Worm castings-based growing media with biochar and arbuscular mycorrhizal fungi for producing organic tomato (*Solanum lycopersicum* L.) in greenhouse

Mohammad Reza Ardakani[1, 2*] and Mehdi Sharifi[2]

1. Department of Agronomy and Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran
2. Canada Research Chair in Sustainable Agriculture, Environmental and Resource Studies Program, Trent University, Ontario, Canada

Abstract

Organic vegetable production has specific research and innovation requirements which are not shared by other parts of the food and farming sector. A pot experiment was conducted to investigate the interactive effects of few permitted organic inputs such as arbuscular mycorrhizal fungi, biochar, and different ratios of peat:worm casting on tomato (*Solanum lycopersicum* L.) growth, mycorrhizal dependency, biomass production, fruit yield, and soil respiration. The experimental design was a factorial arrangement based on completely randomized design with three replicates. Factors included worm casting at three levels (0, 15 and, 30% of the media volume), organic peat-based potting soil at three levels (70, 85, and 100% of the media volume), two *Glomus intraradices* treatments (inoculated at sowing or un-inoculated), and two biochar levels (10% of total weight of the media or unlamented). Results indicated that worm casting × peat combination significantly affected all measured traits except for the number of fruits in plant and mycorrhizal dependency. Mycorrhizal symbiosis had a significant effect only on shoot dry weight and mycorrhizal dependency. Moreover, biochar application significantly affected shoot dry weight, stem diameter and carbon mineralization. Among the different ratios of worm casting and peat in the growing media, 15% worm casting × 85% peat formed the most suitable medium condition for plants and 100% peat without worm casting was the least suitable. The highest fruit fresh weight (228.7 g/plant) was achieved in 15% worm casting × 85% peat and the lowest fruit fresh weight (175.1 g/plant) was achieved in 100 peat treatment.

Key words: biochar; mycorrhizal dependency; organic tomato; soil respiration

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Introduction

Organic vegetable consumption is clearly acknowledged to be an essential element of a healthy diet but the literature on authentic organic management systems is scarce. On the other hand, urban population growth places enormous pressure on both food production and waste management, which in turn impact the planet's ecological health. Composting and vermicomposting can divert a significant amount of waste from landfills and incineration facilities, while providing a nutrient rich growing media for

*Corresponding author
E-mail address: *mohammadreza.ardakani@kiau.ac.ir*
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greenhouse organic vegetable production. Limited information is available on the optimum worm casting ratio in growing media and inclusion of biochar and arbuscular mycorrhizal (AM) fungi in growing media to support growth of tomato plants for a growing season.

Low nutrient content and low mineralization rate of soil organic matter (SOM) are the two major constraints currently encountered in low input and organic agricultural systems (Renner, 2007). These constraints can be addressed by use of nutrient rich high quality organic amendments such as worm castings. Vermicomposting is the decomposition of organic wastes through earthworms to aid the waste stabilization process (Riffaldi and Levi-Minzi, 1983). This process involves interaction between earthworms and microorganisms to produce worm casting. Earthworms process the substrate and boost the biological activity for further biochemical degradation of organic matter by microorganisms (Lim et al., 2015).

In general, vermicompost is physically, nutritionally, and biochemically improved over traditional compost because the mineralization rate of organic matter is accelerated and a higher degree of humification can be obtained through vermicomposting (Lim et al., 2015). Also, in comparison with composting, vermicomposting of organic wastes is a faster process and results in two useful products, earthworm biomass and vermicompost. Earthworm casts are soil conditioners that have high nutrient availability for plant growth (Albanell et al., 1988). As an example, Devliegher and Verstraete (1997) have reported increased availability of phosphate and the elements such as Mg, Ca, Fe, Mn and Cu in the worm-worked soil, compared with initial mineral concentrations in soil.

Arbuscular mycorrhizal fungi are important components of the soil microbial community and are usually found in soils forming a mutualistic symbiosis with the roots of most cultivated plants. Mycorrhizal symbiosis is the most common type of microorganisms-plant relations with various beneficial effects on plants. The most prominent function of this symbiosis to plants is the enhancement of plant root absorptive surface due to the connection of the fungi hyphae with the plant's root hairs, making higher volume of soil available to plant. This enables plants to absorb more water and nutrients and increases plant resistance to drought stress and nutrient deficiency conditions. The AM fungi increase plant productivity and P uptake in P deficient soils or when low to moderate P doses are applied (Hayman, 1983). The fungal hyphae not only absorb phosphate, but also other elements in soils, and translocate them into the plant (Gildon and Tinker, 1983; Faber et al., 1990; Wiedenhoeft, 2006; Smith and Read, 2008; Mardukhi et al., 2015). Addition of AM fungi to the organic-based growing media may improve the release rate and accessibility of the nutrients by plant.

More recently, biochars have been used as soil amendments with promising results (Chan et al., 2007). Biochar is the carbon-rich product obtained by heating biomass in a closed system under limited supply of oxygen intended to be used as a soil amendment to sequester carbon and enhance soil quality. Sustainable biochar is produced from waste biomass using modern thermochemical technologies such as pyrolysis. Pyrolysis involves the heating of organic materials in the absence of oxygen. Addition of sustainable biochar to soil has many environmental and agricultural benefits, including waste reduction, carbon sequestration, water resource protection, and soil fertility improvement (Atkinson et al., 2010). Therefore, the use of biochar as a soil amendment is an innovative and highly promising practice for organic production systems.

Biochar usually has greater sorption ability than natural soil organic matter due to its greater surface area, negative surface charge, and high charge density (Liang et al., 2006). Thus, the addition of biochar to soil offers a potential environmental benefit by preventing the loss of nutrients and thereby protecting water resources. Biochar's high surface area and complex porous structure are hospitable to beneficial bacteria and fungi. Several greenhouse and field studies have been conducted to examine the effect of biochar on crop yields (Glaser et al., 2002; Yamato, et al., 2006; Chan et al., 2007, 2008). In a few cases,

Table 1.

Treatments descriptions

Treatments	Worm Casting %	Peat %	Mycorrhiza	Biochar
a	0	100	NO	NO
b	15	85	NO	NO
c	30	70	NO	NO
d	0	100	YES	NO
e	15	85	YES	NO
f	30	70	YES	NO
g	0	100	YES	YES
h	15	85	YES	YES
i	30	70	YES	YES
j	0	100	NO	YES
k	15	85	NO	YES
l	30	70	NO	YES

either no difference or negative results have been found on the use of biochar as a soil amendment. The varying effects on crop yield appear to depend on factors such as biochar quality, biochar quantities added, soil type, and crop tested. Limited information is available on use of biochar in greenhouse growing media. More research is needed to understand the interactions between worm casting, AM fungi, and biochar and its effect on sustaining tomato growth in a greenhouse setting. Given the fact that the organic sector operates in its own special market and has to comply with specific standards and regulations, it has specific research and innovation requirements which are not shared by other parts of the food and farming sector. Therefore, there is an urgent need for harmonization of scientific research based on organic production, corresponding with standards and regulations.

Under organic growing conditions for tomato when the chemical fertilizers are prohibited according to the standards and regulations, worm casting-based growing media could be a good substitute for chemical fertilizers supplying the nutrient requirements for the plants during their life cycle. Inoculation with mycorrhiza fungi and using biochar will boost the availability of nutrients in worm casting-based growing media.

The main aims of this study were

The overall objective was to develop a worm casting-based growing medium for tomatoes that can support plant growth during its life cycle in a greenhouse setting. The optimum ratio of peat:worm casting and inclusion of biochar and arbuscular mycorrhizal fungi on some tomato agronomic parameters were studied.

Materials and Methods

The experiment was conducted in 18 m^2 Conviron closed-system environmental growth chambers located at the Department of Environmental Sciences, Trent University, Ontario, Canada. Certified organic seeds of tomato (*Solanum lycopersicum,* hybrid, Red Short Vine, Polbig F1.) provided from Johnny's Seed Company were sown in a multi-cellular jiffy container within peat-based jiffy pots. Fourteen days later, seedlings were transplanted to 5 L volume plastic pots containing prepared a growing media mixture (Table 1). Growing conditions remained constant during the course of the experiment: temperatures of 28 °C (day) and 18 °C (night), 40% relative humidity, natural daylight supplemented with HPS lamps (381 $\mu mol/m^{-2}/s$) for a 16 h photoperiod and no CO_2 enrichment.

Worm casting at three levels (0, 15 and 30% of the media volume), organic peat-based potting soil at three levels (70, 85 and 100% of the media volume), mycorrhizal fungi (*Glomus intraradices*) at two levels (with and without application) and biochar at two levels (with 10% of total weight of the media and without application) provided twelve combinations. The experimental design was a factorial arrangement based on completely randomized design with three replicates. Worm casting, the product of Greenscience Technology Co. was used in this experiment [Ammonia 30 mg/kg; Nitrate 420 mg/kg; P (P_2O_5) 970 mg/kg; K (K_2O) 0.34%; Ca 3.1%; Mg 0.42%; S 280 mg/kg; B 14 mg/kg; pH 6.71; EC 1.4 mmhos/cm; organic matter 58.1% and C/N ratio17].

Solid powder of mycorrhizal inoculum which contained *G. intraradices* and produced by Company: MYKE Pro/ PS3, was used as seed inoculation. MYKE® PRO PS3 is a highly concentrated endomycorrhizal inoculant in powder form compatible with most vegetable crops. This product contained 1600 viable propagules per gram of MYKE Pro/ PS3. The inoculum was previously weighted for each mycorrhizal treatment (1 g/pot seed inoculation).

The commercial biochar (Blue leaf® Inc., Drummondville, QC, Canada) which used in this study was obtained using Pyrocycling ™ technology with the method described by Roy et al. (2000) and was derived from softwood bark of balsam fir (*Abies balsamea*), white spruce (*Picea glauca*) and black spruce (*Picea mariana*). Briefly, combustion temperature was set at 475 °C and pressure in the reactor was maintained at 20 kPa. Sunshine #4 Natural & Organic peat-based soil was used in this study, which formulated with Canadian sphagnum peat moss, coarse perlite, organic starter nutrient charge, Gypsum and dolomitic limestone.

Plants were watered every three days after transplanted from jiffy pots to main pots. Tomato plants were supplemented with an OMRI (*Organic Materials Review Institute)* certified organic liquid fertilizer two times a week on December 2013, until the end of the experiment using 2.5% dilution of 2-2-5 (N-P-K; Bio Flores, product of the Netherland). The plants were harvested according to development stage of each treatment continuously until the end of the experiment. Plant height was measured at harvest from soil surface in each treatment. Shoots were harvested above the soil surface. Above organs of each plant separated to shoot (leaves and stems) and fruits, then their fresh weight and total fresh weight of the shoot have been estimated. Dry weight of the shoot organs are also determined after drying in an oven at 50°C for six days. The dependency of a plant species on mycorrhizae was defined as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield, at a given level of soil fertility" (Ortaş 2003). Mycorrhizal dependency (MD) was calculated for each treatment using the following equation given by Hetrick et al. (1993):

MD (%) = [(DW of mycorrhizal plant - DW of non mycorrhizal plant) / DW of mycorrhizal plant] × 100

Table 2.
Analysis of variance table for the effect of treatments on the measured traits

SOV	df	Mean Squares (MS)						
		Plant height	Fruit fresh weight	Fruit in plant	Shoot dry weight	Mycorrhizal dependency	Stem diameter	Carbon mineralization
Replication	2	*	ns	ns	*	ns	ns	ns
WormPeat (WP)	2	**	*	ns	**	ns	*	**
Mycorrhiza (Myco)	1	ns	ns	ns	**	**	ns	ns
WP × Myco	2	ns	ns	ns	ns	ns	*	ns
Biochar	1	ns	ns	ns	*	ns	*	*
WP × Biochar	2	ns	ns	ns	*	**	**	*
Myco × Biochar	1	ns	ns	ns	ns	ns	ns	**
WP × Myco × Biochar	2	ns	ns	ns	ns	**	ns	ns
Error	22	420	92.4	17.9	663.6	1417	11.9	2.05
CV (%)	-	4.37	2.05	0.30	5.49	8.02	0.74	1.68

ns, non-significant; *, significant at P≤0.05; **, significant at P≤0.01.

CO_2 efflux emission from the potting soil, as an indicator of soil biological activity, was evaluated after plants harvested using closed chamber incubation method described by Hopkins (2008). Small plastic cup which contained 20 g soil of each treatment, one vial containing 10 mL of 2*M* NaOH and 10 mL distilled water put into the incubation jars with gastight lids (Mason Jar). The jars then incubated in a dark room with 25°C for 10 days. The vials of NaOH removed from the jars, 1 mL of NaOH (CO_2 trapped in the NaOH during the incubation period) titrated with 0.02*N* H_2SO_4 using a burette with three drops of phenolphthalein as pH indicator after addition of 200 µL of 1*M* $BaCl_2$ and 6.1 mL of 0.5*N* of HCl. Results are expressed in µM C/g soil/h.

Results

Analysis of variance showed the significant effect of worm casting × peat combination on plant height, fruit dry weight, stem dry weight, stem diameter and carbon mineralization (Table 2). Mycorrhizal symbiosis had positive and significant effect on stem dry weight and mycorrhizal dependency. Biochar had also significant and positive effect on stem dry weight, stem diameter and carbon mineralization.

Application of worm casting × peat affected plant height. The highest plant height (64.3 cm) was achieved in 15% worm casting × 85% peat and the lowest plant height (58.7 cm) was achieved in 100% peat treatment (Fig. IA). Like plant height, a similar trend was observed for fruit fresh weight; the highest fruit fresh weight (228.7 g/plant) was achieved in 15% worm casting × 85% peat and the lowest fruit fresh weight (175.1 g/plant) was achieved in 100% peat treatment (Fig. IB).

According to the results of mean comparison, shoot dry weight was the highest in 15% worm casting × 85% peat (35.8 g/plant) and also in 30% worm casting × 70% peat (35.2 g/plant). The lowest value of this trait (28.6 g/plant) was observed in 100% peat treatment (Fig. IIA). The effect of worm casting × peat combination and mycorrhizal symbiosis on shoot dry weight is presented in Fig. IIB. In all combinations of worm casting × peat, shoot dry

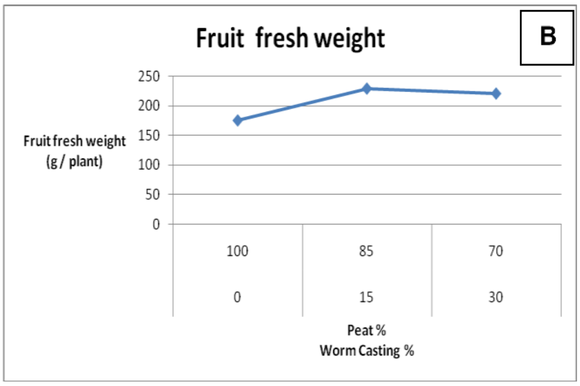

Fig. I. Effect of combinations of worm casting × peat on plant height (A) and fruit fresh weight (B).

weight was higher when inoculated with mycorrhiza. The highest value of this trait was achieved in 15% worm casting × 85% peat × mycorrhizal inoculation (39.8 g/plant) and the lowest value was observed in 100% peat without the inoculation (25.4 g/plant).

In contrast with mycorrhizal symbiosis, application of biochar reduced shoot dry weight. The reduction of shoot dry weight as the result of biochar application was higher in 15% worm casting × 85% peat treatment. The highest shoot dry weight (41.2 g/plant) was achieved in 15% worm casting × 85% peat without biochar application (Fig. II. C). Mycorrhizal dependency increased when the percentage of peat increased; it was the highest (25.98%) in 30% worm casting × 70% peat and the lowest (18.2%) in 100% worm casting treatment (Fig. III. A). The effect of biochar on mycorrhizal dependency was different among the three worm casting / peat ratios. Application of biochar in 100% peat treatment reduced

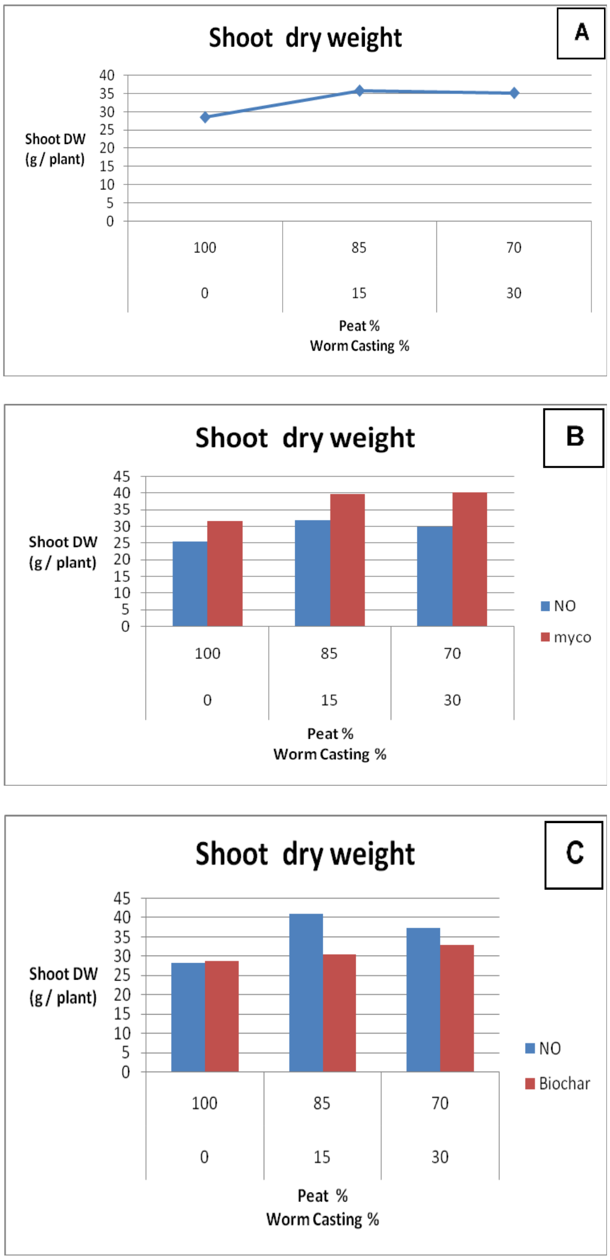

Fig. II. Response of shoot dry weight to combinations of worm casting × peat (A), worm casting × peat × mycorrhizal symbiosis (B) and worm casting × peat × biochar (C)

mycorrhizal dependency; however, it increased mycorrhizal dependency in 15% worm casting × 85% peat and 30% worm casting × 70% peat. The highest value (18.16%) of this trait was achieved in 30% worm casting × 70% peat × biochar (Fig. IIIB).

Stem diameter was affected by the combinations of worm casting × peat; it was the highest (11.98 mm) in both 15% worm casting × 85% peat and 30% worm casting × 70% peat and

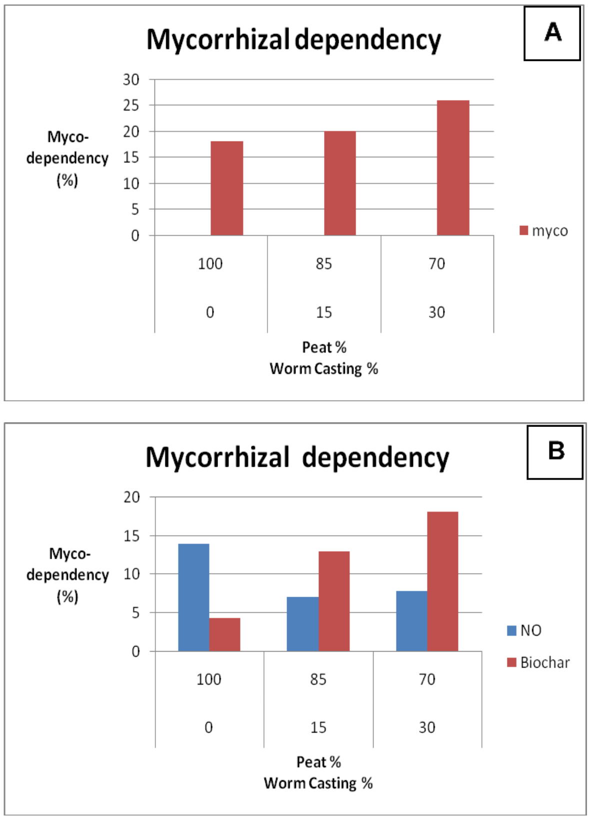

Fig. III. Response of mycorrhizal dependency to combinations of worm casting × peat × mycorrhizal symbiosis (A) and worm casting × peat × biochar (B)

the lowest (11.03) in 100 worm casting treatment (Fig. IVA). Mycorrhizal symbiosis had different effects on stem diameter in different worm casting / peat ratios. Although the symbiosis reduced stem diameter from 11.63 mm to 10.42 mm in 100% peat treatment; however, it increased stem diameter from 11.86 mm to 12.1 mm in 15% worm casting × 85% peat. The effect of mycorrhizal symbiosis on stem diameter in 30% worm casting × 70 × peat was not significant (Fig. IVB). Biochar application also reduced stem diameter in 100% peat and 15% worm casting × 85% peat treatment; however, it increased stem diameter by 5.31% in 30% worm casting × 70% peat (Fig. IV. C).

Mycorrhizal symbiosis had no significant increasing effect on carbon mineralization in 15% worm casting × 85% peat; however, it reduced this trait in other worm casting / peat combinations (Fig. VA). Biochar application increased carbon mineralization in 100% peat (from 0.157 to 0.158 µmol C/g soil/h) and in 30% worm casting × 70%

peat (from 0.159 to 0.162 µmol C/g soil/h); it slightly reduced carbon mineralization in 15% worm casting × 85% peat (Fig. V. B). Results also indicated that in plants inoculated with mycorrhiza, biochar application reduced carbon mineralization; however, in non-inoculated plants, biochar application increased carbon mineralization (Fig. V. C).

Discussion

Results showed that the combination of worm casting × peat in the growing media increased plant height, shoot weight and fruit weight compared with the 100% worm casting treatment. Atiyeh et al. (2000) studied tomato plants in Metro-Mix 360 growing medium in combination with pig manure based vermicompost and sphagnum peat and reported that the shortest seedlings were observed in the medium containing 100% vermicompost and the tallest seedlings were observed in the medium containing a small proportion of vermicompost. They concluded that incorporating high rate of vermicompost in growing medium reduces the aeration and porosity of the medium, increases the concentration of salts, induces heavy metals toxicity and maybe increases the concentration of phytotoxic substances in the growing medium. They also attributed the improved tomato growth in low vermicompost proportion to the improved physical conditions of growing medium, enhanced enzymatic activity, synergistic effects on microorganisms activity and production of plant growth promoting compounds such as the phytohormones (Atiyeh et al. 2000; Atiyeh et al. 2001). Ali et al. (2007) tested the effects of vermicompost and green waste compost on lettuce and found that fresh weight increased in growing medium containing both vermicompost and green waste compost compared with the growing medium containing only the vermicompost.

Hidalgo and Harkess (2002) observed the significant effect of worm casting in growing medium on poinsettia (*Euphorbia pulcherrima*) dry weight. They reported that increasing the proportion of peat in growing medium combination resulted in further enhancement of plant dry weight. In their experiment, the lowest

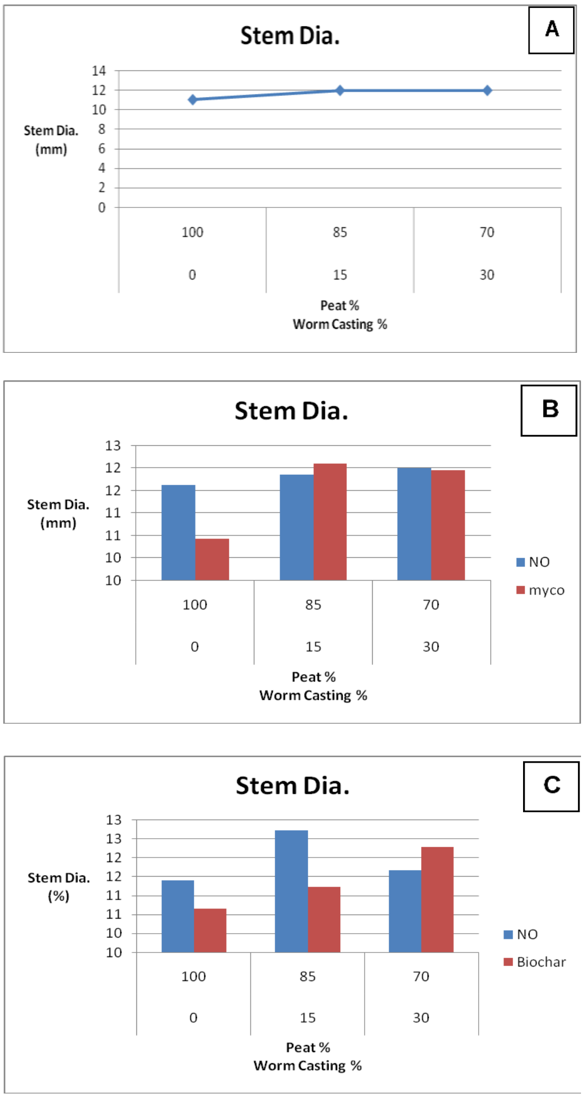

Fig. IV. Response of stem diameter to combinations of worm casting × peat (A), worm casting × peat × mycorrhizal symbiosis (B) and worm casting × peat × biochar (C).

fresh weight was obtained in growing medium containing only peat or only perlite. Atiyeh et al. (2002) studied shoot dry weight of tomato plants in different combinations of growing medium and found that shoot dry weight reduced when the proportion of vermicompost increased. They also found that shoot dry weight was higher in all vermicompost containing treatments compared with the control (vermicompost free Metro-Mix 360 medium). Different results were found in the experiments of Hidalgo et al. (2006) who reported that stem diameter of marigold (*Tagetes*) was higher in growing medium containing only worm casting compared with the medium containing a combination of worm casting and peat moss.

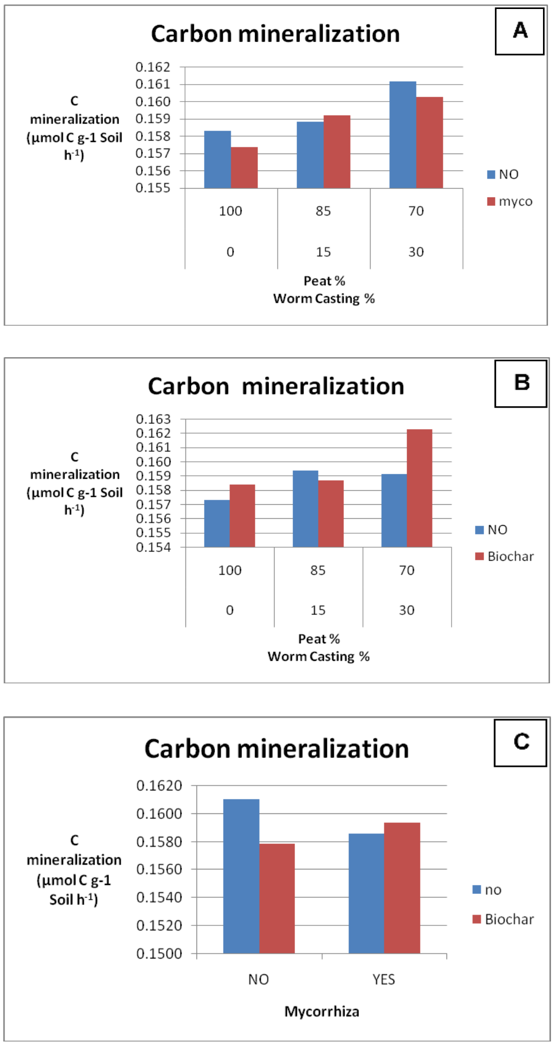

Fig. V : Response of carbon mineralization to combinations of worm casting × peat × mycorrhizal symbiosis (A), worm casting × peat × biochar (B) and biochar × mycorrhizal symbiosis (C).

Results of our experiment show that absence of worm casting in growing medium reduced the value of all measured traits; a worm casting free medium which contains only peat cannot support plant requirements.

Results indicated the effect of AM fungi on growth parameters of tomato. AM fungi are an important component of integrated nutrient management in cost effective sustainable farming systems. Mycorrhizal symbiosis plays an important role in growth and yield improvement of the host plant (Hooker & Black 1995; Miller & Jastrow 2000). Ardakani et al. (2011) reported that inoculating wheat seeds with mycorrhiza (*Glomus intraradices*) significantly increased NPK absorption compared with the non-inoculated control. Salvioli et al. (2008) studied the effect of mycorrhizal symbiosis (*Glomus mosseae*) on tomato plants and reported an increased root and shoot weight, P uptake and fruit production. Mycorrhizal inoculation also increased growth and dry weight of maize and soybean plants compared with the non-inoculated control (Jeong et al. 2006). Nzanza et al. (2012) also reported that inoculating tomato plants with *G. mosseae* increased dry shoot weight by 11% and total plant biomass by 9%.

Results of this experiment indicated the significant effect of biochar on some studied parameters. The effect of biochar application in growing media is well documented. However, the exact mechanisms by which biochar affects plants is not well understood. Biochar application alters soil physical, chemical and biological properties and consequently affecting nutrients absorption and plant growth (Atkinson et al. 2010). Quilliam et al. (2012) found the significant effect of repeated biochar application on soil quality and plant nutrient absorption; although the main function of biochar is the sequestration of carbon. They reported that biochar application increased P, K, Ca, soil moisture, organic matter, EC and mycorrhizal root colonization and reduced saprophytic fungal growth. However, they concluded that the benefits of biochar are transient and repeated application is required. Schulz and Glaser (2012) found that adding biochar to growing medium significantly increased plant growth. They reported that biochar had synergistic effects when applied in combination with mineral fertilizers or different types of composts. The effects of biochar on plants growth is attributed to its high nutrient holding capacity (Glaser et al. 2002). The reduction of plant growth as the result of biochar application mainly happens when it is applied without any other type of organic materials such as vermicompost (Gundale & DeLuca 2006; Gaskin et al. 2010).

Biochar application affects the relation of plant and AM fungi. Results of our experiment showed that application of biochar increased mycorrhizal dependency in worm casting × peat treatments but reduced it in 100% peat treatment. This may be attributed to the adverse effect of biochar on plant nutrient absorption in some cases. LeCroy et al. (2013) tested the effect of biochar, mycorrhiza and chemical Nitrogen fertilizer on sorghum (*Sorghum bicolour* L.) seedling growth and reported that biochar, AM fungi and high nitrogen dose reduced above ground biomass by 42% compared with mycorrhizae and high Nitrogen dose treatment. They concluded that presence of Nitrogen and biochar induces parasitism of the mycorrhizal fungus; reducing plant growth. They also observed that biochar application increased mycorrhizal colonization. Solaiman et al. (2010) reported that mycorrhizal colonization rate and root length increased when biochar was applied to a wheat field.

Results of this experiment indicated that application of worm casting × peat and application of biochar affected carbon mineralization, or on other hand, soil respiration and biological activity. It is generally accepted that soil biological activity is related to carbon mineralization (Fontaine et al., 2003). Liang et al., (1996) tested the effect of composted dairy manure application on soil carbon mineralization rate and observed an increasing effect of the amendment on C mineralization rate. Ouyang et al. (2014) found that biochar application increased soil enzyme activity because biochar increased available nutrients in soil and increased soil dissolved organic C and microbial activity. They also reported that there was a linear relationship between soil enzymes and C mineralization rate. The enhancement of C mineralization as the result of application of organic amendments was also observed in the experiment conducted by Watts et al. (2010). They tested the effect of tillage systems, poultry litter and inorganic fertilizer application in soil and observed that long term application of poultry litter increased C mineralization more than inorganic fertilizer.

Conclusion

The combination of 15% worm casting × 85% peat along with mycorrhiza and biochar application increased growth parameters of tomato in the controlled environment. The 100% peat treatment reduced plant height and shoot weight. Biochar application improved physical conditions of the growing medium and increased carbon mineralization. Our findings suggest that worm casting, peat, mycorrhiza and biochar improve soil biological properties, enhance the availability of nutrients to plant root and consequently increase plant growth and yield. Therefore, worm casting can be an alternative and efficient nutrient source for organic tomato production in greenhouse.

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