AMELIORATIVE IMPACT OF SALICYLIC ACID ON GROWTH OF *ABELMOSCHUS ESCULENTUS* VAR. CLEMSON SPINELESS UNDER ALUMINIUM TOXICITY.

Umebese, C. E. and Fabiyi, O. O.

Department of Botany, University of Lagos, P.M.B. 1029
Unilag Post Office, Akoka-Yaba, Lagos

ABSTRACT

The objective of the present study was to protect the growth of *Abelmoschus esculentus* var. Clemson spineless (okro) using salicylic acid (SA) in soil subjected to aluminium (Al) toxicity and Al chelated with ethylenediaminetetraacetic acid (EDTA). Okro plants were grown in soil contaminated with Al (1.5g kg^{-1}) in the following combinations: Al, Al+SA, Al+EDTA, Al+SA+EDTA and a control (water). The growth parameters were studied over a period of ten weeks while the total sugar and total chlorophyll contents were determined at the 10th week of growth. Al toxicity caused significant reductions (57-84%) in all growth parameters (plant height, fresh and dry shoot weight, fresh and dry root weight, leaf area, stem girth, fruit fresh and dry weight, fruit number) except the Net assimilation rate, Leaf area ratio and Relative growth rate. Treatment of stressed plants with SA improved the growth parameters by 17 - 165%, total soluble sugar by 140% and total chlorophyll by 22%. Plants subjected to chelated Al (EDTA + Al) exhibited much greater reductions in growth than those grown in Al only. Treatment of stressed plants with SA under chelated Al toxicity (Al + SA + EDTA) improved the Growth parameters by 18% - 150%, total sugar by 130% and total chlorophyll by 140% but the impact was less than that of non chelated Al (Al+SA). Al toxicity caused marked reductions in growth parameters, chlorophyll and sugar contents but chelating Al with EDTA resulted in much more decrease in these parameters. SA exhibited higher ameliorative capacity when plants were exposed to non chelated Al toxicity.

Key words: Al toxicity, EDTA, Salicylic acid, Growth, Chlorophyll.

INTRODUCTION

Aluminium (Al) is the most abundant metal in the earth's crust, comprising about 7% of its mass. Since many plant species are sensitive to micro molar concentrations of Al, the potential for soils to be Aluminium-toxic is considerable. Fortunately, most of the Aluminium is bound by ligands or occurs in other non-phytotoxic forms such as aluminosilicates and precipitates. However, solubilization of this Aluminium is enhanced by low pH. Al toxicity is a major factor limiting plant production on acid soils. Soil acidification can develop naturally when basic cations are leached from soils, but it can be accelerated by some farming practices and by acid rain (Kennedy, 1986). Strategies to maintain production on these soils include the application of lime to raise the soil pH and the use of plants that are tolerant to acid soils.

Inhibition of root and shoot growth is a visible symptom of Al toxicity. The earliest symptoms concern roots. Shoots in contrast to the situation observed for Manganese toxicity are less affected (Chang *et al.*, 1999). Root stunting is a consequence of Al-induced inhibition of root elongation. Roots are usually stubby and brittle and root tips and lateral roots become thick and may turn brown (Mossor-Pietraszewska *et al.*, 1997). Such roots are inefficient in absorbing both nutrients and water. Young seedlings are more susceptible than older plants. Aluminium apparently does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Nosko *et al.*, 1988).

The common responses of shoots to Al include: cellular and ultrastructural changes in leaves, increased rates of diffusion resistance, reduction of stomata aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot biomass (Thornton *et al.*, 1986). Accumulating evidence shows that Aluminium affects photosynthesis (Pereira *et al.*, 2000), photoprotective systems (Chen *et al.*, 2005), water relations (Simon *et al.*,1994), carbohydrate content (Graham, 2002), mineral nutrition (Lidon *et al.*, 1999), organic acid metabolism (Watanabe *et al.*, 2002), and nitrogen metabolism (Xiao, 2002) in plant shoot.

Salicylic acid (SA) is considered to be a plant hormone-like substance which plays an important role in the regulation of plant growth and development, seed germination, fruit yield, glycolysis, flowering, and heat production in thermogenic plants (Klessig *et al.*, 1994). The rates of photosynthesis, stomata conductance, and transpiration could also be affected by the application of SA (Khan *et al.*, 2003). Exogenous application of SA induces biotic and abiotic stress tolerance in crops, including increased cold tolerance of germination in pepper (Korkmaz, 2005), chilling tolerance in cucumber (Kang *et al*, 2002) and in maize (Janda *et al.*, 2000), salinity tolerance in barley (El-Tayeb, 2005), improved heat shock tolerance in mustard (Dat *et al.,* 1998), and a decrease in the inhibitory effects of drought stress on tomato, bean, amaranth (Senaratna *et al.*, 2000; Umebese *et al.,* 2009), and wheat (Sakhabutdinova *et al.*, 2003); it also elicits tolerance to toxic metals (Strobel *et al.*, 1995). SA is known to play an important role in modulating the redox balance across membranes, thereby counteracting the negative effects of reactive oxygen species (ROS) generated by oxidative stress (Yang *et al.,* 2004) by increasing the activity of anti-oxidant enzymes such as superoxide dismutase (Singh *et al.*, 2003).

Ethylenediaminetetraacetic acid (EDTA) is a hexadentate (six toothed) ligand and chelating agent often found to be the most effective chelating agent with the ability to sequester metals such as copper, manganese, iron, lead and cobalt (Blaylock *et al.*, 1997). It improves metal build up in the shoot of plants because it develops a metal chelate complex which enhances its movement within the plant, increasing its transport from root to aerial parts (Turgut *et al.*, 2004). EDTA has been shown to induce improved plant growth when used as a chelate for zinc (Salama *et al.*, 2012).

This study investigates the ameliorative impact of salicylic acid on the growth of *Abelmoschus esculentus* in soils contaminated with Al and Al chelate.

MATERIALS AND METHODS

The seeds of *Abelmoschus esculentus* var Clemson spineless (okro) were collected from the Institute of Agriculture Research and Training (IART) Ibadan. Seeds were surface-sterilized with sodium hypochlorite solution (1%) for 15 minutes, and washed thoroughly with distilled water before planting.

Experimental Procedure

The present study was carried out in the Botanic Garden of the University of Lagos from May to July 2013. Seeds were sown in nursery for two weeks before transplanting into pots. 400kg of surface soil (0.15 m) was collected from the agricultural field, air-dried and uniformly mixed with 400 g of NPK. The soil was divided into five batches of 20 pots (19.5cm x 19.5cm) each filled with 4 kg of soil. Treatments were as shown below: Batch 1 was the control where plants were not subjected to Al stress (water only); Batch 2 plants were subjected to 3.35 g Al (30g $AlCl_3.H_2O$); Batch 3 plants were subjected to Al and 0.01 M Salicylic acid (SA). In preliminary investigations, 3.35 g Al together with 3 mM Ethylenediamine-tetraacetic acid (EDTA) killed all the plants. Therefore, in Batch 4 plants were subjected to half concentration of Al (1.675 g) and 3 mM EDTA and Batch 5 plants were subjected to 1.675 g Al + 3 mM EDTA+ 0.01 M SA.

The experimental set up was housed under a transparent plastic shade that permitted good air flow and sunlight penetration but excluded incident rainfall. Plants were arranged in a Randomized Complete Block Design with three replicates. Four plants were transplanted into each pot which was thinned to two plants per pot after the first week. Heavy metal and chelant were applied twice to each pot, three days after transplanting and three days before the final harvest.

Growth Analysis

Three replicates of plants were harvested from each of the batches at 4, 6, 8 and 10 weeks after planting. Growth parameters measured include plant height, leaf area, fresh and dry weights of root, shoot and the whole plant, fruit number, fruit fresh and dry weights, net assimilation rate (NAR), leaf area ratio (LAR) and relative growth rate (RGR).

The leaf area (LA) of plants was determined as outlined by Eze (1965). Each leaf was carefully traced on paper and the leaf traces were weighed. 100 cm^2 of the same paper was weighed to give the standard weight (SW) using Mettler electronic balance. The leaf areas of the leaf traces (LT) were determined using the formula:

$$LA = \frac{W \text{ of LT (g) X standard area (100cm}^2)}{SW \text{ (g)}}$$

Plants were dried in an oven at 80°C for 3 days. Thereafter, the dry weight of the whole plant, shoot and the root were taken. Leaf area ratio (LAR), Net assimilation rate (NAR) and Relative growth rate (RGR) were determined using the appropriate mathematical expression as outlined by Noggle and Fritz (1976)

Determination of Sugar Content
Five hundred milligrams of powdered samples were extracted for 6 h with 50 ml boiling 80% ethanol using a Soxhlet extractor, dried and redissolved in 5ml distilled water. 1ml of 5% (w/v) phenol was added to 1ml extract and 5ml concentrated sulphuric acid was quickly dispensed into the mixture and allowed to cool (Dubois *et al.*, 1956). The optical density was taken at 490 nm using a Corning Spectrophotometer 258.

Estimation of Chloropyll Content
Chlorophyll content of okro was determined using the method of Arnon and Withom (Arnon, 1949; Withom *et. al.*, 1971). Pigments were extracted from 100 mg fresh leaves in 80% acetone and the absorption at 665, 663, 649 and 626 mm were read in a spectrophotometer using the absorption coefficient and the amount of chlorophyll was calculated. The amount of chlorophyll a and b present in the leaf extract was expressed in terms of mg chlorophyll per gram leaves.

RESULTS

The impact of Al toxicity on okro grown in soil treated with toxic concentration of Al and the ameliorative impact of salicylic acid (SA) and chelation with EDTA on plant height, root length, stem girth and leaf area, are shown in Figs. 1, 2, 3 and 4, respectively. Al toxicity caused significant decrease in plant size, when compared with the control. Generally, plants subjected to Al+EDTA exhibited the least size followed by those grown in Al+EDTA+SA even at half strength of Al. SA enhanced the size of plants under Al toxicity both in non-chelate (Al+SA) and chelate (Al+EDTA+SA) batches but the ameliorative impact was greater in the absence of EDTA. However, the size of the control plants was significantly higher (P= 0.05) than all treatments.

The impact of Al toxicity on the plant part dry weights (shoot, root) is shown in Figs. 5 and 6. Weight changes in plant part apparently followed the pattern of size increase in the earlier figures. Plants subjected to any combination with EDTA had the least dry weight. SA-treated plants exhibited significantly improved growth under Al toxicity, with or without the chelate. It appears that the roots of okro are more sensitive to Al toxicity than the shoots as shown by the marked fall in root dry weight in stressed plants.

The impact of Al toxicity on fruit number and fruit weight (Figs. 7 and 8) is not as pronounced as its impact on other plant organs. SA enhanced fruit production in stressed plants, above that of the control. In the presence of EDTA, fruit reduction was significant and the impact of SA on fruit weight was significant.

Table 1 shows the impact of non-chelated and chelated Al toxicity on Leaf Area Ratio, Net Assimilation Rate and Relative Growth Rate of okro and the ameliorative effect of SA. Results show no obvious differences in these parameters between the stressed and unstressed plants.

Figures 9 and 10 show the impact of Al toxicity on the leaf sugar and chlorophyll contents of okro leaves and the ameliorative effect of SA under chelated and non chelated Al. The control had the highest total soluble sugar. The percentage of the total soluble sugar relative to the value of the control is 29%, 69%, 8% and 23 % for Al, Al+SA, Al+EDTA and Al+SA+EDTA respectively. Though SA showed varying ameliorative effects, the values were still significantly lower than the control. Total chlorophyll content in the control, Al+SA and

Al+SA+EDTA showed no significant differences (P=0.05). Plants subjected to Al and Al+EDTA had the lowest total chlorophyll content with 0.2 ± 0.0458 mg g^{-1} and 1.453 ± 0.0322 mg g^{-1} respectively.
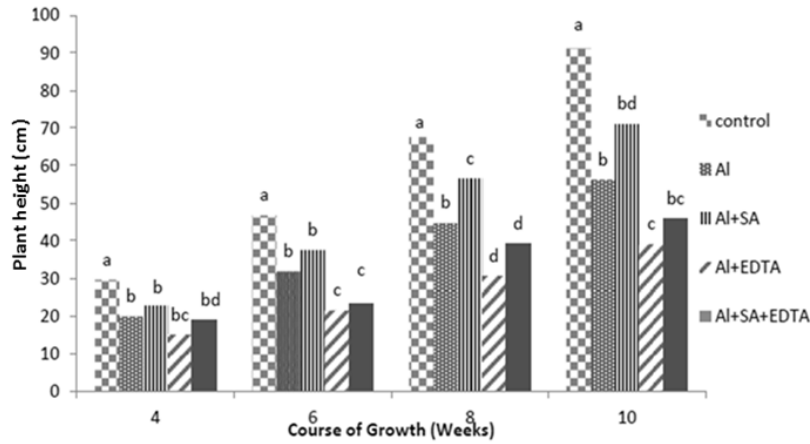

Fig. 1: Plant height of okro plants subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test
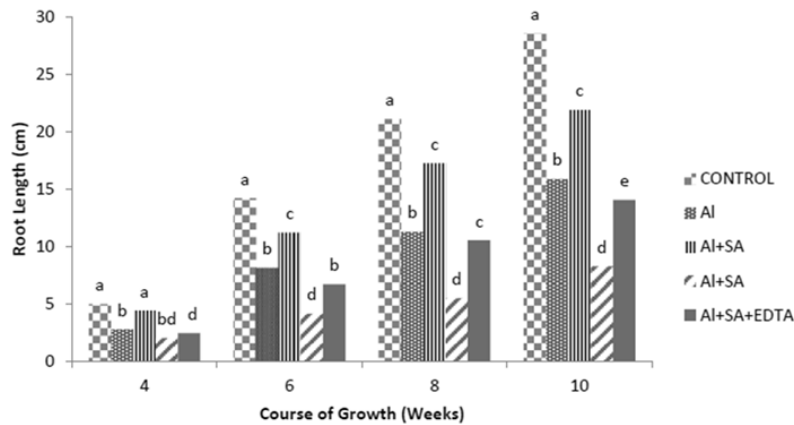

Fig. 2: Root length of okro plants subjected to Al, Al + SA, Al + EDTA and Al + SA + EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
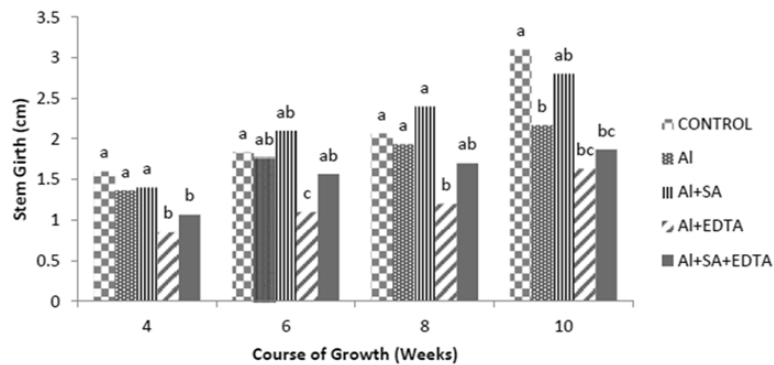

Fig. 3: Stem girth of okro plants subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
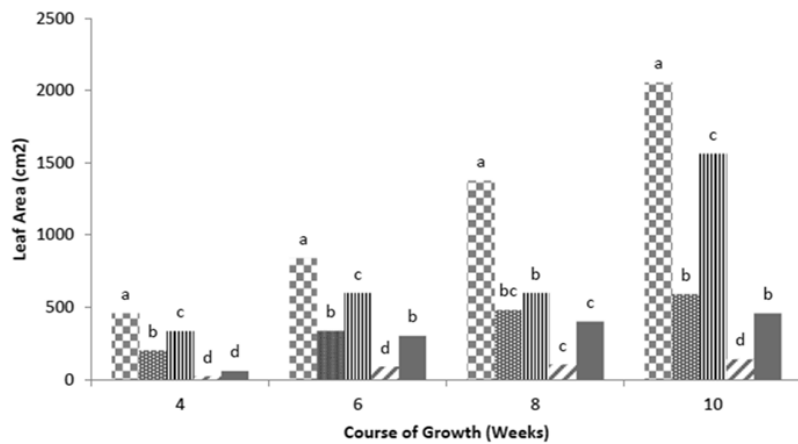
Fig.4: Leaf area of okro plants subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
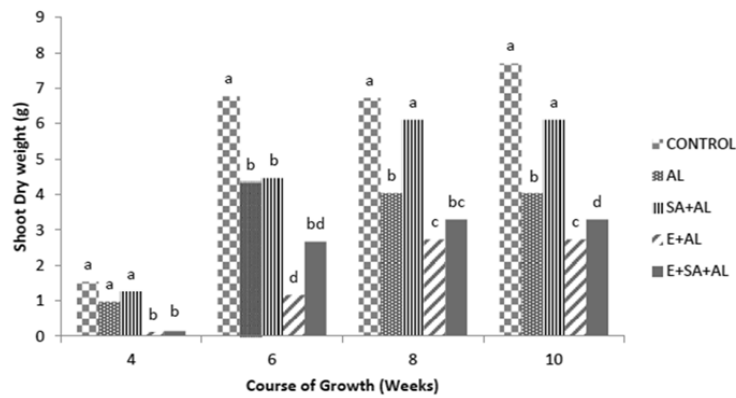

Fig 5: Shoot dry weight of okro plants subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test
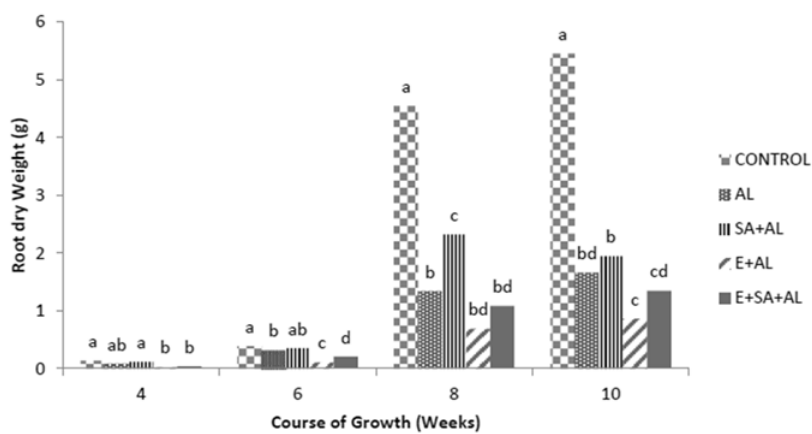

Fig. 6: Root dry weight of okro plants subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
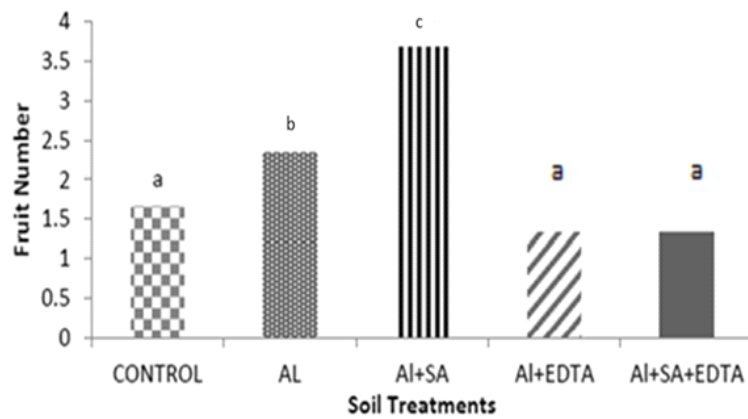
Fig. 7: Fruit number of okro plants subjected to Al, Al + SA, Al + EDTA and Al + SA + EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
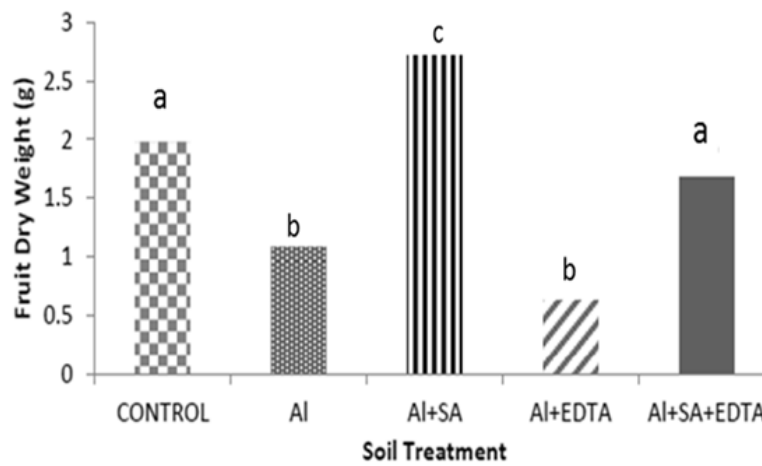

Fig. 8: Fruit dry weight of okro plants subjected to Al, Al + SA, Al + EDTA and Al + SA + EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.

Table 1: Leaf Area Ratio, Net Assimilation Rate and Relative Growth Rate of Okro Plants Subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA.

	CONTROL	Al	Al+SA	Al+EDTA	Al+SA+EDTA
LAR	21.92±7.83a	16.92±5.47a	21.25±7.24a	9.588±5.99a	18.48±8.19a
NAR	0.0143±0.01a	0.01864±0.011a	0.0133±0.01a	0.04881±0.03a	0.02392±0.02a
RGR	0.344±0.34a	0.333±0.25a	0.315±0.30a	0.544±0.52a	0.566±0.72a

Treatments represented by similar letters on the same row are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
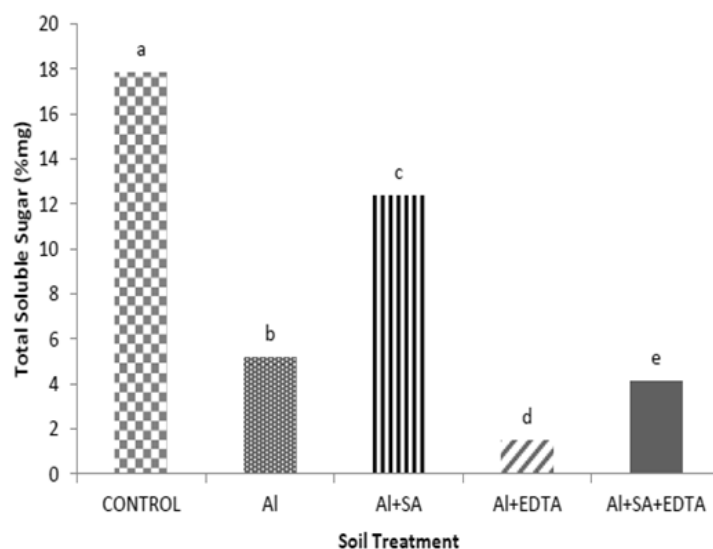
Fig. 9: Total soluble sugar of *Abelmoschus esculentus* treated with Al, Al + SA, Al + EDTA and Al + SA + EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.
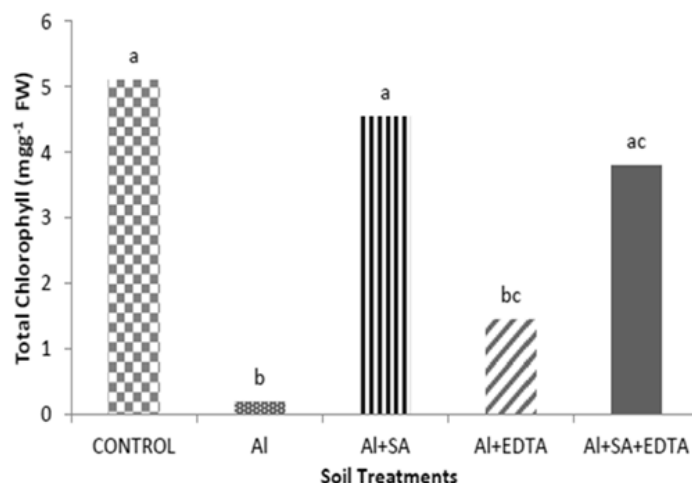

Fig 10: Total chlorophyll content of okro plants subjected to Al, Al+SA, Al+EDTA and Al+SA+EDTA. At each harvest treatments represented by similar letters are not significantly different (P= 0.05) according to Tukeys multiple comparison test.

DISCUSSION

Abelmoschus esculentus is sensitive to high concentration of aluminium (1.5g kg^{-1} soil). Chelating the Al with Ethylenediamine-tetraacetic acid (EDTA) compounded the inhibitory effect of Al on the growth of *Abelmoschus esculentus* var Clemson spineless (okro). This is in agreement with the findings of Yue-bing *et al.* (2009) that the application of EDTA and citric acid had inhibitory effects on plant growth and survival. Besides, synthetic chelating agents at high temperature can also be toxic to plants (Luo *et al.*, 2005).

Aluminium toxicity caused significant reductions in leaf area, plant height, root length, stem girth, root weight and shoot weight and the presence of EDTA in the medium caused more negative impact on these parameters. The reduction of root elongation by EDTA was also observed by Wang *et al.* (2008) and Chen *et al.* (2010). Piechalak (2003) suggested that the presence of EDTA affects negatively the balance of minerals such as

Cu, Zn, Fe and Ca which leads to disturbances in cell metabolism and destabilization of biological membranes. An additional danger is connected with the formation of EDTA chelates with ions of metals necessary in the functioning of plants which may lead to disturbances in basic metabolism. Luo *et al.* (2005) found that the dry biomass of shoots decreased up to 60% and 52% than in the control of corn, and 76% and 61% for beans, respectively, on the 14th day after the application of EDTA and EDDS. The negative impact of EDTA is also supported by Sinhal *et al.* (2010) who showed that Cu, Zn, Pb and Cd combination with 30 mg l^{-1} concentration of EDTA and citric acid caused significant reduction in growth of marigold in terms of plant heights, fresh weight, total chlorophyll, carbohydrate and protein content. Studies also showed the adverse effect of EDTA on the growth of Indian mustard (Vara, 2003).

Ethylenediamine-tetraacetic acid does not stimulate negative impact on plant growth subjected to toxic concentrations of all metals. In some cases, the presence of EDTA stimulates positive amelioration of growth under metal toxicity. Earlier research showed a significant improvement of the growth of *Zea mays* subjected to toxic concentration of Cu chelated with EDTA (Umebese and Alebiosu, 2009). EDTA has also been shown to induce improved plant growth when used as a chelate for zinc (Salama *et al.*, 2012).

Salicylic acid (SA) reduced Al toxicity by stimulating the growth and yield of okro both under chelated and non-chelated Al. The positive effect of SA on different plant growth parameters is supported by several researchers. El-Tayeb *et al.* (2006) showed that exogenous application of SA increased the growth of roots, stems and leaves of both the control and copper-stressed sun flower plants. In agreement with this, Gunes *et al.* (2007) reported that exogenous level of SA increased the dry yield of maize significantly both in saline condition and non saline condition. Increased dry matter of metal-stressed plants in response to SA may be related to the induction of antioxidant responses and protective role of membranes that increase the tolerance of plants to damage.

According to Popova (2009) the protective impact of salicylic acid may be due to any or all of these reasons: SA may prevent cumulative damage development in response to heavy metals, SA may alleviate oxidative damages caused by metals and it may exert a protective effect on the membrane stability.

Aluminum toxicity decreased total sugar content of the leaf as well as the chlorophyll content of okro. The decrease in chlorophyll content may have resulted in the decrease in leaf total sugar content which, correspondingly, resulted in the observed decrease in growth, as a result of the role of chlorophyll in photosynthesis. In previous studies, a significant decrease in chlorophyll content has been reported in flax seedlings exposed to Cd stress (Belkhadi *et al.*, 2010).

Aluminum toxicity caused significant reduction in plant growth and the presence of EDTA in the medium caused more negative impact on these parameters. Thus, EDTA has no protective impact on the growth of the plant. Salicylic acid showed effective amelioration of the impaired growth caused by Al toxicity in okro plants, including those subjected to Al-EDTA chelate, by increasing chlorophyll and leaf total sugar contents.

REFERENCES

Arnon, D. I. 1949. Biochemical methods for Agricultural Sciences. *Plant Physiology* 67: 407- 415.

Belkhadi, A., Hediji, H., Abbes, Z., Nouairi, I., Barhoumi, Z., Zarrouk, M., Chaïbi, W. and Djebali, W. 2010. Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum* L. *Ecotoxicity Environmental* 73: 1004-1011.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D. and Raskin, I. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science and Technology* 31: 860-865.

Chang, Y. C., Yamamoto, Y. and Matsumoto, H.

1999 Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. *Plant Cell Environment*. 22: 1009-1017.

Chen, J. C., Wang, K. S., Chen, H., Lu, C. Y., Huang, L. C., Li, H. C., Peng, T. H. and Chang, S. H. 2010. Phytoremediation of Cr (III) by *Ipomonea aquatica* (water spinach) from water in the presence of EDTA and chloride: Effects of Cr speciation. *Bioreremediation Technology* 101: 3033-3039.

Chen, L. S., Qi, Y. P. and Liu, X. H. 2005. Effects of aluminuim on light energy utilization and photoprotective systems in citrus leaves. *Annual Botany*. 96: 35-41.

Dat, J. F., Lopez-Delgado, H., Foyer, C. H. and Scott, I. M. 1998. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiology* 116: 1351–1357.

Dubios, M., Gilles, K., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Phenol-sulphuric acid calorimetric estimation of carbohydrates. *Annals of Chem.* 28, 350 – 356.

El – Tayeb, M. A. 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regulators* 45: 215 – 224.

El-Tayeb, M. A., El-Enany, A. E. and Ahmed, N. L. 2006. Salicylic acid induced adaptive response to copper stress in sunflower (*Helianthus annuus* L.). *Plant Growth Regulators* 50: 191-199.

Eze, J. M. 1965. *Studies on growth regulation, salt uptake and translocation*. Ph.D thesis. University of Durham, England. pp 31 – 33.

Graham, C. J. 2002. Non-structural carbohydrate and prunasin composition of peach seedlings fertilized with different nitrogen source and aluminium. *Science Horticulture* 94: 21-32.

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G. and Cicek, N. 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity, *Journal of Plant Physiology* 164: 728-736.

Janda, T., Szalai, G., Antunovics, Z., Horvath, E. and Paldi, E. 2000. Effect of benzoic acid and aspirin on chilling tolerance and photosynthesis in yang maize plants. *Maydica* 45: 29 – 33.

Kang, H. M. and Saltveit, M. E. 2002. Chilling tolerance of maize and cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. *Physiology of plants* 115: 571 – 576.

Kennedy, I. R. 1986. *Acid Soil and Acid Rain: The Impact on the Environment of Nitrogen and Sulphur Cycling*. Research Studies Press, Letchworth, UK.

Khan, W., Prithiviraj, B. and Smith, D.L. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *Journal of Plant Physiology* 160: 485 – 492.

Klessig, D.F. and Malamy, J. 1994. The salicylic acid signal in plants. *Plant Molecular Biology*. 26: 1439 – 1458.

Korkmaz, A. 2005. Inclusion of acetyl salicylic acid and methyl jasmonate into the priming solution improves low temperature germination and emergence of sweet pepper seeds. *Horticultural Science*. 40: 197 – 200.

Lidon, F. C., Barreiro, M. J., Ramalho, J. C. and Lauriano, J. A. 1999. Effects of aluminium toxicity on nutrient accumulation in maize shoots: implications on photosynthesis. *Journal of Plant Nutrition* 22: 397- 416.

Luo, C. L., Shen, Z. G. and Li, X. D., 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* 59: 1–11.

Mossor-Pietraszewska, T., Kwit, M. and Fêgiewicz, M. 1997. The influence of aluminium ions on activity changes of some dehydrogenases and aminotransferases in yellow lupine. *Biological Bulletin of Poznan* 34: 47–48.

Noggle, G. R. and Fritz, G. J. 1976. *Introductory plant physiology*. Prentice Hall Inc. New Jersey pp. 688.

Nosko, P., Brassard, P., Kramer, J. R. and Kershaw, K. A. 1988. The effect of aluminium on seed germination and early seedling establishment, growth and respiration of white spruce (*Picea glauca*). *Canadian Journal of Botany* 66: 2305–2310.

Pereira, W. E., de Siqueira, D. L., Martinez, C. A. and Puiatti, M. 2000. Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. *Journal of Plant Physiology* 157: 513-520.

Piechalak, A., Tomaszewska, B. and Baralkiewicz, D. 2003. Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. *Phytochemistry* 64: 1239-1251.

Popova, L. P., Maslenkova, L. T., Yordanova, R.Y., Ivanova, A. P., Krantev, A. P., Szalai, G. and Janda T, 2009. Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. *Plant Physiology and Biochemistry* 47: 224-231.

Sakhabutdinova, A. R., Fatkhutdinova, D. R., Bezrukova, M. V. and Shakirova, F. M. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulgarian Journal Plant Physiology* (Special Issue): 314 – 319.

Salama, Y. A. M., Nagwa, M. K. H., Saleh, S. A. and Zaki, M. F. 2012. Zinc ameliorative effects on tomato growth and production under saline water irrigation conditions. *Journal of Applied Sciences Research* 8: 5877-5885.

Senaratna, T., Touchell, D., Bunn, E. and Dixon, K. 2000. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regulator*s 30: 157 – 161.

Simon, L., Smalley, T. J., Jones, J. B. and Jr. Lasseigne, F. T. 1994. Aluminium toxicity in tomato. Part1. Growth and mineral nutrition. *Journal of Plant Nutrition* 17: 293-306.

Singh, B. and Usha, K. 2003. Salicylic acid induced physiological and biochemical changes in wheat seedling under water stress. *Plant Growth Regulators* 39: 137 – 141.

Sinhal, V. K., Srivastava, A. and Singh, V. P. 2010. EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*). *Journal of Environmental Biology* 31: 255-259.

Strobel, N., and Kuc, J. 1995. Chemical and biological inducers of systemic resistance to pathogens protect cucumber and tobacco plants from damage caused by Paraquat and cupric chloride. *Phytopathology* 85:1306–1310.

Thornton, F. C., Schaedle, M. and Raynald, D. L. 1986. Effect of aluminium on the growth of sugar maple in solution culture *Canadian Journal of Forest Research* 16: 892–896.

Turgut, C., Pepe, M. K., Cutright, T. J., 2004. The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annuus*. Environmental Pollution 131: 147–154.

Umebese, C. E. and Alebiosu, O. M. 2009. EDTA assisted phytoextraction and growth of *Zea mays* cv. EQ89-7 during copper toxicity. *Ecology, Environment and Conservation.* 15: 421-425.

Umebese, C. E., Olatimilehin, T. O. and Ogunsusi, T. A. 2009. Salicylic Acid Protects Nitrate Reductase Activity, Growth and Proline in Amaranth and Tomato Plants during Water Deficit. *American Journal of Agricultural and Biological Sciences* 4: 224-229.

Vara Prasad, M. N. and Freitas, H. M. 2003. Metal Hyperaccumulation in Plants – Biodiversity Prospecting for Phytoremediation Technology. *Electronic Journal of Biotechnology* 6: 285-321.

Wang, K. S., Huang, L. C., Lee, H. S., Chen, P. Y. and Chang, S. H. 2008. Phytoextraction of cadmium by Ipomoea aquatic (water spinach) in hydroponic solution: effects of cadmium speciation. *Chemosphere* 72: 666- 672.

Watanabe, T. and Osaki, M. 2002. Mechanisms of adaptation to high aluminium condition in native plant species growing in acid soils: a review. *Communications in Soil Science and Plant Analysis* 33: 1247-1260.

Withom F. H., Blaydes D. F. and Delvin R. M. 1971. *Experiments in Plant Physiology.* van Nostarand, New York. pp. 245.

Xiao, X. X. 2002. The physiological and biochemical response of longan

(*Dimocarpus longan* Lour.) to aluminium stress and rectification of aluminium toxicity. Ph.D thesis, Fuzhou: Fujian Agriculture and Forestry University (in Chinese).

Yang, Y. N., Qi, M. and Mei, C. S. 2004. Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress.

Plant Journal 40: 909 – 919.

Yue-bing, S., Qi-xing, Z., Jing, A., Wei-tao, L. and Rui, L. 2009. Chelate-assisted phytoextraction is proposed as an effective approach for the removal of heavy metals from contaminated soil through the use of high biomass plants. *Geoderma* 150: 106–112.