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Effects of Soil Supplementation with Organic Fertilization from Earthworm Casts and Inorganic Fertilization from NPK on Growth, Development and Yield of Pepper (*Capsicum annuum* L.) Plants

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Abstract The use of organic source nutrients for quality and quantity development in crop production is gaining global attention. Pot experiments were conducted to investigate the efficacy of soil supplementation with nutrient from enriched earthworm casts, produced under *Gliricidia* seedlings, inoculated with arbuscular mycorrhizae and directly applied inorganic fertilizer (NPK) on the yield of pepper (*Capsicum annuum* L.). Bio-fortified earthworm casts produced by earthworms (*Hyperiodrilus africanus*) fed with leaf mulch of a leguminous plant were applied to pepper. Ten treatments were replicated ten times in Complete Randomized Design. Six weeks old pepper seedlings were transplanted into each pot. Casts from different sources were added to pepper accordingly. Inorganic fertilizer was applied to the ninth treatment while the tenth treatment served as control. Statistical analytical results showed that soil supplementation with earthworm casts promoted vegetative growth and reproductive growth. Casts produced under inoculated *Gliricidia* and mulched soils ($G^+M^+Mu^+$) showed a significant difference ($p < 0.05$) from other treatments and offered a rich potential for organic fertilization. Soil supplementation with earthworm casts produced from mulched, AMF inoculated *Gliricidia* soils supported higher pepper fruits and leaf biomass yield. Thus, nutrient supply by bio-fortified earthworm casts (serving as composite tablets).

Keywords: *soil supplementation, earthworm casts, inorganic fertilization and pepper (Capsicum annuum L.)*

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1. Introduction

The current trend in crop production, in which farmers use synthetic chemical fertilizers for soil amendment is expensive and pose threats to the environment. In addition, inorganic nutrients from chemical fertilizers are released at a rate faster than they can be absorbed by plant roots. A large percentage of consumers are then opting for organically grown vegetables for their benefits such as: pollutant free nature, availability at low or no costs, devoid of any chemical and its potential at enriching the soils. Improvement and maintenance of soil fertility is important for sustainable agriculture. The impact is always positive on crops grown on such soils of organic source.

Capsicum annuum L. belongs to the nightshade family Solanaceae with chromosome number (2n) = 24 [1] *Capsicum baccatum* L (aji) and *Capsicum pubescens* Ruiz and Pay (rocoto) are other domestic species cultivated in Latin America. Botanists regarded Capsicum as fruits because they contain seeds. Capsicums have existed for a long period of time, many historians recorded that *Capsicum* have been cultivated for thousands of years (7000 years). The origin of genus *Capsicum* was traced to central and South America [2] Pepper (*Capsicum annuum* L) has always been one of the most popular vegetables in the home garden [3]. It is easy to cultivate. The varieties of pepper may be sweet or hot, varieties from Mexico, China and Thailland are usually the hottest. Pepper thrives well in a hot weather, moist soil, but avoid wet soil and water regularly in both wet or dry season. The ingredient in pepper that makes the "hot" sensation is called 'Capsaicin' [4]. Thus, a sweet pepper is a pepper that contains low level of capsicin but a sweet green pepper is devoid of this chemical. Pepper is cultivated for its economic, nutritional as well as medicinal values [5,6]. It is also known for its laxative qualities and used to treat chronic rhinitis, it serve as anti-bacterial and anti-fungal, anti-aging, reduce cholesterol, prevents cancers of the bladder, cervix, pancreas and prostate, it prevents blood clot and nose bleeds, eye problems like astigmatism, helps relieve gastrointestinal problems, prevent respiratory problems like asthma, emphysema, wheezing, lung infections, naturally building a good immune system, relieve pain to a certain degree, enhance metabolism and help eliminate a sore throat [5,7]. Improving on production of this crop could best done by application of organic source fertilizer.

Organic fertilizer improves the water holding capacity of soils; as it can hold up to three to five times its own weight in water [8]. Organic fertilizers add humus to the soil, this create a reserve for water and plant nutrients. Humus encourages earthworms and other beneficial soil organisms. Inorganic fertilizers or chemical fertilizers are produced by industries. Some are mined while others are entirely manufactured. Inorganic fertilizers are expensive, not easily affordable by subsistence farmers and can be easily leached or they may react with soil metals to form insoluble compounds. On the contrary, nutrients from organic sources like life mulches are not easily leached; they are slowly released and never fixed in the soil to form apatite [9]. Earthworm cast is a good source of organic fertilizer and it is easy to come by.

Earthworms are everywhere in the world where there is soil and can play a variety of important roles in agro ecosystems [10]. Each species of earthworm is characterized with a distinct size, shape and colour [11]. Earthworms' feeding and burrowing activities help to incorporate organic residues and amendments into the soil; it enhances decomposition and humus formation thereby, playing significant role in nutrient cycling and soil structural development [12]. Earthworm population depends on soil physical and chemical properties like temperature, moisture, pH, salts, aeration and texture as well as available food. As such, they benefit from mulching. The casts produced by earthworms contain organic matter and clay casing which are very essential for plant growth. It has been reported that when added in sufficient quantity, casts can out yield (NPK) fertilizer [13]. However, the amount of nutrients contained in the casts is a function of nutrient content of food materials taken by the worms [14].

Availability of nutrients to the plant is another important phase which could be increased beyond the plant root potential. Mycorrhizae greatly enhance the plants to absorb phosphorus, and sometimes other nutrients that are relatively immobile and present in low concentration in the soil [15]. In contrast, mycorrhizae prevent the uptake of excessive amount of salt and toxic metals in saline, acid and contaminated soil [16]. For these reasons, mycorrhizae is engaged to give additional absorption capacity when inoculated in the soil underneath the plant root. Hence, this research work was therefore designed to investigate and compare the use of earthworm casts produced under inoculated *Gliricidia* seedlings, its leaf mulch and NPK fertilizers on the quality improvement of Pepper (*Capsicum annuum*).

2. Materials and Methods

2.1. Description of Experimental Site

Pot experiment was conducted at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Nigeria. Ogbomoso is on longitude $4^{0}11^{I}$E and latitude 8^{0} 05^{I}N in the Guinea savanna zone of southwest, Nigeria. The temperature ranges from 28°C-33°C with humidity of about 74% all year except in January when there will be dry wind blows. The experimental site lies within the transitional zone of forest and savanna belt of Nigeria. The climate consists of two well defined seasons i.e. rainy and dry seasons. The raining season is characterized by a bimodal rainfall pattern in which a longer spell of rain starts in March or April which peaks in July. This is separated by a short break of uncertain rainfall (August break) that is followed by a short spell of rain which peaks between the end of September and the beginning of October. The dry season sets in towards the end of November and lasts till the end of March [17].

Collection of experimental materials**:** Seeds of pepper and NPK 20:10:10 mineral fertilizer were collected from Oyo State Agricultural Development Programme Office (OSADEP). Ogbomoso, Oyo State, Nigeria.

Nursery preparation**:** Nursery beds were prepared for the seeds of pepper (Chilli type). The seeds were broadcasted directly on the bed of 1m x1m drilling method and highly covered with dry palm fronds to stimulate easy and quick germination and protect the seeds against rain and direct sun. The bed was watered immediately and thereafter as needed. There was no application of chemical fertilizer to comply with requirements of organic pepper production package. The emerged seedlings were allowed to grow for six weeks till they attained the height of 15cm before they were transplanted into plastic pots.

Planting and pot preparation**:** A sub-sample of the soil used for planting pepper was air dried, ground, sieved through 2 mm mesh and set aside for soil nutrient analysis.

The pot experiments were conducted; pots of about 2 kg soil capacity were used. They were perforated at the base to control drainage of excess water and facilitate aeration. There were ten treatments replicated ten times and there were a total of 100 pots. Each pot was filled with 2 kg of air dried soil. The pots were arranged in complete randomized design (CRD) with 10 replicates. The pots were watered and hard materials were placed under the pots to avoid contact with the soil. At transplanting, a seedling of pepper six weeks (42 days) old (about 15 cm in height) was transplanted into each pot. Watering of soil was done before and immediately and thereafter as the need arose. Two weeks after transplanting (WAT), 200 g of casts from different treatments and replicates were applied to the first eighty (80) pots according to corresponding replicates. 24 g N.P.K fertilizer (Equivalent to 120 kg\ha) was applied to the next ten (10) pots while no supplementation was added to the last ten (10) pots. These served as control. Pepper plants were allowed to grow up to maturity.

Treatments involved in the (pot) experiment: $G^{+}M^{+}Mu^{+}$ - Casts from inoculated *Gliricidia* and mulched soils, $G^{+}M^{+}Mu^{-}$ - Casts from inoculated *Gliricidia* and unmulched soils, $G^{+}M^{-}Mu^{+}$ - Casts from uninoculated *Gliricidia* and mulched soils, $G^{+}M^{-}Mu^{-}$- Casts from uninoculated *Gliricidia* and unmulched soils, $G^{-}M^{+}Mu^{+}$ - Casts from inoculated without *Gliricidia* and mulched soils, $G^{-}M^{+}Mu^{-}$ -Casts from inoculated without *Gliricidia* and unmulched soils, $G^{-}M^{-}Mu^{+}$ - Casts from without inoculated *Gliricidia* and mulched soils, $G^{-}M^{-}Mu^{-}$ - Casts from without inoculated *Gliricidia* and unmulched soils, IF - Inorganic Fertilizer and CE - Control experiment. Thus ten (10) treatments replicated ten times were established.

2.2. Data Collection and Presentation

Collection of data started two weeks after transplanting (WAT) and continued every week until plants senescence.

2.3. Seedling Growth Parameters

The stem height, stem girth and number of leaves of pepper seedlings in each treatment and replicate were monitored at weekly interval from the second week after transplantation.

Tape meter was used to measure the height (cm) of pepper plants from soil level to the tip of plants. Vernier calliper was used to measure the stem girth. (cm) while number of leaves was measured by direct counting. The mean values of different growth parameters of each treatment were computed and plotted graphically against weeks after transplantation.

2.4. Reproductive Parameters

The pattern of fruiting of potted pepper plants was monitored; data on number of fresh fruits, its weight as well as total fruit yield were collected. The number of pepper fruits harvested from each replicate and treatment was measured by direct counting, fresh weight of pepper fruits was measured in gram (g) using weighing balance. The total number of pepper fruits produced in each treatment was calculated by the addition of pepper fruits harvested during the period of experiment ditto to total fresh weight of fruits

2.5. Quantification of Arbuscular Mycorrhizal Infection in Pepper Roots

Pepper root samples were collected in three replicates from each treatment with a hand trowel from the soil in the pot at 0.20 cm depth. The fine roots were placed in clean McCartney bottles and labelled. In the laboratory, the roots were washed clear of soil particles after which they were fixed and stored in 50% ethanol. A sub-sample of 2 g fresh weight was taken from the field sample and cleared in 10% potassium hydroxide solution (KOH) and heated by autoclaving at 121°C for 15 minutes. The roots were rinsed in several changes of water to remove KOH. The samples were drained and the acidified roots were stained. The acidified roots were stained using a preparation of acidified glycerol and trypan blue (Water, Glycerine and lactic acid) in which 0.05% Trypan blue solution had been added [18]. Staining was accelerated whenever necessary by heating in an autoclave at 121 for 3 minutes. The stain was poured off and the root samples were covered by a few drops of acidified glycerine at room temp to remove excess stain (de-staining). Assessment of root colonization was done according to the gridline intersect technique of Govanetti and Mosses [19], stained root samples were placed in 1cm grid plastic squared shaped petri-dishes with the gridlines at uniform distances 1 cm apart. Horizontal gridline cross vertical gridlines at intervals forming set of squares like a chequerboard. Both the horizontal and vertical gridlines were viewed with a dissecting microscope at 15-45 x and each time a root was encountered crossing a line, it was scored either infected or uninfected. Infected when hyphae arbuscle or vesicle were found on the root and uninfected when no infection was found.

Earthworm casts contribution (%) to dry matter accumulation (leaf, stem and root dry matter) of pepper plants

Earthworm casts contribution (%) to dry matter accumulation (leaf, stem and root dry matter) of pepper plants

$$\frac{Z-Y}{Y} X \frac{100}{1}$$

Where Z = Total dry matter accumulation (leaf, stem and root dry matter) per treatment

Y= Total dry matter (leaf, stem and root dry matter) in control [20].

Percentage fertilizer contribution to dry matter accumulation (leaf, stem and root dry matter) of pepper plants

Fertilizer contribution (%) to dry matter accumulation (leaf, stem and root dry matter):

$$\frac{A-B}{B} X \frac{100}{1}$$ [20]

Where A= Total dry matter accumulation (leaf, stem and root dry matter) per treatment

B= Total dry matter accumulation (leaf, stem and root dry matter) in control.

2.6. Statistical Analysis

Mean of the data from replicates within each treatment were calculated using excel software The various treatment data were subjected to Analysis of variance (ANOVA) and treatment means were separated using standard error and the significance of the difference between them were determined at $P < 0.05$.

3. Results

3.1. Analysis of Soil and Casts Samples

The analysis of soil samples collected for pepper planting for pH, OC, N, P, Ca, Mg and K. potassium showed that the soil samples used in this study contained less quantities than the earthworm casts irrespective of treatments Table 1.

3.2. Soil Supplementation and Vegetative Growth of Pepper

The result of the present study revealed that growth, development and fruit yield of pepper can be increased through both organic and inorganic fertilizer application. This is due to higher performance of pepper recorded with supplementation (casts and inorganic fertilizer). The plant performance is least in those plants grown on un-supplemented soil. The height of pepper plants and other vegetative growth parameters in soils supplemented with earthworm casts from different sources was significantly high ($P < 0.05$).

The height and number of leaves of pepper plants grown in soils supplemented with casts irrespective of treatments were higher than those supplemented with inorganic fertilizer (NPK). The control (CE) recorded the least Figure 1 and Figure 2 respectively. Casts produced under inoculated *Gliricidia* and mulched soils ($G^+M^+Mu^+$) showed a significant difference P < 0.05 to all other treatments.

Table 1. Soil nutrient content and earthworm casts before the commencement of the trial Soil Sample

Soil Before experiment	Nutrient content						
	N	P	K	OC	Ca	Mg	pH
	g/kg				Cmol/kg		(H_2O)
	0.9	10.51	1.01	14.5	2.78	1.22	6.1
Earthworm casts Treatments							
$G^+M^+Mu^+$	2.4	84.52	4.84	26.2	9.45	3.61	7.3
$G^+M^+Mu^-$	1.9	74.20	2.90	24.3	6.70	2.65	6.9
$G^+M^-Mu^+$	2.2	78.47	3.09	24.8	7.39	2.99	6.9
$G^+M^-Mu^-$	1.9	32.93	2.82	23.8	6.34	2.57	7.0
$G^-M^+Mu^+$	2.2	80.48	3.45	25.2	8.24	3.22	7.0
$G^-M^+Mu^-$	2.1	17.76	2.81	23.6	6.17	2.51	6.9
$G^-M^-M^-u$	2.2	61.22	2.88	24.0	6.90	2.65	6.9
$G^-M^-Mu^-$	1.7	17.3	1.42	23.1	5.01	2.22	6.2

Key:
$G^+M^+Mu^+$: Casts from inoculated *Gliricidia* and mulched soils
$G^+M^+Mu^-$: Casts from inoculated *Gliricidia* and unmulched soils
$G^+M^-Mu^+$: Casts from uninoculated *Gliricidia* and mulched soils
$G^+M^-Mu^-$: Casts from uninoculated *Gliricidia* and unmulched soils
$G^-M^+Mu^+$: Casts from inoculated without *Gliricidia* and mulched soils
$G^-M^+Mu^-$: Casts from inoculated, without *Gliricidia* and unmulched soils
$G^-M^-Mu^+$: Casts from without inoculated *Gliricidia* and mulched soils
$G^-M^-Mu^-$: Casts from without inoculated *Gliricidia* and unmulched soils
Abbreviations: g/kg - Gram per kilogram; Cmol/kg – Centimole per kilogram

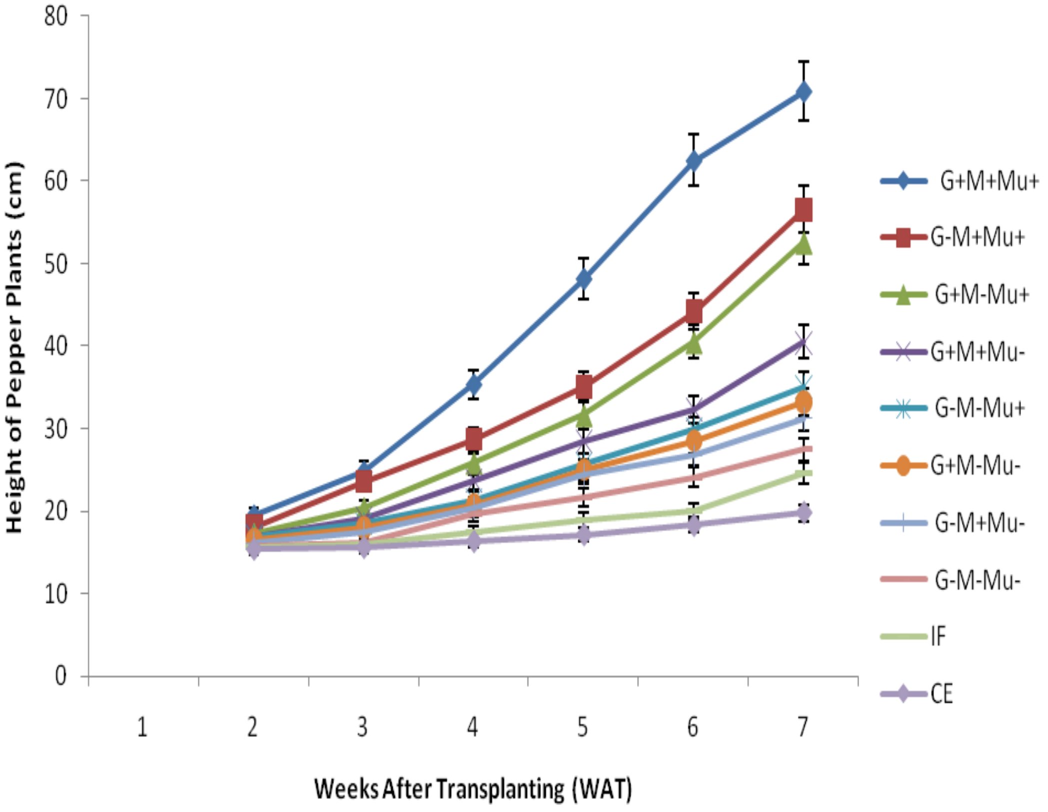

Figure 1. Effects of supplement of soils with earthworm casts produced and inorganic fertilizer (NPK) on the increase in height of potted pepper plants

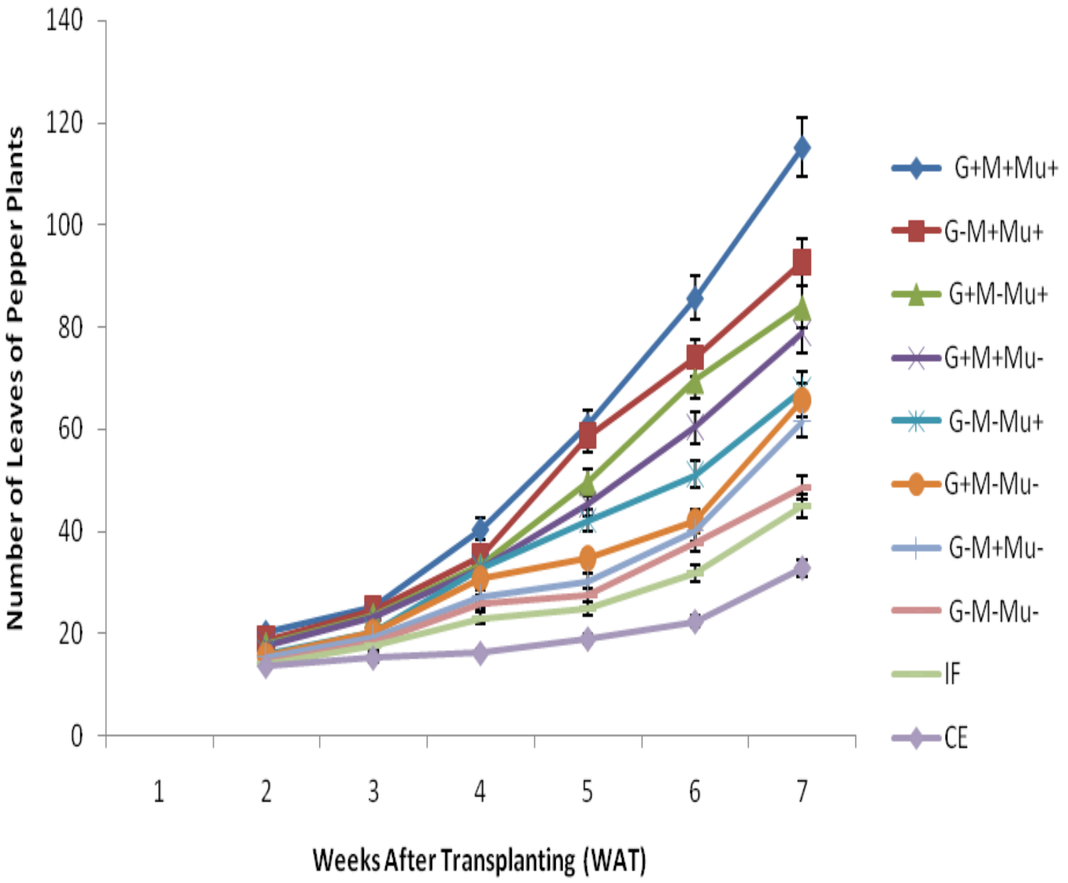

Figure 2. Effects of supplement of soils with earthworm casts produced and in-organic fertilizer (NPK) on the number of leaves of potted pepper plants

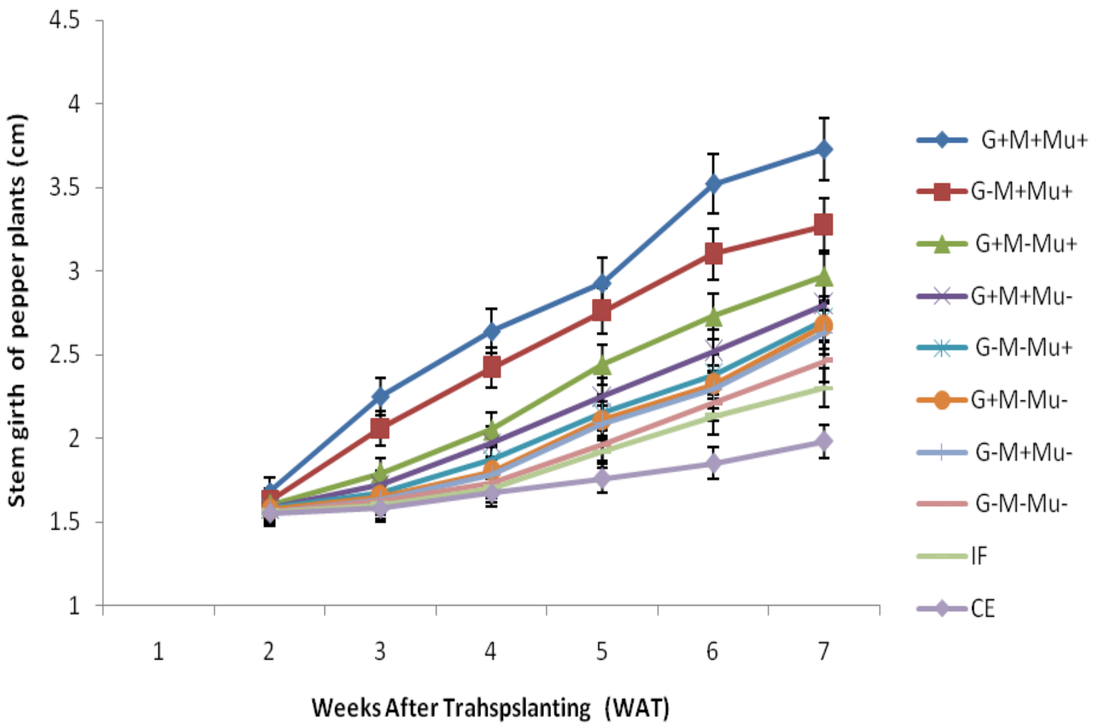

Figure 3. Effects of soil supplementation and inorganic fertilizer (N.P.K) on the stem girth of pepper plants

However, there was no significant difference in the stem girth at the value of $P < 0.05$ in all the treatments (Figure 3). Leaf, stem and root dry matter yield of pepper plants grown in soils supplemented with casts from different sources was higher than those of pepper plants grown in soils with inorganic fertilizer (IF) while pepper plants grown in soils without supplementation (CE) had the lowest. There was no significant difference (at a value of $P < 0.05$) in dry matter accumulation in all the treatments (Figure 4).

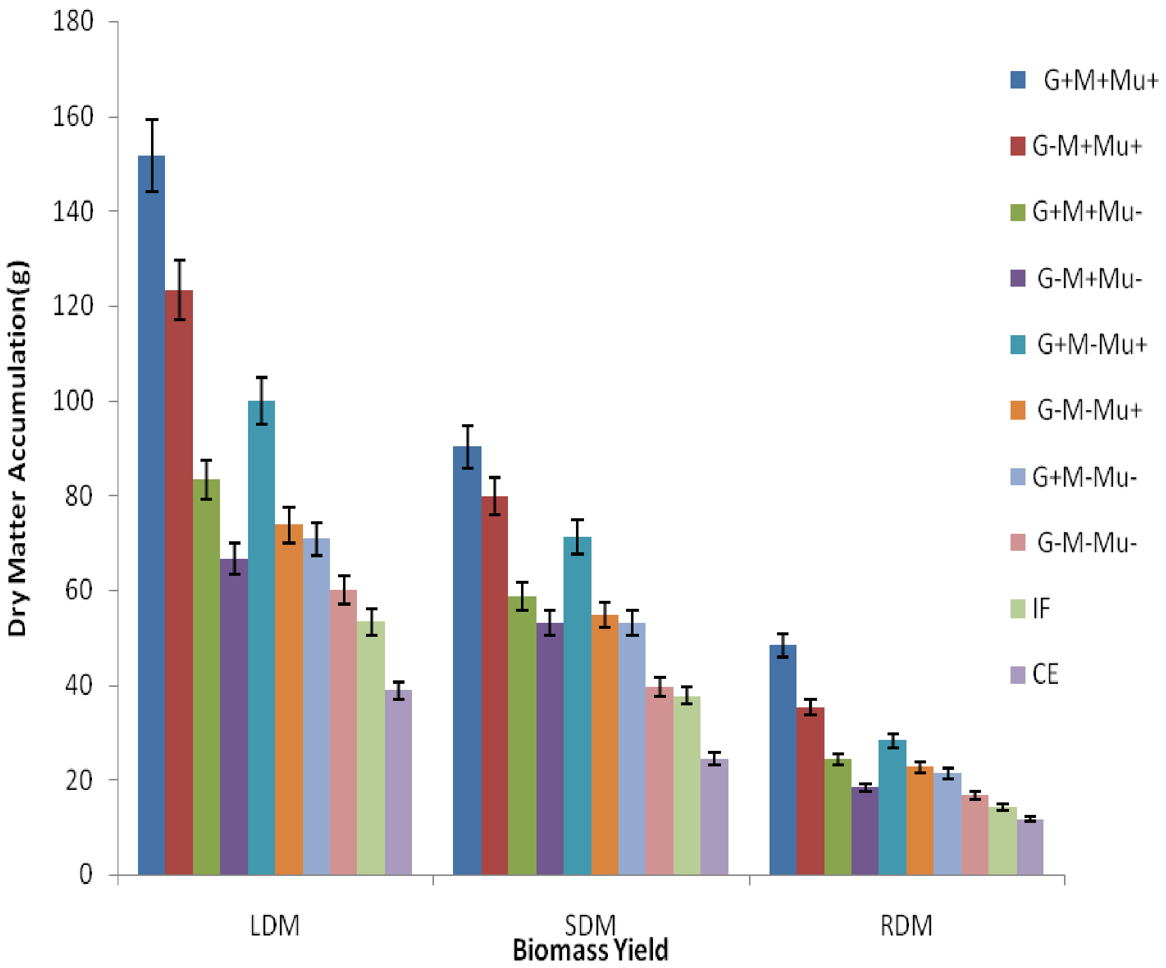

Figure 4. Effects of soil supplementation with earthworm casts and inorganic fertilizer application on leaf, stem, and root biomass yield of potted pepper plants

3.3. Contribution (%) of Earthworm Casts and Inorganic Fertilizer (NPK) to Dry Matter Accumulation (Leaf, Stem and Root Dry Matter) of Pepper Plants

The contribution (%) of earthworm casts to dry matter accumulation of pepper plants was greater than that of inorganic fertilizer irrespective of soil treatments. However, casts from inoculated and mulched soils had the highest contribution followed by those casts from inoculated without *Gliricidia* and mulched soil while those casts from without inoculated *Gliricidia* and unmulched soils had the least contribution (Table 2).

3.4. Soil Supplementation and Total Number and Fresh Weight of Pepper Fruits

The total number of pepper fruits produced from soils supplemented with casts was greater than those produced from soils with inorganic fertilizer, the lowest number of pepper fruits was produced from soils with no supplementation. (CE) (Furthermore, pepper plants grown in soils supplemented with casts from inoculated *Gliricidia* and mulched soils ($G^+M^+Mu^+$) produced highest number of fruits at the value of ($P < 0.05$) while that of control (CE) produced the least. (Figure 5) ditto to total fresh weight of pepper fruits (Figure 6).

3.5. Roots Infection by Arbuscular Mycorrhizal Fungi of Pepper Plants at the End of the

Effects of supplement of soils with earthworm casts produced and inorganic fertilizer (NPK) on pepper stem girth.

Supplemented soils recorded the highest mean vegetative values followed by plants grown on supplemented soils with inorganic fertilizer. Vegetative values include the stem girth of pepper plants in soils supplemented with earthworm casts was greater than those supplemented with inorganic fertilizer. The least mean vegetative values were recorded in plant on un-supplemented soil. Pepper plant stem girth followed the same trend as shown in Figure 3.

Effect of soil supplementation and inorganic fertilizer on number of pepper fruit

The present data on growth and fruit yields of pepper showed significant variation among the treatments examined. Highest number of pepper fruits and fresh weight (yield) were recorded from soils supplemented with casts from different sources. The next higher pepper fruits and fresh weight (yield) was recorded from plant on soils supplemented with inorganic fertilizer and the least number of pepper fruits and fresh weight were recorded in un-supplemented soils (control) Figure 5 and Figure 6 respectively.

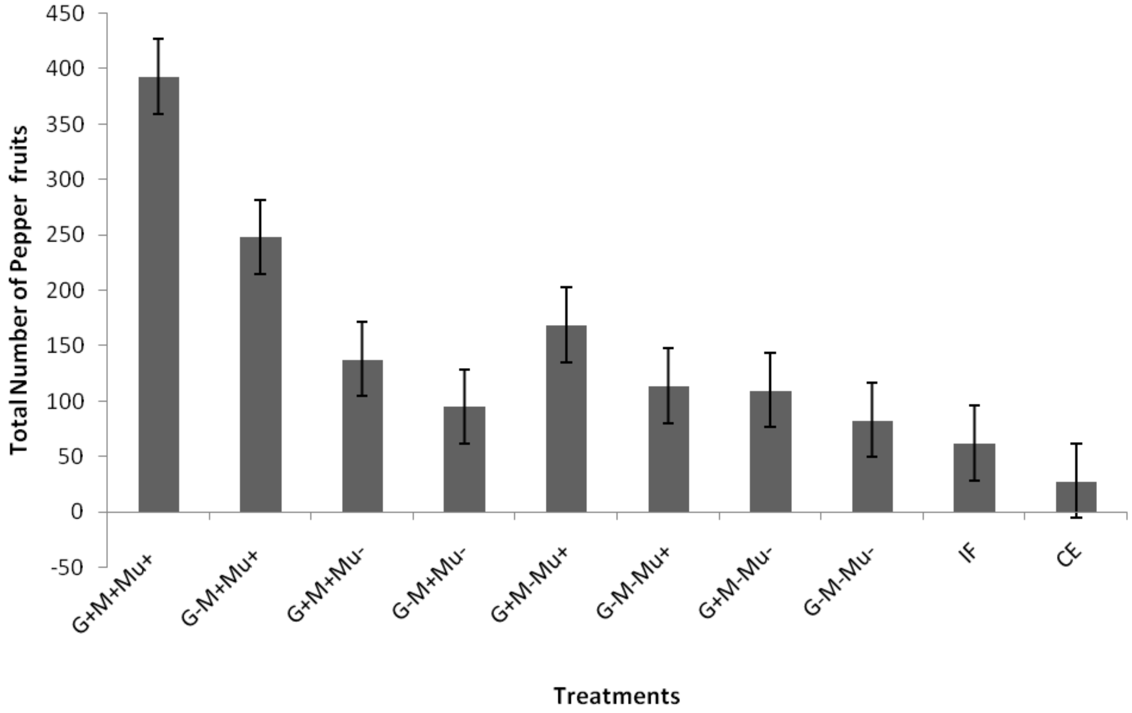

Figure 5. Effects of soil supplementation with earthworm casts and inorganic fertilizer application on the total number of pepper fruits

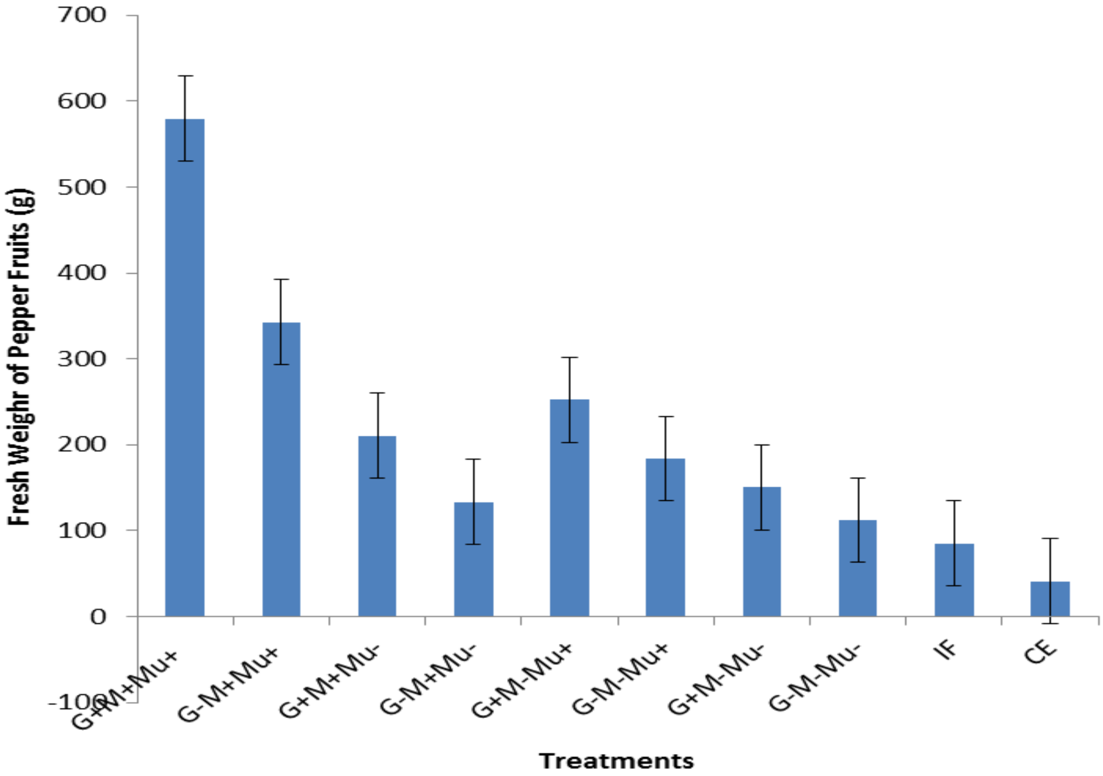

Figure 6. Effects of supplementation of soils with earthworm casts produced and inorganic fertilizer (N.P.K) on fresh weight of pepper fruits harvested at the end of the experiment

Effect of soil supplementation and inorganic fertilizer on root infection

Generally, roots of inoculated pepper were greatly improved when compared to treatments without inoculation. However, plant root infection was always the occurrence when plants are inoculated by arbuscular mycorrhizae. Pepper plant roots inoculated by *Glomus mosseae* was infected and the percentage was highest in soils supplemented with casts from mulched soils with *Gliricidia* sepium ($G^+M^+Mu^+$) while the least root infection was recorded in pepper plants grown on soils without supplementation (control). The root infection of pepper plant by *Glomus mosseae* in soils supplemented with casts from treatment with mulch was higher than its corresponding unmulched treatments. The percentage root infection obtained in casts from soils mulched with *Gliricidia* ($G^+M^+Mu^+$) showed a significant difference ($P < 0.05$) from all other treatments as shown on Figure 7.

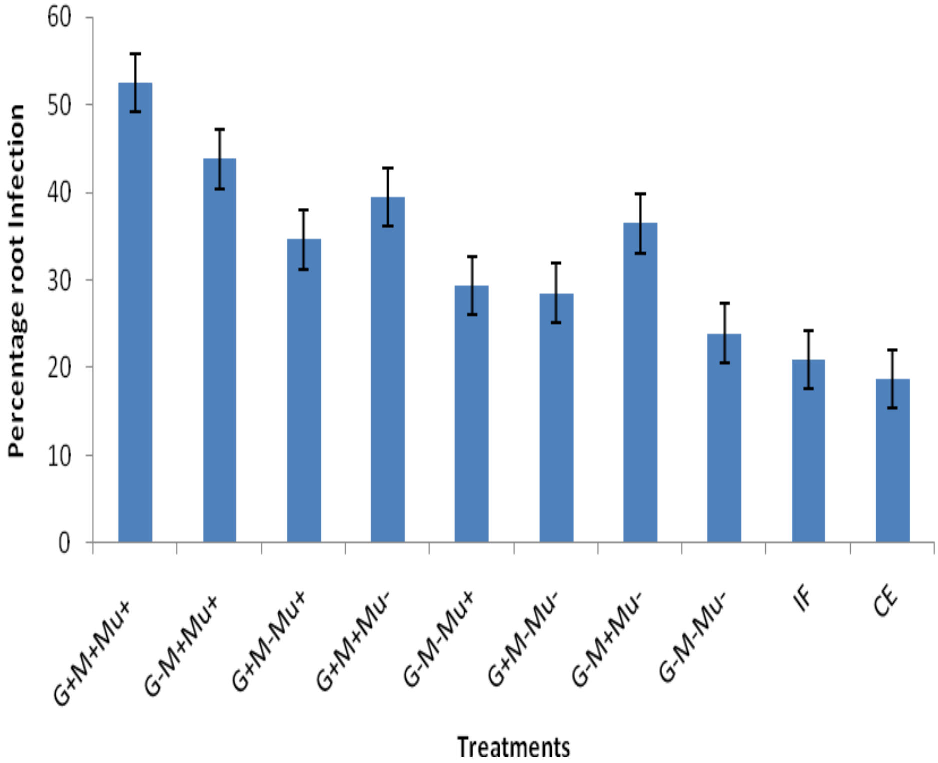

Figure 7. Effect of soil supplementation and inorganic fertilizer on pepper percentage

4. Discussion

The aim of soil supplementation practice generally is to increase the productivity of crops [21,22]. The purpose was the same in this study when earthworm casts was applied to soil for growing pepper, and it was in agreement with those of Moore et al. Madez et al. [9,23]. The purpose is well expressed in this study by significance vegetative performance of pepper plant grown on supplemented with earthworm casts from different sources. Significance performance of both vegetative and yield could be as a result of contribution of casts that improved the soil fertility. The earthworm casts being a pure organic fertilizer was beneficial to the pepper plant and cost effective and environmental saved in this study [24]. However, too low soil fertility status has been reported to have been a common cause of poor growth and yield response in pepper [25]. The sigmoid growth pattern observed in plant height and number of leaves in this experiment is in line with the findings of Ndu et al.; Karim, [26,27]. Highest yields recorded in number of pepper fruit and fresh fruit weight on supplemented soils with earthworm casts conforms to the findings of Uphoff, [28]. Thus, earthworm casts were more efficient than direct application of fertilizer at enhancing growth, development and fruiting of potted pepper.

Root infection of pepper plants by arbuscular mycorrhizal fungi is an indication of symbiotic relationship between the root of pepper and arbuscular mycorrhizal. Highest percentage of root infection of pepper that was observed in pepper plants grown on soils supplemented with casts from mulched soils may be attributed to efficacy of the introduced arbuscular mycorrhizal fungi. Similar report to our results was given on complementary effect of indigenous and introduced arbuscular mycorrhizal fungi [29].

Findings of this study supports the work of other researchers who reported that application of compost (an organic material) could save ~20% mineral N-fertilizer [21,30]. Rizwan et al. [31] reported the same findings in their study even though their own application was in low rate. The result of this study affirmed that soil supplementation with earth worm casts is a type of organic agriculture that holds much promise for vegetable producers interested in gaining premium prices for their crops while lowering input costs [32].

5. Conclusion

In this present study, nutrient supply by bio-fortified earthworm casts was found significantly effective than inorganic chemical (NPK) fertilizer. Earthworm cast serves as composite tablets at improving both quantitative and qualitative productivity of pepper. Moreover, the use of earthworm casts is environmental friendly. It is recommended that earthworm casts (organic nutrient sources) are generated in large quantity for large scale and better crop production without risk of environmental pollution.

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