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Effect of seed depth on germination, growth and chlorophyll contents of *Senna spectabilis*

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ABSTRACT

Senna spectabilis is a medium to large tree belonging to the family fabaceae. It is native to Southern America. The plant has several economic importances such as construction materials and wood carvings. In agroforestry systems, the plant is used as a hedgerow species, shade tree, living fence, fodder, mulch, fuel wood and as a source of honey. Propagation of *Senna spectabilis* is by seeds. Seedling germination and seedling emergence is affected by several factors including soil temperature, light and soil depth. The depth of sowing is one of the important factors influencing seed germination of many plant species. Several researchers have worked on this aspect for other plants, but no research has investigated on the effect of sowing depth on germination and growth of *Senna spectabilis.* The main objective of this study was to determine the effect of different sowing depths on germination, growth and chlorophyll content of *Senna spectabilis.* Seeds of *Senna spectabilis* were obtained from KEFRI Kakamega, Kenya. The seeds were subjected to viability test to ensure that the seeds were viable. Experiment was carried out in a green house at Maseno University botanic garden. The soil that was used was sun dried to kill the pathogens. The experiment consisted of six sowing depths as treatments namely 0, 2, 4, 6, 8, and 12 cm using 10 litres pot, each containing 10 seeds respectively in a completely randomized design with three replications. The seeds were watered daily. Data was collected on the number of seeds germinating each day, plant height, leaf area, leaf number, chlorophyll content, plant fresh weight, and root length. Germination percentage was also calculated. Data collected from the study was subjected to analysis of variance (ANOVA). Treatment means were separated and compared using LSD at ≤0.05.The sowing depth significantly affected germination, growth and chlorophyll content of *Senna spectabilis*. Germination, growth parameters and chlorophyll content decreased with increasing soil depth. 2cm sowing depth had the highest values of germination percentage, seedling vigour, leaf area, leaf number, chlorophyll content and plant fresh weight followed by 4cm. Results indicate that *Senna spectabilis* may be sown at medium depths of 2cm and 4cm in order to obtain the best germination, growth and higher chlorophyll content.

Key words: Sowing depth, *Senna spectabilis*, germination percentage, growth parameters, chlorophyll content

1. INTRODUCTION

Optimum seeding depth is viewed as a desired goal for all crop establishment systems (Karayel and Ozmerzi, 2008). Seeds buried at different depth may experience varying environmental conditions such as change in oxygen and carbon dioxide concentration, temperature, water and nutrient availability whose changes can affect germination, emergence, growth and development. Too shallow sowing depths results in poor germination due to in adequate soil moisture. Deep sowing significantly affects crop emergence and growth (Aikins *et al.,* 2006; Mahdi *et al.,* 1988; Photiades and Hadjichristodoulou, 1984). Aikins and Afuakwa (2008) noted that in cowpea, too shallow sowing resulted to low germination as a result of inadequate soil moisture at the top soil layer. Even though seed germination and vigour of seedling is gene controlled, seed size, viability, sowing depth, soil moisture and soil temperature can all affect seed germination, vigour and chlorophyll synthesis (Thompson, 1993). Deep seed sowing has a number of effects on seedling growth. For instance, there may be an increase in the time between seed germination and seedling emergence which largely determine the ranking of seedlings in the competitive hierarchy for growth resources (Ahirwar, 2015). Both mean percent germination and percent seedling emergence were negatively correlated with depth significantly for each species (Jun Ren et al., 2002).

Senna spectabilis is a rounded deciduous tree that grows to 10m or more metres high and belongs to the family Caesalpiniaceae. Leaves are compound narrowly elliptic and are shed entirely, leaving the tree bare for months. It is quick growing; flourishing even in poor or black cotton soil, but not at its best if the site is too dry. It is spectacular in flower, deservedly popular and widely available in nurseries. *Senna spectabilis* is found in tropical areas in Africa, Asia, Australia, Latino and South America and has been identified as a casual, spontaneous species that survives outside cultivation but does not form self replacing population, and relies on repeated introduction or limited a sexual reproduction for persistence (Chong *et al.,* 2009). *Senna spectabilis* produces large quantities of seeds which can remain viable for up to three years and do not die easily, allowing the species to spread quickly as pods burst and disburse seeds. The seeds can spread further by water (PIER, 2014). In agroforestry, the species is propagated vegetatively via cuttings and stump plants (Irwin and Barneby, 1982). *Senna spectabilis* requires full sunlight, as it does not appear to establish under full canopy forest (PIER, 2014). It requires well-drained soil, has low tolerance for salt, and can tolerate a range of soil types including moist, clay, sand, loam, slightly alkaline, and acidic soils, reportedly able to flourish even in poor, black cotton soils and has been reported to adapt to alkaline soils (Mungatana and Ahimbisibwe, 2010; Gillman and Watson, 2011).

Senna spectabilis is a multipurpose tree providing a wide range of products. It is used in traditional medicine to treat constipation, insomnia, epilepsy and anxiety (Bum *et al.,* 2010). Leaves decoction mixed with soup are drunk to treat and manage internal injuries and tumours (Kokwaro, 2009). The tree also has potential of being used as an agroforestry tree species. However, its use in agroforestry is limited by the low seedling growth and survival at the nursery stage, thus necessitating the need for a study being conducted to evaluate the effect of seed sowing depth on the germination percentage, growth and chlorophyll content of *Senna spectabilis*. Its wood has been used for furniture, as lumber and construction materials, and to make wood carvings. It has been used as a hedgerow species in plantations and agricultural systems (Wakibara and Mnaya, 2002; Mungatana and Ahimbisibwe). *Senna spectabilis* is used in agroforestry as a shade tree and a living fence (Irwin and Barneby, 1982; Missouri Botanical Garden, 2014). The species is considered useful for fodder, mulch, and fuel wood. There is limited literature on the effect of sowing depth on growth, germination and chlorophyll content of Senna *spectabillis*. The main objective of this study was to investigate the effect of sowing depth on germination, growth and chlorophyll content of *Senna spectabilis* seedlings. The data obtained from this study will be important to farmers and agroforesters for faster establishment of the tree seedlings.

2. MATERIAL AND METHODS

Plant material and growth condition

Seeds of *Senna spectabilis* were acquired from Kenya Forestry Research Institute (KEFRI), Kakamega, and transported to the experimental site at Maseno University botanic garden, latitude 0º1'N- 0º12'S and longitude 34º25; E - 34º47'E (Mwai, 2001). The soils are classified as acrisols, deep reddish brown friable clay with pH ranging from 4.5 to 5.5, soil organic Carbon and Phosphorus content are 1.8% and 4.5mg kg^{-1} respectively (Netondo, 1999).

Seed viability test

Seed viability test was conducted in the laboratory. Ten seeds were placed on a Petri dish lined with moist filter paper (plate 1). The seeds were considered to have germinated when at least 2mm of the radical emerged from the seed coat.

Plate 1 Seeds of *Senna spectabilis* germinating on a petri dish

Germination experiment

Seed germination was carried out in a greenhouse at Maseno University Botanic Garden. Eighteen 10 litre pots were half filled with solarised soil from Maseno university botanic field; the pots were perforated at the bottom to allow for the drainage of excess water to avoid flooding, and arranged in a completely randomized design with six treatments. The treatments were 0, 2, 4, 6, 8 and 12cm depths, replicated three times. The accurate depth of sowing the seeds was established by use of wooden boards, 20cm in length with thickness corresponding to the exact planting depth. The wooden boards were pressed into the soil up to their respective depths. Four seeds were then sown in each pot. The pots were provided with water daily.

Determination of days to and completion of germination

The number of days to germination of seeds was determined by counting. A seed was considered to have germinated when the plumule had sprout out of the soil.

Determination of germination percentage

Germination percentage was established by counting the number of seeds germinating every day and calculating the final germination percentage in each treatment according to Abiala *et al.* (2013). The germination percentage was calculated as indicated below:-

Germination % = Number of seedling emerged/Number of seeds sown x100.

Plant growth experiment

Determination of seedling vigour index

The seedling vigour index (S.V.I) was determined using the formula of Abdul-Baki and Anderson (1973). Eight plants per treatment and per replication were sampled for the measurements.

S.V.I= Seedling length (Root + shoot) x Germination percentage

Determination of plant height

The plant height was determined using a metre rule. The plant was measured from the soil surface to the tip. Measurements were carried out on weekly basis on eight plants per treatment and per replication.

Determination of leaf area

Plant leaf area (A_{plant}) was measured according to the method of Coornellisse *et al.* (2005). Eight plants per treatment in each replication were sampled for the measurements. The length and width of the middle leaflet was measured and leaf area was calculated using the equation below;

$$A_{plant} = 0.74 * 3 * N_1 (l * w * \pi/4)$$

Where:

A_{plant} = Plant's leaf area.

L = Length of the middle leaflet (mm).

W =Width of the middle leaflet (mm).

Π = 3.1426

N_1 = Total number of leaves.

Determination of leaf number

The number of fully expanded mature leaves per plant on the main stem and branches were counted and recorded. Eight plants per treatments and each replication were sampled and measurements carried out weekly.

Determination of plant fresh weight

Eight plants per treatment and per replication were uprooted and separated into shoots and roots. The roots were washed in tap water and then dried on blotting paper before weighing using a weighing balance (Denver Instrument Model XL-31000, Germany).

Determination of root length

The root length was measured from the base of the stem, just above the soil surface, to its root apex using a meter rule. Eight plants per treatments and per replication were sampled and measurements carried out.

Determination of chlorophyll content

Determination of chlorophyll content followed the formula of Combs *et al.* (1985). The third fully expanded leaf from the shoot apex was sampled from all the treatments. 0.5g of *Senna spectabilis* was ground in 10ml of 80% acetone using a pestle and a mortar. The resulting substrate was read at 664 and 647 nm using UV visible spectrophotometer. Eight plants per treatments and per replication were sampled for chlorophyll content determination.

Chlorophyll a, b and the total chlorophyll concentration was calculated as follows

Chlorophyll a = 13.19 A 664-2.57 A 647 (mg g^{-1} fresh weight)

Chlorophyll b = 22.1A 647-5.26 A664 (mg g^{-1} fresh weight)

Total chlorophyll = 7.93 A664+19.53 A647 (mg g^{-1} fresh weight)

Where A664 is the absorbance at 664nm, and A647 is the absorbance at 647nm.

Data Analysis

The data collected from this study was subjected to analysis of variance using SAS Statistical Package. Means were separated and compared at P<0.05.

3. RESULTS

Germination percentage

Sowing depth significantly affected germination percentage. Germination percentage decreased with an increase in sowing depth. However, 2 cm sowing depth had significantly higher germination percentage than sowing depth 0, 4, 6, 8 and 12cm. Sowing depth 12cm had significantly lower germination percentage as compared to all the other sowing depths. Generally the control (0cm) and 2cm depth recorded higher germination percentages while the deeper sowing depths recorded lower germination percentages (Table 1).

Seedling vigour

Seedling vigour index was significantly affected by sowing depth. There was a significant decrease in seedling vigour index with an increase in sowing depth except for sowing depth 2cm (Table 1). 2cm sowing depth recorded significantly higher seedling vigour index as compared to 0,4,6,8 and 12cm sowing depth. The lowest seedling vigour was recorded in 12cm sowing depth.

Table 1 Effect of sowing depth on germination percentage and Seedling vigour index of *Senna spectabilis*

Depth of sowing (cm)	Germination percentage %	Seedling vigour index
0	62.40b	1639b
2	69.40a	1830a
4	60.00b	1620c
6	57.00b	1456d
8	56.60b	1408e
12	49.24bc	1278f
LSD	**6.81**	**90.07**

Means with the same letter down the column are not significantly different at $p<0.05$. The table is the mean of the three replicates.

Plant height

Plant height was significantly affected by sowing depth (Table 2). Generally medium sowing depths recorded higher plant height as opposed to very shallow and very deep sowing depths. Sowing depth 2 and 4 cm recorded significantly higher plant height as compared to sowing depth 0, 6, 8 and 12cm. The lowest plant height was recorded at 12cm sowing depth.

Leaf area

Leaf area was significantly affected by sowing depth (Table 2). Generally medium sowing depth of 2, 4 and 6cm recorded significantly higher leaf area as compared to 8 and 12cm sowing depth. Sowing depth 0, 2, 4 and 6cm had no significant difference ($p \leq 0.05$) among them in leaf area however; they were significantly different from leaf area of 12cm sowing depth. Least leaf area was recorded at 12cm sowing depth.

Table 2 Effect of sowing depth on plant height, leaf area, and leaf number of *Senna spectabilis*

Depth of sowing (cm)	Plant height (cm)	Leaf area (cm^2)	Leaf number
0	12.76b	60.75a	12.88a
2	13.56a	70.30a	13.33a
4	14.12a	67.39a	13.22a
6	12.44b	64.53a	13.00a
8	11.84b	60.04ab	12.00ab
12	10.96c	32.92c	11.34b
LSD	0.79	9.96	0.86

Means with the same letter down the column are not significantly different at $p<0.05$. The table is the mean of the three replicates.

Leaf number

Leaf number was affected by sowing depth (Table 2). The highest leaf number was recorded at 2cm sowing depth. This was followed by 4cm and 6cm sowing depths. The deepest sowing depth, the 12cm sowing depth produced the lowest number of leaves per plant. There was statistically significant difference in leaf number among the six sowing depths at 4 weeks after planting. The 0, 2, 4 and 6 cm sowing depths had no significant difference in leaf number. There were significant differences between 12cm sowing depth and other treatments.

Chlorophyll content

The chlorophyll content significantly ($p \geq 0.05$) reduced with increase in sowing depth except for 0cm depth (Table 3). The 2 and 4cm sowing depths had significantly higher chlorophyll content as compared to the other sowing depths treatments. The least chlorophyll content was observed at 12cm sowing depth; however, there was no significant difference in chlorophyll content between sowing depth 8 and 12cm.

Root length

Root length decreased with increase in sowing depth (Table 3). Highest root length was measured at 2cm sowing depth. Root length of *Senna spectabilis* at 0, 6 and 8cm were not significantly different from each other. The 12cm sowing depth had significantly lower root length compared to the other sowing depths.

Plant fresh weight

Shoot fresh weight was generally affected by sowing depth. Sowing depth 2 and 4cm recorded significantly higher shoot fresh weight compared to the other sowing depths. Plant fresh weight values for sowing depth 0, 6, and 8cm were not significantly different from each other, however they were significantly different from those of sowing depths 2, 4 and 12cm. The lowest shoot fresh weight values were recorded at 12 cm sowing depth.

Table 3 Effect of sowing depth on chlorophyll content, root length and shoot fresh weight of *Senna spectabilis*

Depth of sowing (cm)	**Chlorophyll content** (mg g^{-1} fresh weight)	**Root length (cm)**	**Plant fresh weight (g)**
0	20.32b	12.02c	6.30b
2	25.24a	14.81a	7.47a
4	24.07a	13.74b	7.44a
6	20.51b	12.30c	6.40b
8	18.52c	11.76c	6.32b
12	18.02c	11.23d	6.21c
LSD	1.91	**1.06**	**0.65**

Means with the same letter down the column are not significantly different at $p<0.05$. The table is the mean of the three replicates.

4. DISCUSSION

The results indicate that sowing depth greatly influenced *Senna spectabilis* ability to emerge. Similar results have been reported in other plants for example Nabi *et al*. (2001) who found that seedling emergence was decreased with increased sowing depth in cotton and Ahirwar (2015) reported that germination decreased with increase in sowing depth in *Butea frondosa*. According to Minore (1985), the deeper the sowing depth of seeds the fewer seedlings emerge and the greater the number of days to emergence. Germination marks the beginning of the independent growth and existence of seeds or other organs. The depth of sowing seeds is important as it contributes to achieving a good crop stand and establishment and higher yields (Siddig *et al*., 2015). The control (0) depth recorded significantly lower germination percentage than 2cm sowing depth. This may have resulted from the fact that seed depth contributes to the extent of attacks by insects and rodents, especially when unprotected (Adelana, 1980) and also, fluctuations in moisture conditions could be responsible for differences in germination percentages between 0cm (surface) and 2cm planting depth. Moreover, Germinating seeds find it difficult to establish, if sown at the surface of the soil. Aikins *et al*. (2006) observed that too shallow sowing results in poor germination due to inadequate soil moisture at the top soil layer. Deep sowing can significantly reduce crop emergence and yield. The deeper the seed is sown the more strength it needs to push its shoots above the soil surface. The lower germination percentages recorded at deeper depths may be due to the fact that the seeds buried with soil preventing the seeds from accessing optimum water, air, light and temperature, hence decrease in germination with increasing planting depths, as confirmed by Mohammad (2011). Deep sowing causes elongation of stem between the seed and secondary roots. The food reserves of the grain are taxed severely and seedling may even fail to emerge. An increase in hypocotyl or epicotyl length, as noted in deep seedling reduce the probability of the seedlings being capable of overcoming soil strength and render the seedlings more susceptible to attack by pathogens (Yu *et al*., 1990). Depth of sowing affects seedling emergence, growth and yield of crops. Uniform seeding depth is essential towards achieving higher crop yield (Sarkin *et al*., 2015). Proper depth is essential in order to achieve good germination, emergence and high plant population (Srivastava *et al.,* 2006).

There was a significant decrease in seedling vigour index with an increase in sowing depth except at 2cm sowing depth. The results have demonstrated that deeper sowing impacts negatively on seedling vigour. The results are similar to those reported by Roy *et al.* (2011) on wheat plants. The greater the seedling vigor, the higher the number of seedlings that will emerge and become established.

The results indicate that the depth of sowing is important in maximizing the potential of plant height. The tallest plants were recorded at 4cm sowing depth. Higher plant height at medium sowing depths may suggest an increased cell division and enlargement, which could be probably due to high amounts of nutrients and water available at 2 and 4cm depths. Growth involves both cell growth and development which is a process consisting of cell division, cell enlargement and differentiation (Sikuku et al., 2010). The results indicate that medium sowing depth is optimal for higher plant height. The results are in agreement with those of Aikins and Afuakwa (2008) in Cow pea and Pesboilles (2000) for wheat. Umeoka and Ogbonnaya (2016) found that sowing depths significantly reduced cumulative height growth over time in *Telfairia occidentalis*. The deeper sowing depths had lower height; this could have been probably due to inconsistent growth in leaf area and leaf number of *Senna spectabilis* (Tables 2) which may affect photosynthesis and proper growth of plants. Reduced leaf area and leaf number could result to lower photosynthetic capacity of the plants and ultimately limit growth (Ayobola *et al*., 2010).

The results indicate that leaf area and leaf number of *Senna spectabilis* was higher at medium depths of 2 and 4cm as compared to very shallow depth of 0cm and deeper depths of 6, 8 and 12 cm. Similar results have been reported in wheat (Singh and Girish 2013). Singh and Girish, (2013) observed that growth and other yield parameters were influenced bydifferent sowing depths and leaf areaindex and dry matter production were decreased with increasing sowing depths below 2 cm. Siddig *et al.* (2015) noted that number of leaves per plant declined as sowing depth increased in Faba bean seedlings. The results may be attributed to seedlings from deep sown depth producing fewer amounts of leaves and this might have been expected as deep sowing has been shown to have a number of consequences on seedling growth. Leaves are the site of photosynthetic activities of crops through which biomass are produced, partitioned among various parts of crops and stored for crop productivity (Asare *et al*., 2011). The higher the number of leaves, the higher the rates of photosynthesis with resultant increase in carbohydrate, protein and hence increase in food production. Ridge (1991) reported that the number of leaves produced by a plant is directly proportional to the photosynthate produced and Wareing and Philips (1970) indicated that when photosynthesis becomes active in a young seedling, the power of the plant to synthesise new materials is clearly dependent on the amount of leaves exposed to direct sunlight.

The results indicate that chlorophyll was low at the 0, 8 and 12cm depth as compared to 2 and 4 cm depth. The low chlorophyll at 0cm can be attributed to the fact that if shallow-planted seeds survive and germinate, there is the likelihood that the resultant seedlings scavenge for water and mineral nutrients horizontally and not penetrate deeply into the soil. Such behavior may have led to the formation of low quality 'spider' roots as described and illustrated in Roy *et al*. (2003). The root length & root diameter at different growth stages determine the water and nutrient uptake capacity of the plant. Elements such as nitrogen, iron and magnesium play a role in chlorophyll structure. It is possible that uptake of these elements was low at 0, 8 and 12cm depth.

The results indicate that *Senna spectabilis* root length generally reduced with sowing depth apart from the control. Similar results have been reported in Anidaso Soy bean by Aikins *et al.* (2011). Singh *et al*. (1972) in Soybean observed that the main effect of sowing depth was a linear reduction in radical length as the sowing depth increased. Crop yield and plant growth are closely related to root system development (Westesen *et al*., 1987). Roots play an important role in plant survival during periods of drought. 0cm sowing depth may have recorded relatively low root length due to the fact that very shallow seedling may lead to a high incidence of spider roots which are of very low quality. However, provided the radical of the germinating seed penetrates the soil, the seedling will develop normally with a tap root; such seedlings will have contractile roots which ensures that the rhizome and the pen bud are kept below the soil surface. The 2 and 4 cm sowing depth recorded significantly higher root lengths, this may indicate that cell division and elongation were faster at these depths and continued to produce new root hairs for maximum response to nutrients supply (Willumsen, 1993).

There was a significant decrease in plant fresh with increase in depth. Similar results have been reported in Anidaso Soy bean by Aikins *et al*. (2011). According to Umeoka and Ogbonnaya (2016), the performance of any plant in terms of dry matter production is directly dependent on the pattern of its leaf area development in response to solar energy and carbon dioxide perception. In the current study, 2 and 4 cm sowing depth may have recorded higher plant fresh weight due to the larger leaf area and leaf number for photosynthetic activities allowing the plants to accumulate much photosynthates, which translated to higher fresh weight. Increased cell division and cell enlargement may have occurred at 2 and 4 cm sowing depth leading to significantly higher plant height and higher root length of the plants. Higher root length may have conferred the plants with higher surface area for water and mineral salts absorption for dry matter accumulation.

5. CONCLUSIONS

The current experiment was conducted to investigate the effect of sowing depth on germination, growth and chlorophyll content of *Senna spectabilis*. Based on the results, it can be concluded that sowing depth has a significant effect on *Senna spectabilis* germination, growth and chlorophyll content. Germination, growth parameters and chlorophyll content decreased as depth increased. 2cm sowing depth had the highest values of germination percentage, seedling vigour, leaf area, leaf number, chlorophyll content and plant fresh weight. Results indicate that *Senna spectabilis* may be sown at medium depths of 2cm and 4cm in order to obtain the best germination, growth and higher chlorophyll content.

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