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Effects of sowing depth on seed germination and seedling growth of *Aframomum citratum* (Pereira) K. Schum

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Series of investigations have been carried out on the effect of sowing depths on seed germination and early seedlings growth of *Aframomum citratum* and has shown that sowing depth influences germination and early growth of most plant species. Thus, the importance of varying planting depths has received much attention from researchers for some time now. The depths studied in this research were 0, 3 and 6 cm, respectively. Using a split plot complete randomized design (CRD) in a non-mist propagator, a total of 864 seeds (432 fresh and 432 dry) of *A. citratum* were sown with three replicates for each soil type in prepared polyethylene bags. Germination was monitored daily for a period of six weeks while data for germination parameters was collected. Early growth parameters such as average number of leaves (NL), average leaf surface area (SA) and average height of seedlings, (SH) were measured every week for two months. Results revealed that germination started 2 months after fresh seeds were sown in all soil types. Dried seeds being treated with 50% dilution of concentrated sulphuric acid for 20 min did not germinate during the germination period that ranged from 2 to 7 months. Sowing depths significantly affected the cumulative germination percentage and early growth (p<0.05). Thus, the highest percentage of seedlings was produced at 0 cm sowing depth, followed by 3 cm sowing depth and the least was at 6 cm sowing depth. Germination of *A. citratum* seeds can be done based on the information given in this study.

Key words: *Aframomum citratum*, seeds, sowing depths, germination, early growth.

INTRODUCTION

The genus *Aframomum* K. Schum belongs to the Zingiberaceae family and is represented in Cameroon by over 20 species of rhizomatous herbs (Anjah et al., 2015a). It can be propagated by seed and vegetative parts (rhizomes), but by seed is the better way, as virus diseases are not transmitted through seeds (Dawid et al., 2014).

Aframomum citratum is a perennial herb with underground rhizome and stems up to 4 m high which are red at the young stage of growth. Its leaflets have

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petioles of about 2 cm long oblong linear blade and an asymmetric rounded base with inflorescences that originate at the base of the stem and having reddish ovate bracts up to 6 cm wide. It has characteristically trimmer flowers, tubular calyx, oblique truncates, and up to 7.5 cm in length and possess 3-lobed tubular corolla spreading oval with sharp edges, measuring about 7 cm x 5 cm. Further, it has ovoid fruits with capsule 3 cm in diameter, extended by the long tube of the calyx; seeds are small (± 3 mm in diameter), numerous, sub-globulous, contained in a white pulp (MINFOF, 2007); and produces purple coloured flowers which develop into pods that can be as long as 8 cm and about 3 cm wide, with each pod containing numerous reddish brown seeds (can be as many as 300 seeds in one pod).

The works of Lekané (2009) show that the leaves of *Aframomum* spp thanks to their particular aroma are used in packaging of the different traditional dishes; the fruits are sold to generate income both nationally and internationally; and are also being eaten fresh by men, monkeys and rodents. The seeds are used as spices in kitchens for various dishes in Bassa communities in Cameroon (Vivien and Faure, 1995). They are important for soil conservation through their rhizomes and large leaves that protect soils against erosion (Eyob et al*.,* 2008). Nelson et al*.* (2010) demonstrated that seed extract of this plant is a potential antifeedant, which can be used as an environmentally friendly insecticide. Aframomum seed is used in traditional pharmacology to cure cough. They also serve as a thickening agent in medicinal preparations (Laird, 2000). Some local populations in Kenya use stem juice extract and its leaves to treat wounds and intestinal infections (Akendengue, 1994). *A. citratum* plants are exploited for the use of their aromatic essential oils (Amvam et al*.,* 2002). Socio-culturally, the uses of the fruits and plants of Aframomum enter into the rites of enthronement of the traditional chiefs, of succession, and rites of initiation in the secret societies. These seeds are crushed and mixed with a white powder and then used during the ceremonies of valorization of twins. Ingram and Schure (2010) scored *A. citratum* in Cameroon three at a marketable value, indicating that the species is at large-scale trade. According to Tabuna (1999), the fruits of this species were sold in Africa and Europe. *A. citratum* has been designated a non-forest timber product (NTFP) in the Congo Basin and the part exploited are the seeds and leaves (FAO, 2004).

Despite the socioeconomic importance that *A. citratum* represents, it still presents a slow germination process of seeds and seedling development, which can be influenced by a series of factors such as sowing depth, agronomic cultivar and edapho-climatic conditions. The depth of sowing is one of the most important factors with influence on the seed germination of many plant species. Too shallow sowing results in poor germination due to inadequate soil moisture at the top soil layer. On the other hand, deep sowing can also significantly reduce crop emergence and yield. The deeper the seeds are placed, the more time it will take for them to emerge from the soil. The seeds sown deeper should produce shorter seedlings than the seeds sown at recommended depth of the crop (Raju et al*.,* 2017). The germination of *A. citratum* can be slow, surpassing 2 months, which leads to higher susceptibility to pathogens and stresses caused by the environment.

Multiplication through seeds of *Aframomum* spp is very difficult, and this can be related to factors including the soil retention capacity of water and the ability of the embryo to absorb water (Anjah et al., 2015a). Also, the ability of a soil to retain water depends on its texture and topography (Assie et al., 2010).

Another problem with these species is seed dormancy which may be general for all *Aframomum* species since in Cameroon, Anjah et al. (2015b) reported that seeds of dry *Aframomum melegueta*, have trouble sprouting. Dawid et al. (2014) also reported same for *Aframomum cororima*. Although several pretreatments exist, works on the elevation of the dormancy of seeds of *A. citratum* are very rare if not non-existent.

Aframomum spp regenerates easily by rhizome fragments with or without stem. This method of regeneration remains critical because it does not assure the sustainable survival of the species (Eyob et al., 2006), yet regeneration of plant species through vegetative propagation makes it possible to bypass the multiple problems posed by the seed (WAC, 2003).

Irrespective of the very low populations of *Aframomum* species, *A. citratum* in particular is getting to extinction, largely due to difficulties of sustainable harvesting, the economically unviable commercialization, and the lack of biological and ecological information on many other potential uses of the species (Sunderland et al., 2001; Van den Berg et al., 2001; Hyacinthe, 2015).

It is therefore necessary that further studies be carried out to determine the effect of seed sowing depth on the germination and early growth of *A. citratum*. Hence, it is necessary to stimulate public interest on their conservation, sustainable exploitation and possibly cultivation.

MATERIALS AND METHODS

Soil sample collection

This study was carried out in two phases. The first involved the collection of soils from three regions of Cameroon on a one hectare land each.: Buea situated in the Southwest region in latitude 4º10′0″N, longitude 9° 14′ 0″ E and altitude 870 m; Bamenda situated in the Northwest region in latitude 5º 57’ N, longitude 10º 10’ E and altitude 1,614 m and Dschang situated in the West region in latitude 5° 26' N, longitude 10° 26' E and altitude 1,400 m. The zigzag sampling method was used to collect soils (FAO, 2004).

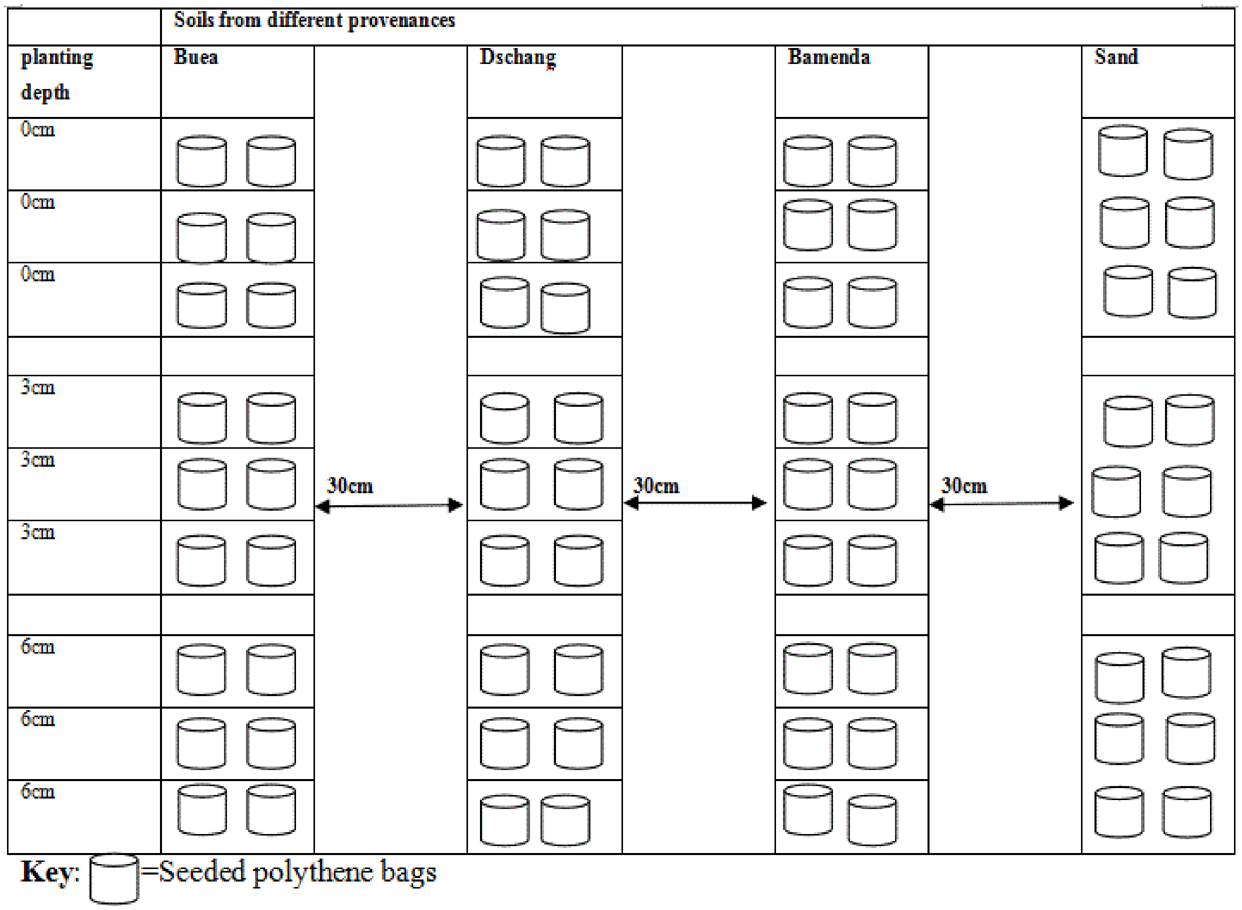

Figure 1. Experimental design for seed germination and seedling growth.

From each site, soils were dug from 13 different points using a soil auger at a depth of 0-20 cm. The soils of all the points were put into a bucket and mixed to have a homogenous mixture, and then put into labelled polythene bags.

The second phase involved observation and monitoring of germination and early growth parameters, by the establishment of a non-midst propagator. The study was conducted in the Nursery Department of Forestry, FASA located in the University of Dschang, Cameroon.

Source of *Aframomum citratum* seeds

Seeds were collected from the natural habitat of Bali Nyonga (Northwest region). The collection was done in May 2016 and kept in paper bag fully labelled.

Germination procedure

Floatation test was done to estimate the percentage of viable seeds in a seed lot. Both fresh and dried seeds of *A. citratum* were soaked into separate containers of water for 5 days. The seeds that floated were not viable while those that settled at the bottom of the container were considered viable to germinate. The germination experiment was conducted in the Nursery of the Department of Forestry, FASA located in the University of Dschang, Cameroon.

The seeds were sown in perforated polythene bags at different depths of soil, that is, 0, 3 and 6 cm, respectively. A total of 864 seeds (432 fresh and 432 dry) of *A. citratum* were used for this research with six seeds sown in each polythene bag randomly. Four replicates were used for each treatment. A total of 144 polythene bags (each batch containing 36 pots) were filled per soil origins and same for sand. After sowing of seed, they were covered with the soil of same composition to the level of required thickness for each treatment. Regular watering was done to maintain the proper moisture content. During the experiments, number of days taken for the emergence of first seedling and completion of germination were recorded in all the treatments. The polythene bags were laid out in a randomized complete block design inside a non-mist propagator of 4 m × 2 m (Figure 1). The 144 pots filled were treated with fungicides (Furaplants) to prevent fungal attack. Each batch of 36 pots filled was then divided into two groups of 18 pots each, in which were sown 108 fresh seeds and 108 dry seeds of *A. citratum.* Using a ruler and the edge of an HB pencil, the depths were determined. The ruler was placed close to the pencil, and the different sowing depths marked on the pencil. Seeds were sown in prepared polyethylene bags, which were treated with a systemic fungicide and nematicide prior to sowing of seeds. At the end of experiment, the following germination and early growth

(a) (b) (c)

Figure 2. Early stage of germination: (a) Bamenda soil; (b) Dschang soil; (c) Buea soil.

parameters for each sowing depth were monitored and calculated every week: shoot height was measured from the base of the plants to the apex, while number of leaves was counted on the stem, germination percentage and leaf surface area.

Data collection

$$\text{Germination percentage (GP)} = \frac{\text{No. of seeds that germinated}}{\text{Total no. of seeds that were planted}} \times 100$$

(Niang et al., 2010).

$$\text{Germination speed (GS)} = \frac{n1}{1} + \frac{n2}{2} + \frac{n3}{3} + \cdots \frac{nx}{x}$$

(Keshava et al., 2014)

Where $n_1 \ldots n_x$ = number of seed germinated per day and 1, 2, 3…x = number of days.

Since all seeds did not germinate at the same time, averagely 2 seedlings of *A. citratum* emerged from each germination bag, having similar morphological characteristics which were selected to study early growth parameters. Early growth parameters such as average number of leaves, average leaf surface area and average height of seedlings, were measured and calculated as follows:

i) Shoot height (SH) was measured from the base of the plants to the apex.
ii) Number of leaves (NL) was counted on the stem every week.
iii) Leaf surface area (SA) = L × W × 2/3 (Raunkiaer, 1934), where L: length of leaf blade and W: width of leaf blade.

Data analysis

The data were subjected to analysis of variance (ANOVA) using XLSTAT software and the treatment means separated using Duncan Multiple Range Test.

RESULTS

Few of the seeds started germinating seven weeks after the day of sowing (Figure 2) and germination was monitored for the next 46 days. Observation and measurements of early growth parameters began 7 months after day of sowing. Out of the 432 fresh seeds and 432 dry seeds sown in the germination test, it was observed that only 200 fresh seeds germinated and dry seeds which were pre-treated for 20 min in concentrated sulphuric acid diluted at 50% did not germinate at all. For this research work, germination was defined as the point at which a seedling appeared at the soil surface.

Sowing depths and germination of *A. citratum* seedlings

The results show that germination was affected negatively by increase in sowing depth, since germination speed was highest at sowing depth of 0 cm (4.43) and lowest at sowing depth of 6 cm (0.97). Also, the number of seeds that germinated decreased with increase in sowing depth, hence germination percentage. As shown in Figure 3, germination percentage decreased with increased sowing depth (Figure 3).

Effects of sowing depth on early growth parameters of *A. citratum* seedlings

Effects of sowing depth on number of leaves

There was a fluctuation in seedling number of leaves throughout the 8 weeks of observation at different sowing depths. At the end of this study, the highest number of leaves were produced by seedlings in Buea soil at 0 cm (11 leaves) while the lowest number of leaves was produced by seedlings in Buea soil at 6 cm (2 leaves). Generally, the seeds sown at depths of 0 cm produced seedlings with highest number of leaves, whereas seeds sown at depths of 6 cm produced seedlings with lower

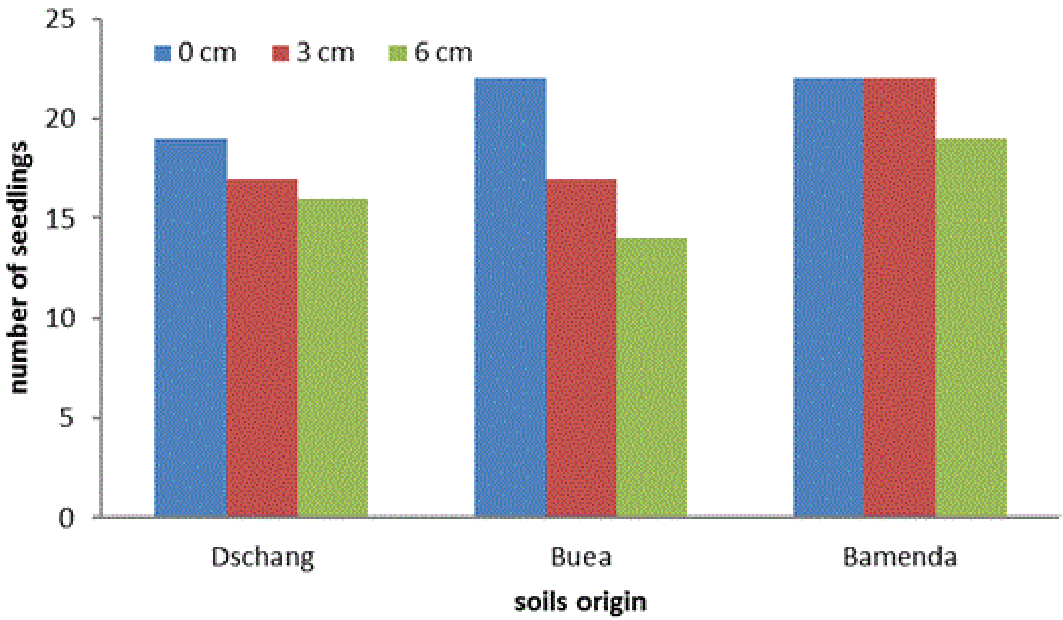

Figure 3. Number of seedlings, sowing depths and soils origin.

Table 1. Effect of different sowing depths on the mean number of leaves.

Sowing depth	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Buea (0 cm)	6.92±1.00[a]	6.92±1.00[ab]	7.50±1.78[a]	7.67±1.67[a]	8.00±1.65[a]	8.42±1.68[a]	8.92±1.68[a]	9.08±2.11[a]
Bamenda (0 cm)	7.42±1.16[a]	7.58±1.16[a]	7.33±1.44[a]	6.83±1.19[ab]	6.92±1.44[ab]	7.50±1.62[ab]	7.50±1.57[ab]	7.58±1.68[abc]
Dschang (0 cm)	5.58±1.16[d]	7.50±0.90[a]	7.17±1.11[a]	6.83±1.53[ab]	7.17±1.34[ab]	7.83±1.40[ab]	7.42±1.56[ab]	8.00±2.13[ab]
Dschang (3 cm)	6.75±0.62[abc]	7.33±0.89[a]	7.25±0.75[a]	6.67±1.44[ab]	7.08±1.31[ab]	7.58±1.68[ab]	7.42±2.07[ab]	7.92±2.15[ab]
Bamenda (3 cm)	6.75±1.36[ab]	7.17±1.03[a]	6.75±1.29[ab]	6.75±1.22[ab]	6.92±1.38[ab]	7.25±1.60[ab]	7.33±1.50[b]	7.67±1.67[abc]
Buea (3 cm)	5.75±1.22[cd]	6.17±0.94[bc]	5.92±1.83[bc]	5.92±1.83[bc]	6.42±1.88[bc]	7.08±1.38[ab]	7.33±2.10[b]	8.25±2.38[ab]
Dschang (6 cm)	5.83±1.40[bcd]	6.83±0.94[ab]	6.58±0.90[ab]	6.33±1.23[bc]	6.42±1.31[bc]	6.75±1.14[bc]	6.83±1.47[bc]	6.92±1.24[bcd]
Bamenda (6 cm)	4.83±1.03[d]	5.33±1.44[c]	5.33±1.44[c]	5.33±1.44[c]	5.42±1.24[cd]	5.75±1.76[cd]	5.75±1.71[cd]	6.25±1.29[cd]
Buea (6 cm)	3.33±1.15[e]	3.75±1.36d[d]	3.92±1.24[d]	3.92±1.24[d]	4.25±1.54[d]	5.00±1.04[d]	5.00±1.71[d]	5.67±1.61[d]

Means in the same week followed by the same letter in the row and column do not differ significantly by Duncan test at 5%.

number of leaves. Statistically, with respect to the different sowing depths, a significant decrease ($p \leq 0.05$) occurred for each soil type as sowing depth increased (Table 1).

Effect of planting depth on plant height

Depth of sowing is an important factor in maximizing the potential of shoot height. The effect of depth of sowing on plant height of *A. citratum* is represented in Table 2. The highest seedling height resulted from Dschang soil at 0 cm (24.03±7.36 cm) while the shortest shoot height (2.42±0.67 cm) resulted from Buea soil at 6 cm. For each soil type, shoot height of seedlings at 0 cm were longer than shoot height of seedlings at 6 cm. There was a significant difference ($p<0.05$) in the decrease of seedling shoot height as sowing depth increased. Meanwhile, seedling shoot height increased throughout the 8 weeks of growth observation (Table 2).

Leaf surface area

From Table 3, it was observed that the leaf surface area showed significant differences (0<0.05) for seedlings at different sowing depths. The leaf surface area for seedlings in Buea soil at 0 cm had the largest surface

Table 2. Effects of different sowing depths on the height of *A. citratum* seedlings in different soil types.

Sowing depth	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Dschang (0 cm)	11.00±2.83^{a}	12.92±3.42^{a}	15.08±4.48^{a}	16.96±5.33^{a}	19.00±6.12^{a}	22.08±6.89^{a}	22.50±7.06^{a}	24.03±7.36^{ab}
Bamenda (0 cm)	11.00±4.00^{a}	12.00±4.11^{ab}	13.50±4.56^{a}	15.50±5.49^{a}	17.92±6.65^{a}	20.10±7.73^{ab}	20.78±7.68^{ab}	21.78±8.07^{abc}
Buea (0 cm)	8.17±3.61^{bc}	10.17±3.61^{bc}	12.17±3.61^{ab}	14.25±3.54^{ab}	17.79±4.83^{a}	20.63±6.10^{ab}	22.84±5.74^{a}	24.49±5.68^{a}
Dschang (3 cm)	10.00±2.95^{ab}	11.83±3.04^{ab}	13.58±3.29^{a}	15.46±3.71^{a}	17.13±4.13^{ab}	18.79±3.95^{abc}	20.15±4.27^{ab}	22.13±4.14^{a}
Bamenda (3 cm)	9.58±3.65^{ab}	10.75±3.62^{ab}	12.25±4.49^{ab}	13.78±5.28^{ab}	15.28±6.10^{abc}	17.23±6.09^{bcd}	18.06±6.63^{abc}	19.46±6.50^{bcd}
Buea (3 cm)	6.25±2.22^{cd}	7.92±2.31^{cd}	9.67±2.61^{bc}	11.54±2.55^{bc}	13.25±3.37^{bcd}	15.22±4.22^{cde}	17.51±5.94^{bc}	18.68±5.16^{cd}
Dschang (6 cm)	4.58±2.23^{de}	6.42±2.68^{de}	8.33±2.93^{cd}	10.04±2.93^{c}	11.53±3.08^{cd}	13.13±3.45^{de}	13.89±3.78^{cd}	15.62±3.80^{de}
Bamenda (6 cm)	4.00±2.04^{de}	5.25±2.34^{e}	6.58±2.47^{d}	8.21±2.87^{c}	9.71±2.91^{d}	10.78±3.80^{e}	11.34±3.79^{d}	12.77±4.11^{e}
Buea (6 cm)	2.42±0.67^{e}	2.58±1.00^{f}	2.92±1.51^{e}	3.85±1.37^{d}	4.22±1.43^{e}	4.92±1.95^{f}	4.67±1.89^{e}	5.42±2.51^{f}

Means in the same week followed by the same letter in the row and column do not differ significantly by Duncan test at 5%.

Table 3. Effects of different sowing depths on the leaf surface area of *A. citratum* seedlings.

Sowing depth	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Bamenda (0 cm)	24.94±11.90^{a}	27.67±13.05^{a}	30.13±13.44^{a}	31.91±13.75^{a}	34.54±15.72^{a}	36.80±16.46^{a}	38.17±15.87^{ab}	40.97±15.53^{a}
Buea (0 cm)	20.62±8.43^{ab}	23.08±7.83^{ab}	24.72±7.99^{ab}	26.79±8.57^{ab}	32.39±9.55^{ab}	36.20±9.46^{a}	39.40±10.53^{a}	41.41±11.31^{a}
Dschang (0 cm)	20.69±9.95^{ab}	23.05±9.98^{ab}	25.33±9.48^{ab}	30.18±10.26^{a}	33.22±11.66^{a}	35.51±12.51^{ab}	37.84±13.11^{ab}	39.85±13.26^{a}
Dschang (3 cm)	18.64±4.10^{b}	21.54±4.27^{ab}	23.85±5.57^{ab}	25.40±5.85^{ab}	28.50±5.00^{abc}	30.70±5.00^{ab}	32.15±5.37^{ab}	34.51±5.46^{ab}
Buea (3 cm)	16.68±6.90^{bc}	17.80±7.00^{bc}	19.09±7.06^{bc}	20.34±7.44^{bc}	23.00±8.51^{cde}	29.45±12.78^{abc}	32.07±13.93^{ab}	34.68±14.59^{ab}
Bamenda(3 cm)	16.11±5.63^{bc}	17.68±5.41^{bc}	19.51±6.71^{bc}	21.52±7.12^{bc}	24.92±9.13^{bcd}	27.22±10.00^{bc}	29.84±10.13^{bc}	33.01±10.58^{ab}
Dschang (6 cm)	11.60±3.34^{cd}	13.69±3.94^{cd}	15.70±3.68^{cd}	17.54±3.80^{cd}	20.34±3.02^{de}	21.19±3.62^{cd}	23.36±4.08^{cd}	26.52±4.08^{bc}
Bamenda (6 cm)	8.00±4.13^{de}	9.43±4.98^{de}	11.15±4.84^{d}	13.53±4.49^{d}	15.64±4.20^{e}	17.13±4.93^{d}	18.25±4.44^{d}	20.88±6.38^{c}
Buea (6 cm)	2.78±1.90^{e}	3.81±2.52^{e}	4.73±3.59^{e}	5.61±3.63^{e}	5.81±3.85^{f}	6.41±4.01^{e}	7.83±5.68^{e}	9.82±6.33^{d}

Means in the same week followed by the same letter in the row and column do not differ significantly by Duncan test at 5%.

area (41.41±11.31 cm) and seedlings in Buea soil at 6 cm had the smallest leaf surface area (9.82±6.33 cm). During this research, leaf surface area decreased with increase in sowing depth and increased from one week to the next (Table 3).

DISCUSSION

In order for established plants to maintain a positive yield, crop seedling germination and growth should be high. Hence, knowing the sowing depth is a useful information for attaining an appropriate stand density and resultant optimum crop performance. The results of the experiments conducted indicate that seeds of *A. citratum* had a very low germination percentage when they were sown at sowing depths of 6 cm. Similar results were observed by Mohammed et al*.* (2004). Germination speed reduced from a mean value of 4.43 at the 0 cm planting depth to a mean value of 0.97 at the 6 cm.

The mass yield of germination decreases whenever small seeds are sown at deep depths. This was the case during our study, in which we observed that *A. citratum* seeds performed better when seeds were sown at 0 cm and 3 cm, meanwhile the lowest means were recorded when seeds were sown at 6 cm. The negative effect of sowing depth was reported by other researchers who found that seedling emergence of cotton seed decreased with increased depth (Nabi et al*.,* 2001). Hojjat (2011) reported that the germination parameters were significantly related by seed weight and large seeds germinated early, showing better germination than small seeds of lentil genotypes. An interaction between seed size and depth of planting indicated that the number of germinated seeds was greatly reduced with increased depth of planting; too shallow sowing results in poor germination due to inadequate soil moisture at the top soil layer while deep sowing can also significantly reduce crop emergence and yield (Aikins et al., 2006).

The observed results with regard to germination percentage are in concurrence with findings of Roozrokh et al. (2005) on chick pea. The negative effect of deep sowing depth was reported by Nabi et al. (2001) who found that seedling emergence decreased with increased sowing depth in cotton. The deeper the seed is sown the more strength it needs to push its shoots above the soil surface. It is suggested that with similar seeds, shallow sowing depths are best. Supporting evidences were also reported by Singh et al. (2017) in *Cinnamomum tamala Nees*.

Conclusion

The results obtained during these studies have greatly shown that the germination of seeds depends on the planting depth. Germination and emergence reduce with increase of planting depth. The seeds that were planted at deeper depths e.g. 6 cm were able to germinate last, as opposed to the ones that were planted at lower depths (0 cm and 3 cm). The number of leaves, shoot height and leaf surface area increased with the number of days when counting was done. Therefore, farmers planting *A. citratum* are advised to have moderate depths for planting. This is to ensure strong anchorage to the soil, and also to ensure faster germination of the seeds.

Recommendation

It is recommended that famers try to sow *A. citratum* seeds at a depth not exceeding 6 cm, possibly between 0 and 3 cm followed by a stable watering regime to obtain good seed emergence and germination in all agricultural undertakings, minimize loss and increase yield. Silviculturalists and famers should be educated on the appropriate agricultural practices regarding suitable depths and how to grow this plant; the farmers should also be advised on how to choose the right soil for growing media and proper watering techniques to ensure high yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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