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Assessing the Potential of Biochar Derived from Tropical Deciduous Tree *Broussonetia papyrifera* for Crop Improvement

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Abstract

In this study, we assessed the effects of biochar prepared from an invasive tree, *Broussonetia papyrifera* on the growth of selected pulse (*Cajanus cajan* and *Vigna radiata*) and cereal (*Oryza sativa* and *Zea mays*) crops under laboratory conditions. Two experiments were designed, one with biochar powder and another with biochar water extract to mimic the environmental conditions of the growing season of the crops. The findings of the study revealed that the seeds of pulse crops responded slower during the initial days (3 to 7 days) of exposure to the biochar powder whereas a marked increase in root and shoot growth parameters was observed with increasing doses of biochar at 15th day, compared with the control. With an initial increase in growth behaviour of pulses (at 3rd day), a stable growth response was observed at 7th and 15th days under control treatments. Both cereal and pulse crops showed better growth responses with increasing dose of biochar, and maximum growth was observed at 1-2% (for powder) and 2-4% (for extract) biochar doses. Application of powdered biochar showed better growth responses than the extract, as it facilitated gradual-release of nutrients, thus allowed the plants to stay healthy for a longer period of time. The results underscore the importance of using biochar based on the specific crop types, seed sizes, and growth stages to fully utilize its potential in sustainable agriculture. Harnessing the beneficial attributes of biochar not only enhances soil quality and crop yields but also presents a promising avenue for environmentally friendly weed control and resource utilization, aligning with broader objectives of agricultural sustainability and food security. Further investigations focused on optimizing biochar formulations and conducting field-scale trials will be essential in realizing its transformative impact on global agricultural practices.

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Introduction

The pyrolysis of biomass under limited oxygen conditions produces a carbonaceous, solid product, popularly called biochar.[1] This material is positively differentiable from other carbon-based products like charcoal owing to its remarkable soil amelioration properties.[1,2] A wide range of materials ranging from municipal sewage waste to crop residues can be used to fabricate biochar and the properties and nutrient content of biochar is thus dependent on the characteristics of initial biomass/feedstock.[3] In addition to carbon, biochar is enriched with traces of calcium, magnesium, phosphorous, nitrogen, oxygen, and sulfur. Biochar supplies bulk of important macro-and micro-nutrients to plants and soil microorganisms.[4] Cycling of nutrients improves the fertility of soil and paves way for the exploration of novel biochars derived from different feedstock materials for the improvement of crop yield. In view of this, recent research trends in the science of biochar includes fabrication of biochar using invasive plant species.[5] Invasive plants interfere with the ecosystem functioning, and inhibit the growth and establishment of native plant species, thus conversion of these weeds into biochar may assist in their judicious management.[6] However, effects of biochar derived from invasive weeds need to be thoroughly assessed under controlled environment before their wider field application.

Laboratory scale experiments can provide insights into the preliminary evaluation of effects of biochar on the crop plants.[7] For instance, in a study by Majidi,[8] the effect of NaCl induced alkalinity was reduced in *Raphanus sativus* L., *Lactuca sativa* L., and *Spinacia oleracea* L. upon addition of dry Oak biochar to Petri plates. In another Petri plate-based study, the biochar from fallen pine needles and dried leaves was found to be capable of improving the germination, growth, chlorophyll, and antioxidant activity in *Triticum aestivum* L.[9] Furthermore, some researchers have conducted the laboratory-based growth studies on biochar powder,[10] however, others have used biochar extracts.[11] Biochar in powder form is capable of trapping nutrients in its porous structure that paves way for their slow release which can be useful for prolonged treatment of soil. In addition, the powder of biochar ensures the effects of surface properties over crops.[12,13] On the other hand, biochar extract provide direct nutrient source to the crop which may help in fast growth responses during the initial days of application. In a study by Ma *et al*.,[14] the application of rice-straw biochar extract demonstrated diverse effects on *Zea mays* L. and *Oryza sativa* L. seeds, and was found to be influenced by the organic compounds present in the extract, such as 2,4-bisphenol and triethyl phosphate. Therefore, the effects of both forms of biochar on preliminary growth parameters of crops needs to be evaluated.

In this study, the effect of biochar prepared from a tropical deciduous tree (*Broussonetia papyrifera* (L.) L'Hér. ex Vent.) was assessed on primary growth responses of some cereal and pulse crops under laboratory conditions. This tree under study is showing rapid invasion in the subtropical parts, particularly urban and peri-urban ecosystems and is posing serious threat to the understorey vegetation by altering soil biophysical properties.[15] Among the different crops grown worldwide, cereals rank first and are followed by the legumes, therefore, evaluation of growth of these plants to different forms of biochar application can be beneficial to the agricultural system.[16] Studies on a wide range of crops are mandatory to validate the responses of biochar on plant growth. Therefore, two experiments were conducted at laboratory scale, where we investigated the effect of (i) biochar powder on pulses (*Cajanus cajan* L. and *Vigna radiata* L.), and (ii) biochar water extract on cereal (*Zea mays* L. and *Oryza sativa* L.) crops, on their growth responses (length and biomass). Effects were observed on at varying time intervals to observe the time-specific responses of biochar application. Powdered (for pulse crop) and water extract (for cereal crops) application of biochar was selected to mimic the initial environmental conditions of the crop growth period.

Materials and Methods

Biochar preparation

Fallen dried leaf litter of *B. papyrifera* was collected for biochar preparation. The litter was oven dried, powdered and pyrolyzed in a muffle furnace at 400 °C for 30 mins hold time at a ramp rate of ~20 °C min^{-1}, and a yield of ~40% was obtained.

Plant Materials

Seeds of pulse crops, viz., *C. cajan* (var. UPHAS 120), *V. radiata* (var. ML-5), and cereal crops, viz., *O. sativa* and *Z. mays* (var. NMH 589 Swarna), were collected from the local market. The seeds were surface sterilized using sodium hypochlorite, and finally washed using distilled water multiple times.

Since the pulses generally grow under dry (summer) conditions, a powder-based laboratory assay was performed for assessing the growth responses of two pulse crops. On the other hand, cereals (rice and maize) are generally cultivated during or at the onset of rainy season. Therefore, to mimic the environmental conditions, a water extract-based laboratory assay was performed to observe the growth responses of cereals in presence of different doses of biochar.

Powder-Based Study: Laboratory Assay 1

The effect of powdered *B. papyrifera* biochar on the growth attributes of pulse crops was evaluated in this assay. An even layer of cotton and Whatman filter paper no. #1 was placed on sterilized Petri plates (Φ = 15 cm). Filter paper was moistened with 5 ml of distilled water and biochar powder at different doses (0, 0.5, 1, 2, 4, and 6%) was evenly spread on the surface of Petri plates. Thereafter, 25 seeds of *C. cajan* and *V. radiata* were placed on respective Petri plates at even distances from each other and 15 ml of distilled water was sprayed using a sprayer. The experiment was performed in triplicate Petri plates for all the treatment combinations for both the plant species. Lids of Petri plates were sealed and seeds were allowed to germinate. The experiment lasted for 15 days in lab conditions at 28 ± 2 °C, 70 ± 2% relative humidity, and a day/night photoperiod of 16/8 h. Five seedings were harvested from each Petri plate on 3rd, 7th, and 15th days after commencement of the experiment, and the observations for seedling's length and biomass (fresh and dry) parameters were recorded using a ruler and weighing balance (ME104; Mettler Toledo), respectively. Mean of five seedlings from each Petri plate was considered as replicate for further analysis.

Water Extract-Based Study: Laboratory Assay 2

This experiment was designed to demonstrate the effects of biochar extract on growth of cereal crops. The water extract was prepared by shaking the biochar (20 g in 500 ml) in distilled water at room temperature for an hour to prepare a stock of 4% biochar extract. The Petri/filter paper bed (as mentioned in assay 1) was moistened using biochar extract (0, 0.25, 0.5, 1, 2, and 4% concentrations). A total of 20 ml biochar extract was provided to each Petri plate at different concentrations mentioned above. Seeds of *O. sativa* (25 per Petri plate) and *Z. mays* (10 per Petri plate) were placed equidistantly in a Petri plate. The experiment was performed in triplicate Petri plates. Experiment was maintained at the same conditions as in Laboratory assay 1. Five seedlings were harvested on 7th day and growth in root and shoot components of both the crops were noted. Mean of five seedlings from each Petri plate was considered as replicate for further analysis.

Statistical Analysis

Analysis of variance (ANOVA) was performed to determine the differences in biochar concentration-wise and day-wise responses of plants in Laboratory assay 1 (two-way ANOVA) and assay 2 (one-way ANOVA).[17] Independent t-test was used to observe the differences between two (7 and 15 days) duration for assay 1 and between two plant species in assay 2 experimental conditions. Significant differences among the treatments were determined by *post hoc* Tukey's test at 95% level of significance ($P < 0.05$). All the statistical analyses were performed in SPSS package (ver. 16). Graphs were prepared in Sigma Plot software (ver. 11.0).[17]

Results and Discussion

Effect of Biochar Powder on Pulse Crop Growth Responses: Laboratory Assay 1

The effects of biochar powder on the growth of *C. cajan* and *V. radiata* were assessed after 3rd, 7th, and 15th days of exposure. Two-way ANOVA results showed a significant ($P < 0.05$) variation in root and shoot length of both the crops at all the three time periods (Table 1). Interestingly, an initial decrease in the growth responses was observed with increasing doses of biochar at day three, whereas a significant ($P < 0.05$) increase in the growth parameters (Table 2) was observed with increasing doses of biochar (upto 2% for *C. cajan* and 1 or 2% for *V. radiata* seedlings) after seven and 15 days (Figs. 1-2). Though the growth responses started declining after 2% biochar doses, but remain higher than that observed for control after 15 days (Figs. 1-2). As compared to day 3 and 7, the application of biochar powder caused a delayed response and a significant ($P < 0.05$) increase was observed in the root and shoot length of both the crops at day 15 (Table 3). However, in case of control there was a non-significant increase in growth parameters (Figs. 1-2). A significant ($P < 0.05$) increase of upto 84% was observed in fresh weight of *C. cajan* and ~250% in case of *V. radiata*. The improvement in biomass observed was in harmony with the observations by Nazir and Batool.[18] The effects were more pronounced in roots and an improvement in biomass was observed at 1 and 2% doses on day 7 as well. The reduced growth during initial days and at higher concentrations (>2%) might be due to alkalinity induced by the alkaline elements present in biochar powder. Further, its hygroscopic nature might have resulted in the binding of water molecules on its surface,[19] thus, comparatively less water availability to the seeds at the starting days of the treatment. However, subsequent release of nutrients and homogenously moistened conditions might have facilitated the growth of seedlings under the biochar applied treatment during later growth stages.[20,21] The crop having comparatively larger seed size showed better growth responses (particularly in case of dry weights) at higher doses of biochar as compared to the small seed size crop. Thus, biochar application upto 2% dose may facilitate in improving the structural development of crop plants by providing resilience to the harsh conditions (Fig. 2). Overall, the effect of biochar powder on plants was found to be species-specific and large sized seed may respond well upto higher doses of biochar as compared to the medium or small sized seeds. Drake *et al*.,[22] also highlighted that the response towards biochar depended upon the type of species. However, such observations need to be verified by conducting such studies on different crop plants.

Table 1: Two-way ANOVA depicting differences between different days (3, 7, and 15) of biochar powder treatment.

Crop	df	F value	
		Shoot length	Root length
Cajanus cajan	5,17	12.542***	3.876**
Vigna radiata	5,17	17.595***	17.228***

, * represent significant variation at $P < 0.01$, and 0.001 levels.

Table 2: One-way Analysis of Variance (ANOVA) depicting variations between different concentrations of biochar treatment.

	Crop	df	F value					
			Shoot length	Root length	Shoot FW	Root FW	Shoot DW	Root DW
DAY 3	*Cajanus cajan*	5,17	43.877***	50.743***	-	-	-	-
	Vigna radiata	5,17	30.545***	48.595***	-	-	-	-
DAY 7	*C. cajan*	5,17	27.296***	7.265**	43.970***	9.074***	25.680***	5.787**
	V. radiata	5,17	88.058***	18.023***	20.739***	10.060***	7.179**	6.892**
	Oryza sativa	5,17	14.403***	8.090**	3.159*	14.912***	8.901***	14.111***
	Zea mays	5,17	43.902***	15.611***	18.375***	9.373***	10.164***	6.713**
DAY 15	*C. cajan*	5,17	13.877***	17.885***	14.637***	7.793**	9.767***	9.215***
	V. radiata	5,17	4.477*	3.818*	20.439***	24.984***	9.367***	14.652***

*, **, *** represent significant variation at $P < 0.05$, 0.01, and 0.001 levels. Here, FW = Fresh weight, DW = Dry weight.

Table 3: t-test depicting variations in day (experiment 1) and crop (experiment 2).

Comparison	df	t value					
		Shoot length	Root length	Shoot FW	Root FW	Shoot DW	Root DW
Cajanus cajan (Day 7 vs. 15)	34	-7.895***	-7.236***	-6.451***	-6.069***	-7.429***	-10.741***
Vigna radiata (Day 7 vs. 15)	34	-8.182***	10.361***	-7.061***	-6.090***	-8.636***	-9.532***
Oryza sativa vs. *Zea mays*	34	6.963***	-12.278***	-11.815***	-14.470***	-16.749***	-17.068***

*, **, *** represent significant variation at P < 0.05, 0.01, and 0.001 levels. Here, FW = Fresh weight, DW = Dry weight.

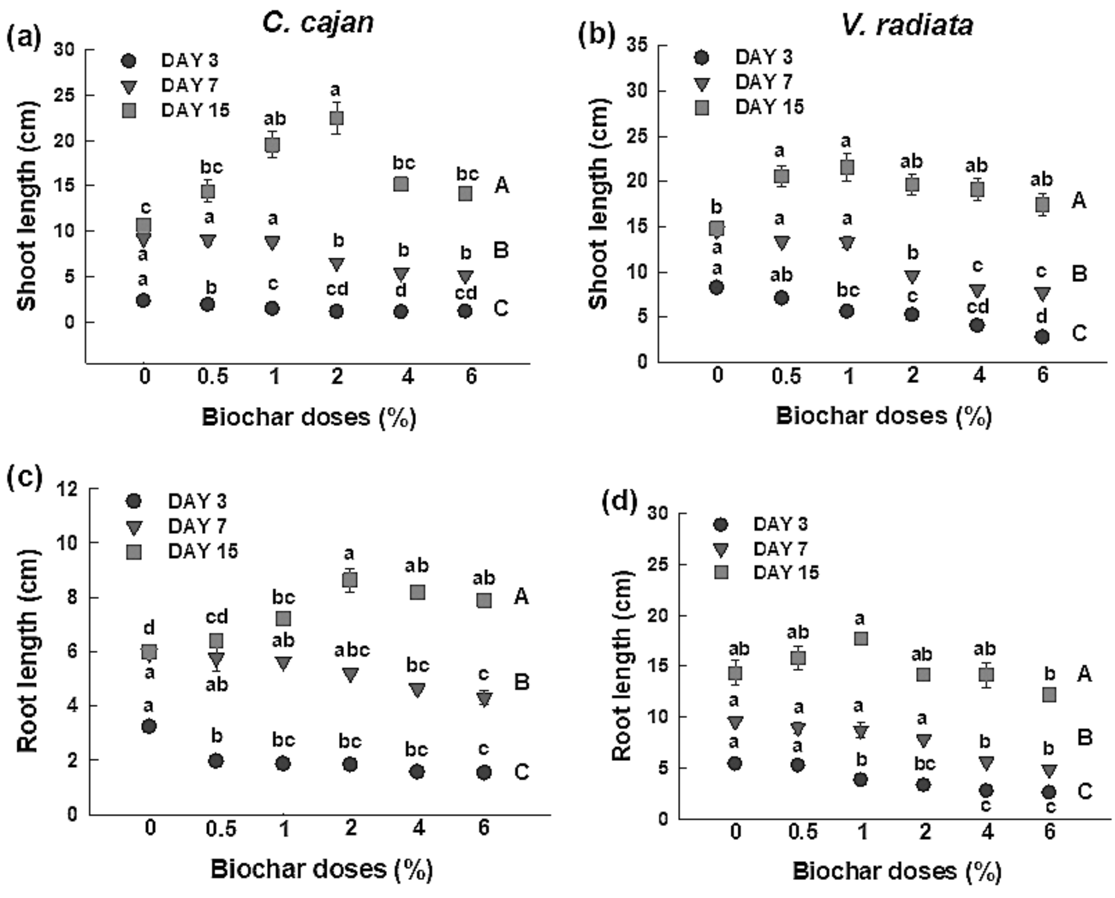

Fig. 1: Variation in root and shoot growth of *Cajanus cajan* (a and c) and *Vigna radiata* (b and d) seedlings after 3, 7 and 15 days of exposure to different doses of *Broussonetia* biochar powder. Different small alphabets represent the significant (P < 0.05) variations among different doses of biochar whereas capital alphabets represent the significant (P < 0.05) variations with days of exposure to the biochar powder based on Tukey's *post-hoc* test.

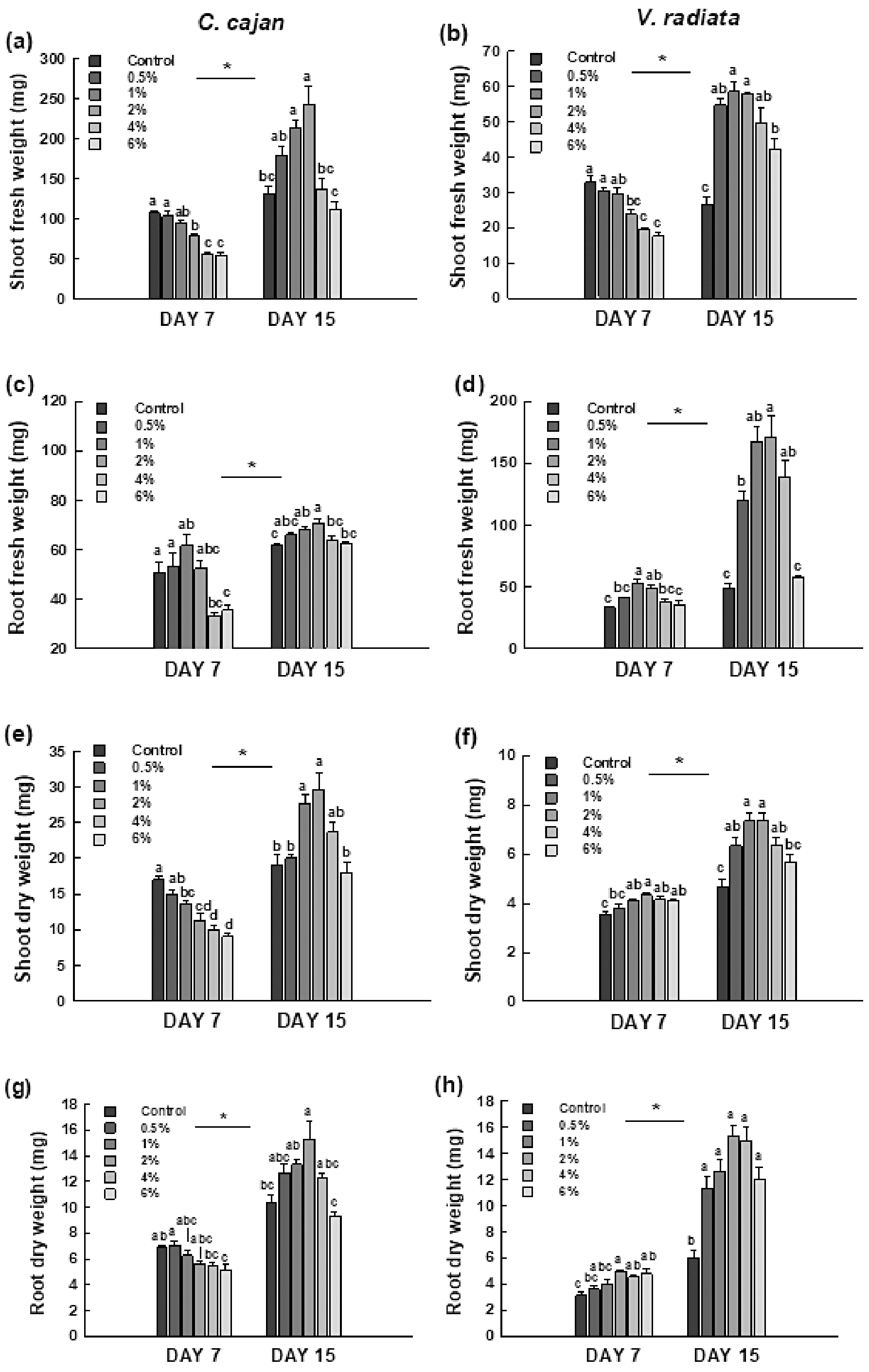

Fig. 2: Variation in root and shoot fresh and dry biomass of *Cajanus cajan* (a, c, e and g) and *Vigna radiata* (b, d, f and h) seedlings after 7 and 15 days of exposure to different doses of *Broussonetia* biochar. Different small alphabets represent the significant (P < 0.05) variations among different doses of biochar based on the Tukey's post-hoc test, whereas '*' represent the significant (P < 0.05) variations between 7 and 15 days of exposure to the biochar based on independent t-test.

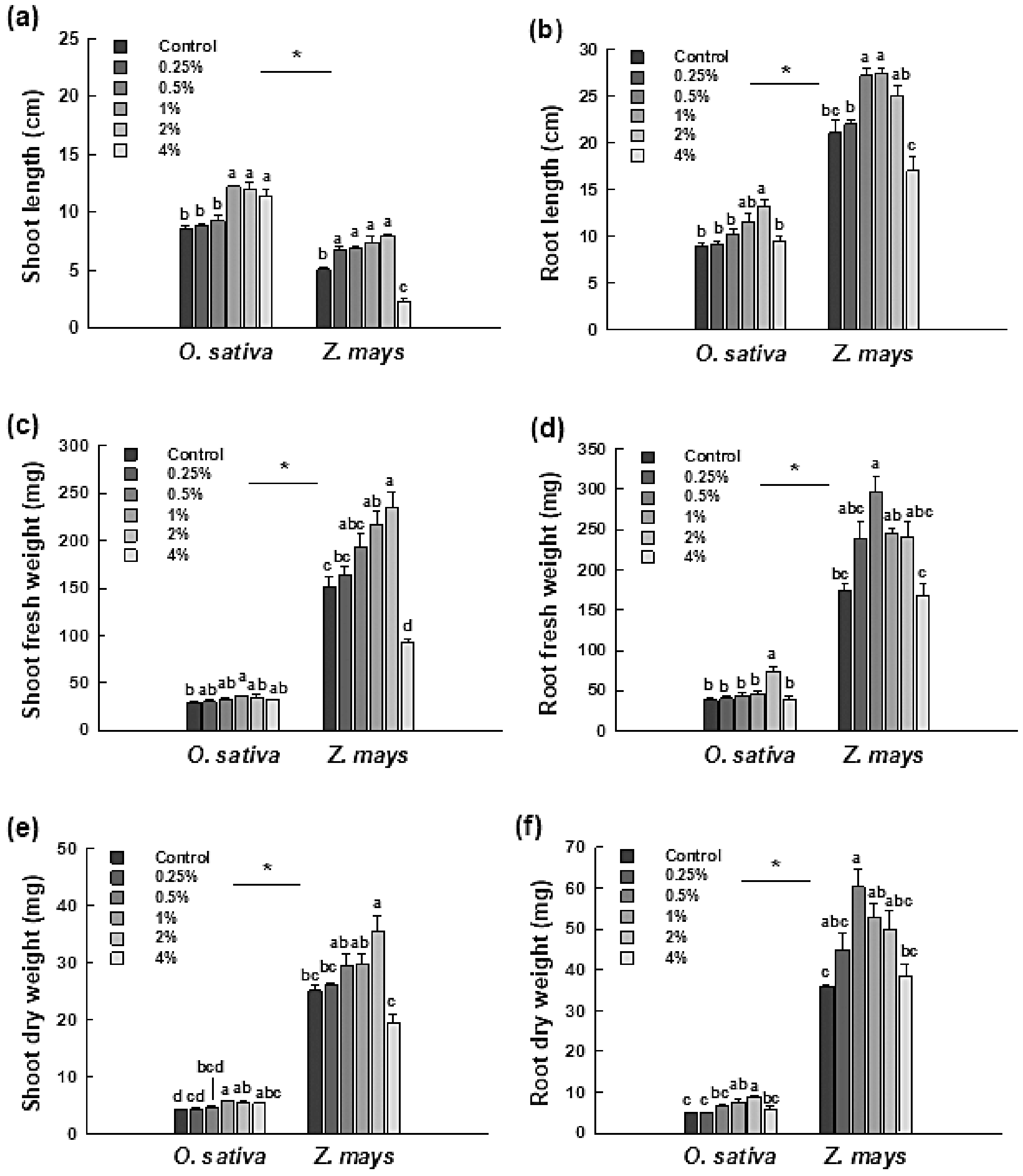

Fig. 3: Variation in shoot (a, c and e) and root (b, d and f) length and biomass parameters of *Oryza sativa* and *Zea mays* seedlings after 7 days of exposure to different doses of *Broussonetia papyrifera* biochar water extract. Different small alphabets represent the significant (P < 0.05) variations among different doses of biochar based on the Tukey's test whereas '*' represent the significant (P < 0.05) variations between the two plant species after 7-days of exposure to the biochar extract based on independent t-test.

Effect of Biochar Water Extract on Cereal Crop Growth Responses: Laboratory Assay 2

A significant ($P < 0.001$) variation in different growth parameters of O. *sativa* and *Z. mays* was observed with different doses of biochar water extract (Tables 2, 3). The seedlings of *O. sativa* and *Z. mays* germinated in biochar extract had significantly ($P < 0.001$) longer roots and shoots than control seedlings germinated in distilled water (Fig. 3). This might be explained by the fact that the biochar extract contains more macro/micro-nutrients in dissolved form than distilled water which may help in providing the initial nutrient supply to the seed germination.[23] In addition to dissolved nutrients, the water extract of biochar contains several bioactive compounds which may facilitate the plant growth and development after application.[24] Here also the effects were species-specific and the t-test revealed significant ($P < 0.001$) differences among growth parameters of *Z. mays* and *O. sativa* (Fig. 3). *Z. mays* showed higher growth as compared to *O. sativa*, when compared to their respective controls. Additionally, as compared to the shoots, the effects of biochar extract dose were more pronounced on the roots of *Z. mays* whereas both root and shoot components showed similar responses in case of *O. sativa*. In *O. sativa*, the shoot fresh and dry weight increased upto 1% dose of biochar extract, whereas in roots, it was maximum at 2% dose (Fig. 3). In case of *Z. mays*, the shoot fresh and dry weight increased in a dose-dependent manner upto 2% dose and an increase of ~55 and ~42% over control was observed at this dose (Fig. 3). The root fresh and dry weight of *Z. mays* was increased by ~40 and 46% at 0.5-1% biochar extract dose. The results corroborate with a study, wherein, Nazir and Batool,[18] reported the improvement in the length and biomass of *T. aestivum* on exposure to the biochar in a laboratory setup.

Differences among Powder and Water Extract Treatments

The effects of *B. papyrifera* biochar varied depending upon the type of treatment conditions applied (i.e., powder or extract). Majority of biochar-based research have reportedly used the powder for plant growth studies. It was observed that plants grown in biochar powder had a longer survival time (15 days) than that of plants germinated in biochar extract (7 days). This might be attributed to the fact that the amount of nutrients extracted for extract preparation gets exhausted, and might have led to the degradation of plants after 7 days of treatment. Conversely, biochar powder allowed the delayed response by slowly releasing the nutrients as per the requirement of the plant, and therefore, plants were able to survive upto 15 days after exposure. The tendency of biochar to slowly release the nutrients has earlier been confirmed by researchers.[25] Furthermore, the effects of biochar are dependent on the type of biochar used and the plant species tested. This has been demonstrated by Jean and Khasa,[26] where the authors observed that the application of biochar from maple bark and hardwood chips improved the germination of *Avena sativa* L., *Festuca rubra* L., *Trifolium repens* L. based on the above stated two factors. Thus, it can be suggested that application of biochar in powder form is better for the growth of crops as it leaves a longer window for plants to grow. On the contrary, the biochar water extract can be applied for growing hydroponic plants, particularly in urban agriculture and indoor-planting systems. However, long-term studies considering a number of plant species with different seed size are needed to reach a concrete conclusion.

Conclusions

The present study was aimed at figuring out the growth patterns of some crop plants towards *B. papyrifera* biochar powder and water extract application. The seedling responses of different crops showed variation in growth with time and species inherent properties such as seed size after biochar application. Biochar powder from *B. papyrifera* leaf litter displayed limited changes in growth after 3 and 7 days of application, however, it caused a substantial increase at 15th day. It induced delayed response by allowing the slow-release of nutrients in powder form. Conversely, in the extract form, plants showed an improvement in growth after 7 days itself. It was also recorded that the growth behaviour of the seedlings to biochar application was dose-dependent and species-specific. Seed size of the crop also found as a major determinant for the dose-specific responses to the *B. papyrifera* biochar. Notwithstanding, detailed studies on various crop plants under different agricultural setups are necessary to ascertain the role of biochar on the growth of crop plants.

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Conflict of Interest

The authors declare that there is no conflict of interest. All the authors have seen the final version of the manuscript and agreed to its submission to the journal.

Statements and declarations

The authors declare that the data presented in this article is their own work.

Data Availability Statement

Relevant data has been presented in the article. The raw data can be made available on request to the corresponding author.

Authors' contribution

IG: Performed the experiment, Data Collection and analysis, Initial drafting and finalization; RS: Designing, Drafting, Analysis, Review and finalization; RC: Analysis and Initial Drafting, AK: Co-supervision, Analysis, Review and finalization; SK: Review and finalization; DRB: Supervision, Conception, Designing, Review and finalization

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