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Effects of Sowing Depths on Seed Germination and Seedling Growth of Custard Apple (*Annona squamosa* L.)

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ABSTRACT
The study was undertaken to determine the effect of sowing depths on the germination and seedling growth of *Annona squamosa* (L.). The experiment was laid out in a Completely Randomized Design (CRD) with 5 treatments: 0. 3, 6, 9 and 12cm depths. Fifteen viable seeds were planted at each of the 5 different sowing depths in 5 plastic rubber containers filled with sandy loamy soil, with a total of 75 seeds planted. Seed germination was observed daily and recorded for a period of 30 days after planting (DAP), germination percentage (%) calculated for each of the sowing depths. Germination percentage was highest (80.00%) at 3 and 6cm depths and lowest (13.33%) at 0cm depth. Evaluation of early seedling growth was based on height, collar diameter and leaf number, 4 weeks after planting (WAP) and continued bi-weekly thereafter up to 14 weeks after planting. Plumule emergence was earliest observed at 10DAP from 0cm depth and latest at the 12cm depth. Highest mean seedling height and collar diameter were observed at 3cm depth while the lowest mean seedling height was observed at 12cm depth at all growth stages 6, 8, 10, 12 and 14WAP. Highest mean leaf number was observed in 3 and 6cm depths at 6 and 8WAP and in 3cm depth at 10-14WAP while lowest mean leaf number was observed in 12cm depth at 6-14WAP. Mean seedling height did not vary significantly ($p>0.05$) among 3, 6 and 9cm depths at all stages of growth. Collar diameter did not vary significantly ($p>0.05$) among 3, 6 and 9cm depths at 12 and 14WAP. Results revealed that seedling growth generally decreased with increase in sowing depth.
Keywords: Sowing, Germination, *Annona squamosa*

INTRODUCTION
Custard apple (*Annona squamosa L.)* is a delicious and important fruit crop which is cultivated in tropical and subtropical climate, the edible fruits of genus annonaceous fruit. There are an estimated 2,200 species of annonaceaein in the world. The high nutrition value of the fruit, its varying distinct flavours, aromas and its attractive shape and colour justifies these efforts. There are three species, *Annona cherimoya*, *Annona muricata* and *Annona squamosa* (Mahdeem, 1994).
Custard apple is typically evergreen or semi-deciduous plant. Native for the new world tropics, the root system is confined to relatively shallow layers and therefore this does not require deep soils. However, it needs well drained soil. The tree withstands high amount of time found in calcareous soil and it is the most tolerant fruit tree.
*Annona squamosa L.*is a native of the west Indians and South America and is now widely grown throughout the tropics at low and medium altitude. Sandy loamy soil is the best for custard apple, well-structured clay loams are also suitable although the main fender roots are relatively shallow. Uniform soil moisture throughout fruit set and fruit development ensures high yields and helps in preventing fruit that is to be grown.

Adeogun *et al.* (2012) stated that one of the problems facing afforestation program in the Sahel is the depth of sowing which imposes encumbering soil pressure during shoot up thrust. It is one of the most common errors that occur in nursery and plantation establishment, depending on the type and size of seed. Agboola (1996) and McWilliams *et al*. (1998) stated that each species has a specified sowing depth requirement base on the type of seed and environmental conditions. Sowing The seed of custard apple fruit contains trapped fatty acids rich in methyl-ester that triggers the production of the gas, countries like Germany and some other European nations use this little wonders for the commercial purpose of production of gas. The seeds are also used as a fish bait in some countries due to toxic in nature.

Shallow depths can result in poor germination due to inadequate moisture at the top soil layer (Desbiolles, 2002) while sowing in deep depths can also significantly reduce germination and growth (Aikins *et a*l., 2006).

The effect of sowing depth on the germination and seedling growth performance of *Annona squomosa* has not been document in this locality. Hence, the need for this study.

Objectives of the Study

The objectives of the study are to:

i. determine the effect of soil depth on the germination of *Annona squamosa*,
ii. determine the effect of soil depth on seedling growth of *Annona squamosa* and
iii. evaluate the best soil depth for the germination and growth of the study species.

Literature Review

Annonaceae is a large family under the division of Magnoliophyta which comprises about 130 genera over 2300 species. The most important genera having a large number of species are Annona (Kulkarni and Chandrasekar, 2011). It is a multipurpose tree with edible fruit and one of the major medicinal plants in Annonaceae family. Some important features of the family are:

- Edible fruits: Custard apple, Sugar apple, Atemoya, Cherimoya, Pawpaw, Soursop
- Medicine: Vermicide, Abortifacient, Insecticide, Extermination of hair lice
- Oils: Soap preparation (Araya, 2004)

Annona squamosa is commonly known as Custard apple or Sugar apple in English, Pommier cannelle in French, Sharifa in Hindi, Sitaphal in Telugu and Sitaphalam in Tamil (Pandey and Barve, 2011). It is distributed in tropical and subtropical trees and shrubs. They range from 10 to 20ft (3-6m) in height with irregular branches and zigzag twigs. The fragrant flowers are borne single or in groups of 2 to 4. The fruit is nearly round, oval or conical which gets separated when the fruit is ripe. The ripe fruit contains creamy white, sweet and delicious flesh (Morton 1987; Yusha *et al*. 2011). The seeds are scattered throughout the flesh, blackish brown, hard and shiny and are poisonous if chewed.

The leaves are used to reduce blood sugar and oil extracted from seed is used to kill spinal diseases while the bark juice is used as an antidote for snake bite (Amita and Kalpana, 2015).

Custard apple appears to possess potent bioactive principles in most of its plant parts (fruits, seeds and leaves). The fruits are mostly consumed as table fruit, mainly used for fresh consumption, generally being considered as a "fruit of poor people" the large aggregate fruits are composed of peel, pulp and seeds. The fruits are also used in ice-cream and preserved as jam, jelly and other products on limited scale.

The pulp is creamed coloured, custard like granular sweet with pleasant flavour and mild aroma, the sweet pulp is edible whereas the bark and leaves are used for the treatment of many ailments like gastro-enteritis, pneumonia and dermatological diseases (Von maydell, 1990).

The seed of custard apple fruit contains trapped fatty acids rich in methyl-ester that triggers the production of the gas, countries like Germany and some other European nations use this little wonders for the commercial purpose of production of gas. The seeds are also used as a fish bait in some countries due to toxic in nature.

Scientific classification of *Annona squamosal* L.

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Magnoliales
Family	Annonaceae
Genus	Annona
Species	*Annona squamosa*

MATERIALS AND METHODS

The study was carried out between May and September, 2019 at the Department of Biological Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria.

The seeds were collected from mother trees of good phenotype at the University of Maiduguri farm. Viability test was carried out using floating method, where seeds were subjected to soaking in cold water overnight, the seeds that sunk were regarded as viable and used for the study, while the seeds that floated were discarded.

The experiment was laid out in a Completely Randomized Design (CRD) with the five treatments (sowing depths) where seeds were sown at 0cm, 3cm, 6cm, 9cm and 12cm depths. A total of 75 randomly selected seeds were used for this study. Each seed was sown in a wide plastic rubber measuring 5cm in diameter and 20cm depth. The wide plastic rubbers with the soil were watered twice daily in the morning and evening. No fertilizers, bacterial or mycorrhiza inoculation was used. Daily record of germination was carried out throughout the period of the experiment.

Data Collection

Seed Germination

Observation on germination of seeds was recorded daily for thirty days. Germination percentage was calculated as:

$$Germination\ percentage\ (GP) = \frac{Total\ germinated\ seeds}{Total\ seeds\ sown}\ x\frac{100}{1}$$

Seedling growth

Seedling growth parameters collected include seedling height (cm), collar diameter and leaf number. The data collection began (30) days after sowing and continued biweekly thereafter for a period of 12 weeks, the seedling height was measured from the soil top level to the tip of the youngest leaf using a meter rule (cm), stem collar diameter (cm) was measured at the collar position using a vernier caliper, while the number of fully expanded leaves was determined by direct counting.

Data Analysis

Data were analyzed using both descriptive statistics (tables and chats) and inferential statistics. One-way analysis of variance (ANOVA) was used to test for significant differences in mean values of the measured seedling growth attributes among the different sowing depths (treatments) at $p<0.05$. Duncan's Multiple Range Test (DMRT) was used for the separation of means where significant differences were observed. The analysis of variance and mean separation were done using statistical package for Social Sciences (SPSS Version 20, SPSS Inc).

RESULTS

Effect of Sowing Depth on the Germination inception and germination percentage of *Annona squamosa*

Plumule Emergence

The inception of plumule emergence for the different sowing depths varied from 26 to 37 days after planting (DAP) (Figure 1). The seeds of *A. squamoa* sown at 0 cm depth exhibited rapid plumule emergence (26DAP); followed by those sown at 3cm (28DAP), 6cm (33DAP), and 9cm and 12cm (37DAP).

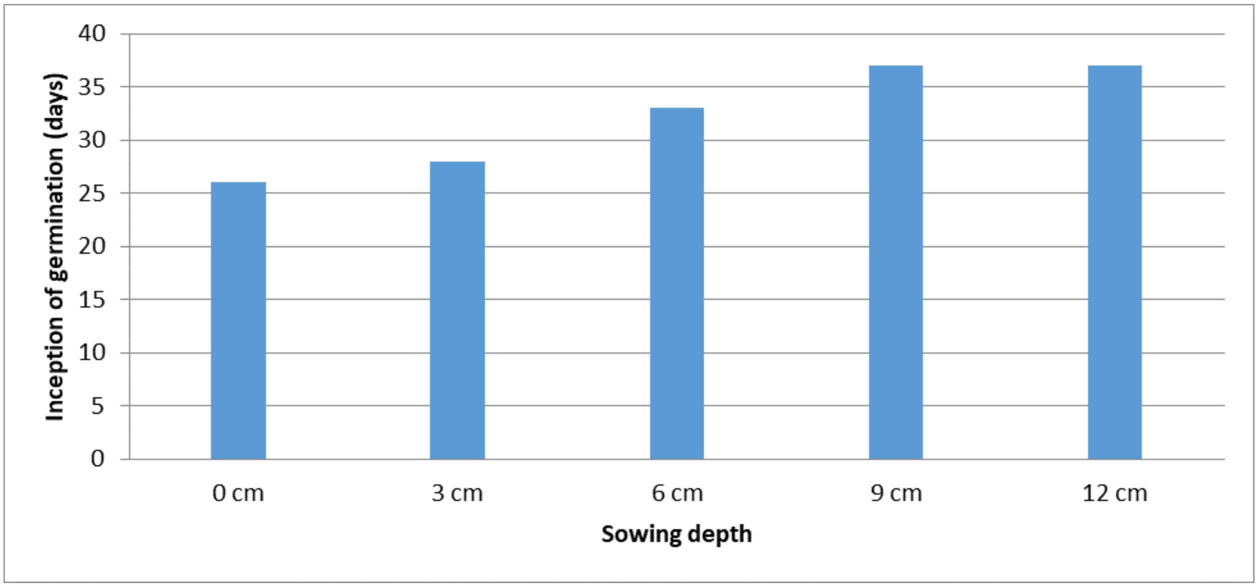

Fig 1: Effect of sowing depth on the germination of *Annona squamosa*

Germination Percentage

Mean germination percentage (GP) varied from 13.33% to 80% (Figure 2). Seeds sown at 3 and 6cm depths had the highest mean GP (80%), followed by seeds sown at 9 and 12cm depths (53.33%) while seeds sown at 0cm depth had lowest GP (13.33%).

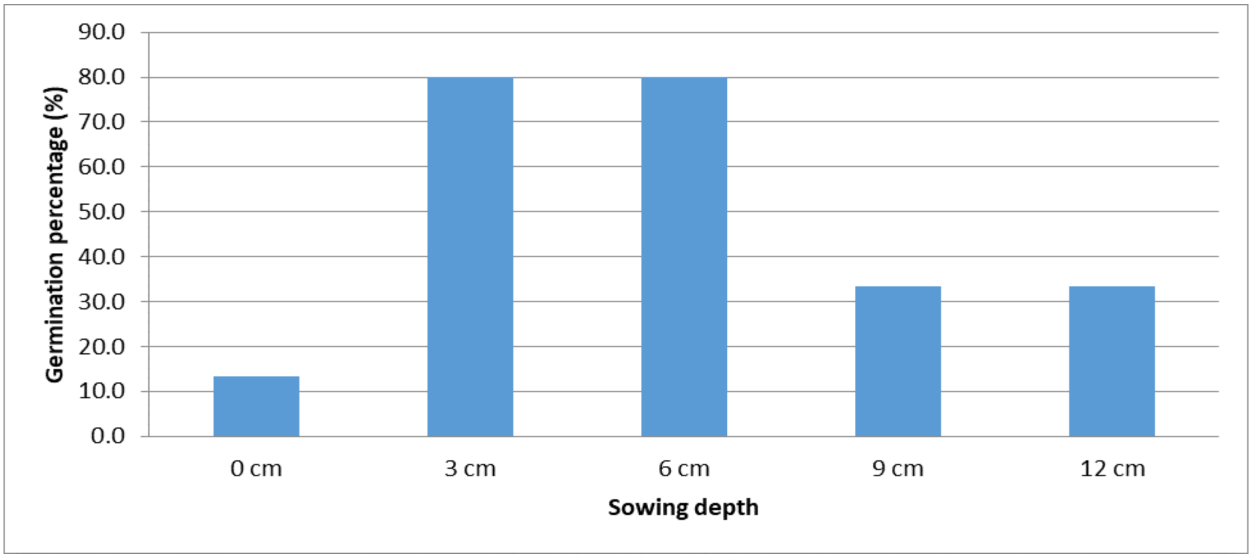

Fig 2: Effect of sowing depth on the germination percentage of *Annona. squamosa*

Effect of Sowing Depth on early growth performance of *Annona squamosa* seedlings

Seedling Height (cm)

Seedlings of *Annona squamosa* subjected to different sowing depths were significantly different ($P \leq 0.05$) in height (Table 1) at 6 to 14 weeks after planting (WAP). Overall mean seedling height 6-14WAP varied from 7.00 cm at 6WAP to 12.76 cm at 14WAP. At weeks 6, 8, 10, 12 and 14, after planting, seedlings from seeds planted at 3cm depth had the highest mean height (10.81, 11.06, 11.33, 11.56, and 12.76cm respectively), followed by seedlings from seeds planted at 6cm depth (9.97, 10.83, 11.16, 11.25 and 12.26cm respectively), while seedlings from seeds planted at 12cm depth had the lowest mean height (7.00, 7.80, 8.27, 8.50 and 8.62cm respectively). However, seedling height did not differ significantly among 3, 6 and 9cm depths at all stages of growth.

Table 1: Effect of sowing depth on mean seedling height (cm) of *Annona squamosa* seeds

Sowing depth(cm)	**Seedling height (cm)**				
	6 WAP	8WAP	10 WAP	12 WAP	14 WAP
3cm	10.81^{a}	11.06^{a}	11.33^{a}	11.56^{a}	12.76^{a}
6 cm	9.97^{a}	10.83^{a}	11.16^{a}	11.25^{a}	12.26^{a}
9 cm	9.57^{a}	10.43^{a}	10.73^{a}	10.80^{a}	10.90^{a}
12 cm	7.00^{b}	7.80^{b}	8.27^{b}	8.50^{b}	8.62^{b}
Mean	9.74	10.40	10.72	10.59	11.24
P-value	<0.001	0.004	0.005	0.005	0.001

Values in the same column with the same letter do not differ significantly ($P \geq 0.05$)

WAP = Weeks After Planting

Collar Diameter

Seedlings of *Annona squamosa* subjected to different sowing depths were significantly different ($P \leq 0.05$) in collar diameter (Table 2) at 6, 12 and 14WAP, but were not significantly different at 8 and 10WAP. Overall mean collar diameter 6-14WAP varied from 0.52mm at 6WAP to 1.15mm at 14WAP. At 6, 8, 10, 12 and 14WAP seedlings from seeds sown at 3cm depth had the highest collar diameter (0.73, 0.81, 0.95, 1.09 and 1.15mm respectively), while seedlings from seeds planted at 12cm depth had the lowest collar diameter (0.52, 0.74, 0.86, 0.95 and 1.01mm respectively). However, seedling collar diameter did not differ significantly among 3, 6 and 9cm depths at 6WAP, among all the treatments at 8 and 10WAP, and among 6, 9 and 12cm depths at 12 and 14WAP.

Table 2: Effect of sowing depth on mean seedling collar diameter (mm) of *Annona squamosa* seeds

Sowing depth(cm)	Collar Diameter (mm)				
	6 WAP	**8 WAP**	**10 WAP**	**12 WAP**	**14 WAP**
3cm	0.73^{a}	0.81^{a}	0.95^{a}	1.09^{a}	1.15^{a}
6 cm	0.72^{a}	0.78^{a}	0.91^{a}	1.00^{b}	1.07^{b}
9 cm	o.68^{a}	0.77^{a}	0.91^{a}	1.00^{b}	1.04^{b}
12 cm	0.52^{b}	0.74^{a}	0.86^{a}	0.95^{b}	1.01^{b}
Mean	0.67	0.78	0.91	1.01	1.07
P-value	0.003	0.551	0.439	0.004	0.008

Values in the same column with the same letter do not differ significantly ($P \geq 0.05$)
WAP = Weeks After Planting

Leaf number

Seedlings of *Annona squamosa* subjected to different sowing depths were not significantly different ($P \geq 0.05$) in leaf number (Table 3) at 6, 8, 10 and 12WAP, but were significantly different at 14WAP. Overall mean seedling height 6-14WAP ranged from 3.67 at 6WAP to 5.57 at 14WAP. At 6 and 8WAP, seedlings from seeds planted at 3 and 6cm depth had the highest mean leaf number (4.00), while seedlings planted at 12 cm depth had the lowest mean leaf number (3.67). At week 10, 12 and 14, seedlings from seeds planted at 3cm depth had the highest mean leaf number (4.29, 4.86 and 5.57 respectively), followed by seedlings from seeds planted at 6 cm depth (4.14, 4,43 and 5.29 respectively), while seedlings planted in 12cm depth had the lowest mean leaf number (3.67, 3.67 and 4.17 respectively). However, seedling mean leaf number did not differ significantly among all the treatments at 6WAP; among 3, 6 and 9cm depths at 8WAP; between 6 and 9cm depths at 10WAP; among 6, 9 and 12cm depths at week 12; and between 6 and 9cm depths at 14 WAP.

Table 3: Effect of sowing depth on mean seedling leaf number of *Annona squamosa*

Sowing depth(cm)	**leaf number (mm)**				
	6 WAP	**8 WAP**	**10 WAP**	**12 WAP**	**14 WAP**
3cm	4.00[a]	4.00[a]	4.86[a]	4.86[a]	5.57[a]
6 cm	4.00[a]	4.00[a]	4.43[ab]	4.43[ab]	5.29[ab]
9 cm	3.83[a]	4.00[a]	4.00[b]	4.00[b]	5.00[b]
12 cm	0.52[b]	3.67[b]	3.67[b]	3.67[b]	4.17[c]
Mean	3.88	3.92	4.04	4.27	5.04
P-value	0.207	0.063	0.069	0.023	<0.001

Values in the same column with the same letter do not differ significantly ($P \geq 0.05$)
WAP= Weeks After Planting

DISCUSSION

Several factors affect emergence as well as germination percentage, and sowing depth has been identified as one of such factors (Minore, 1985; Koger *et al.*, 2004). This explains what happened to the seeds planted. At different sowing depths in this study where emergence was delayed by increasing sowing depth. This agrees with the reports of Koger *et al.* (2004) on *Caperonia palustris,* Arnulfo and Mexal (2005) on *Pinus brutia*, *P. greeggi* and *P. cembroides,* and Koffi *et al.* (2015) on *Lagenaria siceraria*. In their reports they observed that plumule emergence was delayed by increasing sowing depth. The possible reason for the delayed emergence at lower sowing depths could probably be as a result of the long distance the plumule has to contend with before reaching the soil surface (Raju *et al.*, 2017).

Highest germination percentage observed in 3 and 6cm depths than in 0, 9 and 12cm depths implied that sowing at shallow depth generally stimulates more seed germination than when seeds are sown on the soil surface or deeper depths. This is because the former (shallow depth) provides a moist environment around them and prevents seeds and seedlings from drying out, as well as prevent damages by insects (Rusdy and Sjahril, 2015). This result concurs with that of Opande *et al.* (2017) and Chima *et al.* (2017) who noted that germination decreased with increase in sowing depths *in C. brevidens* and *A. muricata,* respectively. Ali and Idris (2015) observed that the deeper the seed is sown, the more strength it needs to push its shoots above the soil surface. According to Aikins *et al*. (2006), too shallow sowing results in poor germination due to inadequate soil moisture at the top soil layer and deep sowing can also significantly reduce plant emergence and yield. This explains the very poor germination percentage observed in seeds sown at 0cm depth and the delayed emergence at 12cm depth in this study.

Seedlings of *Annona squamosa* subjected to 3, 6, 9 and 12cm sowing depths exhibited significant difference in height at 6 to 14WAP, collar diameter at 6, 12 and 14WAP, and leaf number at 14WAP. Adeogun *et al.* (2012) also reported significant difference in seedling height, collar diameter and leaf number of some Sudano-Sahelian tree species subjected to different sowing depths in a containerized experiment. It was observed from the study that seedlings height decreased with increasing sowing depth. This agrees with the findings of Umeoka and Ogbonnaya (2016) who reported that increasing sowing depths significantly reduced cumulative height growth of *Telfairia occidentalis* over time. Lower seedling height at deeper sowing depths might be due to the difficulty encountered by seedlings in pushing their shoots through a thicker soil layer (Rusdy and Sjahril, 2015).

Seedlings from seeds sown at 3cm depth had the largest collar diameter followed by those sown at 6cm, 9cm and 12cm depths respectively. This indicates that an increase in the depth of sowing will result in plants with thinner stems. This agrees with the findings of Adeogun *et al.* (2012) who reported that 3cm depth had better growth parameters than other depths on *Adansonia digitata, Acacia senegal, Delonix regia, Balanites aegyptiaca, Khaya senegalensis, Senna siamea, Prosopis juliflora, and Ziziphus spinachristi* and Koger (2004) who observed that seedling collar diameter of *Caperonia palustri* decreases with increasing sowing depth.

Number of leaves per plant decreased as sowing depth increased. This implies that seedlings from deeper depth produced fewer amounts of leaves. Ali and Idris (2015) also observed a decrease in leaf number as depth increases and noted that deep sowing has been shown to have a number of consequences on seedling growth. Similar observations on leaf number have been reported by Odeleye *et al.* (2007) on *Abelmoschus esculentus.* The reason why the 3 and 6cm sowing depths produced highest leaf numbers could be attributed to the availability of nutrients to the seedling's root within such zone.

CONCLUSION

This study has shown that germination and early seedling growth of *Annona squamosa* are affected by sowing depths. Sowing seeds too deep into the soil has significant negative effects on germination and growth performance of *A. squamosa* seeds and seedlings. The high seedling emergence, germination percentage as well as growth performance of *A. squamosal* at 3cm and 6cm sowing depths, indicate that sowing depths within that range will generate optimum yield and has better silvicultural advantage.

RECOMMENDATION

It is recommended that seeds of *A. squamosa* should be sown at shallow depths (from 3 to 6 cm) since best germination and seedling growths were observed at the two depths.

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