



Impact of *Trichoderma harzianum* on the early growth of *Amaranthus spinosus* under normal gravity and microgravity conditions

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Abstract

This study investigated the use of *Trichoderma harzianum* as bio-fertilizer for the early growth of *Amaranthus spinosus* (spinach) on land and space environment using a simulated microgravity device. White and black spinach seeds were placed in different Petri dishes. The seeds were treated with *Trichoderma harzianum* for at least 24 hours to allow proper absorption by the seeds. The treated seeds were subjected to different growth environments (normal gravity and clinorotated). The treated seeds of spinach were sown inside Petri dishes each containing 10 ml of 1.5% DuchefaBiochemie plant Agar-Agar. The experimental design for each spinach variety was completely randomized design having two treatments (i.e. normal gravity and clinorotated) with three replicates each. This gave rise to a total of six Petri dishes per variety. The clinostat was rotated at a speed of 20 rpm for 72 hours (3 days) and observed. The result shows that the two varieties of spinach responded better in terms of the percentage germination, radicle length, and plumule length under the normal gravity than microgravity environment. However, the seeds of the microgravity germinated earlier than those of the normal gravity. The treatment with *Trichoderma* did not produce any significant effect on the germination and growth of the spinach varieties under both environments. The influence of the biofertilizer could have been more positively seen at the later stage of the growth of the plant.

Keywords: Biofertilizer, Microgravity, *Trichoderma harzianum*, *Amaranthus spinosus*

Introduction and literature review

A bio-fertilizer is a substance containing living micro-organisms which, when applied to seeds, plants surfaces, or soil, colonize the rhizosphere or the interior of the plants and promotes growth by increasing the supply or availability of primary nutrients to the host plant e.g. (*Trichoderma*) (Vessey *et al.*, 2003). *Trichoderma* is a filamentous fungus, opportunistic, and virulent symbionts that are used as biopesticide, biofertilizer or fertility promoter worldwide (Vinale *et al.*, 2008). Application of *Trichoderma* may result in the promotion of plant growth, yield and increase nutrient availability (Bal *et al.*, 2008). In addition, several species of *Trichoderma* are well-known producers of different kinds of secondary metabolites (Reino *et al.*, 2008) that are important for plant growth regulation. Some *Trichoderma* strains may also be able to colonize root surfaces and cause substantial changes in plant metabolism (Harman *et al.*, 2004). Furthermore, *Trichoderma* may produce organic acids that decrease soil pH and permit the solubilization of phosphates, micronutrients and mineral cations like iron, manganese, and magnesium that are useful for plant metabolism (Benitez *et al.*, 2004).

Amaranthus spinosus which is also known as spinach is a superfood. It is loaded with tons of nutrients in a low-calorie package. Dark, leafy greens like spinach are important for skin, hair, and bone health. They also provide protein, iron, vitamins, and minerals (Megan *et al.*, 2018). The possible health benefits of consuming spinach include improving blood glucose control in people with diabetes, lowering the risk of cancer, and improving bone health, as well as supplying minerals and vitamins that can provide a range of different health benefits for overall well-being and bodily functions. Spinach has been used by various cultures throughout history, notably in Mediterranean, Middle-Eastern, and South-East-Asian cuisines. It can be incorporated quite easily into any diet, as it is cheap and easy to prepare (Megan *et al.*, 2018).

Microgravity is a typical space environment, which causes an abiotic stress on living organisms (Akomolafe *et al.*, 2016). The clinostat is one of the various platforms used to simulate microgravity conditions. Several researches have been carried out to investigate the reactions of plants to the condition of microgravity and various altered gravity conditions (Cowls *et al.*, 1984; Fujie *et al.*, 1993; Hu *et al.*, 2007; Mirsandi *et al.*, 2015; Jing *et al.*, 2015; Zheng *et al.*, 2015). The study of the modifications induced by altered gravity in functions of plant cells has been described as a valuable tool for the objective of the survival of terrestrial organisms in conditions

different from those of the Earth. These changes in the gravitational environmental conditions have brought about extensive stress for plant cells (Matía *et al.*, 2009). Plants grown in microgravity or simulated microgravity exhibit spontaneous automorphogenesis (i.e. changes in growth direction) (Hoson, 2014) due to changes in plant hormones such as auxin, gibberellins and ethylene which serve as signal transducers responding to the changes in the gravity vector. Different plants have different responses to the changes in gravity vector. Under microgravity conditions in space, the growth rates of many plant organs were reported to increase (Halstead and Dutcher 1987), but they were not changed or even decreased in some other plants (Kiss *et al.* 1998, Levine *et al.* 2001; Omojola *et al.*, 2018).

The effect of chemical fertilizer is not evident on soil as it has a great buffering capacity. However, overtime the natural balance of the soil element and pH is disturbed and the accumulation of toxic chemicals leads to the toxicity of the food chain. This is caused by fertilizers containing high levels of sodium and potassium. Acidic fertilization results in liming and is the reason for an imbalance in the symbiotic nitrogen fixing. Unprecedented use of fertilizer may also result in nutrient imbalance and disrupts the receiving capacity of agriculture field. Hence, the use of biofertilizer such as *Trichoderma* is encouraged in order to salvage the problems associated with chemical fertilizers (Vessey *et al.*, 2003). Also, the long-term mission of the United Nations Office for Outer Space Affairs (UNOOSA) which is aimed at bringing man to space has encouraged researches on the growth of plants, particularly food crops in the space environment. This then makes it necessary for the investigations of the performance of *Trichoderma* as a biofertilizer in the space environment using a simulated microgravity device.

Due to the medicinal value of the plant spinach and its positive impact on humans as food, spinach is to be considered valuable and most importantly cultivated with the right treatments. In this case, chemical fertilizer is considered harmful to this species. This also calls for the need for biofertilizer (*Trichoderma harzianum*) to be used to ensure the growth and cultivation of this plant (spinach) in a space environment.

Therefore, this study was designed to investigate the use of *Trichoderma harzianum* as bio-fertilizer for the early growth of spinach on land and space environment using a simulated microgravity device. The following are the objectives of this study; to investigate the impact of the biofertilizer on the germination of *Amarathus spinonus* and; to compare the early growth of *Amarathus spinonus* both under gravity and microgravity conditions.

Experimental Methods

Description of the study Area

This experiment was conducted at the Laboratory of the Department of Plant Science and Biotechnology, Federal University of Lafia, Nasarawa State.

Source of materials

Trichoderma harzianum was obtained from Bio-lab Contec Global Agro Ltd, Number 7, Victoria falls street, Maitama Abuja. Spinach seeds were gotten from Lafia modern market, Lafia Nasarawa state. The clinostat device was accessed from the microgravity research group.

Seed treatments

Spinach seeds were placed inside different Petri dishes. Two varieties of *Amaranthus* seeds (black and white) were treated with *Trichoderma harzianum* for at least 24 hours to allow proper absorption by the seeds. The treated seeds were subjected to different growth environments (normal gravity and microgravity conditions).

Germination of seeds.

The treated seeds of spinach were sown inside Petri dishes each containing 10 ml of 1.5% Duchefa Bohemia plant Agar-Agar (Plates 1 A-D). The experimental design for each spinach variety was completely randomized design having two treatments (i.e. normal gravity and microgravity conditions) with three replicates each. This gave rise to a total of six Petri dishes per variety. The seed supporting substrate agar-agar was prepared according to standard method (UNOOSA, 2013). 200ml of 25% Duchefa Bohemia plant agar – agar in tap water (2.5g agar – agar in 100ml of tap water) was prepared. The agar-agar was boiled and stirred until no visible particles were left up to two minutes (i.e. a clear solution).

The solution was allowed to cool down to about 60%. The six Petri dishes were filled with 10ml to 25mL of the agar-agar solution. The right depth of the agar–agar solution is such that the seeds can be embedded only halfway in the agar-agar, thus guaranteeing a supply of oxygen for the seeds. The agar-agar was allowed to cool down and solidify. In each Petri dish, nine seeds of the spinach were planted on the agar – agar by using the tweezers in the same direction in order to identify the micropyle. After seeding the seeds on the agar-agar surface, three of the

Petri dishes were placed vertically using a Petri dish holder as a control, and the remaining three Petri dishes was mounted on the clinostat. The clinostat was rotated at a standard speed of 20 rpm for 72 hours (3 days) inside a growth chamber with standard room temperature and humidity (Akomolafe *et al.*, 2016). The time of germination was recorded, and the germination percentage was calculated after germination. Also, the length of the plumule and radicle after 72 hours of germination was measured.

Data Analysis

All quantitative data were subjected to two-way analysis of variance (ANOVA) with *Trichoderma* treatments as one factor and microgravity conditions as another factor. Significant differences between the means were determined using the least significance difference (LSD) posthoc test at $p \leq 0.05$. All statistical tests were performed using the IBM SPSS version 26 software.

Results and Discussions

The seeds of the different varieties of the plant started germinating at 22 hours after planting for normal gravity while that of the microgravity started 8 hours after planting (Plates 2A and 2B). This indicates that microgravity stimulated the germination of the plant than normal gravity. This agrees with Omojola *et al.* (2018) who also reported an earlier germination in *Corchorus tridens* subjected to microgravity than the normal gravity one. There was visible growth in the microgravity treated samples and different responses by the different varieties (Plates 3A and 3B). Table 1 shows the radicle length of the *Trichoderma*-treated and untreated seeds of black spinach under gravity and microgravity conditions. The results show that normal gravity had a higher radicle length (10.66 cm), which is significantly different from that in microgravity (4.22 cm). Also, the differences in the *Trichoderma* treatments are not significant.

Table 1: The effect of *Trichoderma* treatments on radicle length of black spinach under gravity and microgravity conditions

Treatment	Normal gravity	Microgravity	Mean	
Treated	11.89	5.80	8.84 ^a	LSD _T = 5.32
Untreated	9.42	2.64	6.03 ^a	
Mean	10.66 ^b	4.22 ^a		
	LSD _g = 3.42			

Means followed by the same superscripts within the same row or column are not significantly different ($P>0.05$) or their differences are less than the LSD.

The difference in the plumule length of the *Trichoderma*-treated and untreated seeds of black spinach was observed to be non-significant (Table 2). However, the normal gravity showed a higher plumule length (5.17 cm) which are not significantly different from the microgravity one (2.92cm). The difference in the percentage germination of the *Trichoderma*-treated and untreated seeds of black spinach was observed to be non-significant (Table 3). However, the normal gravity showed a higher percentage of germination (64.75cm), which is significantly different from the microgravity one (27.75 cm).

Table 2: The effect of *Trichoderma* treatments on plumule length of black spinach under gravity and microgravity conditions

Treatment	Normal gravity	Microgravity	Mean	
Treated	5.29	4.08	4.69 ^a	LSD _T = 3.05
Untreated	5.04	1.75	3.39 ^a	
Mean	5.17 ^a	2.92 ^a		
	LSD _g = 2.76			

Means followed by the same superscripts within the same row or column are not significantly different ($P>0.05$) or their differences are less than the LSD.

Table 3: The effect of *Trichoderma* treatments on percentage germination of black spinach under gravity and microgravity conditions

Treatment	Normal gravity	Microgravity	Mean	
Treated	66.60	37.00	51.80 ^a	LSD _T = 31.49
Untreated	62.90	18.50	40.70 ^a	
Mean	64.75 ^b	27.75 ^a		
	LSD _g = 19.33			

Means followed by same superscripts within same row or column are not significantly different ($P>0.05$) or their differences are less than the LSD.

The difference in the radicle length of the *Trichoderma*-treated and untreated seeds of white spinach was observed to be non-significant (Table 4). However, the normal gravity showed a higher radicle length (7.19 cm) which is significantly different from the microgravity one (2.50 cm). The difference in the plumule length of the *Trichoderma*-treated and untreated seeds of white spinach was observed to be non-significant (Table 5). However, the normal gravity showed a higher plumule length (6.43 cm), which is significantly different from the microgravity one (2.04 cm).

Table 4: The effect of *Trichoderma* treatments on radicle length of white spinach under gravity and microgravity conditions

Treatment	Normal gravity	Microgravity	Mean	
Treated	3.09	5.01	4.05 ^a	LSD _T = 6.49
Untreated	11.28	0.00	5.64 ^a	
Mean	7.19 ^d	2.50 ^e		
	LSD _g = 5.71			

Means followed by the same superscripts within the same row or column are not significantly different ($P>0.05$)

or their differences are less than the LSD.

Table 5: The effect of *Trichoderma* treatments on plumule length of white spinach under gravity and microgravity conditions

Treatment	Normal gravity	Microgravity	Mean	
Treated	6.75	4.07	5.41 ^a	LSD _T = 4.74
Untreated	6.10	0.00	3.05 ^a	
Mean	6.43 ^d	2.04 ^e		
	LSD _g = 3.96			

Means followed by the same superscripts within the same row or column are not significantly different ($P>0.05$)

or their differences are less than the LSD.

The difference in the percentage germination of the *Trichoderma*-treated and untreated seeds of white spinach was observed to be non-significant (Table 6). However, the normal gravity showed a higher percentage of germination (46.25cm), which is significantly different from the microgravity one (27.75 cm).

Table 6: The effect of *Trichoderma* treatments on percentage germination of white spinach under gravity and microgravity conditions.

Treatment	Normal gravity	Microgravity	Mean	
Treated	51.80	40.70	46.25 ^a	LSD _T = 27.22
Untreated	40.70	14.80	27.75 ^a	
Mean	46.25 ^a	27.75 ^a		
	LSD _g = 27.22			

Means followed by the same superscripts within the same row or column are not significantly different ($P>0.05$) or their differences are less than the LSD.

Results obtained from the research revealed that the black seeds variety of amaranth responded higher than the white in the microgravity condition while the white responded higher in the normal gravity. For most seeds in the microgravity samples, there were no visible growth which could be due to the change of environmental conditions. This agrees with Matia *et al.* (2009) who observed that these changes in the gravitational environmental conditions have brought about extensive stress for plant cells. There was higher growth rate observed in the normal gravity condition than that of the microgravity. This result agrees with Akomolafe *et al.* (2017) who reported that higher growth rate was observed in the normal gravity than the microgravity which could be that the growth hormones responsible for early growth were affected by microgravity.

Noticeable changes were observed in the seed treated with *Trichoderma harzianum* which agrees with Bal *et al.*, (2008) who reported that application of *Trichoderma* may result in the promotion of plant growth yield and increase nutrient availability. Generally, these results showed that the growth of *Amaranthus* on normal gravity was higher and that of microgravity was mild.

Conclusions

Conclusively, this study has proven that spinach can grow under microgravity condition (zero gravity) using the clinostat due to the fact that visible growth was observed in most of the samples and varieties. The treatment with *Trichoderma* did not produce any significant effect on the germination and early growth of the spinach varieties under both environments. The influence of the biofertilizer could have been more positively seen at the later stage of the growth of the plant.



Plate 1A: Normal gravity condition setup and arrangement

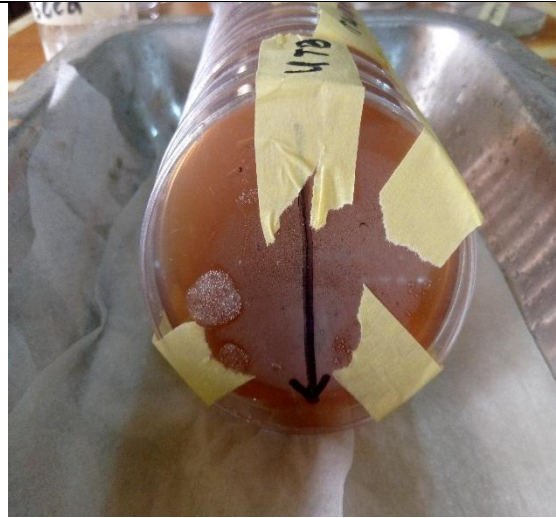


Plate 1B: Proper arrangement with arrows indicating direction of gravity

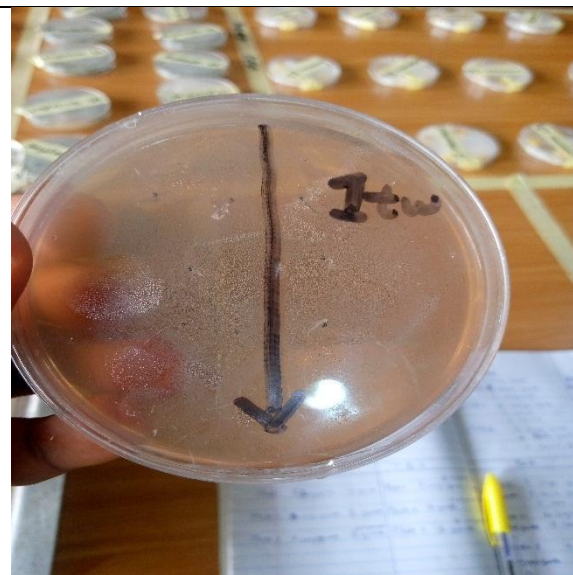


Plate 1C: Treated white seeds (TW) samples first shoot



Plate 1D: Treated black (TB) samples first shoot

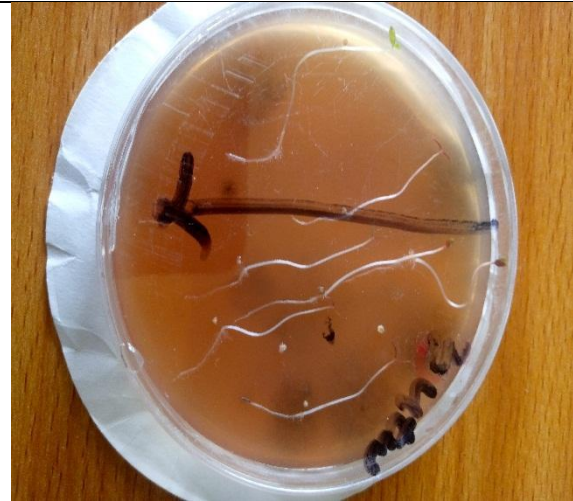


Plate 2A: Germination after 72hours of planting



Plate 2B: Germination after 72hours of planting



Plate 3A: Microgravity device setup with samples under zero gravity condition



Plate 3B: Samples after 72hours of planting

Supplementary Material

Not applicable.

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By checking the following box, the senior author acknowledges that they and the other authors have read, understood, and complied with the Instructions to Authors (ItA).

