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Effect of Biochar on the Abundance of Soil Bacteria

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Authors' contributions

This work was carried out in collaboration between both authors. Author SMIH designed the study, and guided the author TFK who performed the laboratory analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SMIH revised the manuscript before submission. Both the authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

A pot experiment was conducted to study the effect of biochar on the abundance of soil bacteria and compare it with the source biomass. Seven different treatments and a control were used in the experimental set-up. Three different types of biomass were selected and three types of biochar were produced from them. Both the materials were applied to the soil at a rate of 5t/ha. All treatments were incubated for 30, 60 and 90 days. Cultural, microscopic and biochemical tests were carried out to identify the bacterial isolates in soils treated with biochar and its source biomass. Bacterial isolates identified in soil and in some of the biomasses before treatments were applied include *Bacillus badius, Bacillus krulwichiae, Bacillus siralis, Bacillus sylvestris, Bacillus flexus, Aneurinibacillus aneurinilyticus* and *Bacillus thuringiensis* while after incubation periods, seven new isolates were identified. This was true for the biomass treated soils where additional one to two isolates reappeared. Conversely, in the biochar treated soils, most of the isolates disappeared except *Bacillus badius* that survived in all soils till 90 days. Because of its tolerant nature, it was further investigated for cellulase enzyme activity. Interestingly, the isolate did not show any such activity. Conclusively, biochar application may exert negative effect on the distribution and proliferation of soil bacteria with possible effect on soil quality and crop production.

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1. INTRODUCTION

Soil is one of the most important resources of the world. Thus, soil quality determines the nature and capacity of the ecosystem to support plant and animal life on earth. From ozone depletion and global warming to forest destruction and water pollution, the world's ecosystem is impacted by the processes carried out in soil. As a vital component of soil, organic matter determines much of its quality. A soil rich in organic matter is basically rich in quality. Generally, the mass of soil organic matter (SOM) ranges from 1 to 6% of the topsoil [1]. Furthermore, there is a significant correlation between soil quality and soil microbes. Soil microbes are considered as the soul of soil and it is one of the important factors of soil formation. The richness of soil is inseparable from the activities and development of organisms in it. The microbes are crucial in recycling of soil nutrients, decomposing organic matter, storing nutrients, improving soil properties, maintaining soil structure and above all conserving soil quality. Each gram of soil may contain up to 1,000,000,000 or more microbes. As such, for their survival and proliferation microbes need constant supply of organic matter in the form of biomass [2].

Over the last few years, a charred biomass, usually referred to as biochar, has gained much recognition as an effective tool for enhancing soil health. The emerging world of biochar and the entire buzz about its various positive qualities ranges from long term carbon sequestration to increased food security as it is said to be beneficial for agricultural crop production, managing harmful waste, storing soil carbon and combating vulnerable climate change. However, a big disagreement still exists about the potential effects of biochar. The responses of soil microbes due to biochar addition thus need to be investigated. It is imperative to find out whether it is equally good compared to that of biomass. As a part of this approach, bacteria were identified in biomass and biochars treated soils by a number of standard procedures after each incubation periods.

The present research aimed to assess the effects of biochar on soil bacterial abundance leading to soil microbial health (soil bacterial flora and subsequently soil health).

2. MATERIALS AND METHODS

2.1 Sampling Site

A local vegetable field in Jagir Dighulia in Atigram union of Manikganj District in Bangladesh was selected for soil sampling. The georeference of the sampling spot is 23°51.88 N and 90°06.219 E. The soil belongs to the Melandaha soil series; the USDA family code of the soil is Loamy, mixed, non acid, hyperthermic; USDA soil taxonomy code is Typic Endoaquents [3] and the FAO-UNESCO legend is Gleysol (Eutric Gleysol).

2.2 Collection, Preparation and Processing of Soil Sample

The bulk soil sample representing 0-15cm depth from surface was collected by the composite sampling method as suggested by the United States Department of Agriculture [4]. The soil sample was processed following standard procedure as described in [5]. The collected soil sample was air-dried (at ~40°C) after being transported to the laboratory. Soil sample containing the larger and massive aggregates were ground by gently crushing with a

wooden hammer. Ground soil sample was passed through a 2mm stainless steel sieve. The sieved soil sample was then mixed thoroughly and stored in labeled plastic containers for required physical analyses. Likewise, another portion of the soil sample was further ground, passed through a 0.5mm sieve, then mixed thoroughly and finally stored for various chemical and physico-chemical analyses.

2.3 Collection and Processing of Biomass Samples

Three different types of biomass (rice husk, straw and saw dust) samples were collected for producing three different types of biochar. Rice husk biomass was collected from a local rice mills, straw was collected from the local farmers and saw dust was collected from a local timber and saw mill. All biomass samples were oven dried (at 30-35℃) for one day. The straw was cut down into small pieces and ground. All these samples were sieved separately through a 0.25mm sieve and preserved in plastic containers.

2.4 Production and Processing of Biochar

A big earthen pot was taken and metal wires were arranged in a criss-cross arrangement over this pot so that the wires can support smaller pots. Individual biomass was placed in layers in the smaller earthen pots. These pots were covered with earthen lids. 4-5 such smaller pots were placed on the wire arrangement in such a way that pots were uniformly heated from all sides. The fire was enforced time to time by adding wood chips and kerosene oil. Fire was stopped after about an hour, when the biomass turned to biochar. The biochar thus formed was allowed to cool down in the pots with the lids on. Following the cooling of biochar it was sieved through a 0.25mm sieve and then stored in plastic jars.

2.5 Experimental Setup to Observe Changes on Soil Bacteria in Biomass and Biochar

To observe the impact of biomass and biochar on soil bacteria, an *n vitro* pot experiment was carried out in the Department of Soil, Water and Environment, University of Dhaka, Bangladesh. Microbiological studies were performed in the Industrial Microbiology Laboratory, Bangladesh Council of Scientific and Industrial Research (BCSIR).

For incubation study, 21 plastic containers were used. The containers were washed thoroughly and dried at the ambient temperature of the laboratory (~28℃). Biomass (or biochar) was added to soil at the rate of 5t/ha and the treated soils were potted in the plastic containers. Each pot was labeled in accordance with the treatments and was incubated at three different periods *viz.,* 30, 60 and 90 days. The seven treatments were designated as C (control), BM1 (soil + biomass 1- rice husk), BM2 (soil + biomass 2 - straw), BM3 (soil + biomass 3 - saw dust), BC1 (soil + biochar 1 - rice husk), BC2 (soil + biochar 2 - straw), BC3 (soil + biochar 3 - saw dust). Sterilized distilled water was added to the soil to maintain a moist field condition.

2.6 Microbiological Analysis

At the end of each incubation period, soil was collected from each pot and it was subjected to serial dilution to identify the bacteria present in each treatment. It was done both before and after addition of treatments to make a comparative study. All experiments were conducted under aseptic condition.

2.7 Culture and Isolation of Bacteria

Soil samples and 0.85% NaCl solution were mixed in 1:10 ratio (10^{-1} dilution) and from that 1ml solution was transferred to McCartney bottle (10^{-2}) which was further diluted up to 10^{-8}. Each bottle contained 9 ml NaCl solution. From each of the dilutions, 1 ml was placed in the corresponding individual Petri dishes and hot Plate Count Agar (PCA) was poured in the dishes simultaneously, followed by clockwise and anti-clockwise rotations to ensure proper mixing. Once the media had solidified, the dishes were kept in incubator (at 37℃) for 24 hours for the bacteria to grow.

Single bacterial colony was selected randomly and was isolated by streaking on agar plates, followed by incubating at 37℃ for 24 hours (1^{st} sub-culture). These isolates were tested for morphological parameters. 2^{nd} subculture was made on agar slants and verified for their homogeneity until a single isolate was assured, by comparing colony morphological properties *viz.* color, shape, size, elevation, surface, margin, pigmentation and transparency. The isolates were then subjected to biochemical tests for identifying the bacteria.

2.8 Identification of Bacterial Isolates

Along with several biochemical tests, gram and spore staining were done to identify the bacterial strains as described by Cappuccino and Sherman [6]. Biochemical tests included Indole, MR-VP (Methyl Red and Voges Proskauer), Nitrate reduction, Catalase, Oxidase, TSIA (Triple Sugar Iron Agar), KIA (Kligler Iron Agar), LIA (Lysine Iron Agar), Citrate utilization and Carbohydrate fermentation. Following the colony morphology, staining and biochemical tests, isolates of bacteria were identified according to the “Bergey’s Manual of Determinative Bacteriology” [7].

2.9 Cellulase Enzyme Activity

Cellulase enzyme activity was tested to test the cellulose degradation capacity of *Bacillus badius* - the longest surviving bacteria. The assay was done using two different methods (Congo red and DNS), for the isolates of C, M1 and C1 at 30 days of incubation.

A preliminary quantitative analysis (screening) for cellulolytic activity was conducted by the Congo red method as described by Samira et al. [8]. Screening was made on Carboxymethyl cellulose (CMC) agar plates which were then incubated at 37℃ for 3 days. After that, the plates were flooded with 1% Congo red which was poured off and were further flooded with 1M NaCl for 15 minutes. Cellulolytic activity in broth culture was further ascertained by 3, 5-dinitrosalicylic acid (DNS). Sterile production media were prepared in conical flasks and incubated (37℃) at 140rpm for 3 days. One flask contained the CMC and bacterial inoculation while in another flask only CMC was placed. After incubation, 5ml broth was centrifuged (at 1200rpm for 15minutes) from which 1ml broth was taken to test tubes and 3 ml DNS reagent was added to it. The tubes were boiled and allowed to cool.

3. RESULTS AND DISCUSSION

Following streaking and incubating, single bacterial colony was isolated. After that, isolates of bacteria were identified both before and after the incubation periods from the results obtained from colony morphology, gram staining, spore staining, and biochemical characteristics following the procedure as described in “Bergey’s Manual” [7]. Seven different isolates were

identified in the initial materials (Table 1).The soil, rice husk biomass, straw biomass and saw dust biomass possessed 3, 1, 2 and 2 isolates respectively.

Table 1. Bacterial isolates identified in the initial materials

Isolate No.	Name of Sample	Name of isolate
1	Soil	*Bacillus badius*
2	Soil	*Bacillus krulwichiae*
3	Soil	*Bacillus siralis*
4	Biomass-Rice husk	*Bacillus sylvestris*
5	Biomass-Straw & Saw dust	*Bacillus flexus*
6	Biomass-Straw	*Aneurinibacillus aneurinilyticus*
7	Biomass-Saw dust	*Bacillus thuringiensis*

At the end of each incubation periods, soil was collected from each pot and bacteria present in the treatments were identified by the processes described above. It is interesting to note that after the incubation periods, seven new isolates (*Bacillus subtilis, Paenibacillus apiaries, Bacillus alvei, Bacillus sphaericus, Bacillus aneurinilyticus, Xanthomonas campestris* and *Bacillus bataviensis*) appeared along with the old ones.

Results of the bacterial isolates identified in the control, biomass and biochar treated soils at incubation times of 30, 60 and 90 days are presented in Tables 2, 3 and 4.

Table 2. Bacterial isolates identified at incubation of 30 days

Control	M1	C1	M2	C2	M3	C3
Bacillus badius	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*
Bacillus krulwichiae	*Bacillus krulwichiae*	*Bacillus krulwichiae*	*Bacillus krulwichiae*		*Bacillus krulwichiae*	
Bacillus siralis	*Bacillus siralis*	*Bacillus siralis*	*Bacillus siralis*		*Bacillus siralis*	
Bacillus sylvestris	*Bacillus sylvestris*		*Bacillus flexus*		*Bacillus thuringiensis*	
	Bacillus subtilis		*Aneurinibacillus aneurinilyticus*		*Bacillus flexus*	
			Bacillus sylvestris		*Bacillus subtilis*	

Table 3. Bacterial isolates identified at incubation of 60 days

Control	M1	C1	M2	C2	M3	C3
Bacillus badius	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*
Bacillus krulwichiae	*Bacillus krulwichiae*	*Bacillus krulwichiae*	*Bacillus krulwichiae*		*Bacillus krulwichiae*	
Bacillus siralis	*Bacillus siralis*		*Bacillus siralis*		*Bacillus siralis*	
Bacillus alvei	*Bacillus sylvestris*		*Bacillus flexus*		*Bacillus thuringiensis*	
	Paenibacillus apiarius		*Aneurinibacillus aneurinilyticus*		*Bacillus flexus*	
			Bacillus alvei		*Bacillus sphaericus*	

Table 4. Bacterial isolates identified at incubation of 90 days

Control	M1	C1	M2	C2	M3	C3
Bacillus badius	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*	*Bacillus badius*
Bacillus krulwichiae	*Bacillus krulwichiae*	*Bacillus krulwichiae*	*Bacillus krulwichiae*		*Bacillus krulwichiae*	
Bacillus siralis	*Bacillus siralis*		*Bacillus siralis*		*Bacillus siralis*	
Bacillus bataviensis	*Bacillus sylvestris*		*Bacillus flexus*		*Bacillus thuringiensis*	
	Paenibacillus apiarius		*Aneurinibacillus aneurinilyticus*		*Bacillus flexus*	
	Bacillus aneurinilyticus		*Xanthomona scampestris*		*Bacillus bataviensis*	

It is observed that before addition of any treatment, only 3 isolates *viz., Bacillus badius***,** *Bacillus krulwichiae* and *Bacillus siralis* showed prominence in soil. After incubation for 30 days, the untreated soil contained all three bacteria and an additional bacteria, *Bacillus sylvestris*. This additional bacterium might have appeared from the soil itself possibly because of a favorable condition. The biomass rice husk, rice straw and saw dust contained *Bacillus sylvestris*; *Bacillus flexus* and *Aneurinibacillus aneurinilyticus*; *Bacillus thuringiensis* and *Bacillus flexus*; respectively. After adding biomasses and biochars to the soil, some new isolates appeared depending on the source of organic matter. A new isolate *viz*., *Bacillus subtilis* appeared in addition to the four bacterial isolates (*Bacillus badius*, *Bacillus krulwichiae*, *Bacillus siralis* and *Bacillus sylvestris*) in soils treated with rice husk (M1). The fact could be that biomass created favorable conditions for the new bacteria to grow; as well as the bacteria already existed in soil were capable to survive as the biomass did not exert any negative effects on these bacteria. Jangid et al. [9] showed that adding organic amendments results in increased bacterial population; and the nutrients in biomass increase bacterial respiration rates by 2-3 times. Conversely, no new isolate was found in soil treated with biochar of this material (C1) although most of the isolates of soil survived, except for the *Bacillus sylvestris*. It could be said that, C1 had neither antagonistic effect on soil bacteria nor had the ability to create new bacterial growth. Due to conversion of biomass to biochar by pyrolysis, phosphorus, sulphur and potassium are lost in large quantities and are consequently decreased for bacterial utilization. Harmful organic compounds are produced during pyrolysis and hence ready carbon is not easily accessible to the bacteria. Graber et al. [10] found that except some resistant bacteria, most bacteria die in course of time in presence of char but the reason was not clearly defined. They mentioned that chitinase, aminopeptidase and phosphatase enzyme activities were drastically reduced when char was added to soil.

In case of soils treated with straw (M2), two new isolates *viz*., *Bacillus flexus* and *Aneurinibacillus aneurinilyticus* appeared in addition to the already identified four bacterial isolates (*Bacillus badius*, *Bacillus krulwichiae*, *Bacillus siralis* and *Bacillus sylvestris*). These isolates thrived due to the positive effects of biomass which might be for organic carbon accumulation from biomass that ultimately resulted in bacterial abundance, improved microbial community and increased functional diversity. Jangid et al. [9] demonstrated a positive linear regression relating bacterial enzyme activity to the soil organic matter availability. These authors also observed that soil nitrogen regulates the competitive interaction of soil bacteria. Only *Bacillus badius* continued to thrive in the soils treated with

straw derived biochar (C2) but the other bacteria disappeared due to detrimental effects of char.

In the soils treated with saw dust (M3), most of the isolates (*Bacillus badius***,** *Bacillus krulwichiae* and *Bacillus siralis*) survived except one (*Bacillus sylvestris*). Moreover, three new isolates *viz., Bacillus thuringiensis*, *Bacillus flexus and Bacillus subtilis* proliferated. Yet only *Bacillus badius* was able to continue in the soils treated with straw and saw dust derived chars (C2 and C3).

Likewise, after 60 days of incubation, the untreated soil contained all three isolates of the initial soil and an extra isolate, *Bacillus alvei*. Except for the *Bacillus alvei*, rest of the isolates survived in the soils treated with rice husk (M1) along with the newly appeared isolates (*Bacillus sylvestris* and *Paenibacillus apiaries*). In case of biochar (C1), only *Bacillus badius and Bacillus krulwichiae* continued*.* All of the isolates of untreated soil survived in the straw treated soil along with the newly appeared *Bacillus flexus* and *Aneurinibacillus aneurinilyticus.* Like M1, similar occurrence was observed in case of saw dust treated soil (M3). All isolates of soil survived except *Bacillus alvei.* There was also three newly emerged isolates *viz., Bacillus thuringiensis*, *Bacillus flexus,* and *Bacillus sphaericus.* On the contrary, *Bacillus badius* sustained alone in both the char treated soils (C2 and C3). Biomass additions can have indirect long-term effects on soil bacteria by causing changes in pH, physical properties and organic matter of soil. Furthermore, survival of bacteria depends on sufficient moisture in the soil. McElligott [11] reported that improvements in soil water retention by char are only expected in coarse-textured soils or soils with large amounts of macro pores. In sandy soil, char increased available moisture by 18% but, no changes were observed in loamy soil, and soil available moisture decreased by 45% in clayey soil. Homogeneous char with or without N could stimulate loss of soil organic carbon by 8-13% in both agricultural and forest soils. All of these changes in soil adversely affect soil bacterial communities. Moreover, Samdhu and Bawa [12] revealed that char may reduce supply of bacterial available nutrients except a very small fraction of nutrients are retained in char in a potentially extractable form. De Luca et al. [13] showed that some properties of char discourage soil bacterial communities and create microenvironments that is not suitable for colonization at all which lead to reduced proliferation and metabolic efficiency of bacteria amended with maize-derived biochar.

After incubation for 90 days, the untreated soil contained all of the isolates and an additional bacteria, *Bacillus bataviensis.* In the context of soil treated with rice husk (M1), except the *Bacillus bataviensis* all the isolates proliferated along with the three newly appeared isolates (*Bacillus sylvestris, Bacillus subtilis* and, *Bacillus aneurinilyticus*). In case of char treated soil, two isolates *viz., Bacillus badius* and *Bacillus krulwichiae* were able to thrive and the others disappeared. Similar occurrence happened with the straw treated soils. Except for the *Bacillus bataviensis,* all isolates survived with three newly developed isolates (*Bacillus flexus*, *Aneurinibacillus aneurinilyticus and Xanthomonas campestris*). In the soil treated with saw dust (M3), two new isolates (*Bacillus flexus* and *Bacillus thuringiensis*) appeared in addition to the four isolates. Again in both char treated soils (C2 and C3), only *Bacillus badius* existed.

The temperature of the pyrolysis process exerts negative effects on physical and chemical attributes of the char produced which adversely affect soil bacteria. Gaur and Adholeya [14] revealed that total C and N in char does not reflect the actual availability of these elements for microbes to cause immobilization, as char absorbs organic molecules from soil environment.

3.1 Cellulase Enzyme Activity of *Bacillus badius*

In the untreated as well as in the treated soils, a bacterium *viz*., *Bacillus badius* consistently appeared before and after incubation at different days. It appeared that this was the most resilient among all the bacteria found in the present experiment. The assay was done in two different ways for the isolate from 30 days of incubation for C, M1 and C1. No clear zone was observed around the bacterial colonies following inoculation of CMC plates and flooding with Congo red and NaCl. In case of DNS method, the color of the medium was observed after incubation, centrifugation and boiling. The color remained unchanged. Thus, it became apparent that *Bacillus badius* did not have any cellulase activity in the selected treatments *viz*. C, M1 and C1 at 30 days.

The reason could be that, *Bacillus badius* was able to degrade compounds more complex than cellulose. It might be able to extract nutrients in presence of biochar. Samdhu and Bawa [12] reported that cellulose-hydrolyzing bacteria are not able to survive in presence of corncob char though they survive in case of other char. They mentioned that durability of *Bacillus badius* is due to endospore formation and this is triggered when there is depletion of essential nutrients. This bacterium might be able to secrete large quantities of enzymes and hydrolyze complex foods even in the harsh environment.

4. CONCLUSION

Since microbes are the heart of soil, it provides the substratum for soil health and thus soil quality. For the survival and growth of these microbes, organic carbon source is a must. Organic matter (biomass) serves as the source of carbon for the microbes. Although biochar is one of the burning questions of today's world, it is not as effective as the biomass with respect to soil quality. Except the resistant ones, soil bacteria were not able to survive in presence of char due to its antagonistic effects which occurred due to nutrient deficiency, decreased sorption of enzymes as well as binding of essential enzymes. Though produced from biomass, the biochar exerted a negative effect on the abundance and proliferation of soil bacteria. It might be for relative stability, pH and physical properties of biochar; general lack of energy; and loss of readily utilizable carbon sources. Source of biochar is also an important factor which needs to be pondered before using it in agricultural soils.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Troeh FR, Thompson LM. Soils and Soil Fertility. 6th ed. Wiley-Blackwell Publishers, Lowa, Ames; 2005.
2. Mauseth JD. Botany: An Introduction to Plant Biology. Jones & Bartlett Publishers, pp.495.Burlington, Massachusetts; 2012.
3. Imamul Huq SM, Shoaib JU. The Soils of Bangladesh, ISBN-978-94-007-1127-3;97894-007-1128-0(e-Book). Springer Dordrecht Heidelberg. New York, London. 2013;165.
4. USDA (United States Department of Agriculture) Soil Survey Manual. Soil Survey Staff, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Washington, US. 1951;205.
5. Imamul Huq SM, Alam MD. A Handbook on Analyses of Soil, Plant, and Water. BACER-DU, University of Dhaka. Dhaka, Bangladesh. 2005;43-50.
6. Cappuccino JG, Sherman N. Microbiology: A Laboratory Manual. 7th ed. Pearson Education India. 2007;143-193.
7. Bergey DH. Bergey's Manual of Determinative Bacteriology. Wilkins and Wilkins Publishers, Baltimore. United States. 1957;353-413.
8. Samira M, Mohammad R, Gholamreza G. Carboxymethyl Cellulase and Filter Paperase Activity of New Strains Isolated from Persian Gulf. J. Microbiol. 2011;1(1):8-16.
9. Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS. Relative Impacts of Land Use, Management Intensity and Fertilization upon Soil Microbial Community Structure in Agricultural Systems. Soil Biol. Biochem. 2008;40:2843-2853.
10. Graber ER, Meller HY, Kolton M, Cytryn E, Silber A, Rav DD, Tsechansky L, Borenshtein M, Elad Y. Biochar Impact on Development and Productivity of Pepper and Tomato Grown in Fertigated Soilless Media. Plant & Soil. 2010;337(1):481-496.
11. McElligott KM. Biochar Amendments to Forest Soils: Effects on Soil Properties and Tree Growth. MS thesis, University of Idaho, Idaho, US. 2011;10-18.
12. Samdhu DK, Bawa S. Improvement of Cellulase Activity in Trichoderma. Appl. Biochem. Biotechnol. 1992;34-35(1):175–192.
13. DeLuca TH, MacKenzie MD, Gundale MJ. Biochar Effects on Soil Nutrient Transformation. In: Biochar for Environmental Management: Science and Technology. (eds. Lehmann, J. and Joseph, S.) Earthscan, UK. 2009;251–270.
14. Gaur A, Adholeya A. Effects of the Particle Size of Soil-less Substrates upon AM Fungus Inoculum Production. *Mycorrhiza.* 2000;10(1):43–48.

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