

Effects of Sodium Azide and Potassium Chromate on the Morphological Characters of *Colocasia esculenta* (L.) Schott. and *Xanthosoma maffafa* (L.) Schott. Accessions in Nigeria

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Abstract- The morphological assessment of five accessions of *Colocasia esculenta* (NCe 001, NCe 002, NCe 003, NCe 004 and NCe 005) and three accessions of *Xanthosoma maffafa* (NXs 001, NXs 002 and NXs 003) exposed to different concentrations of sodium azide and potassium chromate was carried out using 2.5, 5, 7.5, 10 mg/kg and the unpolluted accessions were used as the control. Results showed that accessions treated with sodium azide had higher mean plant height, mean leaf area and mean yield when compared to the accessions treated with potassium chromate. However, the differences in height, leaf area and girth between treatments were not significant at 5 % but these differences between the accessions were significant at 5 %. Albeit, the difference in yield between treatments was significant at $P=0.05$ but the difference in yield between accessions was not significant at $P=0.05$. Malformed leaves, chlorosis, change in stem colour including plant deaths were some of the variations observed in the accessions treated with these chemicals. Further studies were encouraged for a clearer understanding of the potentials of these chemicals in crop mutation breeding programs.

Index Terms- Sodium azide, potassium chromate, *Colocasia esculenta*, *Xanthosoma maffafa*

I. INTRODUCTION

Oil field chemicals are those chemicals that are in use during oil exploration and production activities. Drilling operations can introduce oil and a wide range of other complex chemical compounds into the environment through drilling fluids and muds (Kloff and Wicks, 2004). When these fluids find their way into the environment the physical, chemical and microbiological properties of the soil are affected (Ekundayo *et al.*, 1989). This discharge has its own peculiar chemistry and plants respond to it differently (Okonokhua *et al.*, 2007); some of such responses could be visible (signs of stress, morphological distortions, fertility and eventually the death of the plant) or invisible (anatomical, physiological, biochemical and genetical compositions).

To examine the impact of oilfield chemicals in the environment, the World Bank recommended Strategic Environmental Assessment (SEA) in the oil exploration and

exploitation locations. Over the years, plants have been used as bioassay to study environmental chemicals with mutagenic/genotoxic potentials (Maluszynska and Juchimiuk, 2005; Nkwocha and Duru, 2010; Olayinka and Arinde, 2012 and Ohanmu *et al.*, 2014). Sodium azide and potassium chromate are two oilfield chemicals of interest to this study. Sodium azide is a biostat used for preventing biofouling of wells. It is used for controlling bacteria that interfere with the production operations of the oil and gas industry; potassium chromate on the other hand, is a corrosion inhibitor that forms a non-reactive thin surface on the metal that prevents access of corrosive substances to the metal surface, thereby inhibiting further corrosion.

Cocoyam (*Colocasia esculenta* and *Xanthosoma maffafa*) belong to the Araceae family and widely cultivated in the Niger Delta region of Nigeria where oil exploration and production activities as well as their associated crude oil pollution frequently take place. In Nigeria, cocoyam is the third most important tuber crop apart from yam and cassava and provides a cheaper yam substitute especially in the eastern part of the country (Azeez and Madukwe, 2010). Apart from being a source of food, cocoyam is used as raw material in food and biotechnology industries (Owusu-Darko *et al.*, 2014), in the production of biofuel (Braide and Nwaoguikpe, 2011 and Adelekan, 2012) and also has therapeutic potentials (Kundu *et al.*, (2012). Ten distinctive groups (7 for *Colocasia sp* and 3 for *Xanthosoma sp*) have been reported in the germplasm of the National Root Crop Research Institute (NRCRI) (Mbanaso, 2010).

This study therefore is limited to investigating the effects of sodium azide and potassium chromate treatments on the morphological characters of cocoyam accessions. This study would also establish if cocoyam can serve as a reliable environmental bioindicator.

II. 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 Abia State, Nigeria. NCe stands for Nigeria *Colocasia esculenta* and NXs means Nigeria *Xanthosoma* species. Each accession was planted in 4 different

concentrations: sodium azide 2.5, 5, 7.5 and 10 mg/kg and potassium chromate 2.5, 5, 7.5 and 10 mg/kg. These chemical concentrations resulted to 0.25, 0.5, 0.75 and 1 % w/w in soil on weight basis. 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, kept in the open and monitored regularly. 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 was in a Randomized Complete Block Design and kept at the Ecological Research Centre of the University of Port Harcourt.

Morphological characters of the control and the treated plants were carried out five months after planting. Plant heights were obtained by measuring the plants from the soil level to the collar of the uppermost leaf. The leaf areas were 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 (Okonokhua *et al.*, 2007). The girths were

also measured, while the number of malformed leaves, leaves with chlorosis and different stem colours were visually scored. At maturity, the corms were harvested and weighed.

Data generated were exposed to two way analysis of variance (ANOVA) and the means were separated using the HSD test at 5 %.

III. RESULTS

Effects on plant height: The effects of sodium azide and potassium chromate treatments on the heights of different accessions were shown in Table 1. The difference in mean plant heights between different treatments was not significant at $P=0.05$ but the difference in mean plant heights between different accessions was significant at $P=0.05$. Investigation also showed that NXs 001 of *X. maffafa* was mostly affected by sodium azide treatment, having a mean height of 23.8 cm while NCe 004 of *C. esculenta* was mostly affected by potassium chromate treatment, having a mean height of 13.88 cm (Figure 1).

Table 1: Effects of sodium azide and potassium chromate treatments on the plant height (cm) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		29.1	34.0	33.8	28.3	31.0	24.5	26.0	30.2
Sodium azide	2.5	33.0	29.0	37.8	–	32.0	27.5	34.5	35.5
	5	41.6	34.0	29.5	35.0	27.8	26.2	34.8	39.3
	7.5	43.8	37.5	38.0	41.5	28.0	22.5	26.5	43.3
	10	43.0	28.2	29.5	29.0	26.0	19.0	27.0	34.5
Potassium chromate	2.5	34.0	30.0	32.8	28.0	26.5	21.7	22.5	34.5
	5	39.0	–	34.3	27.5	23.5	27.5	27.0	36.0
	7.5	36.0	–	35.0	–	26.0	21.0	32.0	34.5
	10	30.0	33.5	26.0	–	29.5	16.0	4.5	36.0

Effects on leaf area: The effects of these oilfield chemicals on the leaf area of various accessions under study were summarized in Table 2. The difference between different treatments was not significant at $P=0.05$ but the difference between difference accessions was significant at $P=0.05$.

Moreover, it was observed that the leaf area of NXs 002 was mostly affected by sodium azide treatment, having a mean leaf area of 146.2 cm² while potassium chromate treatment badly affected NCe 004 (Figure 2), with a mean leaf area of 61.9 cm².

Table 2: Effects of sodium azide and potassium chromate treatments on the leaf area (cm²) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		113.1	139.5	194.5	160.5	201.5	149.5	121.5	201.7
Sodium azide	2.5	72.9	158.4	212.3	–	175.3	172.3	181.6	233.4
	5	154.9	150.5	119.1	203.0	176.7	192.2	167.9	477.5
	7.5	198.7	170.7	223.1	331.1	208.8	133.5	105.4	375.2
	10	227.1	178.1	113.9	150.1	171.7	110.3	129.9	217.0
Potassium chromate	2.5	110.9	132.0	187.3	90.1	190.2	66.2	112.3	246.2
	5	154.3	–	146.5	157.5	145.2	202.5	98.6	229.4

7.5	62.7	–	179.3	–	168.3	107.1	198.5	211.5
10	94.3	105.0	116.9	–	300.9	56.1	11.3	384.8

Effects on girth: The summary of the effects of these chemicals on the plant girth was captured in Table 3. The difference between different accessions was significant at $P=0.05$ but the difference between different treatments was not significant at $P=0.05$. However, analysis showed that the girth of

NCe 001 was mostly affected by sodium azide treatment, with a mean girth of 4.32 cm. On the other hand, potassium chromate treatment badly affected NCe 002 as it had a mean girth of 1.88 cm (Figure 3).

Table 3: Effects of sodium azide and potassium chromate treatments on the girth (cm) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		5.7	6.2	7.2	5.1	5.3	5.7	5.0	6.2
Sodium azide	2.5	3.0	4.0	5.1	–	6.9	5.5	7.5	4.5
	5	4.5	5.5	4.5	6.2	4.5	4.0	7.3	5.3
	7.5	5.3	4.8	6.0	7.5	5.6	4.2	5.0	6.5
	10	4.5	3.5	5.0	6.0	5.9	5.0	6.5	5.5
Potassium chromate	2.5	4.4	4.0	5.0	5.0	6.8	4.6	5.6	5.9
	5	5.0	–	4.5	4.1	6.8	5.3	6.0	4.5
	7.5	3.5	–	5.2	–	4.3	6.0	7.5	5.5
	10	3.0	3.5	4.3	–	7.0	4.5	1.2	6.0

Effects on yield: The effects of sodium azide and potassium chromate treatments on the weight of yield of various accessions were summarized in Table 4. The difference in mean yield between different treatments was significant at $P=0.05$ meanwhile the difference between various accessions was not significant at $P=0.05$. Albeit, NCe 002 had the least mean yield

as it produced 67.5 g and 17.5 g in sodium azide and potassium chromate treatments respectively. Assessment between the two chemicals also showed that sodium azide treatment influenced the accessions to have higher yield than potassium chromate treatments (Figure 4).

Table 4: Effects of sodium azide and potassium chromate treatments on the weight of yield (g) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		150 ^a	150 ^a	100 ^a	75 ^a	100 ^a	50 ^a	25 ^a	200 ^a
Sodium azide	2.5	70 ^a	35 ^a	130 ^a	0 ^a	200 ^a	150 ^a	150 ^a	180 ^a
	5	160 ^a	100 ^a	150 ^a	50 ^a	150 ^a	50 ^a	100 ^a	150 ^a
	7.5	230 ^a	50 ^a	170 ^a	500 ^a	80 ^a	70 ^a	100 ^a	200 ^a
	10	80 ^a	85 ^a	160 ^a	25 ^a	200 ^a	50 ^a	100 ^a	150 ^a
Potassium chromate	2.5	50 ^a	50 ^a	90 ^a	50 ^a	170 ^a	80 ^a	150 ^a	220 ^a
	5	100 ^b	0 ^b	50 ^b	200 ^b	80 ^b	100 ^b	30 ^b	120 ^b
	7.5	50 ^b	0 ^b	60 ^b	0 ^b	100 ^b	50 ^b	40 ^b	150 ^b
	10	40 ^b	20 ^b	80 ^b	0 ^b	100 ^b	60 ^b	10 ^b	50 ^b

Values with different superscripted alphabets within column are significantly different at 5 %

Effects on leaf surface: Potassium chromate treatment induced malformed leaves in NCe 003, NXs 002 and NXs 003 (Plate 1). Interestingly, when these malformed leaves either fell

off or are excised the new leaves that emerged were observed to be normal.

Other effects: Different variations observed on the morphology of various accessions included chlorosis, change in stem colour, change in the number of leaves per plant.



Plate 1: Malformed leaves: a) Control leaf of *Colocasia sp.*, b) Control leaf of *Xanthosoma sp.*, c) NXs 002 treated with 7.5 mg/kg of potassium chromate, d) NXs 002 treated with 5 mg/kg of potassium chromate, e) NXs 003 treated with 10 mg/kg of potassium chromate, f) NCe 003 treated with 5 mg/kg of potassium chromate

IV. DISCUSSION

Plant heights of these accessions responded differently to the chemical treatments. Morphologically, it was expected that the controls would perform better than the treated accessions but it was not so as some of the treated accessions even with higher concentrations had taller plants than the control. Calculating the mean height, it was observed that the total mean height of accessions treated with sodium azide (Figure 1) produced taller plants than with potassium chromate treatment. This trend was also reported by Warghat *et al.* (2011) in *Abelmoschus moschatus*. They observed that treatment with sodium azide even in higher concentrations produced plants that were taller than the control. The significant difference between different accessions indicates that each accession has its own unique chemistry and way of dealing with environmental chemical (Osuji and Nwala, 2015). Increased plant heights in relation to the control were also reported in *Colocasia esculenta* polluted with crude petroleum oil (Bamidele and Sijuade, 2012).

The responses of these accessions to leaf area and girth also follow the trend of the plant height. It was observed that the differences between different treatments were not significant at 5 % but the differences between different accessions to these parameters were. Al-Qurainy (2009) on *Eruca sativa*, also reported increased leaf area even at higher concentration of sodium azide. The comparison between sodium azide and potassium chromate treatments across these parameters showed that sodium azide treated plants had better vegetative attributes. This indicates that potassium chromate is a toxic environmental chemical and, suppresses vegetative vigour of these accessions more than sodium azide. Reduced leaf area has been attributed to limitation of nutrients uptake necessary for expansion of leaf area occasioned by high level of pollutants (Adu *et al.*, 2015).

The mean yield difference between different accessions was not significant but the mean yield difference between treatments was significant. Different accessions treated with sodium azide produced more yield than those treated with potassium chromate, even when compared with the controls (Table 4). Sodium azide with 7.5 mg/kg produced the highest yield in NCe 004 (500 g) and NCe 001 (230 g) as compared with their controls that produced 75 g and 150 g respectively. Meanwhile, potassium chromate treatments produced the highest yield in NXs 003 (220 g) treated with 2.5 mg/kg and NCe 004 (200 g) treated with 5 mg/kg; their controls however, produced 200 g and 75 g respectively. Al-Qurainy (2009) reported that at 3 mM concentration of sodium azide, the yield after 60 days was

found to be very high even when compared to untreated plants. In the same vein, Eze and Dambo (2015) reported that *Zea mays* treated with 0.02 mM of sodium azide produced the best yield in terms of vigour and size when compared to the control. They suggested that sodium azide is a chemical with the potential to induce targeted variability in plants and induces point mutation in plants bringing about robust and improved characters in plants. This suggestion is evident in the responses of these accessions to sodium azide treatments.

Malformed leaves are one of the first signs that indicate a plant under stress; this character was seen in some accessions treated with potassium chromate. Obute *et al.* (2007) attributed the development of malformed leaves to chromosome fracture, decline in the auxin level and change in ascorbic acid concentration. The formation of normal leaves after the excision of the malformed leaves confirmed that potassium chromate had genotoxic potential and the potency of the chemical subsided after awhile. The reduction of the efficacy of potassium chromate after its introduction into the environment was also reported by Ajah and Obute, (2017). Chlorosis and change in stem colour are some of the effects of these chemicals on the accessions. Gnanamurthy *et al.* (2011) and Warghat *et al.* (2011) also reported similar result with sodium azide treatments. They attributed it to decline in the chlorophyll content of plants; this decline causes nutrient restrictions and interferences that decrease the rate of photosynthesis leading to poor yield in plants. The change in the number of leaves was also reported by Adu *et al.* (2015) on *Vigna unguiculata* with spent and unspent engine oil; they credited this phenomenon to the inability of treated plants to absorb water due to a change in the physical, biological and chemical properties of the soil.

Plant deaths were observed in some accessions following treatments with these oilfield chemicals. Potassium chromate and sodium azide treatments caused the death of some accessions: NCe 004 treated with 2.5 mg/kg of sodium azide, NCe 002 treated with 5 and 7.5 mg/kg of potassium chromate and NCe 004 treated with 7.5 and 10 mg/kg of potassium chromate did not germinate at all. Omusun *et al.* (2008) with crude oil on *Amaranthus hybridus* reported plant death and credited the death of plants to poor wettability and aeration of the soil which causes loss of seed viability. The presence of these oilfield chemicals alters the chemistry of the soil, kills the microbes, and truncates the cell division that leads to growth thereby killing the corms which led to lack of germination at all.

This study showed that potassium chromate treated plants have more severe effects than sodium azide treated plants

(Figures 1, 2, 3 and 4). This indicates that potassium chromate is a chemical with xenobiotic potentials and is capable of causing the arrest of enzymatic activities, infertility, decay of vital plant parts and eventually plant deaths. More studies have to be carried out using this chemical so that its potentials can fully be understood. Sodium azide on the other hand, has been applauded by many researchers who advocated inculcating this chemical into plant breeding programs because it has produced robust characters in plants just like colchicine. In this study also, some treated accessions were observed to have higher yields than the controls; this trend is disturbing as the chemical composition of these yields may contain traces of these chemicals. This brings to mind what would happen if some of these yields were consumed by man?

V. CONCLUSION

The effects of these oilfield chemicals have been seen on the morphology of cocoyam but for a more definitive stance on whether to include these chemicals especially sodium azide in mutation breeding program the cuticular, proximate analysis and cytological studies have to be carried out as well. This study has shown that cocoyam is not a good environmental bioindicator because some treated accessions even in higher concentrations had more vegetative vigour than the control accessions. Therefore, it is pertinent to carry out soil assessment/monitoring (physico-chemical analysis) on plots of land where cocoyam would be planted so as to ensure that the soil is not compromised (polluted with chemicals) hence, ensuring the safety of our food source.

APPENDIX

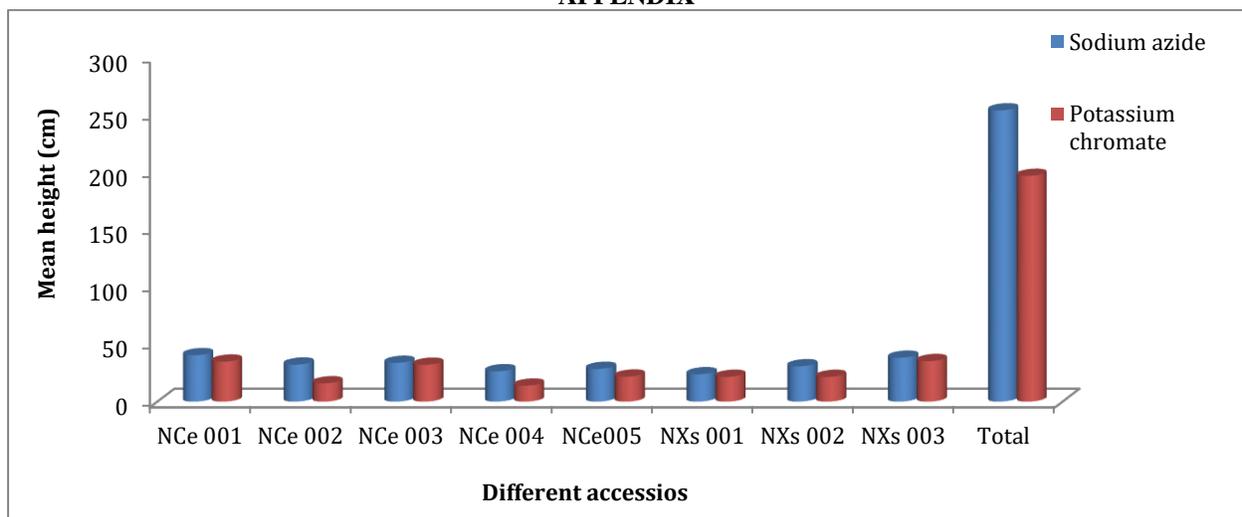


Figure 1: Effects of sodium azide and potassium chromate treatments on the mean height off different accessions

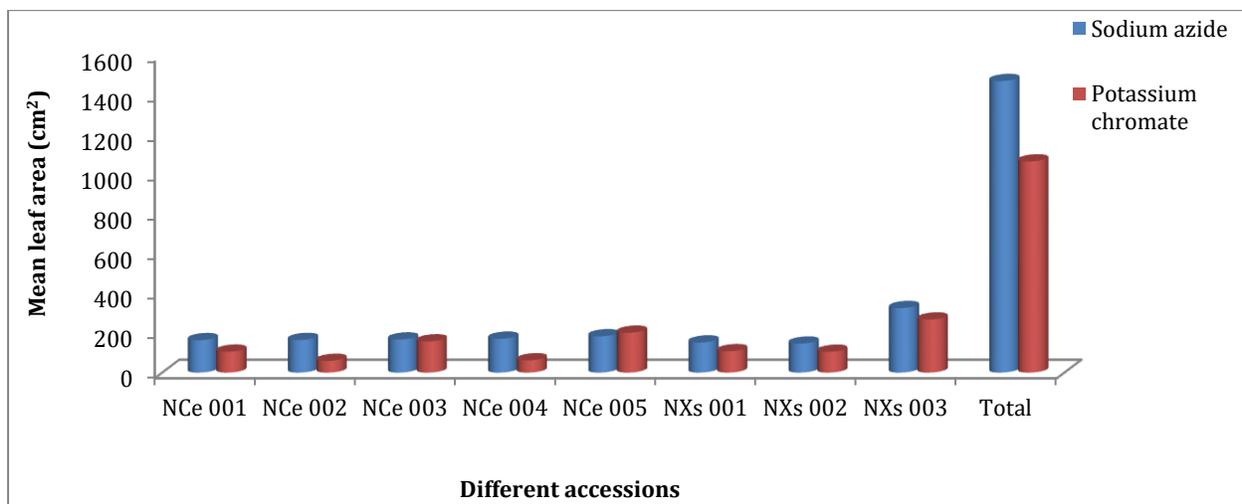


Figure 2: Effects of sodium azide and potassium chromate treatments on the leaf area means of different accessions

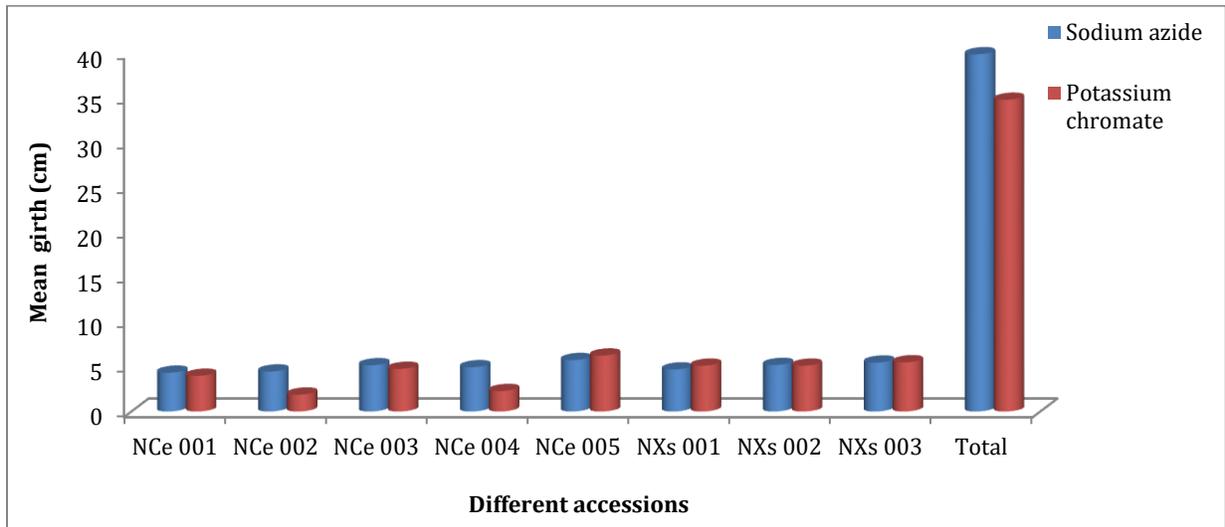


Figure 3: Effects of sodium azide and potassium chromate treatments on the means of girth of different accessions

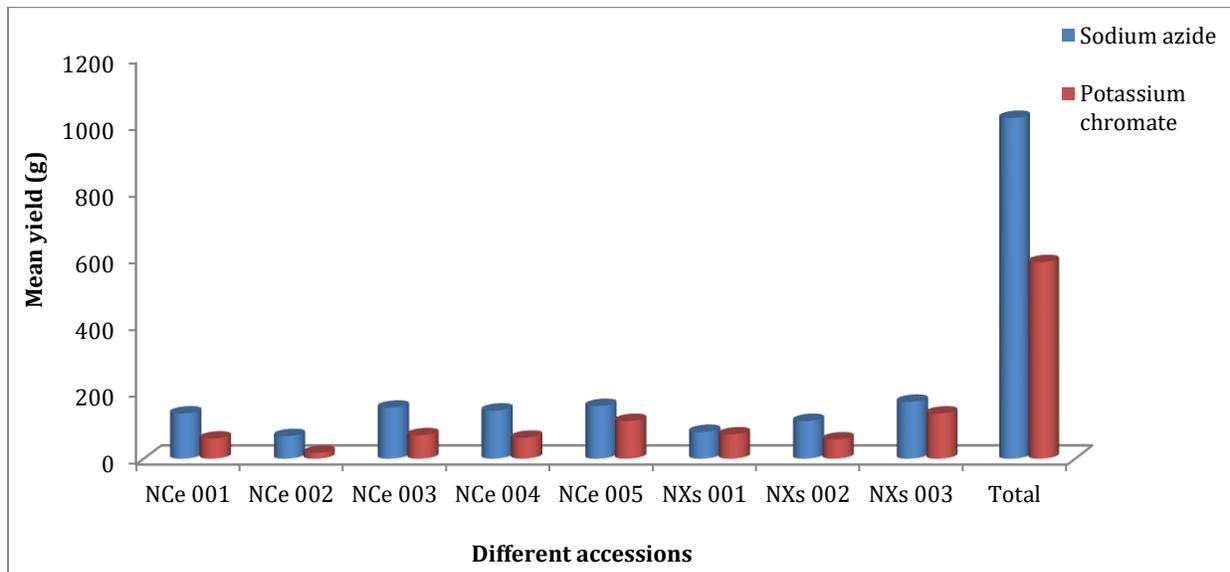


Figure 4: Effects of sodium azide and potassium chromate treatments on the mean yield of different accessions

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