Original Research Article

Early Growth Response of *Annona muricata* (L.) Seedlings to Different Potting Media

ABSTRACT

Aims: The aim of this study was to evaluate the performance of *Annona muricata* under four different treatments of organic manure in a screen house.
Study design: The study was laid out in a completely randomized design (CRD) with four replicates.
Place and Duration of Study: The experiment was carried out in the Screen House of the Department Forestry and Wildlife, Nnamdi Azikiwe University, Awka, from March to August 2018.
Methodology: Four treatments of organic manure were applied to the seedlings of *A. muricata*, namely; T1 (Top soil + River sand (control)), T2 (sheep dung), T3 (decomposed sawdust) and T4 (cow dung). Data on plant height, leaf count, collar diameter, root length and leaf area were collected and analysed using analysis of variance and Duncan Multiple range test (DMRT) at 0.05 level of significance.
Results: The results showed that all the growth parameters improved with increasing time. Plant height and leaf count were significantly higher in T4 with mean value of 11.79c ± 0.29 and 4.58b ± 0.22 respectively and T1 (11.25 ± 0.26 cm and 4.58 ± 0.22 respectively) and lowest in T3 (9.01 ± 0.24 cm and 3.74 ± 0.18 respectively). Leaf area was higher in T4 (10.87 ± 0.43 cm^2) and lowest in T2 (8.55 ± 0.35 cm^2). Root length and collar diameter were highest in T3 (17.15 ± 0.53 cm and 0.25 ± 0.03 cm respectively), root length was least in T1 (12.93 ± 1.72 cm) while collar diameter was lowest in T2 (0.21 ± 0.00 cm).
Conclusion: Cow dung potting media shows greatest potential to promote robust vegetative growth of *Annona muricata* seedlings. Cow dung could be considered an accessible, affordable, and effective alternative to standard potting mixes for nursery propagation of *A. muricata*.These findings contribute valuable insights for optimizing plant growth practices in soursop cultivation.

Keywords: Annona muricata, organic manure, screen house, growth parameters, cow dung decomposed sawdust, seedling growth, organic treatments.

1. INTRODUCTION

Annona muricata L. is a common evergreen tree in the tropics well known across all parts of Nigeria. It is a multipurpose plant valued for its nutritional, medicinal, and economic importance[1]. *Annona muricata* belongs to the family Annonacae. The generic epithet Annona is from the Latin word ‘anon’ meaning ‘annual harvest’ [2], which entails its fruiting pattern. *Annona*, commonly known as the custard-apple genus, graviola, and soursop, consists of about 125 species, with some species widely cultivated for their edible fruits and often becoming naturalized beyond their native range of tropical America and Africa [3].

The height of the tree differs between 4 to 8 meters, which is influenced by various factors such as climate, soil, and crop management (Fig. 1). The tree has a shallow root system, with the tap root growing up to 1.5 to 1.8 meters long and numerous lateral roots [4]. The flowers are hermaphrodite and emerge in clusters of two to four flowers from both branches and trunk, with three green sepals and six petals arranged in two whorls. It has a heart-shaped fruit with spine-like carpels (Fig. 2) with weight varying from 0.9 to 10kg [5]. Soursop fruit is green in colour and soft when ripe with white edible pulp and black or dark brown indigestible seeds (Fig. 3). The sweet pulp is used in making juice as well as candies, sorbets, and ice cream flavouring. In Nigeria, it is served as dessert and a good delicacy for diabetic patients. It is an economic tree well known for its numerous health benefits. Specifically, various studies have shown the medicinal values of this 'miracle plant' to include anti-cancer, anti-tumor, anti-parasitic, anti-viral, and antioxidant properties [6] - [10].

In addition to its medicinal properties, *A. muricata* has economic significance as a tropical fruit tree contributing to the agricultural landscape of various countries. Moreover, the tree's potential for clonal propagation and its growth response to different potting media have been subjects of research, shedding light on its horticultural aspects and cultivation practices.

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The growth and productivity of plants are majorly dependent on the nature of their early stage; these include soil properties, climatic factors, and available nutrients. Organic mixtures present in the soil made available from varying sources, which include remnants of dead plants and animals, plant roots, seeds, and soil organisms, have the ability to stimulate or deter germination rates [11]; [12]. They are revealed to enhance soil physiological and morphological characteristics as well as produce sufficient nutrients for optimum productivity of the soil and plant yield and ensure toxin-free plants [13], [14]. According to [15], preservation of soil fertility remains the principal condition for every effective and permanent system of agriculture and permanent crops such as tree species.

A potting medium is a mixture of various raw materials designed to serve as a substrate to support the roots of cultivated plants in the pots and/or containers. It is a combination of organic and inorganic materials to meet the desired chemical and physical needs of plants to enhance its growth and development [16]. A good potting mixture offers the plant a base for stability, high water capability, and good nutrient uptake, which ensures proper establishment, growth, and yield. These needs and means of balancing them vary and depend on the species grown and the stage of growth. Khan *et al.* [17] asserted that good management of the media is necessary for the production of quality fruit tree seedlings, as vigorous growth is needed to face seasonal hazards in the field. The organic matter in manure is valuable because it improves soil texture, structure, and water-holding capacity; soil aeration lowers and also lowers soil pH.

Despite the diverse uses and rising popularity of *A. muricata*, which underscore its multidimensional importance as a forest tree, the species faces some cultivation challenges. It is characterized by poor and uneven germination, slow early seedling growth, and susceptibility to insect pests. Also, there is increased consumer demand for soursop fruits and other parts of the tree and a rise in popularity due to its medicinal benefits and cure for cancer [18]. One potential solution is identifying optimal potting media compositions to promote vigorous early growth of nursery seedlings. Previous studies have tested the effects of different potting mixtures on tropical fruit trees, but information specific to *A. muricata* is lacking [16], [17].

Fig. 1 Tree of soursop

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Fig. 2 Ripe soursop fruit

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The objective of this study is to evaluate different organic potting media mixtures to determine their effects on the initial growth and development of *Annona muricata* seedlings. Meeting this objective will facilitate plantation establishment to keep up with the increasing demands for soursop fruits and products. It will also reduce the chances of endangering this ecologically and economically important species.

Fig. 3 White pulp of soursop showing seeds

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2. MATERIAL AND METHODS

2.1 Study Area Description

The study site for this experiment was the screen house facility of the Department of Forestry and Wildlife Management in the Faculty of Agriculture at Nnamdi Azikiwe University, located in Awka, Nigeria (6.25° N and 7.12° E and elevation of ~136 m). This region of southeastern Nigeria has a tropical climate marked by distinct wet and dry seasons. Average precipitation is high between 1828 mm - 2002 mm per year, with a mean annual temperature of ~27°C [19], [20].

2.2 Fruit Procurement and Seed Extraction

Fruits of *A. muricata* were purchased from Anambra State Ministry of Environment, Forestry Department, Amawbia. The fruits were depulped and the seeds extracted manually. The extracted seeds were washed with clean water and dried at room temperature for 24 hours. The extracted seeds were subjected to floatation test to determine seed viability. The viable seeds were soaked for 3 days and air-dried for one (1) day to ensure quick and uniform germination.

The mixture of topsoil + river sand at the ratio 2:1 was used to raise the seeds of *Annona muricata* in the germination box. The potting mixture was thoroughly sieved to remove extraneous materials (dead roots, stones, leaves, sticks and other impurities). The seeds were broadcasted in a germination plastic tray covered with black polythene bag to stimulate the temperature needed for quick seed germination; also, watering was done once in three days. Germination of seeds started from 24 to 31 days after sowing.

2.3 Experimental design and layout

The experiment was laid in a Completely Randomized Design (CRD), with four (4) treatments (T1 = Topsoil + River sand (control), T3 = Topsoil + River sand+ decomposed sawdust, T2 = Topsoil + River sand+ sheep dung, and T4 = Topsoil + River sand+ cow dung) and replicated four (4) times. Seedlings were pricked out of the germination bed and transplanted into the 26 x 21 cm poly pots with holes for adequate drainage of water. Watering was done using a watering can on a daily basis. The treatment was replicated 4 times and the seedlings were left to stabilize for two weeks before data collection commenced. The topsoil was obtained from the forest floor. The animal dungs were procured from the abattoir at Amansea, Awka, on the 15th of November, 2017, and sawdust from a sawmill at Eke Awka, Awka, all in Anambra State on the 21st of February, 2018. The topsoil, river sand, and other media were sieved separately and were measured with the aid of a 500 mg cup at the ratio of 2:1 for control and 2:1:1 for other treatments. A shovel was used to thoroughly mix the growth media and a hand trowel was used to fill them into the poly pots. Uniformly growing seedlings were selected and transplanted to the poly pots placed in the screen house.

2.4 Method of Data Collection

Data on growth variables were collected starting from the 14th day after transplanting the seedlings into the different potting mixtures. The following parameters were measured at one-week intervals for all the treatments for eight weeks. The parameters include plant height (cm), leaf count, leaf surface area (cm^2) (done using the grid method), collar diameter (cm), and root length (cm). The destructive sampling method was used to measure the root length. A meter rule was used to measure from the point of cotyledon scars to the root tips.

2.5 Data Analysis

The data collected on growth parameters were analyzed using analysis of variance (ANOVA) techniques performed in R statistical software version 4.3.2 [21]. ANOVA was used to determine if significant differences existed between the means of the treatment groups. Where significant differences were detected at the $p<0.05$ level based on the F-test, post hoc comparison of the means was conducted using Duncan's Multiple Range Test (DMRT). DMRT applies a stepwise algorithm to compare subsets of means while controlling the Type I comparison-wise error rate.

The use of ANOVA and post hoc testing enabled statistical quantification of the impacts of the different potting mixtures on the measured seedling growth metrics. Execution of the analysis in R provided flexible and reproducible data analysis workflows leveraging a popular open-source programming language designed specifically for statistical computing and graphics [21].

3. RESULTS AND DISCUSSION

The result of the one-way analysis of variance (ANOVA) for seedling growth parameters under different potting mixtures is presented in Table 1. The result revealed that there were significant differences (p<0.05) in all the growth parameters except for root length (p>0.05) (Table 2, Fig. 6).

Ta**ble 1: Analysis of Variance for growth parameters of *Annona muricata***

Variables	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Height	Treatment	444.096	3	148.032	3.021	0.000*
	Error	2443.463	380	6.430		
	Total	2887.558	383			
Leaf count	Treatment	43.758	3	14.586	3.681	0.012*
	Error	1505.740	380	3.962		
	Total	1549.497	383			
Collar diameter	Treatment	0.106	3	0.35	2.044	0.011*
	Error	6.557	380	0.017		
	Total	6.663	383			
Leaf surface area (cm^2)	Treatment	177.580	3	59.193	11.925	0.000*
	Error	933.211	188	4.964		
	Total	1110.791	191			
Root length (cm)	Treatment	45.612	3	15.204	3.239	0.060^{ns}
	Error	56.325	12	4.694		
	Total	101.937	15			

** = significant at p<0.05; ns = not significant at p >0.05*

3.1 Plant Height

Mean separation for seedling height using the Duncan Multiple Range Test (DMRT) showed that the plant height varied significantly in all the treatments (T1, T2, T3, and T4) (Table 2). The maximum height was observed in T4 (cow dung) with a mean value of 11.79 ± 0.29 cm, followed by T1 (Topsoil + River sand) with 11.25 ± 0.26 cm, while the lowest mean value was obtained in T3 (decomposed sawdust) which was 9.01cm ± 0.24.

3.2 Leaf count

The mean separation revealed that T3 is significantly different from T1 and T4 but not different from T2 (sheep dung). The maximum mean leaf count was observed in T4 with the value 4.58 ± 0.22 followed by T1 (4.52 ± 0.21). The least mean value was found in T3 which was 3.74 ± 0.18 (Table 2).

3.3 Leaf Area

The highest mean leaf area was observed in T4 with a value of 10.87 ± 0.43 cm2, followed by T1 with a mean value of 9.97 ± 0.23 cm2, while T2 had the least mean value of 8.55 ± 0.35 cm2 (Table 2).

3.4 Collar diameter

In the experiment, the highest collar diameter was recorded in T3 (0.2496 ± 0.025 cm), followed by T4 with a collar diameter of 0.2482 ± 0.005 cm. The least collar diameter was obtained in T2 which was 0.2083 ± 0.004 cm.

3.5 Root length

The results reported that root length showed no significant difference in the four treatments. The highest root length was observed in T3 with a mean value of 17.15 ± 0.54 cm, followed by T2 with a value of 16.87 ± 0.49 cm, while the least mean value was obtained in T1 (12.93 ± 1.72 cm).

Table 2: Mean separation for seedlings of *Annona muricata*

Treatment	Plant height (cm)	Leaf count	Leaf surface area (cm^2)	Collar diameter (cm)	Root length (cm)
T1	$11.25^{c} \pm 0.26$	$4.52^{b} \pm 0.21$	$9.97^{ab} \pm 0.23$	$0.2338^{ab} \pm 0.006$	$12.93^{a} \pm 1.72$
T2	$10.05^{b} \pm 0.25$	$4.15^{ab} \pm 0.20$	$8.55^{a} \pm 0.35$	$0.2083^{a} \pm 0.004$	$16.87^{a} \pm 0.49$
T3	$9.01^{a} \pm 0.24$	$3.74^{a} \pm 0.18$	$8.66^{a} \pm 0.24$	$0.2496^{b} \pm 0.025$	$17.15^{a} \pm 0.54$
T4	$11.79^{c} \pm 0.29$	$4.58^{b} \pm 0.22$	$10.87^{a} \pm 0.43$	$0.2482^{b} \pm 0.005$	$16.10^{a} \pm 1.10$

Values were expressed as Mean ± Standard Error. Mean values with different superscripts within the same column are significantly different (P<0.05)

Fig. 4 Some seedlings of *Annona muricata*

Fig. 5 Seedlings and their roots in ascending order

4. DISCUSSION

The highest mean height was observed in cow dung. This might be due to the fact that cow dung plays a crucial role in providing organic matter as it improves the soil's geotechnical properties such as bulk density, water holding capacity, porosity, and infiltration. Studies have shown that organic manure increased plants height [22], [23], [16]. Cow dung manure is good manure for plant growth as it contains 79.05% moisture, 0.096% phosphorous and 0.520% potassium [24]. Our findings are not in line with the results of [16], who recorded the highest seedling height in fish pond sediments, while cow dung was the third for Dennettia tripetala. Followed by cow dung was topsoil + river sand which may also be due to its good content of organic matter. Aminah et al. [25] suggested that the major component of potting media should be organic matter. Topsoil is the outermost layer of the soil and houses the highest concentration of organic matter where biological activities occur. Also, river sand helps to reduce compaction in the topsoil and, thus, increases soil porosity and aeration. The least growth height was observed in decomposed sawdust which might be attributed to the fact that nitrogen is immobilized due to a high C: N ratio. It retards the rate at which nitrogen is broken down to ammonium and supports the findings of [26] who stated that if decomposed sawdust decreased crop growth, the decrease could be due to a wide carbon/nitrogen ratio in the soil. According to Newton and Daniloff [27], if low nitrogen materials are added to soil, it may result in a decrease in growth and yield. However, this problem can be solved by applying 75 lbs. of nitrate of soda per ton of sawdust used (12 lbs. N/ton).

The highest mean of leaf count obtained in T4 (cow dung) agrees with the findings of [28], who stated that cow dung helps to enhance soil quality by increasing moisture content, microbial activity, and proper aeration, thus making plant nutrients more available for growth and development of leaves. Subhan [29] asserted that the use of organic matter improved the average leaf number in plants. This can be attributed to the ability of top soils to contain a huge biodiversity of life that helps to decompose organic matter in a form that can easily be used by the plant. The lowest mean leaf count in T3 (decomposed sawdust) is in tandem with the insufficient macronutrients in the decomposed sawdust especially nitrogen. Turf [30] reported through greenhouse testing that the negative effects of sawdust in soils with low nitrogen levels could be overcome by adding sufficient quantities of nitrogen, which would provide an equivalent of approximately 2% nitrogen by weight (40 lbs. N/ton). Lunt [31] observed that crop yields were significantly reduced in the year following the addition of decomposed sawdust to soil unless 20 lbs. of nitrogen per ton (or a pound of nitrogen for every 100 lbs. of dry organic matter) was applied.

The maximum leaf area found in T4 (cow dung) could be tied to the fact that cow dung is an important organic manure. Organic manure is essential for maintaining healthy soil and promoting sustainable crop productivity. It improves the physical, chemical, and biological properties of soil, leading to increased crop yields. Recycling organic matter in soil has many benefits, including the overall improvement of soil health and the supply of essential nutrients like nitrogen, phosphorus, potassium, and sulfur. Studies have reported that different levels of organic manure significantly increased plant height [23], [22]. The minimum leaf area in T2 contrasts the result of the studies by [32] and [33] which stated that sheep dung enhanced leaf counts as a result of high water absorption and more uptakes of elements with close association to biosynthesis (N, Mg and Fe), thus increasing the tree growth.

The study shows that T3 (decomposed sawdust) had the highest mean collar diameter, indicating the effectiveness of using wood residues like sawdust and bark for growing ornamental plants in containers. Sawdust has a high water-holding capacity, provides good aeration, and promotes warmth, all of which facilitate germination. Wood residues also contain all the minor elements necessary for plant growth [34]. This finding contradicts a study conducted by [35]. The application of sheep dung improved tree growth and leaf mineral content by creating favorable soil conditions for root growth and nutrient absorption, supplying nutrients, and facilitating the absorption of fixed nutrients by tree roots [36], [35] and [37].

The root length of seedlings raised under T1, T2, T3, and T4 were found to be the same and did not vary significantly, although seedlings raised under T3 had the highest root length mean. According to [38], decomposed sawdust improves growth and crop production. It improves the structure and aeration of heavy soils [39]. It functions on the increase of water infiltration and reduced surface runoff, improved aggregation of surface soil observed by [39]. It enhances moisture conservation through weed control and reduces surface evaporation [40].

The mean trends of the five growth variables across four different treatments (T1 to T4) for the early growth of *A. muricata*, a tropical fruit tree, are shown in Figure 6. For plant height, there is a clear decreasing trend from the control treatment T1 to T2 and T3 before an increase again for T4. This indicates sheep dung and sawdust potting mixtures suppressed height growth compared to the control and cow dung.

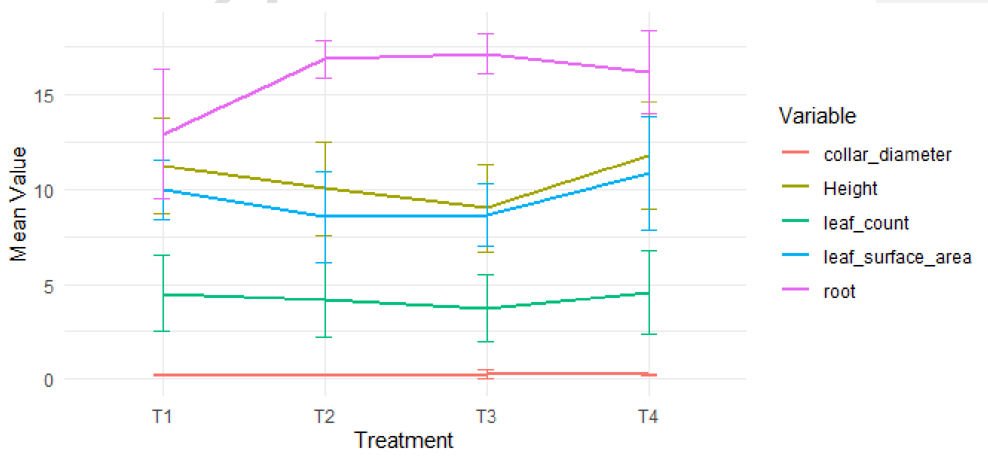

Fig. 6. Trend in mean of growth parameters across treatments

A similar trend is seen for leaf count, with T2 and T3 showing lower means than T1 and T4. This suggests the sheep dung and sawdust treatments reduced leaf production. The pattern is identical for leaf surface area, decreasing from T1 to T2 and T3, then higher for T4. Again, T2 and T3 performed poorly compared to control and cow dung treatments.

Collar diameter does not show a clear increasing or decreasing trend across treatments. This parameter seems unaffected by the potting mixture type. Root length increases progressively from T1 to T4, with the sawdust treatment T3 giving the highest mean. This indicates sheep dung and especially sawdust may promote greater root growth compared to control and cow dung mixtures.

Generally, the trends demonstrate that sheep dung and sawdust potting media limit growth of aerial plant parts like height, leaf count, and area compared to control and cow dung. However, sheep dung and sawdust appear more supportive of extensive root development. Cow dung provides the most balanced early growth across both shoots and roots. These trends provide insight into the comparative effects of different potting mixtures on directing plant growth.

5. CONCLUSION

In this study, the growth and development of soursop were influenced significantly by the application of organic manure to soursop seedlings. Decomposed sawdust and cow dung manure were valuable sources of organic fertilizer for the growth of *Annona muricata* as they greatly improved the growth performance of *A. muricata* over other treatments. However, cow dung manure proved superior to decomposed sawdust because it produced better growth attributes such as plant height, leaf count, and leaf area than its counterpart, which was superior in collar diameter and root length. Thus, their use must be encouraged especially among the farmers in Nigeria for the cultivation of soursop.

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6. RECOMMENDATIONS

Further work should be done on the influence of organic manure on the quantitative and qualitative photosynthetic pigments of *Annona muricata* to ascertain their contribution to the trees' growth. and development. *Annona muricata* in multi-strata systems, vegetative propagation and tree manipulation (pruning, topping, etc.), and increased utilization of the tree in agroforestry systems for sustainable agriculture should also be considered.

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