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Response of *Leucaena leucocephala* (Lam.) De Wit (Leucaena) Provenances to Aluminium in Potted Soil Experiment

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OK and SOG designed the study. Authors OK and AOO conducted fieldwork and managed the analyses of the study, Authors VAP and OK wrote the first draft of the manuscript. Author VAP managed all the correspondence. Author POK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the level of acid or aluminium tolerance provenances in Leucaena (*Leucaena leucocephala*) a favourite agroforestry tree in Kenya.
Study Design: The set up was a 2-factor (provenance-aluminium) experiment in a completely randomized design with three (3) replications and data were subjected to multivariate analysis of variance.
Place and Duration of Study: Potted acid soil experiments were carried out at the Maseno ICRAF/KEFRI centre and Chepkoilel campus farm, Moi University, between June 2009 and July 2010.
Methodology: Potted acid soil experiments were carried out at the Maseno ICRAF/KEFRI centre (pH 4.8) and Chepkoilel campus farm, Moi University (pH 5.0) to assess the effect of varying

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aluminium concentrations on growth of three local leucaena provenances: K156 (Gede), K136 (Kibwezi) and KIT2724 (Kitale). Aluminium was applied at 0, 100, 200 and 300 μM. The number of leaves per seedling, seedling height, root length, root collar diameter and dry weight were recorded at 60 and 120 days after planting.

Results: Generally Aluminium at 100 μM significantly ($p \leq 0.05$) enhanced growth of the seedlings at both sites. However, aluminium at ≥ 200 μM reduced seedling growth.

Conclusion: The *Leucaena* provenance K156 could be used in acid soils because it is tolerant. However, more local provenances should be screened for acid tolerance.

Keywords: Agroforestry; leucaena; root collar diameter; tolerant.

1. INTRODUCTION

Leucaena leucocephala (Lam.) de Wit (leucaena) is an important tree in agroforestry systems in many parts of the world. It is currently being utilized for this purpose in Western and Central Kenya, including areas that have low soil pH, such as Maseno, in Kisumu district and Uasin Gishu district [1,2]. The plant is a shrubby leguminous multipurpose tree used in soil fertility improvement owing to its ability to fix atmospheric nitrogen. It is used as a source of fodder, browse, mulch, firewood, and poles [3]. However, most genotypes of *L. leucocephala* do not grow well in acid soils and under such conditions their full potential in biomass production is not realized [4,5].

Acid soils inhibit plant growth through toxicities of aluminium (Al), manganese (Mn) and hydrogen ions (H^+) and deficiency of essential elements, such as phosphorus (P), molybdenum (Mo), calcium (Ca) and magnesium (Mg) [6-7]. For most plant species, the effect of low soil pH which is often associated with Al toxicity is manifested in reduction of root growth which leads to increased shoot to root ratio, and reduced mineral ion and water absorption from the soil [8-11]. Al toxicity interferes with the growth of *L. leucocephala,* and other tree legumes, either directly by reducing its root volume in the soil, or indirectly by suppressing biological nitrogen fixation (BNF) [3]. Photosynthesis is also affected, resulting in reduced biomass and general poor plant growth [12].

Plant species and genotypes within species also differ significantly in tolerance to acid soil stress [13-15]. Progress in breeding for acid soil tolerance in *L. leucocephala* in Hawaii has been reported [16]. Similarly, acid tolerant *Rhizobium* isolates that can nodulate *L. leucocephala* have also been isolated from Kenyan acid soils [17], [2]. But, the selection of acid tolerant genotypes of *L. leucocephala* for use with the locally available acid tolerant *Rhizobia* has not been adequately accomplished in Kenya [17,2].

Some researchers have observed significant variation in low pH tolerance among *L. leucocephala* germplasm grown in acid soils. However, it is not known which of the *L. leucocephala* provenances that are currently grown in various localities in Kenya are acid tolerant. If some of the local germplasm of *L. leucocephala* in Kenya are tolerant to low pH, then such genotypes could be adopted for use in acidic soils. Likewise, matching acid tolerant genotypes of *L. leucocephala* with tolerant *Rhizobium* could increase productivity of *L. leucocephala* in acid soils and hence lead to the realization of their potential in agroforestry systems [18].

2. MATERIALS AND METHODS

Seeds of the three randomly selected *L. leucocephala* provenances were obtained from KEFRI seed bank in Muguga, Kenya. They comprised bulked local seed collections from Gede (Kilifi District), Kitale (Trans Nzoia District) and Kibwezi (Makweni District), which have been designated as K156, KIT2724 and K136, respectively. The accession name for the seeds that were collected from Kitale could not be established hence the shortened form (KIT) for Kitale and test number (2724) has been used in this study as KIT2724 to represent the accession from Kitale.

2.1 Germination of *L. leucocephala* seeds

All the seedlings for the experiment were pre-germinated in the laboratory as described by Muok [2]. Seeds of the three provenances were surface sterilized in 2% sodium hypochlorite for 10 minutes and thoroughly washed with deionized water. They were nipped and soaked in distilled water for 45 minutes to imbibe.

Imbibed seeds were placed on trays of moist filter paper and incubated at 26ºC - 28ºC for two days to germinate. Successful germination was determined as emergence of the radicle. Two day old uniform pre-germinated seedlings were selected and immediately transferred to plant pots and subjected to different test treatments. It was assumed that the seeds derived from individual maternal parent that formed the "bulked seeds", had equal germination capacity and thus the seedlings formed a fair representation of each of the provenances.

2.2 Soil Analysis

Soil analysis for selected attributes was done for each experimental site before the soil sampling was done for the pot experiment. Soil samples were collected at a depth of 20-cm (using a soil auger) from the fields at Chepkoilel Campus farm, Moi University and Maseno ICRAF/KEFRI centre. Five soil samples were collected from each of the 30 sub-plots and bulked forming a composite sample, and then five representative sub-samples were withdrawn from the composite sample after thorough mixing. The sub-samples were air-dried in the laboratory and ground to pass through the 2 mm sieve. The samples were then analyzed for pH, cation exchange capacity (CEC), organic carbon (C), exchangeable Ca and Al, Olsen phosphors (P), and total nitrogen (N) using standard procedures [19]. The exchangeable Al was measured using Atomic Absorption Spectrophotometer (AAS).

The aluminium solutions were prepared from analytical grade Aluminium potassium sulphate ($AlK(SO_4)_2.12H_2O$). A 0.01 M Al stock solution was prepared by dissolving 5.1642 g of dry salt in 500 ml of distilled water and diluted to 2 litres in a volumetric flask with more distilled water. Working concentrations were prepared by serial dilution and 500 ml of respective aluminium concentration (0 μM, 100 μM, 200 μM and 300 μM) was applied to the appropriate pots at the inception of treatment. Plant characteristics (plant height, root collar diameter, root length, number of leaves per plant and plant dry weight) were recorded at 60 and 120 days after potting.

2.3 Experimental Design

The set up was a 2-factor (provenance-aluminium) experiment in a completely randomized design with three (3) replications and data were subjected to multivariate analysis of variance, using a computer programme (SPSS version 7.5; SPSS® Inc). Means were separated using Tukey HSD test. Differences were accepted as significant at $p \leq 0.05$. The fixed factors were aluminium and provenances, and dependent factors included plant height, root collar diameter, root length, number of leaves per plant and plant dry weight.

3. RESULTS AND DISCUSSION

3.1 Chemical Properties of Chepkoilel and Maseno Soils

The selected soil chemical properties of the Chepkoilel campus, Moi University and Maseno ICRAF/KEFRI experimental sites at the beginning of the experiment are presented in Table 1. The soils are acidic with $pH \leq 5$ although Chepkoilel soils had slightly higher pH than Maseno soils. The concentration of organic carbon was similar in both sites. The soils at Chepkoilel had significantly ($p \leq 0.05$) higher CEC than Maseno soil while the concentration of calcium was the same in both sites. Maseno soils had slightly lower concentration of aluminium compared to that of Chepkoilel, however % Al saturation in Maseno was more than double that at Chepkoilel. The soils at Chepkoilel had higher phosphorus and lower nitrogen but Maseno soils had significantly ($p \leq 0.05$) lower phosphorus and higher nitrogen.

3.2 Number of Leaves Per Seedling

All the provenances had significantly ($p \leq 0.05$) more leaves at 100 μM Al concentration but significantly lower at higher Al concentrations at 60 and 120 day old potted (DAP) at both sites. K136 at 60 DAP had the highest number of leaves at Chepkoilel while K156 had the highest number of leaves at 120 DAP in Maseno (Figs. 1 and 2).

3.3 Seedling Height

A 100 μM Al level significantly ($p \leq 0.05$) increased seedling height of all the provenances at both 60 and 90 DAP except for K156 at 60 DAP. However, higher aluminium concentrations above 100 μM reduced seedling height in all provenances at both sites (Figs. 3 and 4) the seedlings grew faster at Maseno than at Chepkoilel site.

Table 1. Selected chemical properties of soils at Maseno and Chepkoilel experimental sites at the time of planting

Soil properties	Mean values Maseno site	Chepkoilel site
pH (1 soil: 2.5 water)	4.8[b]	5.0[a]
CEC (Cmol/kg)	8.9[b]	11.6[a]
Organic Carbon (%)	1.9[a]	2.0[a]
Calcium (me/100 g)	2.0[a]	2.0[a]
Exch. aluminium (me/100 g)	0.2[b]	0.9[a]
Aluminium saturation (%)	16.7[a]	7.1[b]
Available Phosphorus (ppm)	2.6[b]	4.9[a]
Nitrogen (%)	1.1[a]	0.2[b]

Key: - Means followed by the same letter in each row are not significantly different ($p \leq 0.05$) from each other according to Tukey HSD test

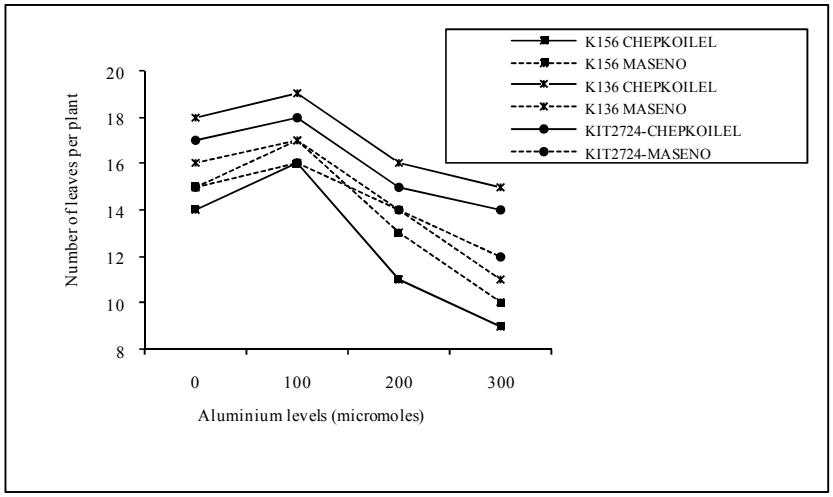

Fig. 1. Number of leaves per plant of 60-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

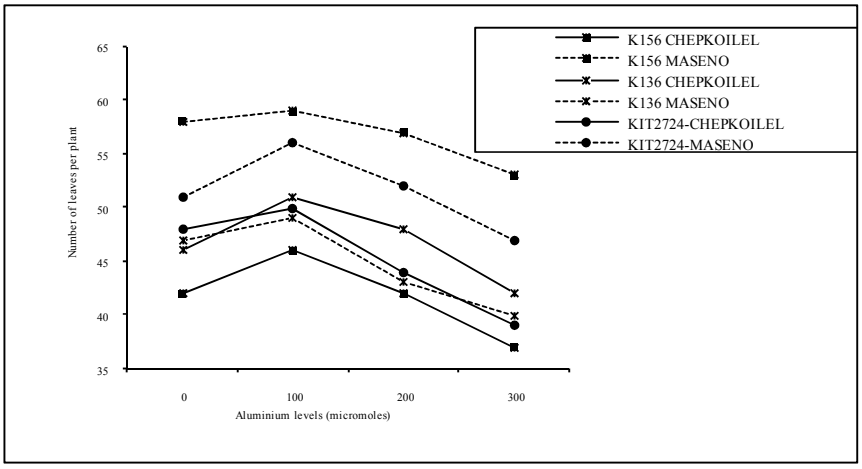

Fig. 2. Number of leaves per plant of 120-day old, potted *L. leucocephala* at varying aluminium levels at Chepkoilel and Maseno sites

3.4 Seedling Root Length

At 60 DAP, aluminium induced a steady decrease in seedlings root length at Maseno while at Chepkoilel, there was a slight increase in root length at 100 μM Al followed by a steady decrease in root length at Al concentrations above 100 μM (Fig. 5). At 120 DAP aluminium concentration of 100μM induced a slight increase in root length of all the seedlings at both Chepkoilel and Maseno, but higher aluminium concentrations reduced the root length of all the provenances at both sites (Fig. 6). K156 was the least affected provenance at both sites while K136 was the most affected and KIT2724 showed intermediate response between the two provenances.

3.5 Seedling Root Collar Diameter

The seedling root collar diameter of all the provenances was significantly higher at 100μM Al at both 60 DAP (Fig. 7) and 120 DAP (Fig. 8). However higher Al concentrations reduced root collar diameter in all the provenances. In general the seedlings had significantly ($p \leq 0.05$) larger root collar diameter at Chepkoilel than in Maseno at 120 DAP.

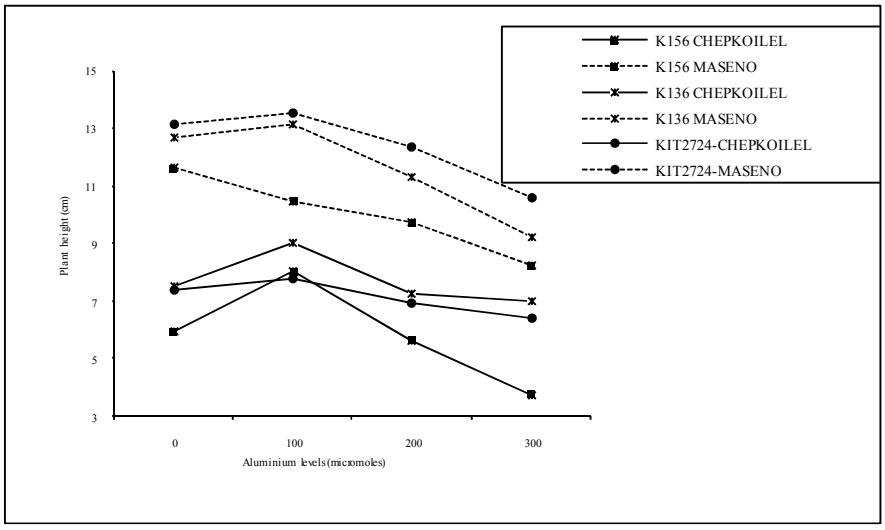

Fig. 3. Plant height of 60-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

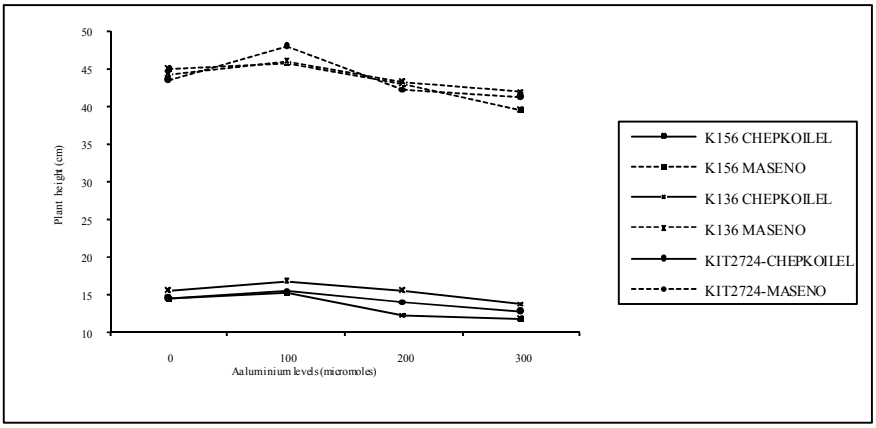

Fig. 4. Plant height of 120 days old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

3.6 Seedling Biomass

At both 60 and 120 DAP aluminium concentration of 100μM induced an increase in seedling biomass of all the provenances at Chepkoilel. At Maseno, the seedling biomass significantly decreased in response to increasing aluminium concentration (Figs. 9 and 10). The seedlings suffered a large reduction in biomass at 120 DAP than at 60 DAP. The significance due to interaction effects varied according to interaction orders as well as the growth parameters assessed at different DAP.

4. DISCUSSION

The soils in the two sites are acidic (pH≤5). However soils at Maseno were slightly more acidic (pH 4.8) than the Chepkoilel soils (pH 5.0). The pH value in these two sites is below the critical value for optimal growth and development of *L. leucocephala*, which has been suggested as 6.8 [4-5,16]. It is therefore anticipated to affect the growth and establishment of *L. leucocephala* directly through aluminium and H^+ stresses and phosphorus and calcium deficiency, as has been stated by other workers [20-23,7]. The low pH in the soils may indirectly affect the growth and development of *L. leucocephala* by affecting the BNF process. The values of CEC obtained in this study for Maseno (8.9 Cmol/kg soil) and Chepkoilel (11.6 Cmol/kg soil) sites were quite low according to [32,19]. This is an indication that the soils are highly leached. The CEC of nitisols and ferralsols is significantly influenced by pH because of the nature of major clay particles in them [24-26]. The low concentration of exchangeable aluminium in the soils (Maseno, 0.2 me/100 g soil and Chepkoilel, 0.9 me/100 g soil), was not expected. This result contrasts with the findings of others like [27] that worked in the same site (Chepkoilel) and obtained Al concentration of 4 me/100 g soil [27]. Used titration method to determine the level of exchangeable Al as opposed to the Atomic Absorption Spectrophotometer (AAS) analysis used in this study. The differences in these results might also reflect heterogeneity of the soils in this site (Chepkoilel) because the two experiments [27] were conducted in two different plots that are 800m apart. The difference in Al concentration could also be due to other intrinsic chemical soil properties. Percentage aluminium saturation was equally low, 7.1% in this study compared to 44% reported by [27]. The low exchangeable Al or percentage saturation in the soils could explain why addition of 100μM Al level still promoted growth of *L. leucocephala* in the pot experiment. Such wide differences observed in % Al saturation suggest that more study consisting of several samples from this site should be undertaken to resolve the discrepancy.

The available phosphorus in the soils (Maseno 2.6 ppm, Chepkoilel 5.0 ppm) was extremely low. Interpretation of [28-30] indicates that less than 15ppm P is too low for proper plant growth and development. These soils have been reported to be generally low in Olsen P [31,1,27]. It is important to note that, these two sites are low in

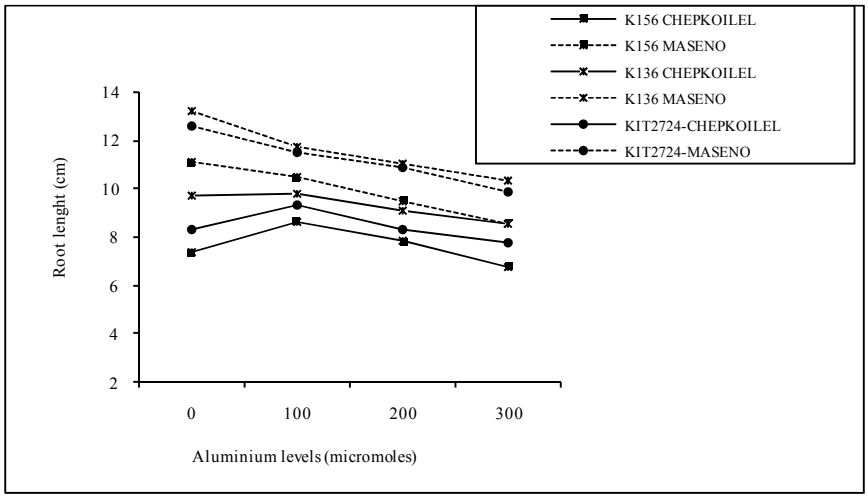

Fig. 5. Root length of 60-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

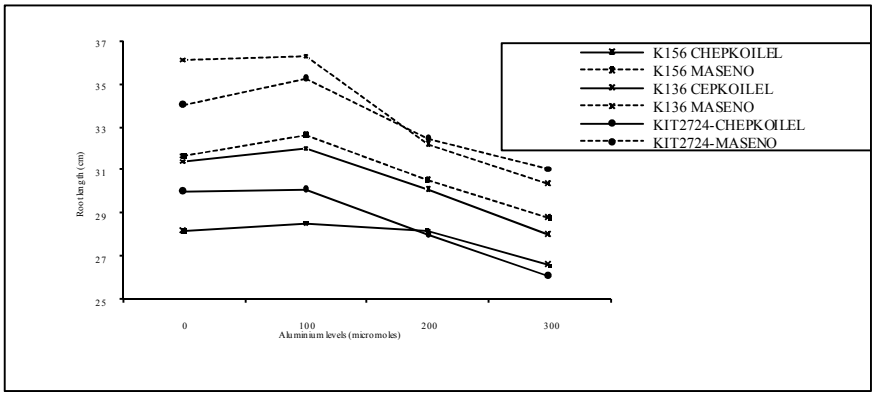

Fig. 6. Root length of 120-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

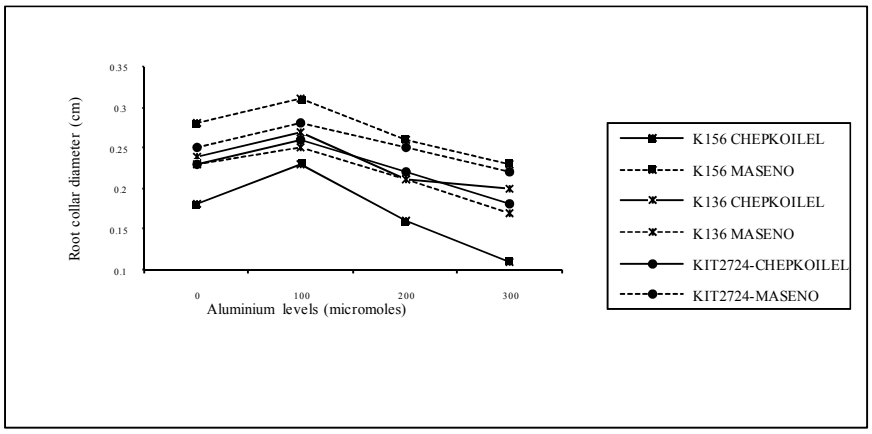

Fig. 7. Root collar diameter of 60-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

available P and this element should be applied to the soil to avoid P-related problems in the interpretation of the results. The amount of nitrogen in Chepkoilel soils (0.2%) was considered to be low according to Metson (1961) who rated 0.1% – 0.2% total N as being low. The low nitrogen content of the Chepkoilel soils may have been due to reduced microbial activity in the soil caused by low pH, which in turn minimized the breakdown of organic matter [32] to release nitrogen. However, Maseno soils had moderately higher level of nitrogen (1.1%). Thus, explains why the seedlings at Maseno had better growth and establishment compared to the ones at Chepkoilel. The soils in the two sites had similar concentrations of calcium and organic carbon. The concentration of Ca (2 me/100 g soil) in both sites is regarded to be low according to the description of [33-35] who considered Ca levels of 0.2 me/100 g to be very low for optimal growth of crops. The percentage organic carbon in the Maseno soils (1.9) and Chepkoilel soils (2.0) was also low according to the broad rating by Metson (1961), in which soils with $<$2% was considered to be very low in carbon content. Overall, the nutrient status of the soils in these two sites can be regarded as low and for optimal growth of plants including *L. leucocephala*, application of organic fertilizers or manure seems to be mandatory for high biomass production.

Response of *L. leucocephala* seedlings to Al treatment varied amongst the three provenances tested as well as between the two sites. Higher concentrations of Al (>100 μM) reduced growth at both sites. However, Al treatment at 100 μM enhanced the growth of seedlings. The root length, and even other growth attributes (height, number of leaves, collar diameter and dry weight) of *L. leucocephala* seedlings were

adversely affected by soil acidity related factors. The basis of this positive response to Al treatment at concentrations below 100 μM is not fully understood at present but similar observations have been reported by other authors [36-37]. For instance, [36] reported that the growth of *L. leucocephala* and mineral uptake, particularly N, P and K, were stimulated at low concentrations of Al. In this experiment the uptake of N, P, K was not measured. Aluminium is not classified as one of the mineral nutrients utilized by plants and therefore this observation is unique and the possible reasons for it is yet to be fully explained. According to [36], *L. leucocephala* was capable of tolerating low Al toxicity because of "exclusion mechanism(s)".

This mechanism involves the release of organic acids from the plant roots that act as Al binding ligands such as malic and citric acids. When these ligands are released into the rhizosphere, they effectively chelate Al3+ and prevent its entry into the root [38]. The authors then concluded that growth stimulation by Al application was ascribed not only to the alleviation of H^+ toxicity but also to the increased root activity for P uptake. [37], [39] reported a similar finding in several seedlings including *Hordeum vulgare*, *Acacia mangium* and *Melastoma melabathrium*. Hutton [23] also reported that Al toxicity to *L. leucocephala*, is mediated through poor uptake of Ca. This suggests that so long as leucaena plants can absorb adequate levels of Ca in high

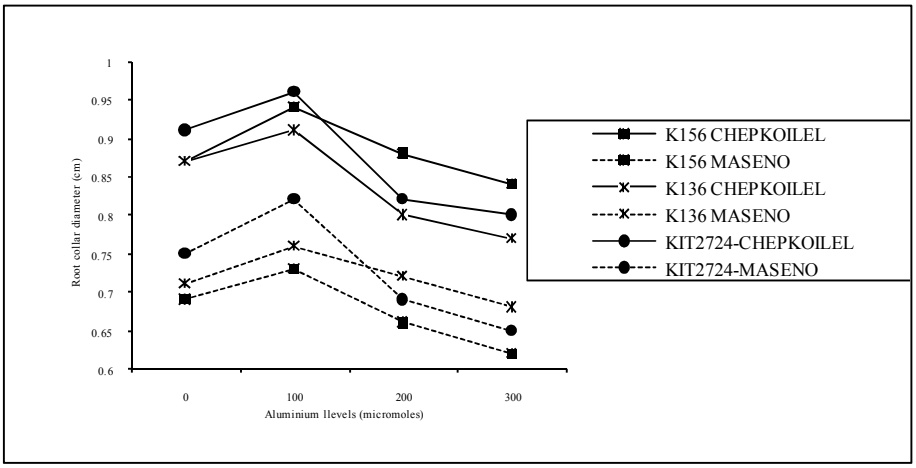

Fig. 8. Root collar diameter of 120-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

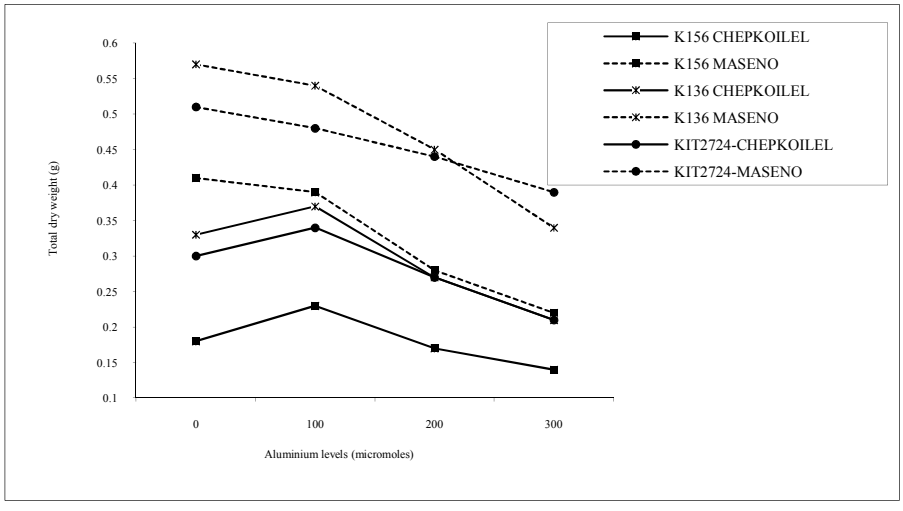

Fig. 9. Dry weight of 60-day old, potted *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

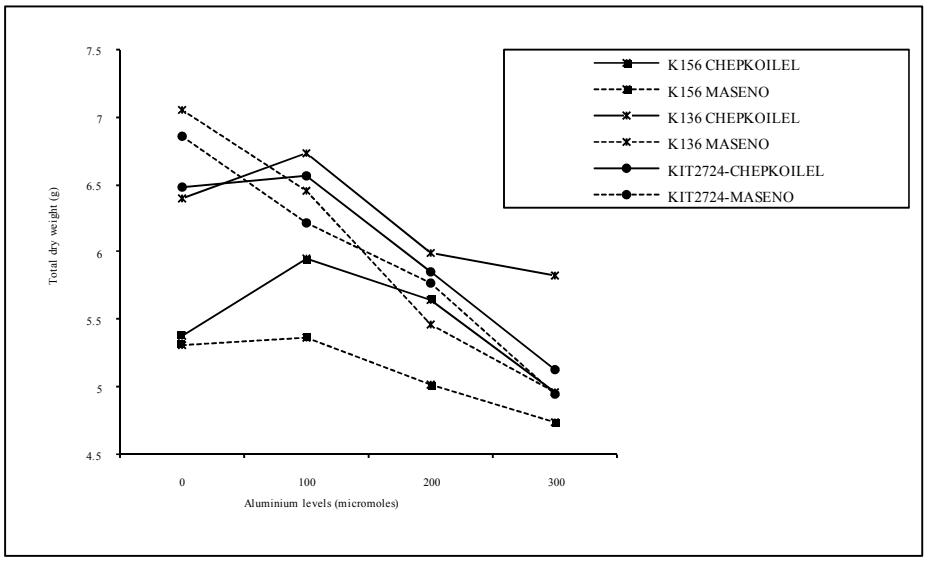

Fig. 10. Dry weight of 120-day, potted-grown *L. leucocephala* seedlings at varying aluminium levels at Chepkoilel and Maseno sites

Al soils, their growth and establishment would not be significantly affected. The differential response of the provenances to aluminium stress could be used as a guide to breed for high biomass yielding *L. leucocephala* for acid soils.

It is recommended that *Leucaena* provenance K156 could be used in acid soils because it is tolerant. However more provenances of *L. leucocephala* from different localities in Kenya and elsewhere should be screened for tolerance to soil acidity and aluminium toxicity to obtain provenances that could be used in acid soils.

5. CONCLUSION

The study found out that; Low aluminium concentrations (100 μM) improved growth but high Al levels (≥200 μM) severely reduced root length and other growth parameters. This led to overall poor seedling growth. The three provenances of *L. leucocephala* showed significant differences in response to aluminium application indicating differences in genetic potential to withstand Al toxicity. There were low concentrations of Al in the soils used, which imply that soil acidity stress on plants in these localities may be due to other related soil acidity factors and probably not Al^{3+} toxicity alone. Tolerance to Al was noted in K156 however; K136 and KIT2724 seemed to be less tolerant. However, there is need to establish the exact soil acidity related factor that induces stress to plants in these two sites because % Al saturation appears to be low. Provenance variation in response to Al toxicity has been shown in this study. It is therefore important to conduct specific studies to explain the mechanisms of tolerance and genetic control of this tolerance in *L. leucocephala.* Long term responses of *L. leucocephala* to Al also need to be studied.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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