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A STUDY OF THE EFFECT OF MICROGRAVITY ON GERMINATION OF MILLET SEED.

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ABSTRACT

This paper focused at studying the world beyond the outer space. Thus, plants grow through light stimulus and effect of gravity had been neutralized by clinostat in order to create microgravity environment. Microgravity environment will make plant respond to light stimulus, through this plant will grow faster. These are the reasons for faster germination in microgravity environment.It is on this note that this study therefore aims to empirically examine, (i.e. main aim) using the clinostat, how plant seeds, small organisms or small samples from material sciences react to simulated microgravity conditions with a view to promote space education and research in microgravity.

1.0 Introduction

Environment on earth is quite different from Environment in outer space. Outer space begins at an altitude of 100km above the sea level. There are some important factors that can be used to differentiate environment on earth from environment in Outer Space. Among these factors are: Pressure (Air),

Temperature (varies depending on location or layer of the atmosphere) and Gravity.

Gravity is always present on earth; however, the influence of gravity can be modified or, in some cases, compensated for (Uchegbu, 2003). The phenomenon of **'Zero-Gravity'** does not imply complete absence of gravity, but an object under zero-gravity is subjected to other forces in different directions, thereby cancelling out the effect of gravity. Thus, Zero-gravity is an alternative term for weightlessness. The Zero-Gravity Instrument Project is aimed at opportunities to perform experiments under simulated microgravity conditions. This will inspire scholars to become interested in what life is like in space by demonstrating the influence of a unique environmental stimulus—gravity—which is used by organisms for their spatial orientation and growth direction (Perbal, 2001).

Although experiments in real microgravity in space are rare and expensive, similar experiments can be conducted on the ground (Herranz, 2013). This study will provide straightforward approach to perform experiments on plant growth using the clinostat, an experimental device which can create simulated microgravity conditions in a laboratory.

Gravity is the stimulus that a plant uses to grow its root in the direction of the gravity vector (down), anchoring the plant in the ground, and to grow the shoot in the opposite direction of the gravity vector (up), out of the soil in the direction of the sun. To understand "up" and "down" is mandatory for the survival of plants on earth (Blancaflor& Masson, 2013). It is also indispensable for all life on Earth because photosynthesis is needed for food and oxygen production. By growing plants, one can easily observe the impact of gravity on orientation and growth. Considerable progress has been achieved with regard to basic knowledge of gravity-sensing in plants and their final responses in the form of gravitropism (Sack, 1997). Thus, experiments performed in microgravity have greatly contributed to the understanding of how plants sense the direction of gravity and respond to it. However, the complete signal transduction process is not yet understood in detail.

This study focuses on plant research with respect to gravity (plants in space). Plants were chosen as test systems since they are

easily available and their experiment demands are quite easy to meet. Plants are very important for spaceflight since they provide us with information on fundamental biological processes. Understanding the molecular and cellular basis of mechanisms in plants for responding to gravity is important with respect not only to plant breeding and agriculture on earth but also to growing plants in space (space farming) and ensuring a supply of oxygen and food during long-term space missions (Ferl, 2002).

In the light of the aforementioned, this study will project helped those involved to answer questions on what some people thought will never happen years before this time. Questions such as: "How can microgravity be simulated on earth? Can the problem of feeding be solved for those scientists living in outer space? Can plant or crop be cultivated in outer space?" These questions were earlier thought unanswerable. However, this research has proved beyond every reasonable doubt that these questions are indeed answerable by simulating microgravity or weightlessness.

The Clinostat is a device used to simulate microgravity on earth. This instrument can be used to observe the growth of seedlings in a microgravity environment (simulated in the laboratory by the clinostat) which is similar to gravitational environment of outer space. A single-axis clinostat only produces the effect of weightlessness along the axis of rotation (Brown *et al*, 1976). The clinostat can cancel out the effect of gravity in all directions (UN-OOSA, 2013).

Plants will respond to light, temperature, gravity, pressure and water (Leather *et al.*, 1972). These are phenomena regulating the development of root and the possibility to orient their growth. The effect of the prolonged rotation of the clinostat on the germination and root cap anatomy of seedlings of Millet (Pennisetum Glaucam) were examined in this paper. Millet was selected for this study because of the short germination period and the size of the seed.
The broad aim of this present study is to examine the effects of microgravity on germination of millet seed with a view to promote space education and research in microgravity.

The rationale/specific objectives of the research are to:

a. Examine physical phenomena and the reaction of indigenous plant seeds, small organisms or small samples

from material sciences, in simulated microgravity conditions.

b. Appraisethe environment of outer space, with special emphasis on the concept of microgravity. It will also explore the basic principle of operation of the Clinostat, as a Zero-Gravity Instrument to promote space education and research in microgravity.

c. Upgrade and augment the space science curriculum for schools in Nigeria.

2.0 The Expected Contribution of ZGIP Research to Knowledge

In addition to advancing the frontiers of knowledge in Space education, this study will contribute to knowledge in understanding gravitational biology such as the influence of gravity on the spatial orientation and growth direction of plants. Finally, the findings of this study will contribute to the existing literature and analytical techniques on the subject matter by contributing to a deeper understanding of the plant experiment in microgravity.

3.0 Conceptual Issues & Operational Definitions of terms in Zero Gravity Instrument Project (ZGIP)

Basic conceptual issues and operational terms relevant to this empirical discussion include; Gravity, Mass, Weight, Microgravity, Gravitropism, Hypergravity.

- **Gravity:** It is a natural phenomenon by which all physical bodies attract each other. Gravity is the force that gives weight to physical objects and causes physical objects to fall toward the ground when dropped from a height.
- **Mass**: It is amount of matter in an object or how heavy an object is. Unit is Kg/ Pounds
- **Weight**: It is a force experienced by an object due to gravity. Unit is Newton.
- **Microgravity:** Microgravity is a unique environment in outer space where the effects of gravitational force are minimized. Very small compared to gravity on the surface of the earth. Described generally as acceleration of less than 1g. Microgravity conditions can be found on sounding rockets, spacecraft, a satellite orbiting the earth and the International Space Station (ISS).
- **Gravitropism:** Gravity is the stimulus that a plant uses to grow its root in the

direction of the gravity vector (down), anchoring the plant in the ground, and to grow the shoot in the opposite direction of the gravity vector (up), out of the soil in the direction of the sun/ light.The primary root of a plant grows in the direction of the gravity vector, while the shoot grows in the opposite direction. The growth and orientation response of sessile organisms to gravity is called ***Gravitropism.***

- **Hypergravity:** An acceleration force larger than 1 g is called hypergravity. It can be generated in a laboratory by means of a centrifuge, which can simulate the acceleration and deceleration forces that occur during the launch and landing of space vehicles. In addition, hypergravity experiments help to detect and understand gravity-related phenomena, thereby contributing to studies in microgravity.
- **Clinostat:** The Clinostat is an experimental device which can create simulated microgravity conditions in a laboratory on earth. This instrument can be used to observe the growth of seedlings in a microgravity environment (simulated in the laboratory by the clinostat) which is similar to gravitational environment of outer space. The Clinostat enables the constant rotation of an object, such as a plant, biological systems, ranging from bacteria and cells to small organisms, around a perpendicular to the force of gravity thereby cancelling the effect of gravity by equalizing the gravity vector around the horizontal axis. (Brown *et al* 1976). For the Zero-Gravity Instrument Project, a one-axisclinostat will be used.

4.0 Materials and Methods

The materials used during the experiment included: the clinostat, petri dishes, parafilm tape, double-sided sticky tape, glass beaker, weighing balance, hygrometer, an improvised wet chamber, agar-agar substrate, a heating source and a magnetic stirrer.

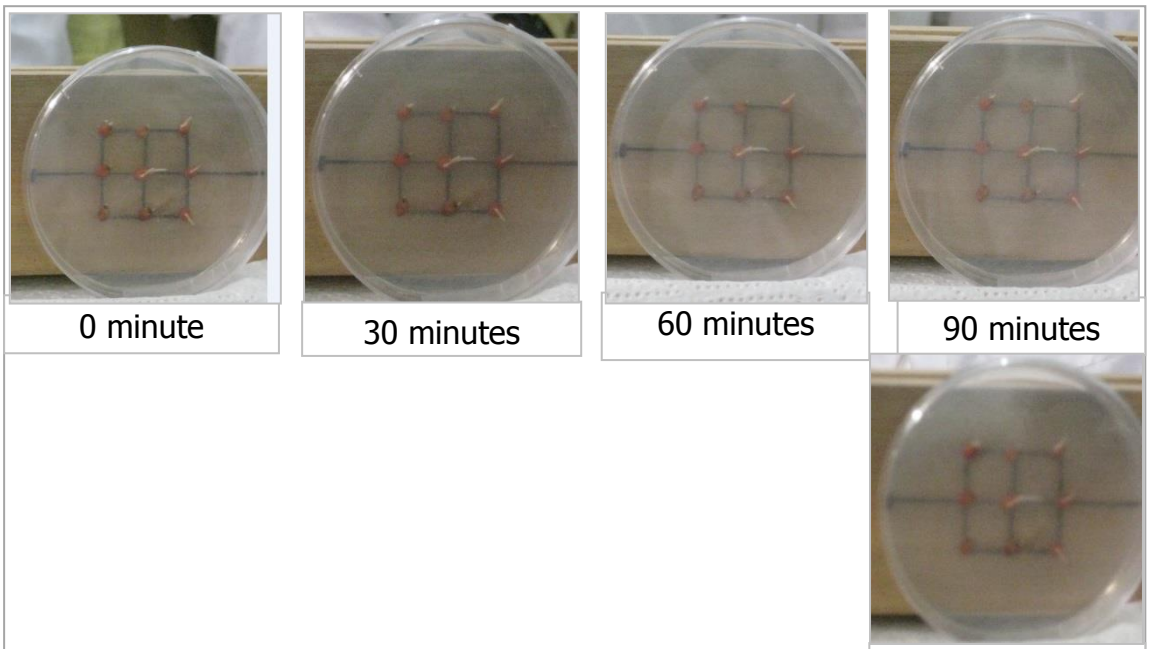
Each petri dish was prepared by drawing a reference line which indicated the gravity-vector. The mass of the petri dish was measured, and then it was used to measure 1.5g of Agar-Agar substrate. The substrate was poured into 100ml of water and the solution was heated on the heating source. A magnetic stirrer was dropped into the solution to prevent the formation of lumps, after which the solution was equally divided into four petri dishes and allowed to cool for

some time. For each petri dish, nine millet seeds were planted into the substrate with their micropyle facing one direction. After the vapour had been tapped out, the petri dishes were covered and partially sealed with parafilm tape. The petri dishes were then arranged in the petri dish holder and placed in the wet chamber with a hygrometer for a period of 30 – 40 hours. When the petri dishes were removed from the chamber, some of the seeds had germinated. One of the petri dishes was placed on the laboratory bench with the vector line pointing downwards (1g sample). Another was placed with the vector line oriented at 900. The clinorotated sample was mounted on the clinostat with the double-sided tape and the clinostat was set to rotate at a speed of 10 r. p. m. A digital camera, located on a stationary stand was used obtain photographs of the seedlings at intervals of 30 minutes.

The Image J software was used for data analysis (Ferreira & Rasband, 2012; ARCSSTE-E, 2014). To measure the growth rate, the picture showing the millet seedlings was opened in image J, and after setting the scale, the segmented line from the tool bar of Image J was selected to measure the increase in length of the root of the germinated millet seedlings. This was done for all the seedlings in the clinorotated petri-dish sample and also for the 1g sample. The angle tool was used to measure the root curvature of the millet seedlings in the clinorotated and 900 - turned sample.

5.0 Results

The photographs obtained for the 900-turned sample, the 1g sample and the clinorotatedsample at 0, 30, 60, 90, 120 minutes are shown in figures 1 to 3 respectively.



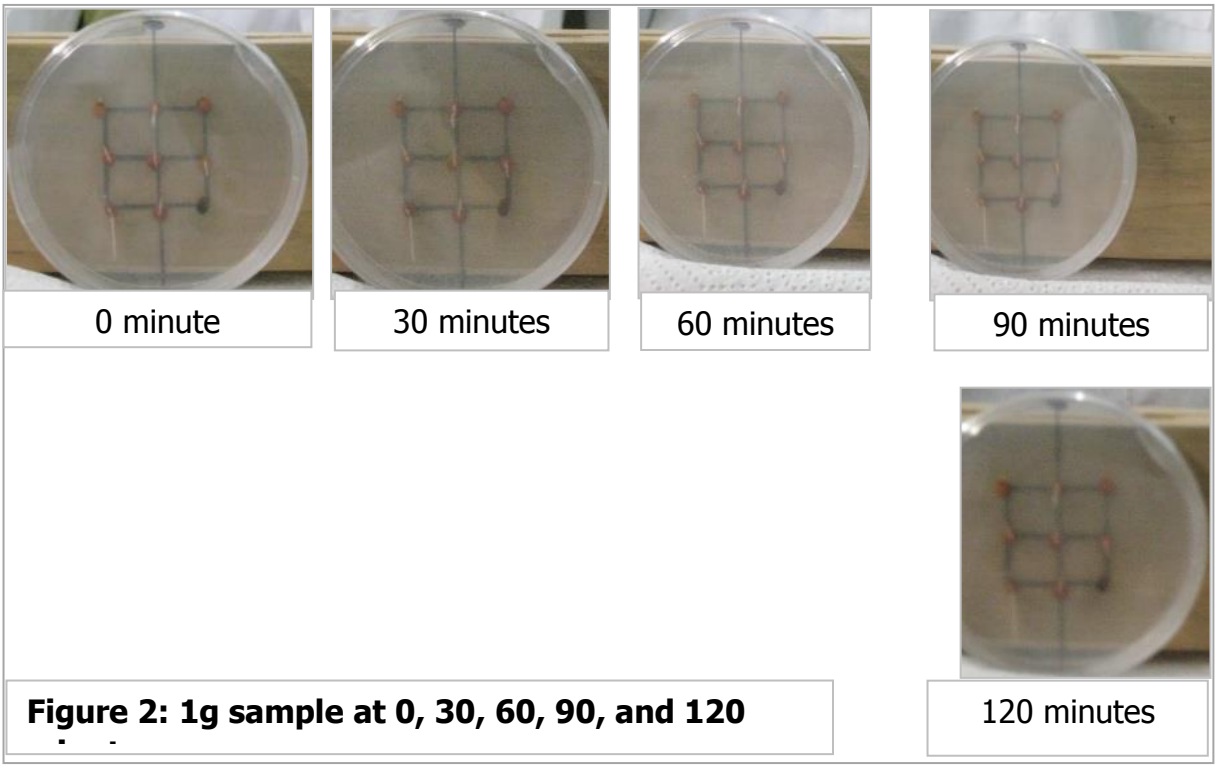


Figure 2: 1g sample at 0, 30, 60, 90, and 120

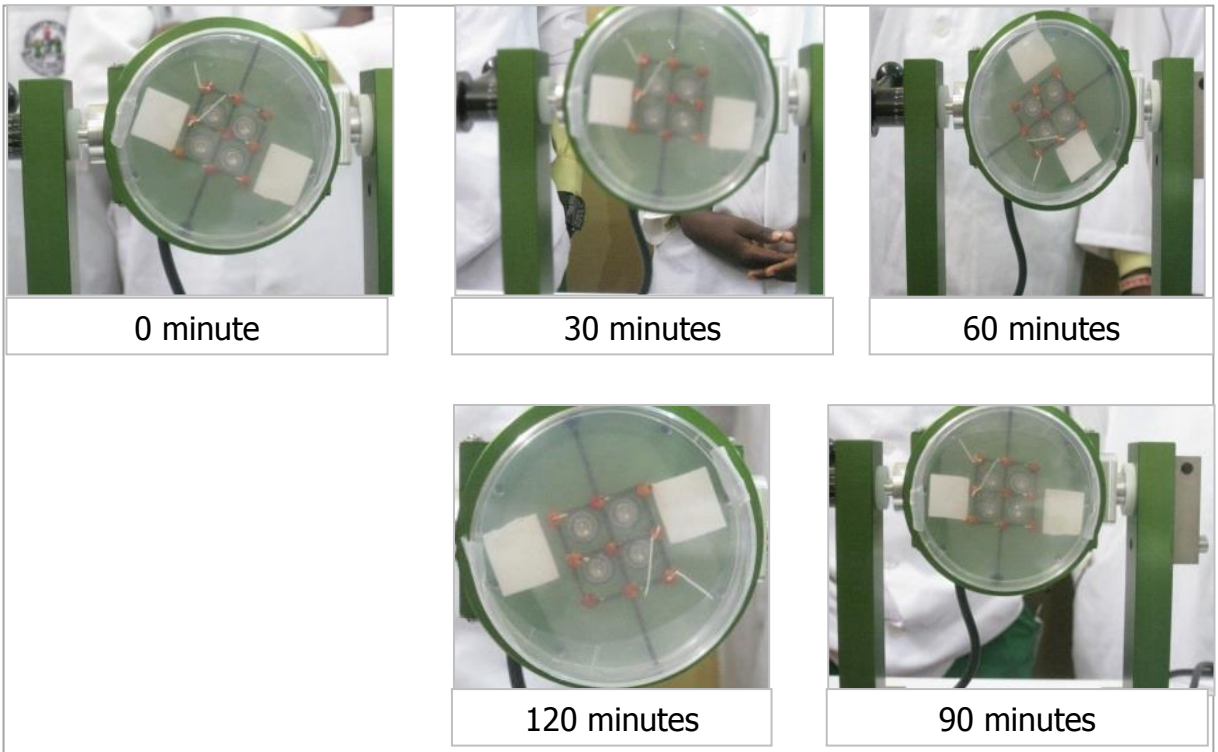


Figure 3: Clinorotated sample at 0, 30, 60, 90, and 120 minutes

Table 1: Root Growth Observation

Sample	Time (in minutes)	Root Curvature (in degrees)	Length of the root (in cm)	Increase in Length of root (in cm)	Growth Rate (GR) (in cm/min)
90^0-turned	0	126.850			
	30	124.850			
	60	123.796			
	90	121.973			
	120	118.327			
Clinorotated	0	126.850	1.550	0.000	
	30	132.480	1.953	0.403	0.0134
	60	135.310	2.198	0.245	0.0082
	90	138.640	2.224	0.026	0.0009
	120	150.470	2.275	0.051	0.0017
					Average GR=
					0.0061
1g	0		1.550	0.000	
	30		1.640	0.090	0.0030
	60		1.664	0.024	0.0008
	90		1.708	0.044	0.0015
	120		1.718	0.010	0.0003
					Average GR =
					0.0014

Source: Authors' Laboratory Experimental Work, 2016

Table 2: Increase in Length of Root (cm) with increase in Time

Time (min)	Clinorotated (cm)	1g (cm)
0	0	0
30	0.403	0.09
60	0.648	0.114
90	0.674	0.158
120	0.725	0.168

Source: Authors' Laboratory Experimental Work, 2016

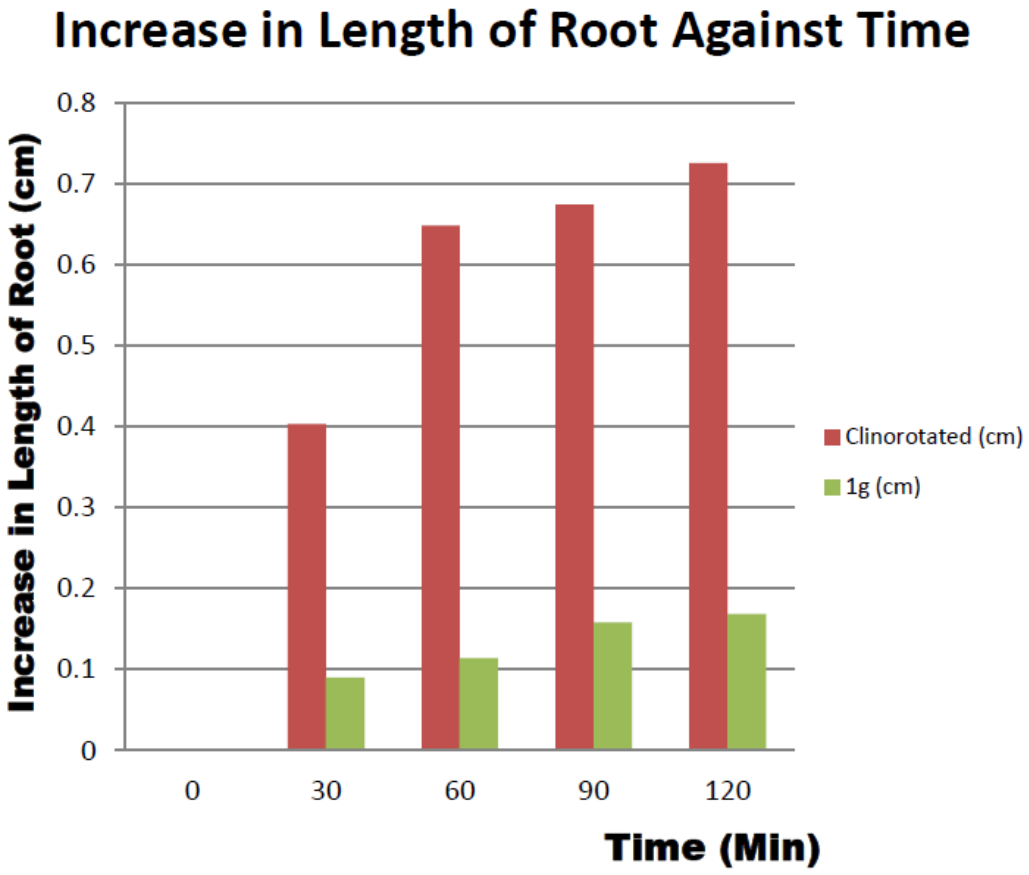


Fig 1.1: The Bar Chart Showing Increase in Length of Root with Time

Table 3: Increase in Root Curvature with Time

Time (min)	Root Curvature (in Degrees)	
	Clinorotated	90°-turned
0	126.850	126.850
30	132.480	124.850
60	135.310	123.796
90	138.640	121.973
120	150.470	118.327

Source: Authors' Laboratory Experimental Work, 2016

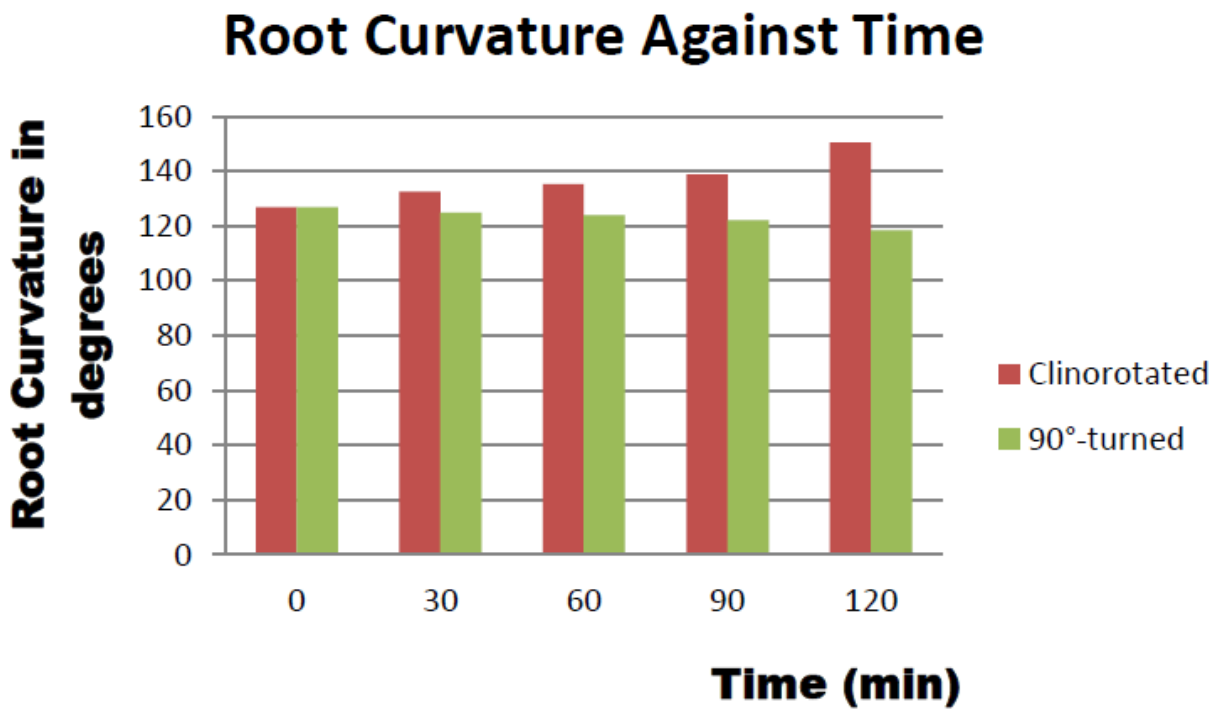


Fig 1.2: The Bar Cart showing Increase in Root Curvature with Time

6.0 Discussion and Conclusion

From table 1, the average growth rate of germination of millet seedlings (pennisetumglaucam) on the clinostat (clinorotated sample) was 0.0061 cm/minute while that of

1g was 0.0014 cm/minute. This difference was significant. Roots grown on the clinostat did not show any preferential growing direction while 1g roots were developed perfectly vertical, according to the gravity vector.

Angle of curvature formed in the clinorotated samples increased with change in time while angle of curvature formed in the 900-turned sample decreased with change in time.

In figure 1.1, the bar chart showed that clinorotated samples grew faster than 1g. Figure 1.2 showed that the angle of curvature of the clinorotated samples increased while that of the 900-turned sample decreased. This result is positive. It showed that the germination and development of good and viable seeds is possible in a few days both in 1g and in simulated microgravity environments.

Conclusively, the overall analysis suggested that the germination of millet seeds was slightly affected by the simulated microgravity environment created by the rotation of the clinostat. If enabling environment like that of agar-agar substrate can be created in the outer space, plant can germinate and grow to the point of maturity and fruitfulness in space and this will solve the problem of feeding the scientists and other inhabitants of the outer space.

During the course of the zero gravity instrument projects, some challenges were encountered. Three different seeds were planted (rice, soya beans and millet seeds) but one (millet seeds) out of the three seeds germinated. Using power point to prepare poster was a little bit difficult but with the help of the poster manual we overcame the problem.

The organizers of this project, ARCSSTE-E, should try to increase the number of participants and go from school to school to publicize or enlighten people about the project already done and the forthcoming ones. They should also assist the participants in the area of transportation and mobility. The Nigerian government should provide

funds and an enabling environment so as to encourage a project of this kind.

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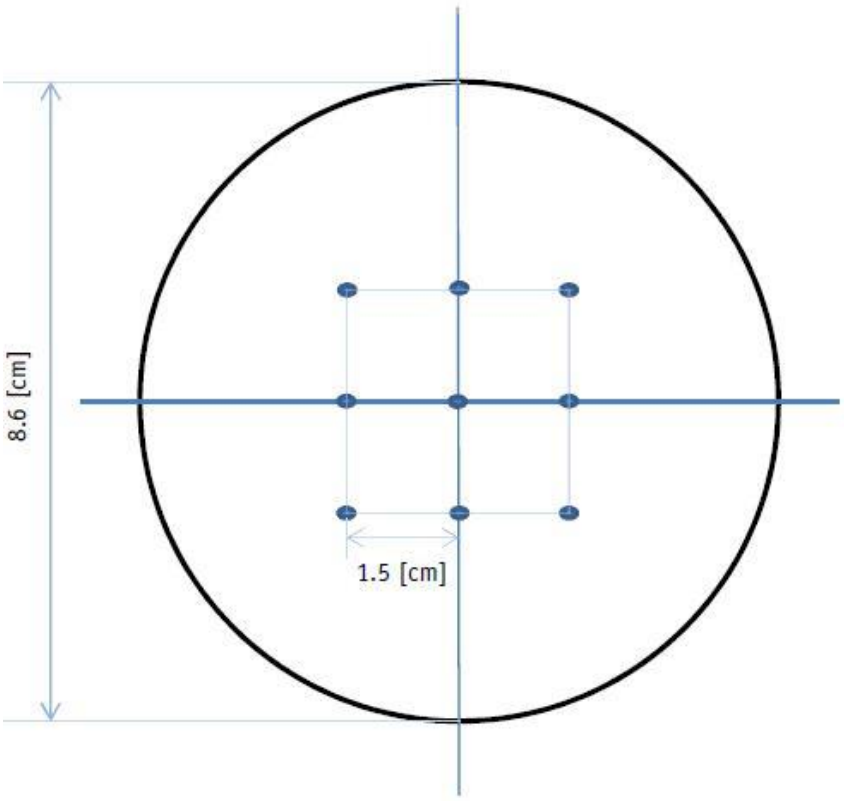
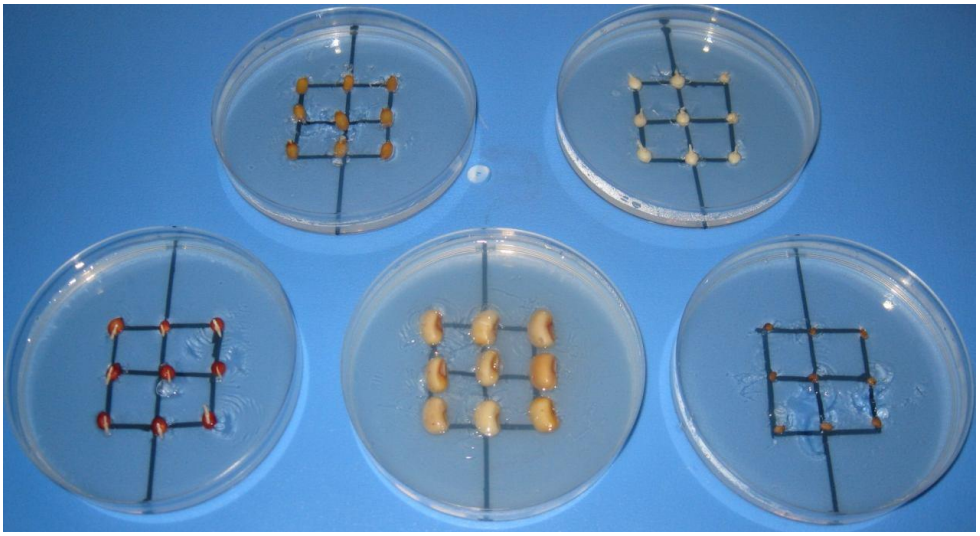
Appendix: Supported Photographs for the ZGIP Research

Below is the photograph showing laboratory equipments available for the Clinostat project:



Preparation of container to plant seed in - Template for the seed placement

Soak the seeds over night (to aid fast germination)



Seed Germination: Plant the seed in a very fertile environment Placing the planted seed in a very moist environment inside a wet chamber to create relative humidity of 60-100%

Clinorotated Sample **90^0 – Turned and 1g (control) samples**