mean number of epidermal cells on the adaxial epidermis (Figs. 3 and 4). Furthermore, the treatments generally increased the number of epidermal cells in all the accessions when compared with their controls; the accessions with decreased epidermal cells when compared to their controls were NXs 003 and NXs 002 (Table 1b). However, in NXs 002 only the sample treated with 2.5 mg/kg of sodium azide had increased number of epidermal cells when compared with the control. Increased epidermal cell numbers due to pollution was also reported by Rai and Mishra [11] in *Pongamia pinnata* and Selebatso et al. [30] in *Arabidopsis thaliana*. Statistically, the differences in the number of epidermal cells between the treatments (Table 6) were not significant at 5% but the differences were significant at *P*=.05 between various accessions.

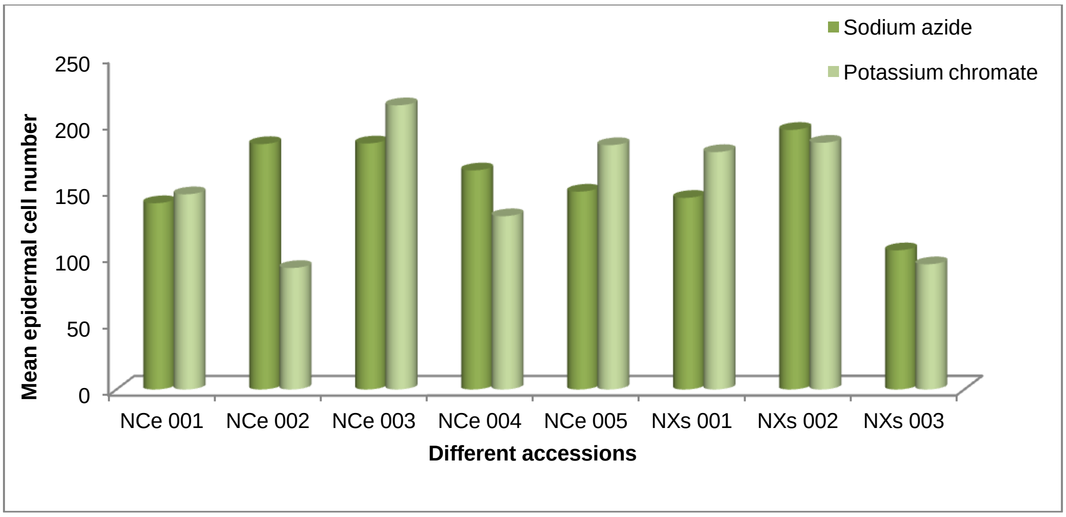

Fig. 3. Effects of sodium azide and potassium chromate on the mean number of epidermal cells of the adaxial epidermes in different accessions

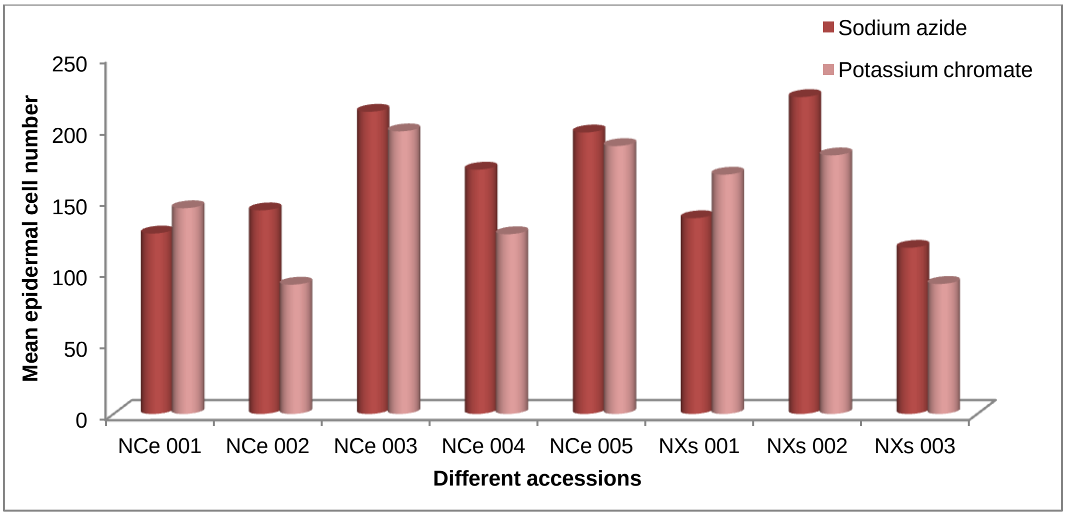

Fig. 4. Effects of sodium azide and potassium chromate on the mean number of epidermal cells of the abaxial epidermes in different accessions

Table 3. Different types of stomata present in the epidermes of different accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Sodium azide	2.5	Adaxial	B, BP	B, BP	B, BP	_	B, BP	B, AB	B	B, BM
		Abaxial	B, BP, BM	B, BP, BM	B	_	B, AB, BM, BP	B, AB, BM	B, AB	B, BP
	5	Adaxial	B, BP, BM	B	B, AB	B, AB, BP	B	B, BM	B, AB	B
		Abaxial	B, AB, BP, BM	B	B, BP	B, AB, BM, BP	B, BM, BP	B	B, AB	B, BP
	7.5	Adaxial	B, BP	B, BP	B	B, BP, BM	B, BP	B, BM	B	B
		Abaxial	B, BP	B, BP	B, BP, BM	B, AB, BM, BP	B, BM, BP	B	B	B, AB
	10	Adaxial	B, BP, BM	B	B, BP, BM	B, AB, BM, BP	B, BM, BP	B	B, AB	B, BM
		Abaxial	B, AB, BP	B, BP	B, AB, BP	B, AB, BP	B, AB, BM, BP	B, BM	B,BM	B, BP
Potassium chromate	2.5	Adaxial	B, BP	B, BP	B, BP	B, BP	B, BP	B, AB	B	B, AB, BM
		Abaxial	B, BP	B, BP, BM	B	B, BP	B	B, AB, BP	B, AB	B, AB
	5	Adaxial	B, BP	_	B	B, AB	B, BM, BP	B, BP	B, BM	B, AB
		Abaxial	B, BP	_	B, BP	B, AB, BP, BM	B, BM, BP	B, AB, BP	B, BP	B
	7.5	Adaxial	B	_	B	_	B, BM, BP	B	B, AB, BM, BP	B, AB
		Abaxial	B	_	B, BP, BM	_	B	B, BP, BM	B, AB	B
	1 0	Adaxial	B	B	B	_	B, BP	B, BP	B	B
		Abaxial	B, BP	B, BP	B, BP, BM	_	B, BM, BP	B	B, AB, BP	B
Control		Adaxial	B, BP	B, BP, BM	B, BP	B, BP	B, BP	B, BP	B, BM, BP	B, BM, BP
		Abaxial	B, BP	B, BP	B, BP	B	B, BM, BP	B, AB	B, AB, BM	B, AB, BM, BP

(Keys: B= Brachyparacytic, AB= Amphybrachyparacytic, BP= Brachyparatetracytic, BM= Brachyparahexacytic-monopolar, BD= Brachyparahexacytic-dipolar)

Table 4. Nature of anticlinal walls present in the epidermes of different accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Sodium azide	2.5	Adaxial	Straight	Straight	Straight	_	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	_	Straight	Straight	Straight	Straight
	5	Adaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
	7.5	Adaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
	10	Adaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Potassium chromate	2.5	Adaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
	5	Adaxial	Straight	_	Straight	Straight	Straight	Straight	Straight	Straight
		Abaxial	Straight	_	Straight	Straight	Straight	Straight	Straight	Wavy
	7.5	Adaxial	Straight	_	Straight	_	Straight	Straight	Straight	Straight
		Abaxial	Straight	_	Straight	_	Straight	Straight	Straight	Straight
	10	Adaxial	Straight	Straight	Straight	_	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	_	Straight	Straight	Straight	Straight
Control		Adaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
		Abaxial	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight

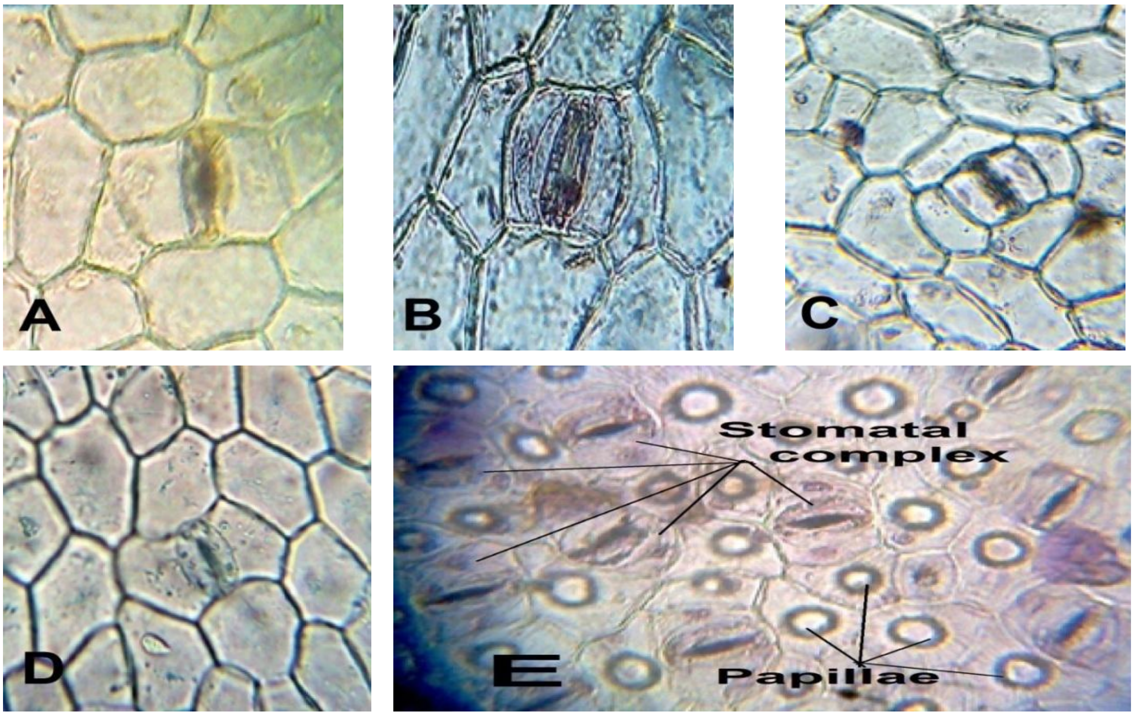

Plate 2. Different types of stomata: A) Brachyparacytic stomata, B) Amphibrachyparacytic stomata, C) Brachyparahexacytic-monopolar stomata D) Brachyparatetracytic stomata, E) Stomatal complexes and papillae

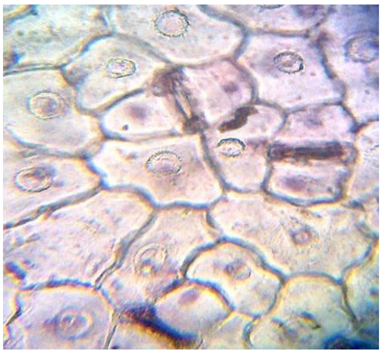

Plate 3. Wavy anticlinal walls of abaxial epidermis of NXs 003 with 5 mg/kg treatment of potassium chromate treatment

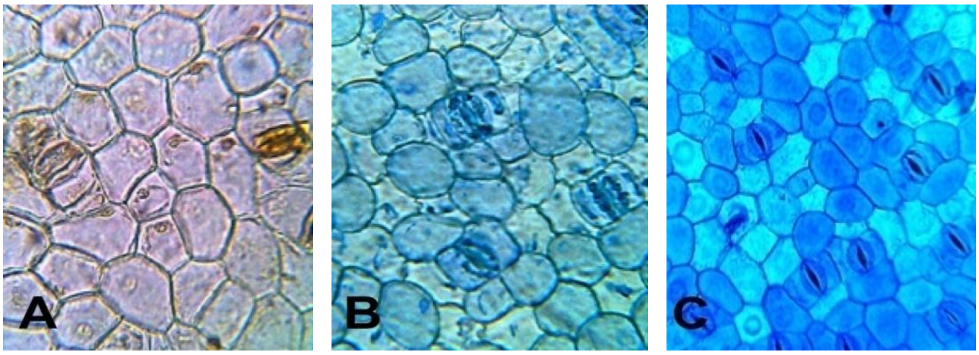

Plate 4. Isodiametric and circular shaped epidermal cells: A) Adaxial epidermis of NXs 002 treated with 7.5 mg/kg of potassium chromate, B) Adaxial epidermis of NXs 003 control, C) Abaxial epidermis of NXs 003 control

Table 5. Number of subsidiary cells present in the epidermes of different accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Sodium azide	2.5	Adaxial	4-7	6-7	7	_	6-8	5-8	5-7	4-5
		Abaxial	4-7	6-8	5-9	_	5-7	5-6	4-5	4-6
	5	Adaxial	4-7	7-9	5-7	6-7	6-8	4-6	4-7	5
		Abaxial	5-7	5-7	5-9	6-8	5-7	6	4-6	5-6
	7.5	Adaxial	6-7	6-7	6-8	6-8	6-8	5-6	5-7	4-7
		Abaxial	5-6	5-7	5-8	6-8	6-8	6-7	5-6	4-5
	10	Adaxial	6-7	6-8	5-8	5-6	5-8	5-7	5-7	4-6
		Abaxial	5-7	5-9	5-7	5-7	5-7	5-6	4-5	4-5
Potassium chromate	2.5	Adaxial	6-8	6-10	5-8	6-9	6-7	5-7	4-8	4-6
		Abaxial	5-8	6-8	6-7	5-7	5-8	5-6	5-7	4-5
	5	Adaxial	6-10	_	5-7	6-8	4-10	5-6	5-7	4-6
		Abaxial	6-8	_	5-9	4-8	6-9	5-6	5-6	4-6
	7.5	Adaxial	5-7	_	5-7	_	5-11	5-7	4-7	4-6
		Abaxial	5-8	_	5-7	_	4-6	4-7	4-7	4-6
	10	Adaxial	6-7	5-8	5-7	_	6-8	4-7	5-6	4-6
		Abaxial	6-7	6-8	5-8	_	4-7	5-6	4-6	4-5
Control		Adaxial	6-8	6-7	6-8	6-9	6	5-7	5-7	5-8
		Abaxial	5-8	5-7	5-8	6-8	6-8	5-6	5-6	6-7

Table 6. Analysis of variance showing mean square of effects of the treatments on stomatal indices

Source of variation	df	MS	F	F crit
Treatments	17	3877.15	1.21	1.70
Accessions	7	18935.12	5.91**	2.08
Error	119	3210.94		
Total	143			

***= indicate significance at P=.05*

4. CONCLUSION

This study shows that potassium chromate and sodium azide are capable of inducing variations in the epidermal characters of *C. esculenta* and *X. maffafa* accessions. From the study, it can be deduced that sodium azide is a more powerful chemical than potassium chromate; as most of the variations observed were more pronounced with sodium azide treatments. Though a lot of scholars have called for the use of sodium azide in plant mutagenic breeding programs, the authors wish to state that before that could be done; thorough physico-chemical and cytological analyses must be carried out on such plants to ascertain their safety levels. Furthermore, since oil workers are frequently exposed to these chemicals, proper handling of these chemicals should be stressed to ensure the safety of lives and the environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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