Advances in Modern Agriculture (2023) Volume 4 Issue 2
doi: 10.54517/ama.v4i2.2367

Asia Pacific
Academy of Science Pte. Ltd.

ORIGINAL RESEARCH ARTICLE

Growth of horned melon (*Cucumis metuliferus*) under different levels of *Trichoderma*-based bio-fertilizer

Moses Mutetwa[1,*], Tuarira Mtaita[2], Wonder Ngezimana[3], Paul Chaibva[1]

[1] Department of Agronomy and Horticulture, Faculty of Agriculture, Environment and Natural Resources Management, Midlands State University, P.O Box 9055, Gweru, Zimbabwe

[2] Department of Agricultural Sciences, College of Health, Agriculture and Natural Sciences, Africa University, P Bag 1320, Mutare, Zimbabwe

[3] Department of Horticulture, Marondera University of Agricultural Sciences and Technology, P.O Box 35, Marondera, Zimbabwe

*** Corresponding author:** Moses Mutetwa, mosleymtetwa@gmail.com

ABSTRACT

Implementing practices such as organic agriculture, sustainable agriculture, or ecological agriculture can greatly reduce and eliminate the harmful effects of synthetic fertilizers on both human health and the environment. In efforts to promote a more environmentally friendly approach, this study was conducted at the Department of Horticulture and Agronomy, Midlands State University in Zimbabwe. The focus was on the use of *Trichoderma* bio-fertilizer at various levels to determine its impact on the growth of horned melon (*Cucumis metuliferus*) in a greenhouse setting. The experiment followed a Complete Randomized Design (CRD) and included four different *Trichoderma*-based biofertilizer treatments, as well as a control treatment [0 g/pot (control), 0.1 g/pot, 0.2 g/pot, 0.3 g/pot, and 0.4 g/pot], all replicated four times. The research findings indicate that the biofertilizer utilized had a significant impact ($p \leq 0.05$) on vine length, number of leaves, and branches for growth characteristics. However, the biofertilizer did not have a significant effect ($p \geq 0.05$) on stem girth, chlorophyll content, or branching pattern. This study reveals that the horned melon plants treated with the *Trichoderma*-based biofertilizer exhibited noticeable changes in their vegetative growth, flowering patterns, and fruiting features at different application levels. Further investigation is required to fully understand the potential benefits of using *Trichoderma*-based biofertilizer in horned melon cultivation.

Keywords: *Cucumis metuliferus*; *Trichoderma* spp.; biofertilizer; inoculation; growth morphology; sustainable agriculture

1. Introduction

The expansion of the population requires a corresponding increase in the production of food crops to satisfy the continuously rising need for sustenance. In developing nations, a viable solution to meet this demand is to enhance the productivity of food crops. Utilizing chemical techniques has proven effective in boosting food crop output by stimulating plant growth and managing plant diseases. Adequate and appropriate application of fertilizers, in terms of quantity and quality, plays a crucial role in the growth, yield, quality, and overall health of the soil. The application of excessive fertilizers has resulted in numerous ecological issues,

ARTICLE INFO
Received: 13 November 2023 | Accepted: 15 December 2023 | Available online: 22 December 2023

CITATION
Mutetwa M, Mtaita T, Ngezimana W, Chaibva P. Growth of horned melon (*Cucumis metuliferus*) under different levels of *Trichoderma*-based bio-fertilizer. *Advances in Modern Agriculture* 2023; 4(2): 2367. doi: 10.54517/ama.v4i2.2367

such as water eutrophication, degradation of the rhizosphere microecological environment, loss of biodiversity, global warming, and depletion of the stratospheric ozone layer. Furthermore, certain fertilizers containing heavy metals can have adverse effects on soil and plant health and may even enter the food chain through soil absorption[1–4].

In response to growing concerns around food quality, environmental safety, and soil preservation, there has been a notable rise in the adoption of sustainable agricultural methods[5]. This has led to a shift towards alternative practices, such as the use of microbial fertilizers, which offer a more sustainable and environmentally friendly approach to plant nutrition[6].

In contrast, inadequate utilization of inorganic fertilizers in sub-Saharan Africa (SSA) has been a major obstacle due to difficulties in accessibility, leading to nutrient depletion and subsequent land degradation[7]. However, the availability of bio-fertilizers could provide a potential answer to this issue, as they are recognized for their cost-effectiveness[8]. These types of fertilizers can be beneficial for all types of crops in various agro-ecologies[9]. As a result, the consistent use of bio-fertilizers supports the growth and development of microbial populations in the soil, ultimately aiding in the maintenance of soil fertility and promoting sustainable agriculture[10,11].

The use of bio-fertilizers is considered environmentally friendly as it reduces the reliance on chemical fertilizers in crop production worldwide. However, they cannot fully replace the essential role that chemical fertilizers play in achieving maximum crop yields. These biofertilizers have the potential to greatly improve crop output[12] by controlling plant diseases, increasing phosphorus availability, excreting ammonia, promoting plant hormone production, fixing nitrogen, and forming siderophores[13,14]. They are specifically defined as products containing naturally occurring microorganisms that are artificially cultivated to enhance soil fertility and crop productivity[15]. The term ‘biofertilizer’ refers to preparations containing live cells of efficient strains of microorganisms that fix nitrogen, solubilize phosphorous, or decompose cellulose and have the ability to enrich soil fertility either independently or in partnership with host plants. The term biofertilizer describes the utilization of all biologically derived nutrients to fuel plant growth[16].

Trichoderma, a diverse genus of multifunctional fungi found in various ecosystems, is a prime example of this concept[17]. These free-living fungi are known for their interactive nature in foliar, soil, and root environments[17], and they have become a valuable resource for natural growers due to the availability of a wide range of *Trichoderma*-based biopesticides used in disease management for plants[18,19]. *Trichoderma* has been found to offer numerous benefits, including enhancing plant growth, increasing root development, promoting plant maturity, improving seedling strength, facilitating seed germination in unfavorable soil conditions, enhancing nutrient absorption, producing enzymes and phytohormones that assist in phosphorus solubilization, providing resistance to abiotic stresses, inhibiting the growth of harmful root microflora, and increasing yield parameters[20–25].In spite of the potential, smallholder farmers in SSA are not fully utilizing bio-fertilizers[26]. The need to comprehend the obstacles and also emphasize the prospects is long overdue in order to aid policy-making decisions.

Researchers are captivated by the use of *Trichoderma*-based products as a microbial inoculant and are motivated to explore its untapped benefits[27]. As a result, this study aims to examine the impact of *Trichoderma* biofertilizer and the possibilities it presents for organic production of horned melon in Zimbabwe, post-adoption of bio-fertilizers. Based on this context, the present investigation formulates the hypothesis that the utilization of *Trichoderma*-based biofertilizer has an effect on the performance of horned melon.

2. Materials and methods

2.1. Description of research site

The Department of Horticulture and Agronomy at Midlands State University in Zimbabwe oversaw this greenhouse study. The university is situated in Natural Agro-Ecological Region III of Zimbabwe at latitudes 19°50′ S and 29°84′ E (**Figure 1**), just 10 km southeast of Gweru Central Business District. The soils in this region are sandy loams with kaolinite clay minerals, originating from the fersialitic group. The weather during the trial period is as shown in **Table 1**.

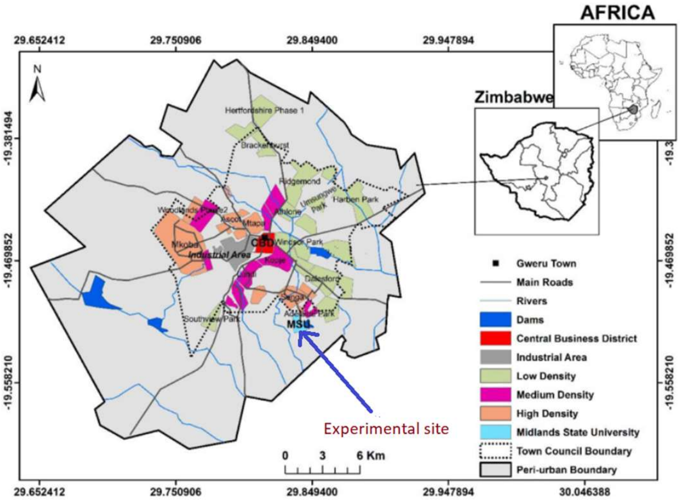

Figure 1. Experimental site.

Table 1. Weather at the experimental site during the trial period, 2021.

		J	F	M	A	M	J	J	A	S	O	N	D
Temp/°C	Min	16.3	16.1	15.3	12.9	10.2	8.1	7.4	9.6	12.5	14.8	16.1	16.5
	Max	25.3	25.2	24.5	23.1	21.8	19.8	19.4	23.0	26.9	28.4	27.4	25.9
	Avg	20.8	20.7	19.9	18.0	16.0	14.0	13.4	16.3	19.7	21.6	21.8	21.2
Rainfall, (mm)		173	126	88	33	8	4	4	1	5	27	100	183
Rainy days, (d)		12	10	8	4	1	1	1	0	1	4	9	13

2.2. Experimental procedure

In the greenhouse, the experiment followed a complete randomized design with four replications and included four different bio-fertilizer treatments as well as a control treatment. The treatments consisted of varying application rates of the bio-fertilizer: 0 g/pot (control), 0.1 g/pot, 0.2 g/pot, 0.3 g/pot, and 0.4 g/pot. 200 g of FYM was thoroughly mixed with 3 kg of soil in polyethylene pots, and water was added to reach field capacity. The selected biofertilizer treatments were also incorporated during transplanting. Throughout the experiment, no mineral fertilizers were utilized. At 4 weeks, healthy seedlings of horned melon were chosen and transplanted into the treated media, followed by watering.

The maintenance of the pots involved regular watering and the application of karate, copper oxychloride, and ridomil gold to prevent any infestation or disease.

After transplanting, data collection commenced after a period of two weeks. For this particular study, a commercial bio-fertilizer was chosen that contained at least 1.0×106 colony-forming units per gram of dry weight of *Trichoderma harzianum*. The seeds used were acquired from fruits purchased at the commercial

Fruit and Vegetable Market in Sakubva, Zimbabwe. The seedlings were then cultivated in the greenhouse using floating trays.

2.3. Data collection

The following growth parameters were recorded at different times during the growth of the plants, starting 3 weeks after transplanting.

Vine length and number of leaves on the main stem were recorded weekly from 21 days after sowing (DAS) up to 42 days after sowing. Measurements for vine length were taken from the lowest rudimentary leaf to the tip of the vine using a meter rule, and leaves were physically counted as well.

The branching pattern was recorded at the initiation of the blossoming of the plants.

Stem girth was recorded using a vernier caliper at the time of flower initiation.

Relative chlorophyll content was recorded using a hand-held chlorophyll meter, SPAD-502 Plus (Konika-Minolta), in a non-destructive manner. The meter determines the relative quantity of the photosynthetic pigment present by measuring the leaf absorbance in two wavelengths (red and near-infrared) regions.

2.4. Statistical analysis

GenStat 18th edition was used for statistical analysis, and to compare interaction effects, the least significant difference (LSD) test was conducted at a 5% level of probability. Fischer's protected $LSD_{0.05}$was utilized to separate any treatment means that showed significant differences.

3. Results and discussion

3.1. Effect of *Trichoderma*-based biofertilizer on vine length

There were significant ($p < 0.05$) differences between varying *Trichoderma* levels on horned melon vine length at 28, 35, and 42 days after sowing (**Figure 2**). At 42DAS, vine length registered significant ($p < 0.05$) differences with plants from 0.3 g/pot recording the longest (62.3 cm) but did not differ from 0.4 g/pot (53.2 cm) and 0.2 g/pot, while 0.1 g/pot recorded significantly ($p < 0.05$) the shortest. Vine length increased with an increase in the quantity of biofertilizer applied. The mean vine length recorded was 47.12 cm.

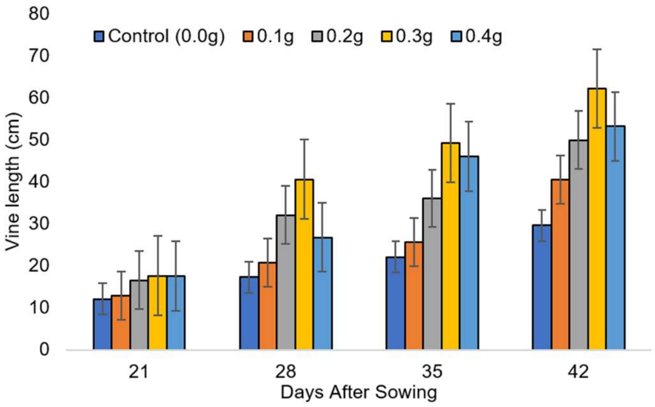

Figure 2. Influence of *Trichoderma*-based biofertilizer on vine length.

3.2. Effect of *Trichoderma*-based biofertilizer on number of leaves

As shown in **Figure 3**, the data regarding the number of leaves affected by the application of the *Trichoderma*-based biofertilizer was significant ($p < 0.05$) statistically. Treatments that received the biofertilizer had a greater number of leaves than the control treatment. The control (0.0 g/pot) treatment recorded consistently the lowest number of leaves from 21DAS through to 42DAS followed by 0.1 g/pot. At 42DAS 0.3 g/pot and 0.4 g/pot registered significantly ($p < 0.05$) the highest number of leaves, 17.8 and 17.5 leaves, respectively. The mean number of leaves recorded was 15.95.

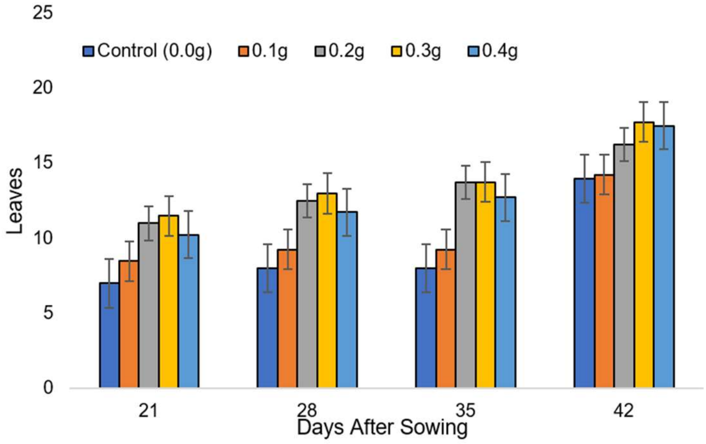

Figure 3. Influence of *Trichoderma*-based biofertilizer on number of leaves.

3.3. Effect of *Trichoderma*-based biofertilizer on number of branches

The summary of the analysis of variance shows that the means for the number of branches did not differ strongly among treatments (**Figure 4**). The treatments that received the biofertilizer did not differ ($p > 0.05$) from one another, except for the control treatment. However, the trend was that with an increasing amount of biofertilizer applied, the number of branches was increasing numerically. The mean number of branches recorded was 4.35.

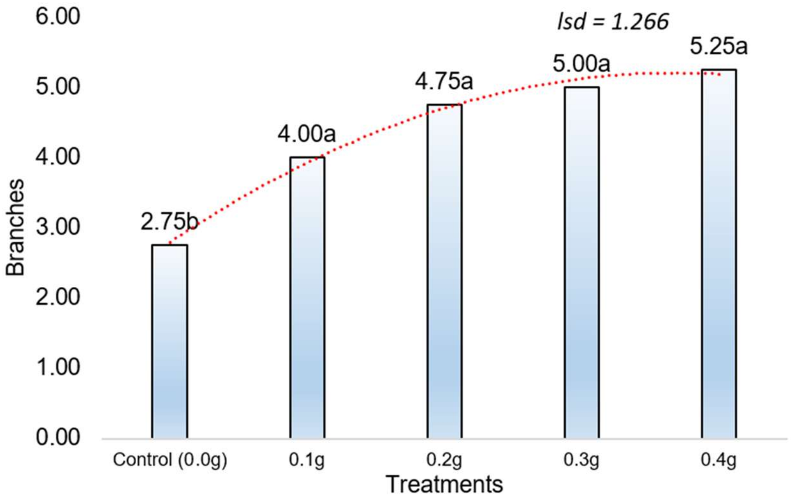

Figure 4. Influence of *Trichoderma*-based biofertilizer on number of branches.

Note: Figures not sharing a common letter in a column differ significantly at 0.05 probability.

3.4. Effect of *Trichoderma*-based biofertilizer on stem girth

The summary of the analysis of variance regarding the stem girth as affected by the application of *Trichoderma*-based biofertilizer is shown in **Table 2,** where there were no significant ($p > 0.05$) differences in the means between the applied treatments. But numerically, the data shows that the means for the stem girth were increasing with an increase in the amount of biofertilizer applied. The means of stem girth recorded from treatments that received the application of the biofertilizer were numerically higher than the average (5.33) for all the treatments.

Table 2. Influence of *Trichoderma*-based biofertilizer on stem girth, chlorophyll content and branching pattern.

Treatment	**Girth(mm)**	**Chlorophyll content**	**Node number**		
			1st Branch	**2nd Branch**	**3rd Branch**
0.0 g	4.96	34.20	1.50	2.50	3.50
0.1 g	5.38	35.08	1.50	2.50	3.50
0.2 g	5.43	35.62	1.50	2.50	3.50
0.3 g	5.46	36.43	1.75	2.75	3.75
0.4 g	5.44	37.25	1.75	2.75	3.75
Mean	5.33	35.72	1.60	2.60	3.60
Significance $LSD_{0.05}$	ns0.693	ns4.693	ns0.808	ns0.808	ns0.808

ns denotes non-significance at 0.05 probability.

3.5. Effect of *Trichoderma*-based biofertilizer on chlorophyll content

There were not significant ($p > 0.05$) differences between the applied treatments on chlorophyll content (**Table 2**). However, like the trend that was shown for stem girth, the chlorophyll content was numerically increasing with increasing application of the biofertilizer to the horned melon. The means for 0.3 g/pot (36.43) and 0.4 g/pot (37.25) were numerically higher than the average (35.75) for all the treatments applied in the investigation.

3.6. Effect of *Trichoderma*-based biofertilizer on branching pattern

Data patterning for the branching appearance on the main stem is shown in **Table 2**. The node number from which the 1st branches appeared to be influenced by the application of the *Trichoderma*-based biofertilizer did not differ statistically ($p > 0.05$) between the treatments. The same trend was shown even for the 2nd and 3rd branches; the node number from which they appeared was not significantly ($p > 0.05$) influenced by applying the biofertilizer at varying levels. The control treatment and the treatments with biofertilizer did not differ ($p > 0.05$) statistically from one another regarding the branching pattern.

4. Discussion

Plant growth stimulation by *Trichoderma* spp. in addition to other microbes, has been reported in several crops[22,28]. Furthermore, Sani et al.[24] had reported an increase in plant height, number of leaves, number of branches, shoot dry matter weight, and root dry matter weight with microbial biofertilizer. The positive influence on vine length, number of leaves, and branches could be due to the improved uptake of mineral nutrients as a result of improved root hairs in the soil rhizosphere. More mineral nutrients for photosynthesis could have resulted in the horned melon growing more nodes and ultimately a greater number of leaves. With more nutrients as well, the plants were stimulated to grow more shoots, resulting in a greater number of branches with the increasing application of the *Trichoderma*-based biofertilizer.

The significant effect of biofertilizer application on vine length, number of leaves, and branches might be ascribed to the plant growth-promoting fungi (PGPF), which improved plant growth by synthesizing plant growth-promoting hormones[29] or averting plant diseases[30]. This result is consistent with several research studies that also observed enhanced plant growth[31,32]. El-Mansi et al.[33] also reported the increase in branches due to the application of biofertilizers. The higher above-ground growth as a result of greater vine length, number of leaves, and branching in treatment with *Trichoderma*-based inoculum may be because of PGPF-amplified N uptake, solubilized P, produced siderophores, and secreted phytohormones required to chelate Fe and make it accessible to plants for advanced growth.

With regards to relative chlorophyll content, the absence of a main effect response to *Trichoderma*-based inoculation in the current study contrasts Bashan et al.[34] and Mahato and Neupane[35] who reported higher photosynthetic pigment quantities in wheat seedlings and maize, respectively, following inoculation. In contrast to the current findings, Chirino-Valle et al.[36] noticed a greater chlorophyll concentration when a treatment containing *T. Atrobrunneum* and *T. harzianum* was applied. However, Liu et al.[37] found that plants inoculated with three types of *Trichoderma*-enriched biofertilizer had lower chlorophyll content compared to the control treatment. This could be attributed to the fact that *Trichoderma* biofertilizers may have a negative effect on the expression of genes involved in chlorophyll synthesis during the middle and late stages of leaf development. It is possible that *Trichoderma* biofertilizers upregulate chlorophyll synthesis genes in the early stages of leaf development, but downregulate them later on.

Application of *Trichoderma*-based biofertilizer could not influence the node from which branching occurred. The probable reason for this finding could be that this attribute may be influenced genetically, apart from the type or quantity of fertilizers applied. So, since the same variety of horned melon was used in this study for all the treatments applied, the biofertilizer could not cause any effect in this regard.

The non-significant differences between the means for stem girth as affected by varying application levels of the *Trichoderma*-based biofertilizer are similar to findings by Mahato and Neupane[35], who did not observe a positive effect of *Trichoderma* on stem girth in maize. The *Trichoderma*-based biofertilizer has an inhibiting effect on stem girth, probably owing to the interference of this fungicide with arbuscular mycorrhizal fungi (AMF) or the problem of its competitiveness with rhizospheric microorganisms or with obtainable key nutrients. Even though *Trichoderma* has seldom been viewed as a parasite, there are some reports indicating the pathogenicity of *Trichoderma* to plants[38–40].

The presence of *Trichoderma* spp. has a positive impact on plant growth by establishing a symbiotic relationship with roots, resulting in increased secondary roots and overall leaf area, as well as improved root system structure[41,42]. *Trichoderma* not only promotes plant growth through its own metabolic processes but also through the release of secondary metabolites in the rhizosphere, which has been documented in previous studies[43,44]. In addition, *Trichoderma* has the ability to reduce soil components that hinder plant growth[45–47]. Further research has shown that *T. harzianum* 1295-22 is capable of enhancing nitrogen utilization and solubilizing difficult-to-absorb nutrients such as Cu^{2+}, Fe^{3+}, Mn^{4+}, and others, resulting in enhanced plant growth and development[48]. Therefore, it can be inferred that one or more mechanisms may be at play in regulating the growth of horned melon when using *Trichoderma*-based biofertilizers.

5. Conclusion

Taking into account the results, it can be inferred that implementing *Trichoderma*-based biofertilizers proved to be effective in impacting vine length, leaf count, and branch count of horned melon plants. Therefore, incorporating this treatment in the cultivation of commercial horned melons could lead to sustainable crop productivity while promoting positive plant growth and ensuring environmental safety. Nevertheless, further comprehensive and systematic research is essential to fully comprehending the advantages of using *Trichoderma*-based biofertilizers in enhancing horned melon production.

Author contributions

Conceptualization, MM; methodology, MM; software, MM; validation, TM and WN; formal analysis, MM and PC; investigation, MM and PC; data curation, PC; writing—original draft preparation, MM and PC; writing—review and editing, MM and TM; supervision, TM and WN; project administration, MM;. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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