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Effects of carbonized rice hull and arbuscular mycorrhizal fungi application on potting media chemical properties, growth and nutrient uptake of Falcata (*Paraserianthes falcataria* L.)

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Abstract

A pot experiment was conducted to evaluate the effects of carbonized rice hull (CRH) and arbuscular mycorrhizal fungi (AMF) inoculation on potting media chemical properties, growth, N and P uptake of *Paraserianthes falcataria*. The plants were grown in a soil-sand potting media amended with CRH at the rates of 2%, 5%, 10%, 20% and 40% (w/w). A control treatment (soil-sand) with and without AMF inoculation was included. The experiment was laid out in RCBD with 3 replications. Plants were grown for 90 days. Soil samples were also analyzed for the changes in chemical properties. While N and P nutrition were determined through plant tissue analysis. The results indicated that charcoal application can significantly reduce soil pH in potting media. In contrast, charcoal application positively increased soil OC, total N, extractable P and exchangeable K compared to unamended pots. The increase in the available nutrients was in proportion to the rate of CRH application. The highest significant increase was consistently observed in potting mixes amended with 40% CRH. Despite the improvement in soil chemical properties, CRH and AMF inoculation did not positively influence plant height, stem diameter, and total dry matter production. Similarly, comparable results were observed on the N and P concentration and uptake of plants amended with CRH and AMF and the control treatments. Results of the experiment demonstrate the ability of CRH and AMF to influenced soil chemical properties. However, both amendments failed to have a significant improvement on the growth, biomass production, and nutrition of *P. falcataria* seedlings.

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Introduction

Paraserianthes falcataria or locally known as falcata is a fast- growing legume tree species grown commercially in the southern part of the country. In Caraga region (considered as the Timber Corridor in the Philippines), falcata is considered as one of the most important plantation tree species that is widely grown primarily as raw wood materials.

In 2011, a national greening program (NGP) has been launched by the Philippine government. NGP is a massive forest rehabilitation program which targets to grow 1.5 billion trees including native, exotic and fast-growing trees covering about 1.5 million ha of public lands nationwide (Aquino and Daquio, 2014). Several tree species have been massively produced under nursery condition. The success of reforestation programs is largely dependent on the quality of planting material and thus-on a high quality of potting media.

A common potting media used during nursery production is the soil-sand mixture. Although this type of potting media offers some advantages such as easy to obtain, have good aeration and water drainage, however, for large-scale production the use of such materials is too heavy requiring a large amount of topsoil for media preparation. In addition, issues such as lack of nutrients, prone to compaction, the presence of weed seeds and plant-borne diseases has often been reported (Wilkinson *et al.,* 2014). Hence, utilization of locally available farmyard waste materials as potting media component has been largely practiced (Bhardwaj, 2014, Prasanna Kumar and Raheman, 2012).

One of the processing by-products of rice milling is rice hull. In rice production, rice hull accounts for about 20 %. In the Caraga region alone, about 56, 000 metric tons of rice hull is generated annually and are left as waste in various rice millers (Baconguis, 2007). This large volume of waste materials could potentially be used as potting media component by converting into carbonized materials. Carbonized rice hull (CRH) is made from the incomplete or partial burning of dried rice hull under a controlled burning process. CRH is sterile, highly porous, low bulk density material and made up of a recalcitrant form of carbon which is more resistant to microbial decomposition.

The use of CRH as soil amendments has been well recognized. In the study conducted by Ogbodo (2011), rice hull charcoal application resulted in a large increase in soil nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and superior growth of plants. Higher soil organic carbon (OC) and available potassium (K) and increased below ground biomass production of the plant amended with rice hull charcoal was also reported by Abrishamkesh *et al.* (2015). A similar improvement in the availability of plant nutrients with rice hull charcoal application was also obtained in the study of Rollon *et al.* (2017). Charcoal application improves soil physico-chemical properties, stimulates the growth of beneficial microorganisms and enhance nutrient availability leading to better plant growth.

Microbial inoculant such as arbuscular mycorrhizal fungi (AMF) is a beneficial fungus that forms a symbiotic association with most tropical tree species including falcata (Wulandari *et al.,* 2016). Among its benefits is the increase in plant absorption for an immobile nutrient such as P, copper (Cu) and zinc (Zn). AMF is also known to increase water uptake, protects the plant against fungal diseases and reduce heavy metal uptake of plants (Willis *et al.,* 2013). In exchange, the host plant provides a continuous supply of carbohydrate as a source of energy for the fungi. Under nursery condition, AMF inoculation can greatly improve plant growth and vigor of seedlings to cope with transplantation stress. Hence, AMF inoculation is necessary for the production of high-quality seedling materials.

Despite the multiple benefits obtained from using CRH as potting media component, however, no standard potting ratio has been established particularly in falcata seedling production. Moreover, utilization of such materials in combination with AMF inoculant in the region is still underexplored.

If the addition of CRH as potting media component improves both physical and chemical properties of potting media while AMF enhances plant nutrient absorption. Thus, a combination of CRH and AMF could have a synergistic effect which might lead to better growth and survival of tree seedlings. Hence, the overall objective of this study is to evaluate the effects of CRH application at different concentrations and AMF inoculation on soil-sand media chemical properties, seedling growth, biomass production and nutrient uptake of *P. falcataria* and to determine the appropriate potting mix ratio for *P. falcataria* seedling production.

Materials and methods

Experimental design

A randomized complete block design (RCBD) experiment with seven treatment combinations and three replications was conducted under screen house condition of the College of Agricultural Sciences and Natural Resources (CASNR), Caraga State University (CSU), Ampayon, Butuan City, Philippines from December 2016 to May 2017. The treatment combinations were the following: T_1= 60 % soil : 40 % sand (control) ; T_2= 60 % soil : 40 % sand + AMF ; T_3= 60 % soil : 40 % sand + 2 % CRH + AMF ; T_4= 60 % soil : 40 % sand + 5 % CRH + AMF ; T_5= 60 % soil : 40 % sand + 10 % CRH + AMF ; T_6= 60 % soil : 40 % sand + 20 % CRH + AMF ; T_7= 60 % soil : 40 % sand + 40 % CRH + AMF.

Soil-Sand Collection, Preparation and Analyses

Bulk soil samples were collected from the soil surface (0-20 cm depth) from CASNR Experimental site, CSU. On the other hand, sand was collected from San Mateo, Cabadbaran City, Philippines. Both soil and sand were air dried and sieved at 2-mm. Sand was washed 20 times with tap water. The sieved soil and sand were pasteurized separately at 325 C for three consecutive days at three hour per day. Subsamples were subsequently taken for chemical analyses (pH, OC, total N, extractable P and exchangeable K, Ca, Mg and sodium (Na)) at the Regional Soils Laboratory of the Department of Agriculture, Taguibo, Butuan City, Philippines and the rest were prepared for bagging.

Carbonized Rice hull Production, Characterization and Application

Rice hull was collected from the Municipality of Remedios T. Romualdez, Agusan Del Norte, Philippines. Carbonized rice hull (CRH) was produced using an open-type fabricated carbonizer developed by Phil Rice Research Institute, Agusan Del Norte. A sieved (2-mm) subsamples of CRH were taken and submitted to Regional Soil Laboratory for chemical analysis (pH, total OC, N, P, K, Ca, Mg and Na). The remaining sieved CRH was thoroughly mixed with soil-sand potting media at 2 %, 5 %, 10 %, 20 % and 40 % concentration (w/w) and incubated for 20 days prior to planting. Moisture content was maintained at 60 % moisture content of field capacity in CRH amended and unamended soils.

Seeding and AMF Inoculation

To avoid cross-contamination, seeds for the control treatment and the seeds for AMF inoculated were separately grown in pre-germinating trays. For AMF inoculated plants, 30 grams of mycovam was applied in the middle part of pre-germinating trays where seeds were sown. Mycovam is a commercial mycorrhizal inoculant produced by Biotech Department, University of the Philippines, Los Baños, Laguna, Philippines. The inoculant is a soil based containing spores of AMF *Glomus etunicatum, Glomus macrocarpum,* and *Gigaspora margarita* and infected roots of Bahia grass (*Paspalum notatum*) as a trap plant.

Transplanting

A total of 21 polyethylene pots were used in the study. Each pot was filled with 2 kg of a soil-sand mixture at 60 %: 40 % combination. Pre-germinated seeds were sown in a cell tray (100 cells) filled with sterilized soil. Twenty days after potting media incubation, a 25 days old seedling with similar height was transplanted in each pot. The seedlings were allowed to grow for 90 days. Blanket application of 15 mg each of N, P_2O_5 and K_2O kg^{-1} soil as starter fertilizer using urea, solophos, and muriate of potash was done one week after transplanting. Urea and muriate of potash were applied as an aqueous solution while solophos was applied as granules.

Moisture content in each pot was maintained at 60 % of its field capacity. Weeds and insects in each pot were removed by handpicking.

Harvesting

Twelve weeks (90 days) after transplanting, plant in each pot was harvested. During harvest, final plant height and stem diameter were determined. Each plant were cut close to the soil surface. Soil particles adhering to the roots were removed carefully.

The shoots and roots were washed with tap water, rinsed with distilled water and blot-dried using a paper towel. The shoots and roots were then air-dried for two days and oven dried for three days or until constant weight is obtained using a forced draft oven set at 70° C.

After oven drying, the shoots and roots were weighed separately. Total dry matter was obtained by combining the weight of the shoots and roots per plant.

Plant Tissue and Soil Analysis

After obtaining the plant dry weights, the plant samples were sent to Regional Soil Laboratory for N and P analyses.

The amount of N and P uptake (mg plant^{-1}) was determined by taking the product of total dry matter yield per plant and their respective N and P contents. Soil samples on the other hand after harvest were air dried and sieved (2-mm). About 300 g of each soil sample was sent to the same Laboratory for soil pH, OC, total N, extractable P and exchangeable K analyses.

Statistical analysis

All data gathered were subjected to analyses of variance using the STAR (v2.0.1) program. Comparison of treatment means was done using Tukey's Honest Significant Difference Test at $p<0.05$. Simple correlation analysis was also performed using STAR in order to analyze the relationship between the selected variable.

Results and discussion

Initial chemical properties of soil and sand

Table 1 presents the initial chemical analysis of soil and sand used as potting media. Analysis showed that both media were slightly alkaline in pH with very low OC and total N. The P and K content in soil was sufficient while sand was deficient. Generally, the soil had higher N, P and K content over sand. Meanwhile, the concentration of basic cations such as Mg and Na both in soil and sand was extremely high, except for Ca.

Table 1. Initial chemical properties of soil and sand before experiment.

Property	Soil	Sand
pH (1:5 soil to H_2O)	7.34	7.54
OC (%)	0.58	Trace
Total N (%)	0.11	0.01
Extractable P (mg kg^{-1})	58.00	1.00
Exchangeable (mg kg^{-1})		
K	291.00	190.00
Ca	Trace	Trace
Mg	19,600.00	37,000.00
Na	600.00	600.00

Chemical properties of carbonized rice hull

General properties of CRH are presented in Table 2. The derived CRH had a strongly alkaline pH with very high OC and high total N, P and K. The Ca content in CRH was low with a medium level of Mg and high Na concentration.

Effects of CRH and AMF on potting media chemical properties after harvest

Chemical analysis of potting media after harvest as influenced by CRH application and AMF inoculation are presented in Table 3. Potting media pH ranged from 6.27 to 7.03 (slightly acidic to neutral pH) with maximum and minimum pH recorded in sole AMF inoculated pots without CRH (T_2) and 40 % CRH + AMF media (T_7), respectively. Addition of CRH in potting media regardless of the rates significantly reduced soil pH at harvest.

Table 2. Chemical characteristics of carbonized rice hull (CRH).

Property	CRH
pH (1:5 soil to H_2O)	8.89
OC (%)	28.03
Total (%)	
N	0.55
P	0.07
K	0.48
Ca	Trace
Mg	0.01
Na	0.03

On the average, potting media pH in CRH amended pot was 6.49, lower by 0.49 units than the average potting media pH (6.98) in CRH unamended pots. In general, increasing rates of CRH application decreases potting media pH. Soil pH reduction observed in charcoal amended pots could have been attributed to the production of acidic material as a result of charcoal oxidation. Our observation conforms to the findings of Liu and Zhang (2012) who reported a decreasing pH trend to increasing charcoal application rates. Similarly, Abrishamkesh *et al.,* (2015) and Satriawan and Handayanto (2015) found the same pH reduction in rice hull charcoal amended soil compared to the untreated soil. Charcoal is not totally inert material and can undergo oxidation in soil, through chemical and microbial activity.

Table 3. Means for the effects of CRH application and AMF inoculation on potting mix pH, OC, total N, extractable P and exchangeable K at harvest.

Treatment	pH	OC	Total N	Extractable P	Exchangeable K
		(%)		(mg kg^{-1})	
T1= 60 % soil: 40 % sand (control)	6.93a	0.49d	0.07c	24.75c	221.50e
T2= 60 % soil: 40 % sand + AMF	7.03a	0.46d	0.07c	23.75c	214.00e
T3= 60 % soil: 40 % sand + 2 % CRH + AMF	6.79ab	0.56d	0.08c	23.25c	257.50de
T4= 60 % soil: 40 % sand + 5 % CRH + AMF	6.63bc	0.58d	0.08c	24.25c	346.50d
T5= 60 % soil: 40 % sand + 10 % CRH + AMF	6.43cd	0.85c	0.12bc	31.75b	567.25c
T6= 60 % soil: 40 % sand + 20 % CRH + AMF	6.33d	1.06b	0.17ab	33.75b	824.25b
T7= 60 % soil: 40 % sand + 40 % CRH + AMF	6.27d	1.39a	0.22a	40.50a	1205.25a
P value	**	**	**	**	**

Means in a column followed by common letters are not significantly different at 5 % level of significance; ns=not significant;

*= significant at $p<0.05$; **= significant at <0.01.

In contrast to the decreasing pH with increasing charcoal addition, soil OC showed an opposite trend. Wherein, soil OC increases with increasing rates of charcoal application. Analysis revealed that CRH amended potting media had significantly more soil OC than the unamended soils and CRH was more effective in increasing soil OC. Moreover, the increase in soil OC was in proportion to the rate of CRH application. The increase of soil OC in the CRH amended potting media could be a result of the high amount of OC in the CRH. Also, the OC in CRH is more recalcitrant, thus, leaving a large amount of residual OC in the potting media where CRH had been added. High OC in soils treated with charcoal has been also reported by Koyama *et al.,* (2016) where the addition of 20 g pot^{-1} and 40 g pot^{-1} rice husk charcoal significantly increased soil OC content by 550 % and 815 % over the control pot, respectively. Milla *et al.,* (2013) also had similar findings where OC content in soil was increased with the addition of rice hull charcoal.

Table 4. Means for the effects of CRH application and AMF inoculation on plant height, stem diameter and biomass production of *P. falcataria* at harvest.

Treatment	Height (cm)	Stem diameter (mm)	Dry weight (g plant^{-1})		
			Root	Shoot	Total
T_1= 60 % soil: 40 % sand (control)	62.80	5.00	1.10	8.83	9.93
T_2= 60 % soil: 40 % sand + AMF	65.33	4.88	1.26	9.04	10.29
T_3= 60 % soil: 40 % sand + 2 % CRH + AMF	65.40	4.50	0.94	8.00	8.94
T_4= 60 % soil: 40 % sand + 5 % CRH + AMF	55.78	3.88	0.80	6.86	7.66
T_5= 60 % soil: 40 % sand + 10 % CRH + AMF	63.78	4.50	0.86	7.13	7.99
T_6= 60 % soil: 40 % sand + 20 % CRH + AMF	61.68	4.63	1.11	7.96	9.06
T_7= 60 % soil: 40 % sand + 40 % CRH + AMF	61.60	5.13	1.13	8.34	9.47
P value	ns	ns	ns	ns	ns

Means in a column followed by common letters are not significantly different at 5 % level of significance; ns=not significant;
*= significant at $p<0.05$; **= significant at <0.01.

A similar trend was observed in total N, extractable P and exchangeable K where nutrient availability increases in proportion to the CRH application rates. For total N, the highest value was recorded at the highest application rate (T_7) with a 214 % increase over the control treatment. Addition of CRH at the highest rate (T_7) also resulted in a 64 % increase in extractable P and 444 % increase in exchangeable K over the control pots, respectively. If the total N, available P, and K values in CRH amended potting media were average, the values were 0.13 % N, 30.7 mg kg^{-1} P and 640 mg kg^{-1} K, respectively. On the other hand, the mean N, P and K content in pots without CRH application was 0.07 % N, 24.25 mg kg^{-1} P, and 217.75 mg kg^{-1} K, respectively. Clearly, the nutrient content in potting media with CRH was higher than the control media. Despite the significant increase in the available N in potting media with CRH application, the N levels still fall under the low category. This was already expected since both potting media (soil-sand) was deficient with N. Moreover, P levels are within the medium range while K values are above the sufficiency range. The large increase particularly in P and K in potting media with CRH application was not surprising.

As pointed out in Table 2, CRH had initially high P and K, therefore, it is reasonable the potting media treated with CRH also had a high P and K. Charcoal ash contains a large amount of soluble nutrients and provides nutrient directly into the soil. Also, it holds nutrients through the negative charge on the charcoal's surface area. On the other hand, sole AMF inoculation (T_2) did not positively influence potting media nutrient content. Hence, it is un-doubtable that the increase in soil fertility in potting media was mainly due to CRH application, with the highest improvement observed in potting media amended with CRH at highest application rate (T_7).

These results are in agreement with those of Nurhidayati and Mariati (2014) who reported a significant increase in the availability of soil N, P, and K with rice husk charcoal application. Similarly, Masulili *et al.,* (2010) reported that rice husk charcoal addition significantly increased soil P and K concentration. The said increase was attributed to the addition of plant nutrients from charcoal and the increase in nutrient retention in the soil.

Table 5. Means for the effects of CRH application and AMF inoculation on total N and P concentration and uptake of *P. falcataria*.

Treatment	Concentration (%)		Uptake (mg plant^{-1})	
	N	P	N	P
T_1= 60 % soil: 40 % sand (control)	3.25	0.51	322.04	49.84
T_2= 60 % soil: 40 % sand + AMF	3.01	0.71	310.43	76.86
T_3= 60 % soil: 40 % sand + 2 % CRH + AMF	3.14	0.50	278.45	43.98
T_4= 60 % soil: 40 % sand + 5 % CRH + AMF	3.13	0.42	236.09	33.97
T_5= 60 % soil: 40 % sand + 10 % CRH + AMF	3.57	0.63	286.43	51.17
T_6= 60 % soil: 40 % sand + 20 % CRH + AMF	3.67	0.54	327.75	52.23
T_7= 60 % soil: 40 % sand + 40 % CRH + AMF	3.76	0.76	357.82	72.19
P value	ns	ns	ns	ns

Means in a column followed by common letters are not significantly different at 5 % level of significance; ns=not significant; *= significant at $p<0.05$; **= significant at <0.01.

Effects of CRH and AMF on plant height, stem diameter and biomass production of P. falcataria

Table 4 shows the different plant measurements recorded at harvest. All plant parameters measured did not differ significantly among treatments. Plant height at harvest in CRH amended potting media with AMF inoculation were not statistically different with the control treatment. Similarly, no significant differences were also detected among treatments on stem diameter. Results further reveal that potting media amended with CRH were shorter and have smaller stem diameter over potting media that were not amended with CRH.

Addition of CRH at different rates and AMF inoculation did not significantly increase plant dry biomass at harvest. There was even a decreased in total dry biomass observed in CRH amended potting media.

Table 6. Pearson correlation matrix for the selected parameters.

	pH	OC	Total N	Extractable P	Exchangeable K	N uptake	P uptake
Root biomass	0.23^{ns}	0.14^{ns}	0.08^{ns}	0.12^{ns}	0.02^{ns}	0.67^{**}	0.68^{**}
Shoot biomass	0.32^{ns}	0.01^{ns}	0.01^{ns}	-0.03^{ns}	-0.06^{ns}	0.83^{**}	0.71^{**}
Total biomass	0.31^{ns}	0.03^{ns}	0.03^{ns}	-0.01^{ns}	-0.04^{ns}	0.83^{**}	0.73^{**}

ns= not significant; * significant at p-0.05; **significant at <0.01.

The average total dry weights in CRH potting media was 8.62 g which was lower by 13 % and 16 % than in control (T_1) and sole AMF inoculated pots (T_2), respectively. Addition of CRH may have increased nutrient content of potting media, however, it fails to have significantly influenced on plant dry biomass. On the other hand, sole AMF inoculation (T_2) slightly increased plant root, shoot and total biomass. However, the said increase was not statistically significant. The present results are in contrary with the previous findings where charcoal application improved plant biomass production (Carter *et al.,* 2013; Milla *et al.,* 2013).

The observed reduction in total dry matter production could have been attributed to the total amount of N and P absorbed by the plant (Table 5).

Correlation analysis showed a strong positive and significant relationship between total dry matter and N uptake ($p<0.01$; $r=0.83$) and P uptake ($p<0.01$; $r=0.73$) (Table 6).

Thus, a decrease in the amount of N and P absorbed by the plant would lead to low the dry matter production.

Effects of CRH and AMF on tissue N and P concentration and uptake of P. falcataria

Total N and P concentration and nutrient uptake of *P. falcataria* are presented in Table 5. Total N concentration in plants ranged from 3.01 % to 3.76 % which is below the sufficiency range values (Owen and Kissel, 2018).

This was already anticipated since the potting media had low N content (Table 1). On the other hand, P tissue concentration which ranged from 0.42 % to 0.76 % is considered sufficient. Numerically, plants in the CRH amended potting media at higher application rates had more N and P than the control treatment. However, neither CRH application nor AMF inoculation has any significant on N and P concentration.

Similarly, CRH application and AMF inoculation had no significant effect on the tissue N and P uptake. However, sole AMF inoculation (T_2) increased tissue P uptake more than the other treatments but the values were not statistically significant. Application of CRH on potting media may have increased the N and P levels in the soil. However, the said increase was not enough to raise the N and P concentration in plant tissue at a significant level.

Conclusion

Application of CRH and AMF on soil-sand potting media positively influenced soil OC, total N, extractable P and exchangeable K while significantly reduced soil pH. In contrast, CRH and AMF inoculation on soil-sand potting mixes did not enhance the growth, biomass production and N and P nutrition of *P. falcataria*. Thus, an appropriate potting mix ratio for *P. falcataria* seedling production using CRH and AMF was not achieved in this study.

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