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Rooting African Sandalwood stem cuttings using low-cost technology employed in the commercial propagation of *Camellia sinensis* in Kenya

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***Osyris lanceolata* (African Sandalwood) is endangered in the wild due to poor natural regeneration and over-exploitation for the highly valued sandalwood oil. The domestication of *O. lanceolata* is hampered by erratic and unreliable supply of seeds. A study on the rooting of *O. lanceolata* stem cuttings was conducted using a low-cost technology employed in commercial rooting stem cuttings of tea bushes (*Camellia sinensis)* in Kenya. Four locally available soils of different pH (3.81, 4.33, 5.46 and 6.03) were tested as rooting media at incubation time of 60, 90, 120 and 150 days under a polyethylene tunnel. The effect of rooting media pH and incubation time were significant for rooting (p< 0.001 and p< 0.001), number of roots (p< 0.001 and p< 0.001), length of the longest root (p< 0.001 and p< 0.001), height of the tallest shoot (p=0.004 and p< 0.001) and the number of shoots (p=0.002 and p<0.001). The best rooting achieved was 37% at incubation of 120 days in the rooting medium of pH 5.46. Further, the cuttings rooted in the rooting media of pH 5.41 and 6.03 were superior in all the parameters assessed.**

Key words: African sandalwood, stem cuttings, rooting media pH, rooting, incubation time, polyethylene tunnel.

INTRODUCTION

The African Sandalwood, also known as East African Sandalwood (*Osyris lanceolata* Hochst. & Steud. ex A. DC.) is a multipurpose, drought-tolerant (Mugula et al*.,* 2021) and hemi-parasitic tree (CITES, 2013). It belongs to the semi-parasitic plant family Santalaceae (Polhill, 2005). The African Sandalwood produces fragrance-scented wood and essential oil (Walker, 1966; Iyengar, 1968; McKinnell, 2008; Mukonyi et al*.*, 2011). It has emerged as a potential commercial species in Africa due to significant decline in original source of sandalwood oil (*Santalum album* L.) from India in the 1990s, and the increasing demand for sandalwood oil over the years in global market (Subasinghe, 2013; Mugula et al., 2021). Dwindling of the species populations in Africa is attributed to over-exploitation and some populations in Uganda, Kenya, Tanzania and South Sudan may have completely

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disappeared due to illegal harvesting and smuggling of tree logs (Mugula et al., 2021). *Osyris lanceolata* has a wide geographical distribution and has been reported in Africa, Asia and parts of Europe (Polhill, 2005; Maundu and Tengnas, 2005; IPNI, 2022). In Africa, it ranges from Algeria to Ethiopia and south to South Africa; in Europe it occurs in the Iberian Peninsula and Balearic Islands; in Asia from India to China, and also on Socotra (Polhill, 2005). In Kenya, *Osyris lanceolata* is known by various local names in the areas of natural occurrence such as Oloseseiyet (Masai), Mûthïthïi:(Kikuyu), Murmurwo (Pokot) and Kijulu (Taita) (Maundu and Tengnas, 2005; Beentje, 1994). *Osyris lanceolata* grows at an altitudinal range of 900 to 2550 m above sea level (Maundu and Tengnas, 2005; Beentje, 1994). Traditionally the tree has different uses among different tribes in Kenya, including making of red dye, smoking milk containers, use of bark powder to heal wounds, treating stomach-ache, diarrhoea, ulcers, snakebites and rashes (Maundu and Tengnas, 2005, Beentje, 1994). The global annual aggregate demand for various Sandalwood trees is estimated to be 7,000 tons against supply of 3000 tons (Subasinghe, 2013). The projected demand by 2025 is 20,000 tons (Global Risk Insights, 2017).

Sandalwood is harvested in the wild by uprooting (CITES, 2013). This interferes with its natural regeneration and is not sustainable, hence conservation and domestication of the African sandalwood is a priority. Success in the propagation of *O. lanceolata* is often faced by various constraints especially lack of understanding of its silvicultural requirements. Although propagation through seed is feasible (Kamondo et al*.,* 2014), it is hampered by unavailability of adequate seeds as the species is a poor seeder and only seeds well after some years. Moreover, there are no secure seed sources in the country. The *O. lanceolata* seeds also have a short shelf life of about one year (Ruffo et al*.,* 2002; Kamondo et al., 2018). This calls for development of an alternative method of raising *O. lanceolata* seedlings to complement the available seeds. Vegetative propagation through rooted stem cuttings is one of the most viable options for propagating plants. Already many plant species from tropical and temperate regions are commercially produced through rooted stem cuttings (Mesen, 1993; Shiembo et al*.,* 1996; Larsen and Guse, 1997; Takoutsing et al*.,* 2014). Tea bushes (*Camellia sinensis*) is one of plants propagated commercially through rooted cuttings. Compared to other species, the commercial propagation of *C. sinensis* through rooted stem cuttings is relatively simple and cost effective since most of the materials required are available locally (Kamunya et al*.,* 2003; Teshome et al*.*, 2016). Studies on rooting of *C. sinensis* established that pH is an important factor. The cuttings should be planted soon after the excision in the propagation beds having a good soil with adequate water holding capacity and a low pH as the high pH induces over callusing and may delay rooting in the nursery plants (Hamid et al., 1991). Rooting media are primarily the local soils of low pH and good drainage (Hamid et al., 2006). There are, however, some minor variations in the composition of the rooting media to suit the prevailing local conditions; in Australia for example, a mixture of sub-soil, composted pine-bark and sand has proved to be suitable for good rooting and growth of green tea cuttings (Angela, 1999), whereas in India, soil thoroughly mixed with cattle manure and rock phosphate is the recommended medium (Barbora et al., 1996). In East Africa and Malawi, a layered profile with sub soil on top of surface soil is the preferred rooting and growing medium (Green, 1964). The cuttings are incubated under polyethylene tunnels and shaded using local materials such as bamboo mats or branches of trees. The objective of the research reported in this paper was to determine the applicability of the technology used in the commercial production of tea bushes through rooted stem cuttings in the propagation of *O. lanceolata* and the effect of time on rooting.

MATERIALS AND METHODS

Study sites

The study was undertaken at the Kenya Tea Development Agency's (KTDA) Kangaita Tea plantation nursery. Kangaita is in Kirinyaga County in central Kenya and it lies on the western side of Mt. Kenya on latitude 0˚30'S, longitude 37˚16'E and altitude 2180 m a s l. The climate is cool and calm with a weakly bimodal rainfall distribution of 2040 mm annually with peaks in April/May and October/November. Kangaita has a mean annual temperature of 15.5°C (Mutuku et al., 2016). The soils in the catchments are deep well drained brown to dark brown Nitrosols and Andsols with acidic humid top soils (Sombroek et al*.,* 1982).

Plant material and development of rooting media

Osyris lanceolata stem cuttings were harvested from seedlings hedge that had been raised in a glasshouse at the Kenya Forestry Research Institute (KEFRI), Muguga Research Nursery from June 2012. The seedlings hedge had been sprayed with systemic fungicides once a week for three weeks prior to harvesting to reduce endogenous fungal load in the cuttings as per recommendation of Machua et al*.* (2008). Two fungicides were used; one a carbendazim-based and the other a dithiocarbonate/ pheylamide, at a concentration of 3 g L^{-1}. The average size of each cutting was 10 cm long and 3 to 4 leaves with surface area approximately of 25 cm^2 were left intact. After this preparation, cuttings were surface sterilized using a suspension of carbendazim-based fungicide prepared by mixing 3 g of fungicide in one liter of water and immediately inserted into the respective rooting media. The rooting media were contained in clear polyethylene tubes measuring 10 cm × 25 cm. The rooting media consisted of top and sub-soils collected at different sites within Kangaita tea plantation and in the adjacent natural forest that had prior been established to have different soil pH levels. The rooting media pH levels were; 3.81 (top soil from tea plantation), 4.33 (subsoil from field covered by grass/fern in natural forest), 5.46 (top soil from field covered by grass/fern natural forest) and 6.03 (topsoil from a site of former

Table 1. Chemical characteristic of rooting media of different pH levels.

Field pH of rooting media	E.C. (mS/cm)	C (%)	N (%)	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
3.81	0.089	5.27	0.825	55	136.215	379.138	1354.024	4.319	4.112	124.264	255.006
4.33	0.139	2.9	0.5	21	86.492	251.053	258.937	4.380	3.886	69.715	239.330
5.46	0.071	3.17	0.62	19.5	903.389	482.332	2559.768	5.493	2.379	Traces	165.199
6.03	0.093	6.37	0.95	53	982.176	544.737	1380.843	25.115	5.006	126.81	482.959

Source: Author's nutrient analysis of the rooting media

cattle shed). The chemical properties for each rooting medium were determined prior to setting the experiment (Table 1).

Experimental design

The experimental layout was a randomized complete design in a split-plot structure. The main plot treatment was the incubation time (60, 90, 120 and 150 days) and sub-plot rooting media of different pH levels. Each rooting medium was replicated three times in each incubation time and each replicate had 30 leafy stem cuttings. The experiment was covered with polyethylene tunnel to create mist condition (Plate 1). The polyethylene tunnel had four partitions corresponding to each incubation. The height of the tunnel was 1.2 m at the ridge and a width of 1.2 m. Each partition was 4 m long. The whole experiment was shaded with bamboo mats. The experiment was monitored fortnightly for fungal infestation and watering. Since *O. lanceolata* cuttings are highly prone to fungal attack (Machua et al*.,* 2008), the experiment was set in a way that minimized exposure of the cuttings during assessment by ensuring that at any given assessment, only the cuttings whose assessment was due had the polyethylene tunnel exposed and conclusively evaluated. Data was collected at the end of each incubation period. A cutting was scored as having rooted if it had a root of at least 0.5 cm long. The temperatures and humidity inside the polyethylene tunnel were recorded automatically after every ninety minutes using data logger model (SATO.SK-L200THα).

Data collection and statistical analysis

Data was collected on the number of cuttings that rooted number of roots per rooted cutting and the length of the longest root (centimetres). Data on the number of new shoots (sprouts) and the height of the tallest shoot per every sprouting cutting were also captured. The whole set of data was organized using Ms-excel and analysed using GenStat 18th edition. Analysis of variance (ANOVA) was done to determine the significant differences between rooting media pH levels and different incubation periods on the proportion of the cuttings that rooted, number of roots per rooted cutting, length of the longest root, and number of new shoots and height of the tallest shoot per every sprouting cutting. For the proportion of cuttings that rooted, the data was arcsine transformed before subjecting to ANOVA. Data on the number of roots and number of sprouts was log transformed too before being subjected to ANOVA. Mean separation was done using Tukey post hoc test. Significance differences were declared at 5% for the pH level and incubation time.

RESULTS

The mean weekly temperatures range in the polythene tunnel from the onset of experiment in June to November 2015 was 15.44 to 17.65°C while the mean relative humidity was 83.77 to 86. 12% (Figures 1 and 2). The absolute minimum temperature was 8.8°C and maximum was 26.1°C, while the corresponding values for relative humidity were 35.3 and 99.9% (Figures 1 and 2).

Rooting of the stem cuttings

The highest mean rooting percentage was 37.8 recorded at 120 days in rooting medium of pH 5.46 followed by 31.11% recorded in rooting medium of pH 6.03 at 120 and 150 days (Figure 3). The rooting trend for the cuttings in the rooting media of pH 5.46 and 4.33 recorded an increasing trend to 120 days and then a drop at 150 days (Figure 3).

The overall rooting success at the closing of the experiment was significantly different in respect to incubation time ($p<0.001$) and rooting media pH ($p<0.001$) but their interaction was not significant ($p= 0.214$) (Table 2). After conducting mean separation, the rooting percentage at 60 days was significantly lower compared to rooting at 90-, 120- and 150-days incubation time while the rooting at 90 days was significantly lower compared to rooting at 120 days but was similar to rooting at 150 days. The rooting percentage in the rooting media of pH 5.46 and 6.04 was similar but both were significantly higher compared to rooting in the more acidic media (pH 3.81 and 4.33) (Figure 3).

The number of roots per stem cutting

The number of roots per rooted stem cuttings were significantly affected by incubation time ($p=0.001$) and pH ($p=0.001$) but their interaction was not significant ($p=0.506$) (Table 2). The highest mean number of roots (6.1) was recorded at 150 days in pH 6.03 followed by 5.8 recorded at 120 days in pH 5.46 and the lowest was 1 recorded at 90 days in pH 3.81 and at 60 days in medium of pH 6.03. Overall, the mean number of roots was significantly higher at 150 days incubation time compared to 60 days (Figure 4) but was similar to the number recorded at 90 and 120 days. The mean number of roots in cuttings rooted in rooting media of pH 3.81 and 4.33

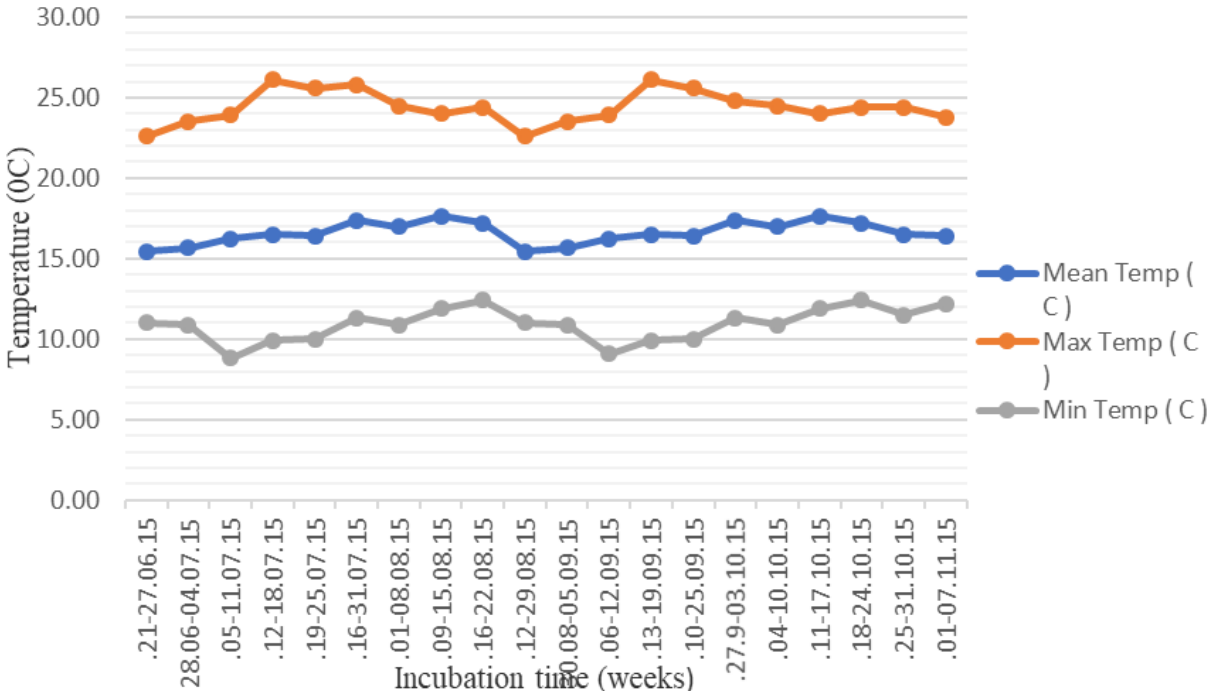

Figure 1. The weekly maximum, minimum and mean temperatures in the polyethylene tunnel to 150 days.
Source: Author's recorded environment data.

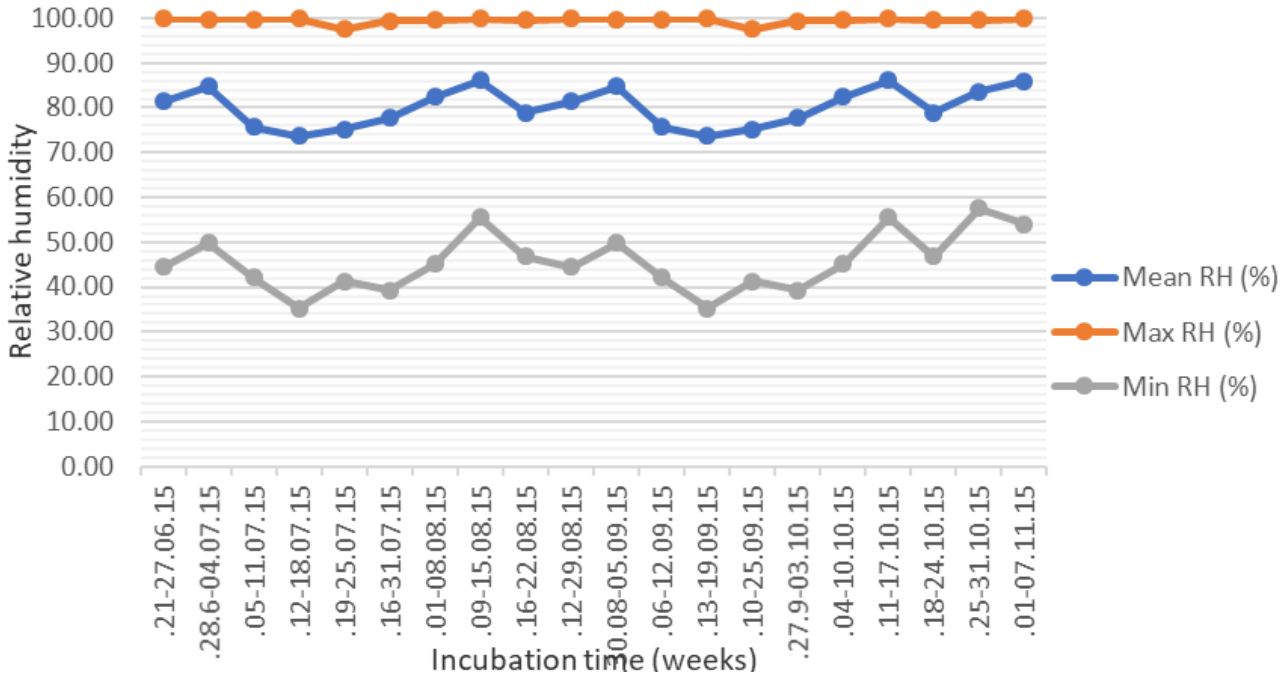

Figure 2. The weekly maximum, minimum and mean relative humidity in the polyethylene tunnel to 150 days.
Source: Author's recorded environmental data.

was similar to 150 days. Similarly, there was no significant difference in the mean number of roots in cuttings rooted in the rooting media of pH 5.46 and 6.03. However, the mean number of roots in the cuttings incubated in the

Table 2. Anova on the effect of pH and incubation time on rooting success, number of roots and shoots and the longest root and tallest shoot at 150 days.

Parameter	Source variation	df	ss	ms	v.r.	F pr.
Rooting (%)	Time_days	3	1.18490	0.39497	26.09	<.001
	PH	3	1.00130	0.33377	22.05	<.001
	Time_days.pH	9	0.19595	0.02177	1.44	0.214
	Residual	32	0.48443	0.01514		
	Total	47	2.86657			
Number of roots	Time_days	3	10.9002	3.6334	5.48	0.001
	PH	3	14.3886	4.7962	7.24	< 0.001
	Time_days.pH	9	4.1825	0.5975	.09	0.506
	Residual	212	140.4983	0.6627		
	Total	225	166.4926	0.7400		
Longest root	Time_days	3	1303.73	434.58	28.35	< 0.001
	PH	3	1945.24	648.41	42.30	< 0.001
	Time_days.pH	9	242.76	34.68	2.26	0.031
	Residual	206	3157.99	15.33		
	Total	219	6155.56	28.11		
Number of new shoots	Time_days	3	18.0635	6.0212	21.53	<.001
	PH	3	4.1223	1.3741	4.91	0.002
	Time_days.pH	9	2.4532	0.2726	0.97	0.459
	Residual	1289	360.5504	0.2797		
	Total	1304	385.1894			
Height of the new shoots	Time_days	3	761.540	253.847	120.87	<.001
	PH	3	28.201	9.400	4.48	0.004
	Time_days.pH	9	48.482	5.387	2.57	0.006
	Residual	1287	2702.914	2.100		
	Total	1302	3541.138			

Source: Author's analysis and field experiment.

rooting medium of pH 3.81 was significantly lower compared to those incubated in media of pH 5.46 and 6.03 (Table 2).

Length of the longest root

The length of the longest root per rooted cuttings was significantly influenced by incubation time ($p<0.001$), rooting media pH ($p<0.001$) and the interaction between incubation time and rooting media pH ($p<0.031$) (Table 2). There was no significant difference in the mean root length of the cuttings rooted in the rooting media of pH 3.88 and 4.33, however, their respective means were significantly lower compared to that of cuttings rooted in the rooting media of pH 5.46 and 6.03 (Figure 5). Similarly, there was no significant difference in the mean root length in cuttings rooted in the rooting media of pH 5.46 and 6.03. The mean root length of the rooted cuttings at 150 days was significantly longer compared to the mean root length at incubation times of 60 and 90 days but not at 120 days (Figure 5). Further, majority of the roots on the rooted cuttings in the more acidic media (pH 3.81 and 4.33) were short and thick whereas the growth of roots in the rooting media of high pH levels was normal. (Plate 2)

Number of new shoots

The number of new shoots formed was significantly influenced by time ($p< 0.001$ and the pH levels of the rooting media ($p=0.002$) but their interaction was not significant ($p=0.459$) (Table 2). The least mean number of new shoots was recorded in the rooting medium of pH 6.03 and it was significantly lower compared to the mean

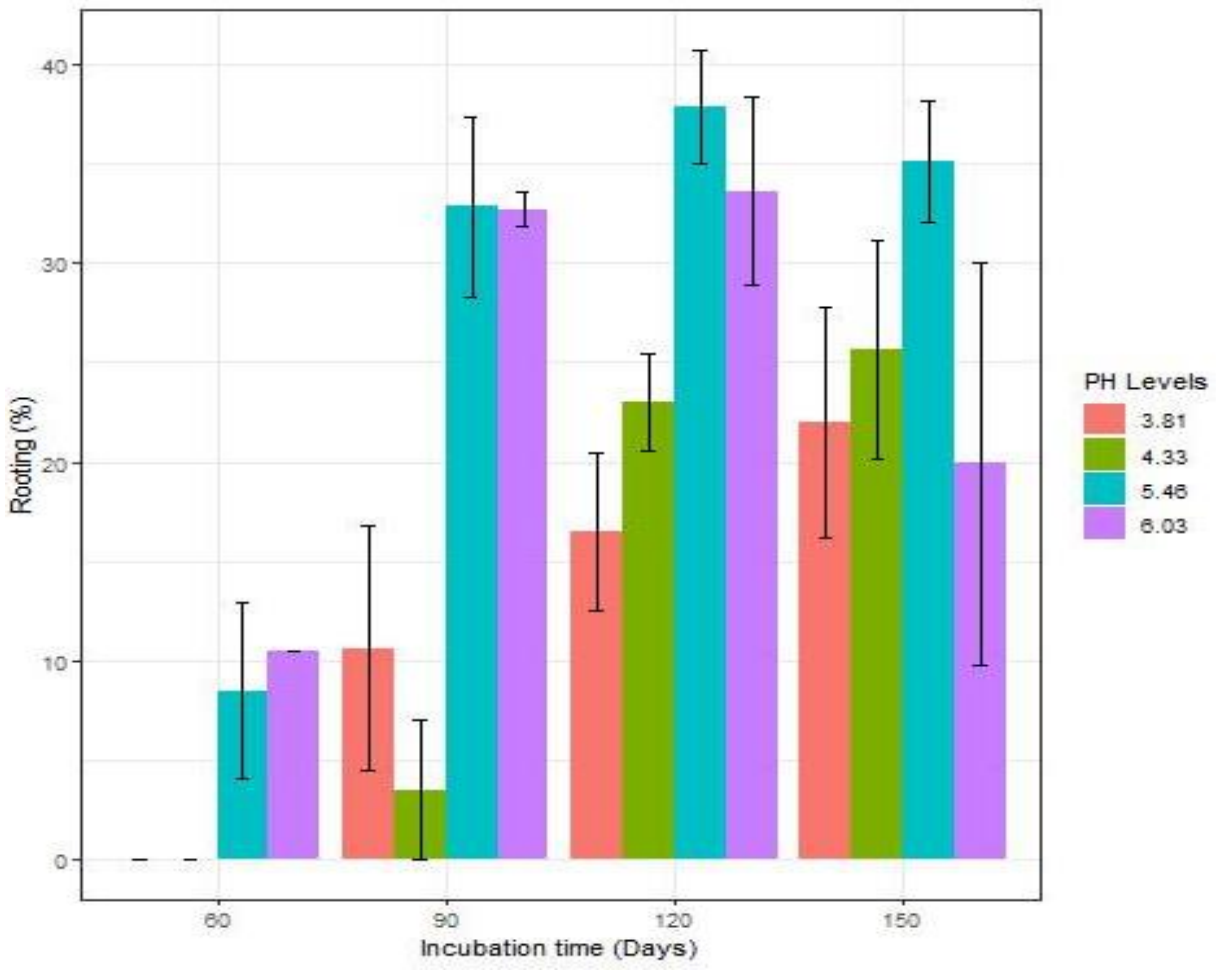

Figure 3. The rooting trend of *O. lanceolata* stem cuttings in rooting media with different pH values at different incubation time to 150 days.
Source: Author's analysis and field experiment.

shoot number recorded in the rooting media of pH 5.46 and 4.33 but was similar to the mean shoot number in the rooting medium of pH 3.81. The mean number of new shoots at the close of the experiment was significantly higher compared to all the other incubation times, whereas the mean number of shoots at 90- and 120-days incubation periods was similar.

Height of the longest new shoot at the end of the experiment

The height growth of new shoots was significantly influenced by incubation time ($p<0.001$), rooting media pH ($p=0.004$) and the interaction between incubation time and rooting media pH ($p<0.006$) (Table 2). The lowest mean shoot height was about 1 cm recorded in rooting media of pH 3.81, 4.33 and 5.46 at 60 days incubation time and the highest was 3.67 cm followed by 3.57 cm in rooting media of pH 5.46 and 6.01 respectively at 150 days (Figure 6). The mean height growth of new shoots was significantly higher at each incubation time compared to the preceding one. The mean height growth of new shoots in rooting media of different pH values was not significantly different with exception of cuttings incubated in rooting medium of pH 6.03 which was significantly taller compared to that rooted in rooting medium of pH 4.33 (Figure 6).

DISCUSSION

The result shows that it is possible to root *O. lanceolata* stem cuttings employing low-cost technology used in the commercial production of *C. sinensis* seedlings through rooted stem cuttings. The use of stem cuttings from a seedlings hedge raised in the glasshouse and frequently sprayed with systematic fungicide effectively lowered the fungal load that is reported by Machua et al. (2008) to cause near total decay of cuttings obtained from naturally

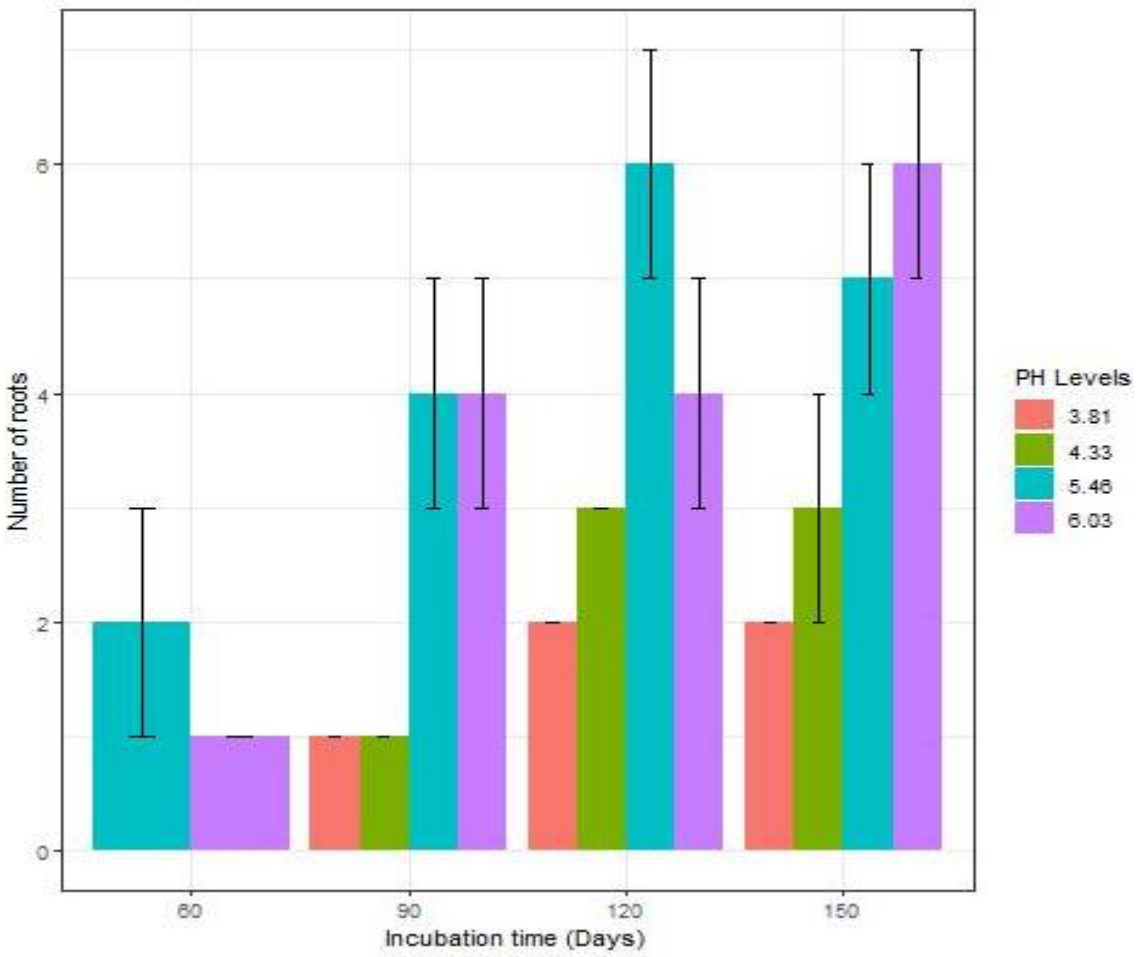

Figure 4. Effect of rooting media pH on the number of roots at different incubation time to 150 days.
Source: Author's analysis and field experiment.

growing trees in the field.

The *O. lanceolata* stem cuttings rooted poorly in the rooting media of low pH (4.33 and 3.81) compared to those rooted in the rooting media with high pH values (5.46 and 6.03). It appears that low pH inhibits rooting in *O. lanceolata* stem cuttings. The low pH could have adversely affected the physiological processes that influence root initiation and growth thus reducing the number of roots formed and possibly slowing down their growth and development. Further, low pH could have directly or indirectly inhibited formation of a number of other roots altogether. According to Visser (1959), Banerjee (1992) and Hamid et al. (2006), stem cuttings of *C. sinensis* incubated for rooting in rooting media of pH range outside 4.5 to 4.8 induced over callusing and suppressed rooting. Hamid et al*.* (2006) further indicates that for the roots of *C. sinensis* to grow and develop well, acidic growing medium is essential; this is contrary to what was observed with *O. lanceolata* stem cuttings. The stem cuttings of *O*. *lanceolata* rooted best in less acidic media which also promoted vigorous root growth compared to the more acidic media. The rooting trend in *O. lanceolata* stem cuttings was also opposite to that of *Rhododendrons spp.* cuttings in which a rooting of 85 to 100% was recorded in a rooting medium of pH 4.1 to 4.5 while no rooting was obtained at pH of 7 to 8 in a non-mist propagator unit (Thomas et al*.,* 1998). Further, majority of the roots on the rooted cuttings of *O. lanceolata* in the rooting media of low pH values were short and thick whereas the growth of roots in the rooting media of high pH was normal. The abnormal morphology of roots in the cutting rooted in the more acidic media could be attributed to toxicity from Aluminum, Iron and manganese ions which are usually present in large quantities in acidic soils (Cook and Ellis, 1987; Millar, 2004). It can be deduced that *O. lanceolata* stem cuttings do not tolerate acidic conditions unlike *C. sinensis* stem cuttings (Hamid et al*.,* 2006).

The length of the roots was highest in the rooting media with high pH than in the more acidic media. This may indicate that the physiological processes influencing root growth and elongation in *O. lanceolata* operate best in

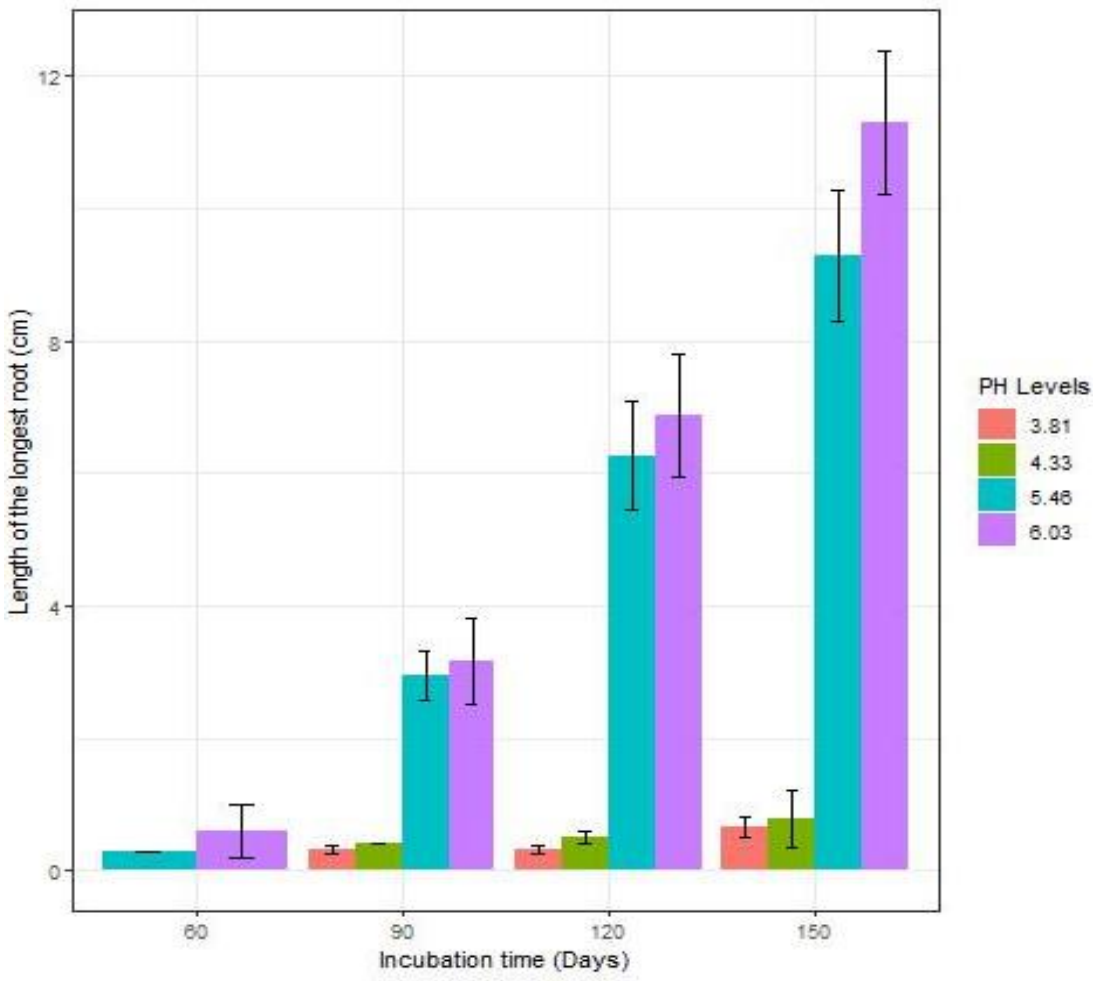

Figure 5. The trend of growth on the length of the longest root in the rooted cuttings in rooting media of different pH values and incubation time to 150 days.
Source: Authors analysis and field experiment (2022).

the media with high pH compared to the more acidic ones. Similar observations were made where high pH was positively correlated to the root elongation in rice seedlings (Azura et al., 2011). This observation is however contrary with what has been reported by a number of authors where high pH is negatively correlated with root elongation (Tang et al., 1996; Edwards and Scott, 1974; Camargo et al, 2004). The root growth of *O. lanceolata* cuttings in the rooting media with high pH developed rapidly with time attaining a mean value of 11 cm at 150 days from just 0.6 cm long at 60 days in rooting medium of 6.01. This growth was comparable to a mean of 10 to 12 cm reported for the control in rooting experiments testing the effect collecting cuttings in different seasons of year, application of IBA and cutting type in *O. lanceolata* in Tanzania (Teklehaimanot et al., 2004). However, the main difference is that the reported growth for the work done in Tanzania took only forty days compared to 150 days in the present study. The rapid growth of the roots in the rooted cuttings of *O. lanceolata* can be partially attributed to the evolutionary strategy for survival in the root semi-parasites. Semi-parasitic plants develop root system very fast in search of potential host plants to attach to for continued growth and survival (Westwood et al., 2010). The highest rooting in *O. lanceolata* stem cuttings was achieved after incubation of 120 days. This was a much longer period compared to 40 days reported by Teklehaimanot et al. (2004) in Tanzania in which the highest rooting of 54% was recorded in cutting harvested in September 2000 and treated with 50 ppm IBA while the control of the same material had a rooting success of 30%.

Though the mean number of new shoots was affected by both rooting media pH and incubation time there was no clear observable trend. This could be due to the fact that formation of new shoots on severed stem cuttings is primarily influenced by the level of stored food in the stem cuttings rather than external environment (Ofori et al.,

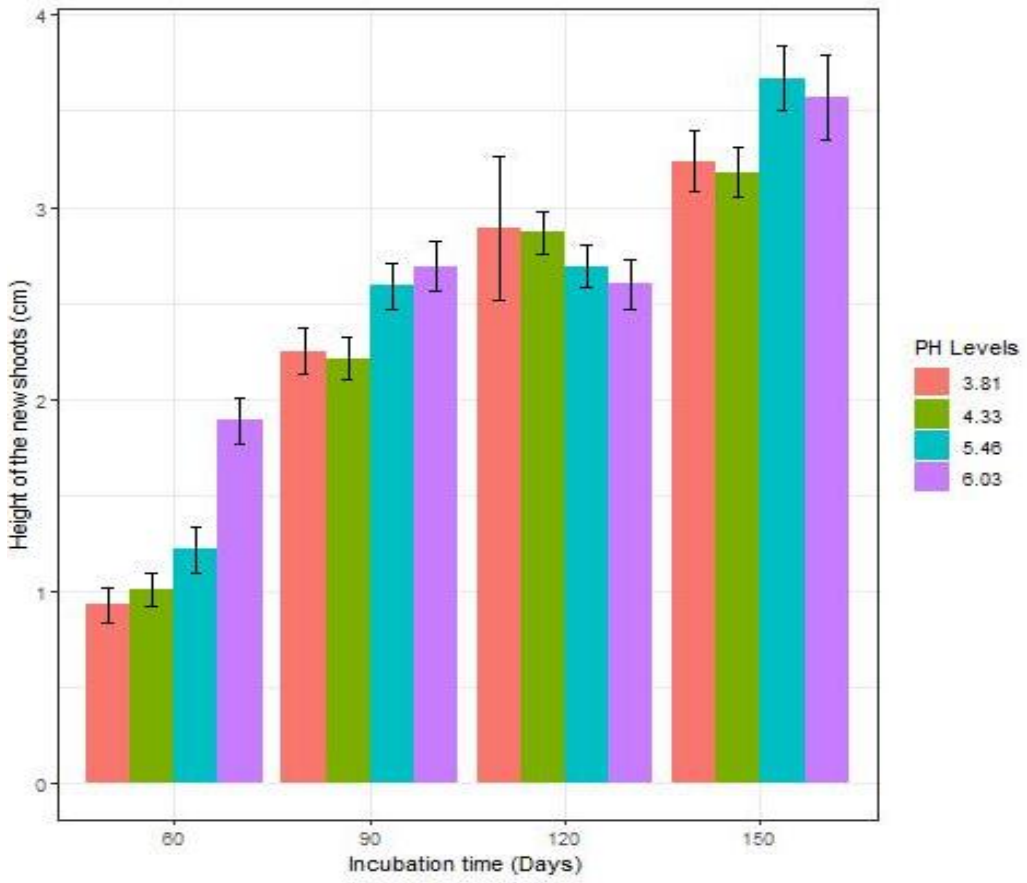

Figure 6. The trends in height growth of longest new shoot in rooting media of different pH levels at different incubation time to 150 days.
Source: Authors analysis and field experiment (2022).

Plate 1. Rooting experiment under polyethylene tunnel shaded with bamboo mat. (Giathi, 2015a)
Source: Giathi (2015a).

1996; Hartmann and Kester, 2002). In addition, the new shoots formed could have developed apical dominance thus inhibiting formation of more shoots. This could have masked the effect of time.

Plate 2. Rooted O. lanceolata stem cuttings that were incubated in the less acidic media (5.46 and 6.03 pH) (Giathi, 2015b).
Source: Giathi, (2015b).

The growth of new shoots increased significantly with time in all the rooting media but it was higher in the less acidic ones. This could be due to availability and ease of absorption of some macro nutrients such as nitrogen, potassium, sulphur, calcium and magnesium and micro-nutrients such as boron, copper and zinc by the roots of cuttings and their translocation to the shoots in the less acidic rooting media (Cook and Ellis, 1987; Millar, 2004; Nabahungu et al., 2007).

CONCLUSION AND RECOMMENDATIONS

O. lanceolata stem cuttings rooted best in rooting media consisting of ordinary soils of pH 5.46 and 6.03. The highest rooting was achieved after an incubation period of 120 days. The stem cuttings of *O. lanceolata* can be rooted using ordinary soil of Kangaita with pH range of 5.4 to 6.0 and incubated for 120 days. Further research should be conducted to test the suitability of ordinary soil with higher pH levels than 6.03 and the changes of pH with incubation time should be monitored.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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