Longer periods of charcoal incubation exerting deleterious effect on vegetative growth of rice

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ABSTRACT: Charcoal application as a soil amendment in paddy does not improve rice growth and yield of the first crop, but it does in the following crops. Pre-incubation of charcoal prior to application to transplanted rice may enhance the synchronization of nutrient release and rice uptake. This study aimed at evaluating effect of charcoal incubation periods before rice transplanting on soil property and rice vegetative growth. A pot experiment was conducted under a greenhouse condition at the Field Research facility, Sakon Nakhon Rajabhat University during June - September 2019. Five pre-incubation periods of rice husk charcoal (RHC) before the rice transplanting were tested including no RHC with watering 0 day before rice transplanting and RHC with watering 0, 15, 30, and 60 days before rice transplanting (DBT). Soil pH and leaf number of rice were examined as preliminary data of the treatment effects on soil and plant. RHC amendment with watering 0 DBT had significantly higher soil pH and leaf numbers over other treatments. The longest the pre-incubation periods of RHC brought about the lowest rice leaf number. Nutrient deficient and volatile-matter-derived allelopathic effects were proposed as the mechanisms responsible for the deleterious effects of the RHC pre-incubation on the rice plants.
Keywords: Biochar, Nutrient deficiency, *Oryza sativa*, Soil pH, Synchronization

Introduction

Poor soil fertility produces low rice (*Oryza sativa*) yield in Northeast Thailand (Vityakon, 2007). Charcoal used as a soil amendment, namely biochar, has attracted much interest both in scientific and on-farm levels due to its soil fertility and crop yield improvement (Latawiec et al., 2017). Charcoal was proved to have agronomic benefits in soil properties (e.g., increases in soil pH and nutrient availability, and decreases in Al and Mn toxicities) and plant (e.g., tomato and corn) growth (Hossain et al., 2010; Butnan et al., 2015). Amongst many soil properties, a rise in soil pH is the most crucial role of charcoal, because pH is the master variable regulating all other soil properties (Bloom and Skyllberg, 2012).

Charcoal application to paddy rice did not improve yield in the first application, but it did in later seasons (Liu et al., 2016; Wang et al., 2018). Synchronization between nutrient release from charcoal and their uptake by rice plant could be the problem issue. Kongthod et al. (2015) demonstrated that the peak of NH_4^+ concentrations in a loamy-sand Nampong soil was at 60 days after additions of rice husk and cassava charcoal and that of NO_3^- was at 90 days. These peaks of inorganic N release do not synchronize with nutrient requirement of rice, as shown by Murayama (1967) who reported that the maximum nutrient requirements of rice are in the tillering (35 – 40 days) and panicle formation (55 – 70 days) stages. Charcoal does not provide nutritional, especially N, benefit to first crop of rice. As a consequence, investment

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in charcoal amendment does not provide a pay off for farmers during the main rice-growing season (rainy season). Meanwhile, off-season rice incurs high production costs, for example average of 8,600 THB/rai in Ubon Ratchathani province (Srisurin et al., 2017).

This study therefore aimed at estimating the effect of periods of charcoal incubation before rice transplanting on soil pH and rice vegetative growth. We hypothesized that the more extended incubation periods prior to the transplanting would bring about higher rice growth.

Materials and methods

The soil used was Roi Et series collected from 0 - 15 cm-depth from a paddy field in Sakon Nakhon (17° 20' 36.7" N; 104° 02' 52.6" E). Charcoal was produced from rice husk under a kiln modified from the 200-liter metal cylindrical-shaped tank. A completely randomized design with three replications was conducted under a greenhouse in the Field Research facility of Plant Science section, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University. Data measurements were performed from June to September 2019. Treatments were composed of (i) no rice husk charcoal (RHC) amendment with watering 0 day before rice transplanting (No RHC + 0 DBT) and (ii) RHC amendment with watering 0 (RHC + 0 DBT), (iii) 15 (RHC + 15 DBT), (iv) 30 (RHC + 30 DBT), and (v) 60 (RHC + 60 DBT) days before the transplanting (**Figure 1**).

Each pot (height = 23.5 cm, top diameter = 19 cm, bottom diameter = 15 cm, v = 6,823 cm^3 in a inverted truncated-pyramid shape) was filled with 6 kg air-dry soil and mixed thoroughly with RHC of 120 g/pot equal to 2% w/w according to Butnan et al. (2015). Prior to rice transplanting, each pot was watered to 70% of water holding capacity at the days of their respective treatments, except No RHC + 0 DBT and RHC + 0 DBT which were flooded to 3 cm-depth from the date of rice transplanting (**Figure 1**). Rice variety RD22 was seeded and nursed for 30 days in a nursery tray before transplanted to the pots.

Chemical fertilizers, graded 46-0-0, 0-0-60, and 18-46-0, were applied to all pots to achieve N, P, and K at the rates of 28.8 kg N/rai, 9.6 kg/ P_2O_5/rai, and 14.4 kg K_2O/rai (Sun et al., 2015). Fertilizer application was divided into three periods − 50% of the total amount was at the rice planting, and the latter applied twice to 25% were at 20 and 40 days after the transplanting (Ly et al., 2015). Soil pH and rice leaf numbers were examined after rice transplanting till the end of the vegetative growth stage of the rice plant (72 DAP). Soil pH was measured using a portable pH meter. Sampling intervals of both parameters were at 44, 51, 65, and 72 days after rice planting.

Repeated measure analysis of variance based on the completely randomized design was used to evaluate the effects of periods of RHC incubation before rice transplanting on soil pH and leaf number of rice. Mean comparisons were performed using Fisher's least significant difference. Relationships between soil pH and

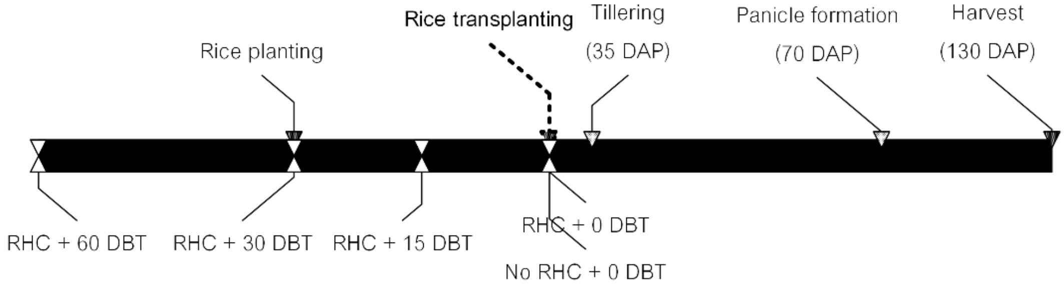


Figure 1 Timeline of rice growth stage and treatment managements; DAP = days after rice planting; DBT = days of pot watered before rice transplanting; RHC = rice husk charcoal.

Table 1 Repeated measure analysis of variance pertaining to the effects of incubation periods of rice husk charcoal before rice transplanting (Treatment) and sampling intervals (Time) on soil pH and leaf number per hill of rice over 72 days of rice planting.

Source of variance	degree of freedom	P-value	
		Soil pH	Leaf number/hill
Treatment	4	**	***
Time	3	ns	***
Treatment x Time	12	*	***

* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; ns = not significantly different

rice leaf numbers were assessed using Pearson's correlation. Significant differences were at $P \leq 0.05$. The statistical analyses were performed by the SAS software version 9.1 (SAS Institute, Cary, NC, USA).

Results and discussion

Periods of RHC incubation before rice transplanting significantly affected soil pH and vegetative rice growth (leaf number per hill) (**Table 1**). Incubation of RHC at the transplanting date (RHC + 0 DBT) brought about significantly higher soil pH over other treatments at 72 DAP (**Figure 2a**). The highest leaf numbers in RHC + 0 DBT over other treatments were found from 44 to 72 DAP (**Figure 2b**). Soil pH significantly correlated to rice leaf number at 65 and 72 DAP (**Table 2**).

Charcoal constituted ash, volatile matter, and fixed carbon contribute to increases in soil pH (Butnan, 2015). Ash is composed of high content of alkalis, e.g., sylvite (KCl), calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), and magnesium carbonate ($MgCO_3$) (Yuan et al., 2011). Meanwhile, fixed carbon and volatile matter contain negative-charged functional groups which are attributed to high pH, e.g., carboxyl ($-COO^-$), carbonyl ($-CO^-$), and oxyl ($-O^-$) (Yuan et al., 2011). In this study, higher soil pH of RHC + 0 DBT exerted increases in plant nutrient availability compared to treatments possessing longer periods of RHC incubation. Also, alkalis contain basic cations which are necessary to plant growth, e.g., K^+, Ca^{2+}, and Mg^{2+} (Butnan et al., 2015). Acid neutralization enriched the content of NH_4^+ which is the most favorite N form for rice uptake

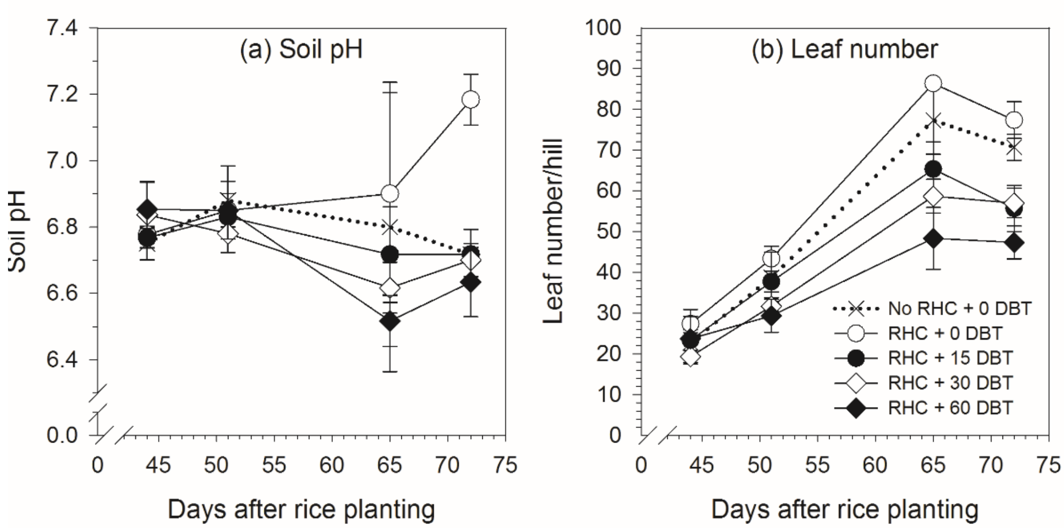
Table 2 Pearson correlations between soil pH and leaf number of rice in different time intervals after rice planting (n = 15).

	Leaf number/hill			
	44 DAP	51 DAP	65 DAP	72 DAP
Soil pH	-0.151^{ns}	0.207^{ns}	0.444*	0.687**

* = $P \leq 0.05$; ** = $P \leq 0.01$; ns = not significantly different
DAP = days after rice planting

due to the enhancement of ammonification of charcoal-derived N and soil native N (Chen et al., 1998).

On the contrary to RHC + 0 DBT, the extended periods of RHC incubation prior rice transplanting, i.e., RHC + 15 DBT, RHC + 30 DBT, and RHC + 60 DBT, showed lower soil pH (**Figure 2a**) and rice leaf number (**Figure 2b**). Insufficient nutrients to support rice growth could result in the deleterious effects of the longer pre-incubation periods on the rice plants. Lower soil pH of the longer pre-incubation periods was a consequence of decreases in negative-charged organic anions in the decomposition process (Zimmerman, 2010; Dai et al., 2014). This acidified soil rendered lower nutrient availability (Mengel and Kirkby, 2001). In addition, the lower rice growth may be driven by unavailability of ash-derived cations such as Ca^{2+} and Mg^{2+}. These basic cations could be hydrolyzed (Dai et al., 2014) and precipitated with RHC-derived silicon via the reaction: $Ca^{2+}/Mg^{2+} + SiO_4^{4-} = Ca/MgSiO_3 \bullet H_2O + H_2O$ (Morita et al., 2016; Antonangelo et al., 2017), prior the transplanting. Besides, N deficiency, which was triggered by N losses via ammonia volatilization and denitrification before having rice plants in the pots, may stunt rice growth after the transplanting. This was a consequence of neutralization under the pre-incubation treatments leading to acceleration of N mineralization and consequently N losses



Treatment	DAP 44	51	65	72
No RHC + 0 DBT	ns	ns	ns	b
RHC + 0 DBT	ns	ns	ns	a
RHC + 15 DBT	ns	ns	ns	b
RHC + 30 DBT	ns	ns	ns	b
RHC + 60 DBT	ns	ns	ns	b

Treatment	DAP 44	51	65	72
No RHC + 0 DBT	ab	ab	a	a
RHC + 0 DBT	a	a	a	a
RHC + 15 DBT	ab	ab	b	b
RHC + 30 DBT	b	bc	bc	b
RHC + 60 DBT	ab	c	c	c

Figure 2 Incubation periods of rice husk charcoal (RHC) treatments affecting soil pH (a) and leaf number of rice (b), i.e., no RHC with watering 0 day before rice transplanting (No RHC + 0 DBT), and RHC amendment with watering 0 (RHC + 0 DBT), 15 (RHC + 15 DBT), 30 (RHC + 30 DBT), and 60 (RHC + 60 DBT) days before rice transplanting. Vertical bars are the standard deviation. The accompanying tables beneath the graphic data demonstrate mean comparisons within time intervals (days after rice planting, DAP). The similar letters within a DAP are not significantly different ($P \leq 0.05$; Fisher's least significant differences).

(Dai et al., 2014).

In addition to pH effects, pre-incubation treatments may produce allelopathy to hasten the rice growth. These charcoal derived-allelopathic compounds included hydroquinones, benzoquinones, catechols, resorcinol, gallic acid, ethylene, acetylene, muconic acid, oxalic acid, maleic acid, malic acid, malonic acid, fumaric acid, formic acid, tartaric acid (Spokas et al., 2010; Olivier, 2011). Certain compounds were derived from both original pyrolysis and decomposition processes, e.g., quinones, catechols, ethylene, acetylene, gallic acid, and tartaric acid (Olivier, 2011; Kaal et al., 2012). Phenols, catechols, gallic acid, and quinones were reported to depress the rice growth (Djanaguiraman et al., 2005) via interfering various biochemical processes, for examples, phosphorylation pathway, ATPase activity, cell division, mineral uptake, and syntheses of carbohydrates, proteins, and nucleic acids (Einhellig, 2004).

Conclusions

The results of this study were opposite to our original hypothesis. The longer periods of incubation of rice husk charcoal before rice transplanting led to more deleterious effects on rice vegetative growth. Nutrient deficient and allelopathic effects were mechanisms detrimental to rice plants. The results clearly showed not to have pre-incubation of rice husk charcoal for paddy rice production. Data on the grain yield are necessary to validate the findings of this study.

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