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Potential for Biochar as an Alternate Carrier to Peat Moss for the Preparation of *Rhizobia* Bio Inoculum

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Carrier based preparations of *Rizobium* inoculant, developed using two different rice biochar preparations (biochar alone and biochar- vermiculite 50:50) were evaluated in comparison with peatmoss based carrier (peatmoss: vermiculite 50:50) for their suitability as a best alternate of peatmoss for commercial production of *Rizobia*. The three different carriers were evaluated over a period of 180 days for its moisture content, pH, survival of the microbial inoculant and respiration rate. At the end of storing period (180 days) different carrier materials were used to inoculate kidney bean seeds in a pot experiment conducted in sandy soil to ensure the viability of survived *Rizobia* in different carrier preparations. In addition, the ability of carrier materials to ameliorate the antimicrobial effect of seed diffusate was also assessed. Among the different carriers, bio char based carrier recorded a maximum population of log 9.98 cfu g^{-1} of carrier on 180 days after inoculation with a maximum moisture content of 20%. A slight decline in pH was recorded at the end of storing period. It was also found that kidney bean seeds inoculated with bio char based inoculant gave the highest nodule dry weight, plant dry weight, plant height. In addition, biochar was found to ameliorate the antimicrobial effect of *kidney bean* seed diffusate.

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1. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are widely used as biofertilizers to enhance crops productivity [1,2]. One of the most serious challenges facing the development of commercial biofertilizers is ensuring a consistent survival rates of the inoculum. Carrier materials are a limiting factor in success of inoculation process by providing protective niche or microenvironment for microbe and also by enhancing soil structure, perhaps making it more suitable for microbial colonization [3].

The production of bio-inoculant in Egypt depend mainly nowadays on beat moss and vermiculite mixture as a carrier. Beat moss is not an Egyptian product and imported for several purposes including bio-inoculant manufacture. A trials for finding local and economically effected alternatives for beat moss is occurred including the use of compost and other agricultural residues. A good microbial carrier should possess essential characteristics, as the capacity to deliver the suitable number of viable microbial cells in vital physiological state at the right time [4]. 'Biochar' is a carbon rich product produced when burning organic matter like wood, grasses, manure or agricultural residues under conditions of low oxygen (pyrolysis) and it sounds more 'clean and green [5]. Biochar characterize by a porous structure in addition to high surface area enabling it to attract and retain water and nutrients, such as nitrogen and phosphorus. This is beneficial for growth of bacteria and fungi [6].

Another advantage for using biochar is its relative recalcitrance against microbial decay and thus on its slower return of terrestrial organic carbone as carbon dioxide (CO_2) to the atmosphere [5] hence it acts as an efficient carbon sequester.

In Egypt, there are greater prospects for the production of biochar, since natural agricultural legnocellulitic resedues like rice straw is available in plenty, most of it is burned under non controlled conditions causing environmental and health risk. Hence, there is an urgent demand to make the best use of these valuable resources that are rich in nutrients, which are available cheap in abundance in the form of biochar. Because of all the supporting benefits and efficient chance of the utility of biochar for bioinoculant preparation, the present study was proceeded to evaluate the potential of biochar as a carrier substrate for *Rhizobia* production as an alternate to beat which imported from abroad.

2. MATERIALS AND METHODS

2.1 Carrier Materials

Peat moss and vermiculite was kindly supplied by microbiology department, Soil, Water and environmental Research institute, sakha, Egypt. Biochar materials were prepared via slow pyrolysis in a 2128 cm3 steel cylinder within a 42 x 19 x 14 cm3 muffle furnace fitted with an inlet for N_2 gas (flow rate 0.5 LPM) and were left at the highest treatment temperature for 1-1.5 h (600°C). The rice straw used in this study was generated as waste products in rice farms of sakha research station production sector.

Carrier's pH was measured using a 1:5 carrier material to water ratio with a pH meter. Organic carbon was determined by the Walkley-Black procedure [7]. Moisture content, ash content, total carbon, total nitrogen, water holding capacity and bulk density were also determined.

2.2 Production of Carrier Based Inoculum

2.2.1 Preparation of broth

Yeast mannitol broth was inoculated with *Rhizobium phaseoli* in 250 ml Erlenmeyer flasks, incubated at 30°C for 72 hours in a shaker incubator at 100 rpm. The culture containing a bacterial population of about 25.6×10^{11} cells ml^{-1} was used for mixing with the carrier preparations.

2.2.2 Preparation of carrier materials

The carrier materials were dried, powdered and sieved through 150 µ sieve, neutralized using lime if acidic or HCl if alkaline and packed in opaque low density polypropylene bags with thickness of about 75 µ and then sterilized according to the procedure reported by [8].

2.2.3 Inoculation loading of bacterial culture in the sterile carrier preparations

Broth bacterial culture with a cell density of 25.6×10^{11} cells ml^{-1} in late log phase was inoculated in carrier preparations aseptically to obtain 35%

moisture content. The treatments which do not receive *Rhizobia* where moistened with sterilized broth of required quantity.

2.3 Analysis of the Carrier Based Inoculant

The inoculated pages were stored at room temperature and screened for viable cell respiration, cell population, moisture content and pH at 15 days interval up to six months of storage.

2.4 Measurement of Respiration Rate

Carrier respiration was estimated according to [9] as follow:

10 g of inoculated carrier was placed in 50 ml plastic tube, and then plastic tubes were fitted into a Duran bottle containing 25 ml 0.05 NaOH (prepared with CO_2 free distilled water) where the tubes were kept at the neck of the bottle. The same system but without carried was used as a blank and system with uninoculated carrier as control. Bottles were incubated for 72 h at room temperature. After incubation, plastic tubes were removed and 5 ml of (0.5 M) barium chloride was add to NaOH solution followed by a few drops of phenol phethalin, Mixture was titrated with (0.05 M) HCl with continuous stirring until red color turned to colorless.

The rate of respiration was calculated according to the following relationship:

$$CO_2 \text{ (mg) } CW/t = \frac{(V0-V)X\ 1.1}{d.wt}$$

CW is the amount of carrier dry weight in gram
t is incubation time (h)
V0 is volume of HCl titration of blank
V is volume of HCl titration of sample
d.wt is dry weight of 1 g moist carrier
Conversion factor (1 ml 0.05 M NaOH = 1.1 mg CO_2
CO_2 (mg) $10g^{-1}72h^{-1}$

Treatment details:

T1- Rice straw based biochar + Vermiculite + *Rhizobia*
T2- Rice straw based biochar + Vermiculite
T3- Peat moss + Vermiculite + *Rhizobia*
T4- Peat moss + Vermiculite
T5- Rice straw based biochar + *Rhizobia*
T6- Rice straw based biochar alone

2.4.1 Seed defusate

Kidney bean seeds were surface sterilized according to the method described by [10]. Sterilized seeds were rolled in sterilized carrier materials and then embedded into yeast mannitol agar plates seeded with *Rhizobia*, kidney bean seeds without carrier were used as control, plates were incubated at 8°C for 2h then 28°C for 48 h. Average diameter of inhibition zones was recorded [11].

2.4.2 Pot experiment

A pot experiment was conducted in plastic pots containing 1 kg sand soil to evaluate the potential of different inoculated carrier preparations stored for 180 days to form effective and suitable number of *Rhizobial* nodules on the kidney bean plant roots. Pots ware irrigated with distilled water at 60% of field capacity for sandy soil, hougland solution was used to irrigate plants once a week. Experiment was continued for 35 days then number of nodules, nodules dry weight, plant dry weight, and plant height and nitrogen in plant were measured.

3. RESULTS

Most partially decomposed organic matter such as bio char and compost are known to support survival of bacterial inoculum. The porous structure nature of materials such as charcoal biochar and compost provide an excellent environment for carrying bacteria [12]. Beside the previous character of biochar, it is also a nutrient rich material that provides a good microbial habitat and increase their survival and enhancing the crop growth through plant microbial interaction [13]. Biochar possess elevated water holding capacity which increases survival chances of soil microbes. The physicochemical characters of different carrier preparations were recorded in Table 1.

In this study, all carrier preparations have been able to support the survive of the mean bacterial count of inoculum up to 10^9 viable cells. The population remains nearly constant or decline slightly during the first 60 days in case of the two types of biochar based carrier and 45 day in case of beat based carrier. Carrier consists from biochar only recorded higher *Rhizobia* count of log 9.98 cfu g^{-1} of carrier after 180 days of inoculation (Fig. 1) followed by Biochar-Vermiculite of log 9.61 cfu g^{-1} of carrier then peat- verm of log 9.45 cfu g^{-1} of carrier. This

result was compatible to many reports. Biochar was used by [14] as a carrier material for both *Rhizobia* and Arbuscular Mycorrhizal (AM) fungus. Other reports in Japan [15] and Syria by [16] also proved that biochar is an efficient carrier for symbiotic nitrogen fixing root nodule bacteria *(Bradyrhizobium Rhizobium* and *Mesorhizobium*).

Biochar was proved to act as a suitable alternate to peat based carrier because of its higher surface area and micro nutrient availability which enhance survival of the microbe for prolonged period [5].

The initial pH for all three carrier materials was adjusted near neutrality and the effect of storing period on pH change was recorded Fig. 2. Gradual decline in inoculated peat- Vermiculite carrier pH was observed along storing period where the pH decreased from 7 to 6.4 while pH of control (un inoculated peat- Vermiculite) remain constant the reason for this decline in pH can be attributed to the metabolic activity of inoculant under limited conditions of oxygen in combination with the original acidic nature of beat (4.5) which moved to neutrality by calcium carbonate. In case of bio char based carrier (Bio char: Vermiculite and bio char) slight decrease in pH was detected at the end of storing time as shown in (Fig. 2) The slight decline in pH value for all carrier materials may be attributed to the production of metabolites with acidic nature by bacteria. In addition, acidic compounds may be released from bacterial cell after death. This naturally will lower the pH value of the carrier material and hence lower the viable bacterial count [6]. Although this slight decline in pH, it remains in acceptable level suitable for survival of inoculant and could not cause severe death in bacterial population inhabits the carrier.

Table 1. Physiochemical properties of the carriers used in this study

Properties	Biochar-vermiculite	Peat moss- vermiculite	Biochar
Water holding capacity %	233.5	203.7	350.9
Moisture %	11.55	12.41	10.2
Bulk density	0.53	0.59	0.43
pH	8.3	5.1	8.1
Total carbon %	14	24	26
Total nitrogen	0.32	0.43	0.42
Ash	73.5	43	56
C/N ratio	43.7	55.8	61.9

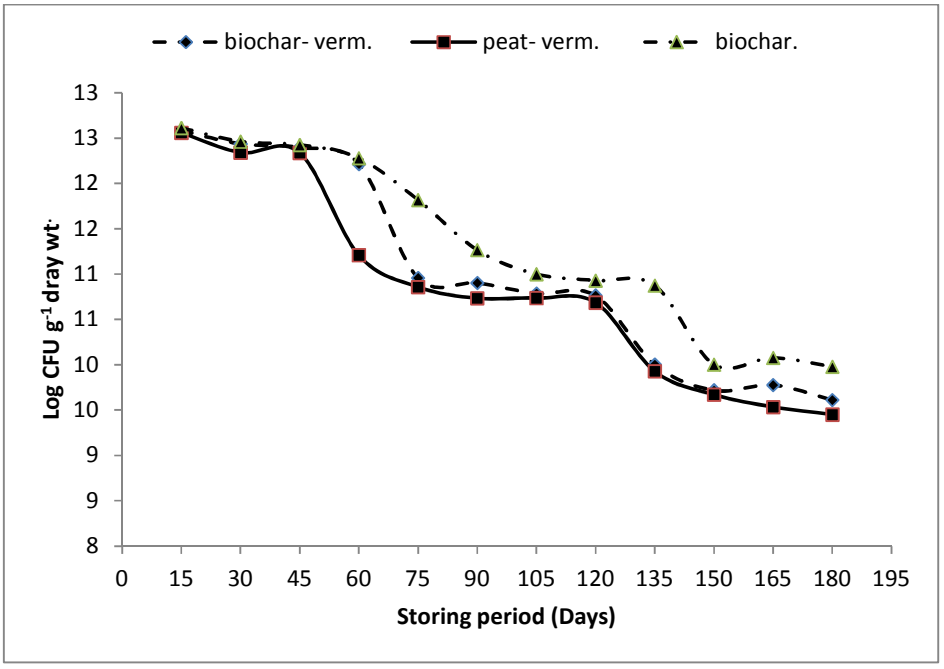

Fig. 1. Effect of different carrier preparation on log CFU of *Rhizobia* during storing period

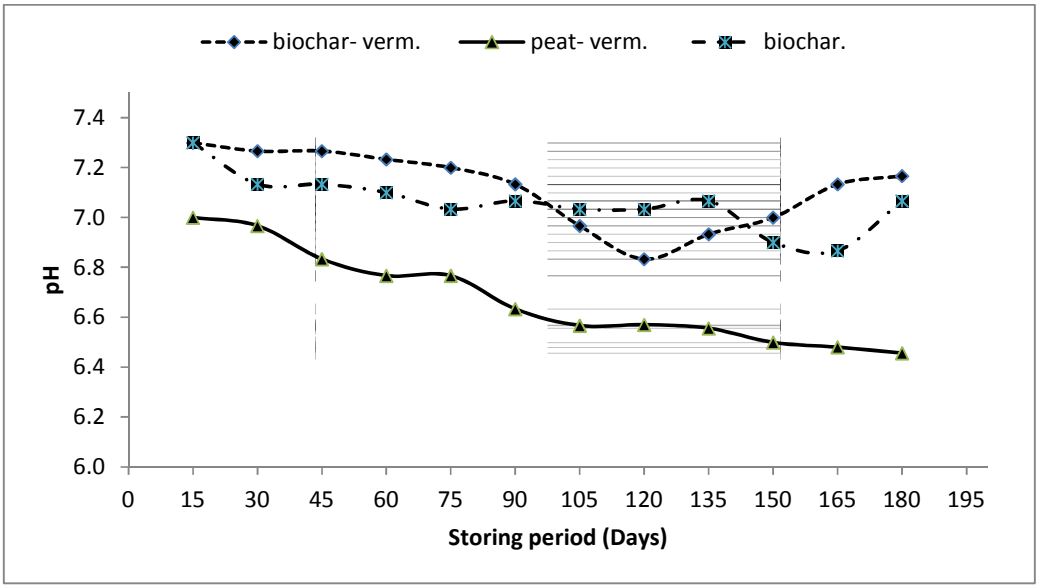

Fig. 2. Effect of different carrier preparation on pH of inoculated carriers during storing period

3.1 Effect of Different Carrier Preparations on the Moisture Content of *Rhizobium* Inoculants

The effect of different carrier preparations on the moisture content of *Rhizobium* inoculant was studied (Fig. 3) the moisture content of *Rhizobium* inoculant for all carrier materials was 35 at the beginning of experiment and thereafter a gradual decrease was observed in all preparations. Bio char based carrier retained the higher moisture content of 20% at the end of storing period, while biochar: vermiculite based carrier retained 13.42% moisture content. A sharp decline in moisture content was noticed in peat: vermiculite based carrier to 12.43%.

The results confirmed that bio char possess high water holding capacity as a result of slow release of water from its sponge like structure [17]. Moreover the smaller particle size and higher surface area of biochar enabling it to chelate micronutrients and act as nutrient rich pool for the microbes [18]. Higher survival of *Rhizobium* under treatment receiving bio char based carrier was due to its higher water potential.

3.2 Respiration Rate

In all carriers, respiration rate was declined slightly during the first 60 days then began to decrease sharply and remained nearly constant for about another 60 days then decreased again (Fig. 4). This behavior may be attributed to the residual nutrients present in culturing media in addition to the bio-degradable component present originally in carrier materials which responsible for the differences in respiration rate between different carrier preparations. This behavior in respiration rats followed the same trend of log CFU.

The summery of changes in all carrier systems represented by initial, final and reduction % was recorded in Table 2. Data of reduction % were subjected to the analysis of variance and treatments means were compared using the L.S.D. method according to [19]. The results indicated that the effect of different carrier preparation was highly significant on log cfu, pH and moisture content while non-significant on respiration rate.

3.3 Seed Diffusate

One of the most important problems rendering nodulation process in legumes is antimicrobial effect of seed diffusates liberating during legume seed germination. So the ideal carrier should possess adsorption ability to protect *Rhizobia* cells. Bio char have characteristics that conducive for use as inoculum carriers, including high internal porosity, large specific surface area, and the ability to adsorb organic compounds and bacteria [20,21,22,23]. The results indicated that all carrier materials could ameliorate the anti-microbial effect of germinating seed (Fig. 5). In addition, these results were confirmed by a pot experiment.

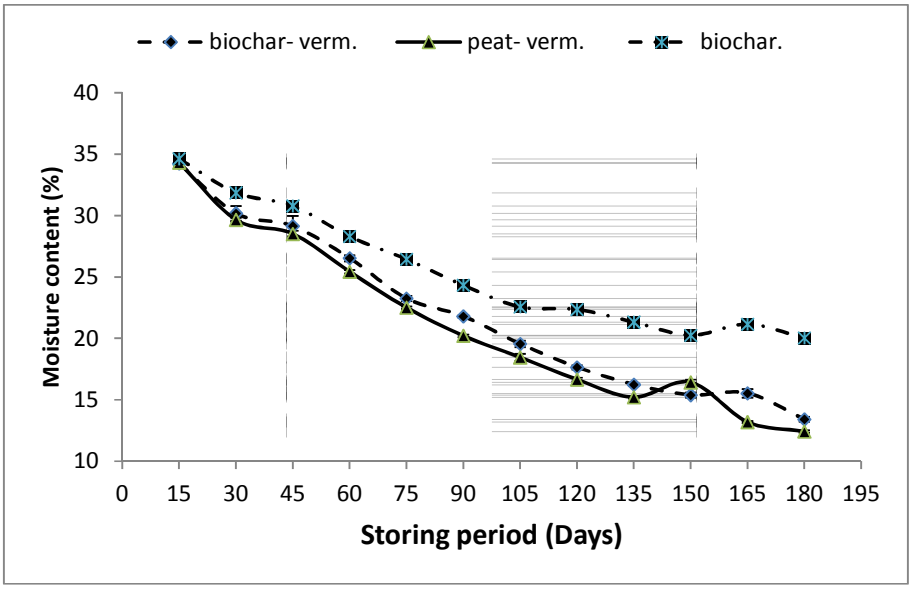

Fig. 3. Effect of different carrier preparation on moisture content of inoculated carriers during storing period

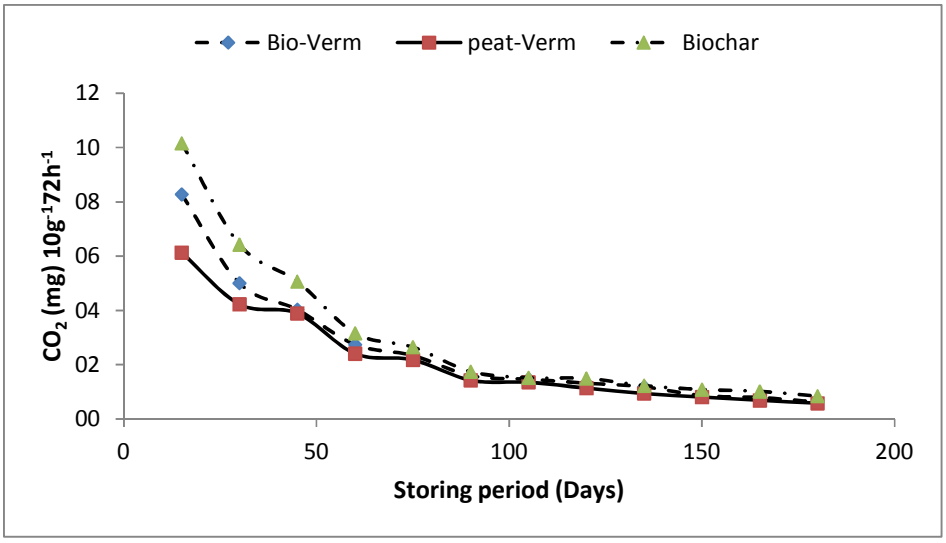

Fig. 4. Effect of different carrier preparation on respiration rate of inoculated *Rhizobia* during storing period

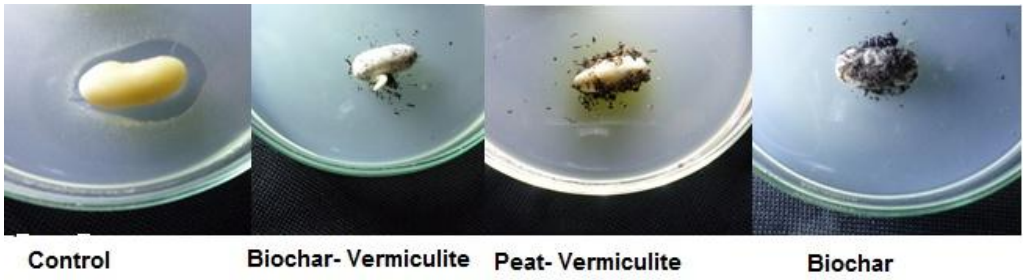

Fig. 5. Effect of different carrier preparation on protection of rhaizobia against kidney bean seed diffusate comparing to control

Table 2. Summery of changes in carrier systems at the final stage of storing period

	pH			Moisture content			Log CFU			Respiration rate		
	Initial	**Final**	**Reduction %**	**Initial**	**Final**	**Reduction %**	**Initial**	**Final**	**Reduction %**	**Initial**	**Final**	**Reduction %**
T1	7.30	7.17	1.8b	34.3	13.4	60.8d	12.58	9.61	23.61b	8.27	0.62	92.41
T2	7.30	7.27	0.46c	34.1	12.7	62.6c	-	-	-	-	-	-
T3	7.00	6.33	9.5a	34.3	12.4	63.8b	12.56	9.45	24.75a	6.13	0.58	90.51
T4	6.97	6.77	2.9b	34.4	11.9	65.3a	-	-	-	-	-	-
T5	7.30	7.07	3.2b	34.6	20.0	42.1f	12.61	9.98	20.88c	10.16	0.84	91.62
T6	7.30	7.10	2.7ab	34.6	17.7	48.9e	-	-	-	-	-	-
F test			**			**			**			N.S
LSD 0.05			1.16			0.01			0.67			--

T1= Bio char: Vermiculite inoculation; T2= bio char: Vermiculite control; T3= peat: Vermiculite inoculation; T4= peat: Vermiculite control, T5= Bio char Inoculation and T6= Bio char control

Fig. 6. Nodules formed on kidney bean plant root when inoculated by different carrier preparations after 180 days storing period

Table 3. Effect of different carrier preparations on nodule formation parameters and plant vegetative parameters

Treatments	Dry weight of plant (g/plant)	Plant height (cm)	No. nodules/ plant	Dry weight of nodules (g/plant)	N% in plant
T1	3.27a	19.67ab	83.33	0.095b	1.73b
T2	2.73b	14.67b	--	--	1.40d
T3	3.30a	19.33ab	82.00	0.090b	1.59c
T4	2.50b	15.17b	--	--	1.44d
T5	3.47a	21.67a	78.33	0.125a	1.84a
T6	2.73b	14.67b	--	--	1.40d
F test	**	**	N.s	*	**
LSD 0.05	0.32	3.65	--	0.03	0.11

T1= Bio char: Vermiculite inoculation; T2= bio char: Vermiculite control; T3= peat: Vermiculite inoculation; T4= peat: Vermiculite control, T5= Bio char Inoculation and T6= Bio char control

3.4 Pot Experiment

The results obtained from the pot experiment were in complete compatibility with the results of carrier evaluation in laboratory. This compatibility reflected in the success of inoculated treatments to form considerable and effective nodules (Fig. 6). The treatments inoculated with bio char carrier recorded the highest nodule dry weight, plant dry weight, plant height and nitrogen in plant (Table 3 above). It was obvious that the nodules were formed as clusters in the main root when bio char was used. This may be due to the ability of bio char to efficiently adsorb seed diffusate liberating during seed germination.

4. CONCLUSION

Based on this study, bio char can be used as a best alternate carrier material to peat moss for the preparation of *Rhizobia* inoculant in Egypt. The use of rice straw based bio char was found to increase survival of *Rhizobia* inoculant up to 180 days since it is available in plenty, everywhere. Moreover, bio char doesn't have any storage problems due to its limited moisture content and low degradability. More studies should be proceeded to find a novel methods capable of produce large quantities of rice straw bio char with constant chemical composition.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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