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Biochar optimum speed increases productivity and conserves residues of glyphosate in field soils

Md. Abu Sayem Jiku[1], Md. Arifur Rahaman[1], Muhiuddin Faruquee[2], Shata Rupa Sinha[3], Md. Ashraful Alam[4], Ashutus Singha*[1]

[1]*Institute of Environmental and Sustainable Development in Agriculture, Graduate School of Chinese Academy of Agricultural Science, Beijing, China*

[2]*Department of Crop Genetics and Breeding, Institute of Crop Sciences, Graduate School of Chinese Academy of Agricultural Sciences, Beijing, China*

[3]*Department of Horticulture, Bangladesh Agricultural University, Mymensingh, Bangladesh*

[4]*Institute of Agricultural Resources and Regional Planning, Graduate School of Chinese Academy of Agricultural Science, Beijing, China*

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Abstract

Biochar assists to reclaim the soil environment through absorbing the toxic compounds and its optimum application has a significant role to detoxify the glyphosate residues from soil that improves soil properties. Therefore, this study was carried out to detoxification of glyphosate soil residues by biochar amendments and to finding the suitable biochar application rate in crops. In this study, successfully applied of glyphosate to the upper 2-3cm of the top soil display the moderate toxicity for seedling growth but this negative effect has been mitigated by 5% biochar application. In control, leaf chlorophyll content was higher and showed better performance than biochar treatment. Among the all biochar treatments, Gly+ch10% indicates slightly higher shoot fresh biomass than all biochar treatments but no significant difference found in shoot dry weight. In root morphology, the biochar amendment and glyphosate treatment did not show significant difference in fine roots production. For instance, optimum application of biochar influences to enlarge the total root length, which has positive effect to uptake the mineral nutrient from the deeper part of soil. On the other hand, higher rate of biochar application has negative impact on shoot and root growth. These findings are suggesting that biochar amendments (5-10% v/v) can mitigate absorbs effects of herbicidal residues and there is no toxic effect of glyphosate resides. For a successful introduction of biochar application in agriculture field acts as a huge amount of carbon sink and increased crop production as well as positive effect to mitigate climate change.

***Corresponding Author:** Ashutus Singha ✉ ashutus.iwm@sau.ac.bd

Introduction

Glyphosate is a major water polluting herbicide and biochar is being effectively used to remove Glyphosate from soil residues. In long term application of high residues of glyphosate confirmed delayed degradation and are harmful for the crop. Biochar can be used to remove or bind herbicide residues in soil at the time of seeding (Hossain *et al.,* 2020). It effectively absorbing of phytotoxic glyphosate residues in soils is still debated but few studies recommended that the poisonous effects of glyphosate in the soil as well as crops (Dallegrave *et al.,* 2003; Gasnier *et al.,* 2009).

High residues of glyphosate confirmed delayed degradation and these residues are harmful to the crops and soils environment (Neumann *et al.,* 2012). Soil amendment of biochar is a promising technique to bind pollutants makes them very suitable for remediation of agricultural soils (Beesley *et al.,* 2011; Kookana, 2010). Besides, glyphosate is a major water polluting herbicide and active charcoal is effectively used to delaying degradation and its harmful impact on the crop (Neumann *et al.,* 2012). Conversely, biochar has an effect on soil texture, structure, porosity, density and particle size distribution. Additionally, biochar contains highly condensed aromatic structures which resist decomposing in the soil and could mitigate up to 12% of current anthropogenic CO_2 emissions (Lehmann, 2006; Waiman *et al.,* 2012; Woolf *et al.,* 2010). Biochar influences soil bulk density (Major *et al.,* 2010), changes soil pH, cation exchange capacity (CEC), electrical conductivity (EC) and nutrient levels (Amonette *et al.,* 2009; Gundale *et al.,* 2007; Lehmann *et al.,* 2011; Liang *et al.,* 2006). Macro and microstructure of soil particles with biochar (Downie *et al.,* 2009) effect reduce the concentration of soluble compounds (Gundale *et al.,* 2007) and also changes of chemical (Nguyen *et al.,* 2010) and physical properties (Downie *et al.,* 2009) of soil. Biochar application helps to enhance plant growth yield, reduce leaching of nutrients, increase the retention capacity of fertilizer in soil (Xu *et al.,* 2012) and have a large surface area to reducing pollutant from soil environment (Beesley *et al.,* 2011).

Glyphosate phytotoxicity causing the impairment of overall vital metabolic processes, such as protein synthesis and photosynthesis (Bott, 2010a). It is rapidly translocated to stems, leaves, and roots of the entire plant and ultimately accumulating in young growing tissues furthermore meristematic regions of the roots, shoots, rhizomes, tubers, stolons of plants (Bingham et at. 1997; Sprankle *et al.,* 1975). It is reported that half-life times of glyphosate range varied from 1-197 days but in agricultural soil less than 60 days (Giesy *et al.,* 2000). Moreover, glyphosate adsorption is mainly depending on soil pH and soil organic matter (SOM) buts it's has a dual role in soil sorption capabilities (Gerritse *et al.,* 1996; Gimsing *et al.,* 2007; Gimsing *et al.,* 2004; Gimsing *et al.,* 2007a). Several investigations show soils can exhibit great variability in their ability to degrade glyphosate (Franz, 1985; Mamy *et al.,* 2005; Sørensen *et al.,* 2006). Glyphosate herbicide might be occurring degradation and these residues are harmful to the crop. As per recommendation, glyphosate is applied pre-sowing and it must be degraded or bind before seeding. As bio-charcoal is being used to remove herbicide, it can be used to remove or bind herbicide residues in soil at the time of seeding. For this reason, a hypothesis that biochar amendments can minimize the glyphosate residual effect on plant growth and carried out the research for detoxification of glyphosate soil residues by biochar amendments and to finding the suitable biochar application rate in crops. The main aim of this study was to assess the 1) testing of perspectives for detoxification of glyphosate residues by biochar amendments, 2) determination of optimum dose of biochar,

Materials and methods

Pot experiments, as a complement to field measurements, allow the investigation of the plant under semi-controlled conditions without distracting effect of heterogeneous environmental factors such as types of farming, soil biota, microorganism, and microbial activities in the soil environment. The experiment was carried out at open field condition on the 15th of March 2015. Wheat (*Triticum aestivum*) was used as a test plant. The soil was collected from

Tahirpur Upazilla, Sunamganj district, Bangladesh with fallow land. To get a homogeneously substrate, the soil was sieved by hand using a quadratic sieve of 45cm side length and a mesh diameter of 2mm. Roundup Ultramax® formulation as active ingredient glyphosate was used for all the experiments.

Biochar was collected from Shibalaya Upazilla of Manikganj District, Bangladesh. Glyphosate solution was applied directly to the soil and homogenously mixed to the used soil volume. Depending on the aim and approach of the experiment, a waiting time has been given for the glyphosate 24 hours before sowing of the wheat seeds. After waiting times 10 seeds of wheat (Triticum aestivum cv.) were sown into each pot at sowing depth of 2.5cm then fine sand was used to cover the top layer of pot to reduce evaporation.

Experimental design and treatment

The experiment was laid out in Completely Randomized Design (CRD) with four treatments and four replications. Roundup Ultramax® was applied in soil with or without different doses of biochar in different treatments. The soil was homogeneously mixed and prepared a pot (400 g/pot). The treatment was Soil Mixed with Roundup Ultramax® 6L/ha (Gly); Roundup Ultramax® at 6L/ha dose with 5% v/v Biochar (Gly + Biochar 5%); Roundup Ultramax® at 6L/ha dose with 10% v/v Biochar (Gly + Biochar 10%) and Roundup Ultramax® at 6L/ha dose with 20% v/v Biochar (Gly + Biochar20%). From this solution applied dose was 10mL-kg soil (Bott, 2010b). After filling, pots were incubated for 24 hours before sowing, and then 10 seed of winter wheat were sown in each pot. After every 24 hours pots were watered (soil moisture level 70%) and randomized, after 48 hours data were recorded and photos was taken. Plants were removed from the pots and washing out the root systems with water at the end of each experiment. Finally, roots and shoots were separated and took the fresh weight. Shoots were dried at 60°C for 24 hours and weighed for shoot dry weight. Roots were preserved in 20% alcohol solution for further root morphology study then roots were also dried at 60°C for 24 hours and dry weight was taken.

Data collection from the experiment

Germination% Calculation: Number of seeds germination, out of 10 seeds sown, was recorded for each treatment, after 24-hour interval and percentage were calculated using the following formula:

$$\text{Germination\%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

SPAD value measurement

SPAD value of wheat leaves was collected from each plant and measured to determine the nutrient status of the plants. The chlorophyll meter SPAD-502 Plus was used to measure the SPAD value. The SPAD value was taken from each youngest fully developed leaves and finally got an average value of chlorophyll content.

Fresh and Dry biomass of Shoot and Root: After harvesting shoots were cut above the top soil level and weighed the fresh biomass. The fresh shoots were dried in oven at 40°C for 3 days and dry matter was determined by weighting. In case of root biomass, the same method took place after carefully washing soil and removal of all organic and biochar particles.

Root morphology: Before oven dry, roots were preserved in 20% ethanol solution. The root system was distributed on the scanner plate and scanned with a scanner (Epson Perfection V700 Photo, Epson, USA) of the image of each treatment. The image was analyzed with WinRHIZO software to observe the root morphology. Root length was measured considering the diameter classes (0.0-0.2mm, 0.2-0.4mm, 0.4-0.6mm,0.6-0.8mm,0.8-1mm, 1-1.2mm and >1.2mm) of the total root system. Total root length and total root average diameter were also measured.

Statistics analysis

Statistical analysis of variance was performed by using Sigma plot 12 statistics software package by comparing means through one-way-ANOVA (Sigma plot, Systat Software. Inc. U.S.A).

Results

Emergence% of seedlings

Seeds emerged after 4th days of seeding. The treatments among different biochar content did not show any significant differences in emergence percentage of seeds per unit of time. Gly+ch10% treatment showed a better emergence rate than other treatments. At first control, treatment showed lower emergence percentage, but after 6th-day control, gly+ch5% and gly+ch20% treatments showed the similar result of emergence percentage of seeds. Among biochar amendment treatments, slower germination percentage was found for gly+ch5% treatment at the beginning of emergence. After 7th days all values reached above 90% at the end of emergence, whereas the highest value was revealed for gly+ch10% that was higher than 97% in emergence (Fig.1).

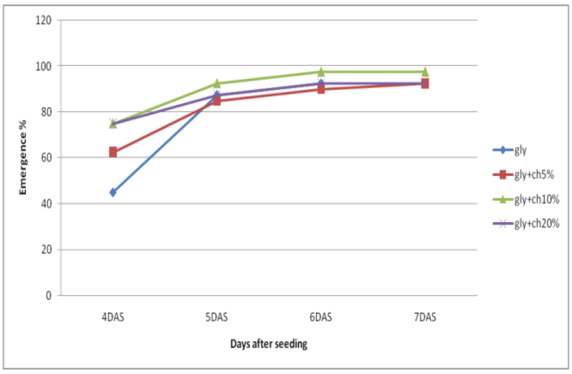

Fig. 1. Percentage of emerged wheat seeds among different biochar amendment treatment per day after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Gly=soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, gly+ch10%=glyphosate with biochar amendment of 10%, gly+ch20%=glyphosate with biochar amendment of 20%.

Leaf chlorophyll content

SPAD value was taken 7th days after seeding and different treatments showed different patterns of chlorophyll content. Glyphosate treated control showed significantly better performance compared with an additional application of 5% biochar treatments. Glyphosate treated control performed higher SPAD value than all the other treatments. Among glyphosate with biochar treatment, Gly+ch5% treatment was performed poorly compared to gly+ch10% and gly+ch20% treatments respectively.

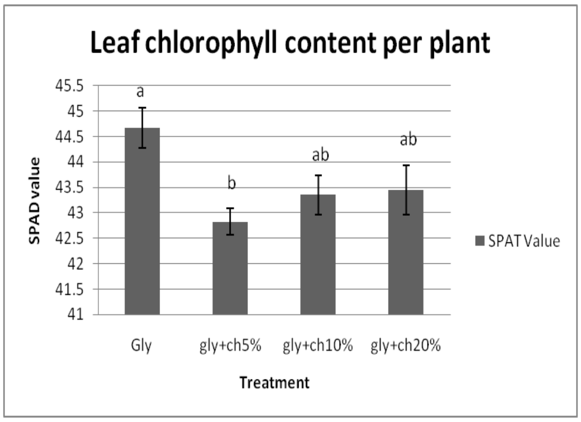

Fig. 2. Soil Plant Analysis (SPAD) values showing leaf chlorophyll content of winter wheat seeds (cv. Isengrain) after 7th of seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: gly= soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, gly+ch10%= glyphosate with biochar amendment of 10%, Gly+ch20%=glyphosate with biochar amendment of 20%. Error bars indicating standard error. Different letters above the bar indicating significant differences (α = 0.05).

Fresh and dry biomass of Shoot

Fig. 3. Shoot developmental stage of winter wheat (cv.Isengrain) seedling after 7days of emergence compared with the glyphosate-treated control, shoot fresh weight was no significant difference at Gly+ch5% and Gly+ch10% biochar amendment treatment. Gly+ch5% and Gly+ch10% biochar amendment treatment showed significant difference with gly+ch20% treatments. In the comparison of biochar treatments, biochar with addition 10% treatment seemed to increase shoot fresh weight compared to all other biochar treatment.

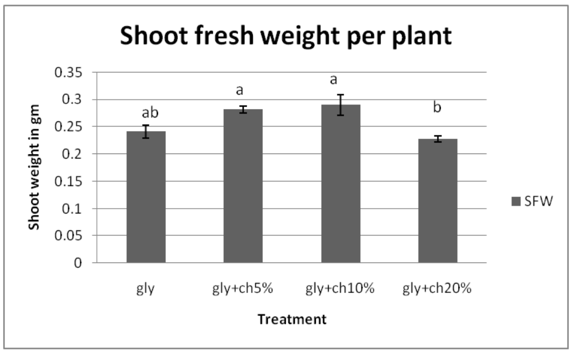

Fig. 4. Shoot fresh values of winter wheat of different treatments 7th days after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont= soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, gly+ch10%=glyphosate with biochar amendment of 10%, gly+ ch20% =glyphosate with biochar amendment of 20%. Error bars indicating standard error. Different letters above the bars indicating significant differences (α = 0.05).

Shoot dry weight showed a similar pattern to fresh weight, besides Gly+ch10% treatment was performed slightly higher than all other treatments. Among biochar amendment treatments, gly+ch5% was shown significant differences to Gly+ch20%. Whereas Gly+ch20% was performed lower production of shoot fresh biomass than all other three treatments.

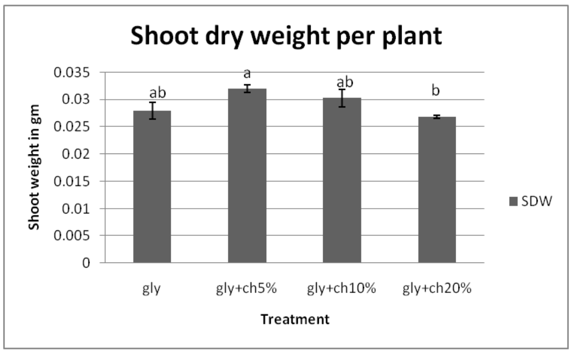

Fig. 5. Shoot dry weight values of winter wheat of different treatments 7th days after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont= soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, gly+ch10%=glyphosate with biochar amendment of 10%, gly+ch20%=glyphosate with biochar amendment of 20%. Error bars indicating standard error. Different letters above the bars indicating significant differences (α = 0.05).

Fresh and dry biomass of root

Compared with the glyphosate-treated control, root fresh weight was significantly increased in the variant with additional application of 5% biochar. No significant differences were detectable for the remaining treatments. Among biochar treatment, Gly+ch5% performed better root fresh weight and Gly+ch5% showed worse root weight than other biochar treatment.

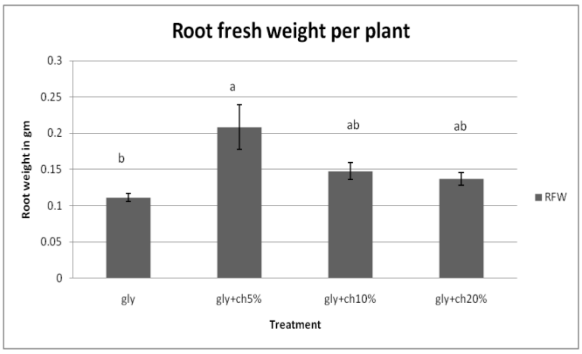

Fig. 6. Root fresh weight values of winter wheat of different treatments 7th days after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont= soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, gly+ch10%=glyphosate with biochar amendment of 10%, gly+ch20%=glyphosate with biochar amendment of 20%. Error bars indicating standard error. Different letters above the bars indicating significant differences (α = 0.05).

In case of root dry weight, the difference among different biochar amendment and without biochar amendment had almost disappeared. Besides Gly+ch5% treatment that had higher root dry weight than other treatments. On the other hand, glyphosate treated control without showed worse performance in case of root dry weight than other treatments.

Root morphology

Root morphology of the experiment did not show any significant difference among all treatment in the diameter range 0.0 to 0.5 mm. Biochar treatments did not perform mitigation effect in fine root production compared to glyphosate control treatments.

However, biochar amendment treatment showed better result in root length in comparison to glyphosate control in coarse diameter class.

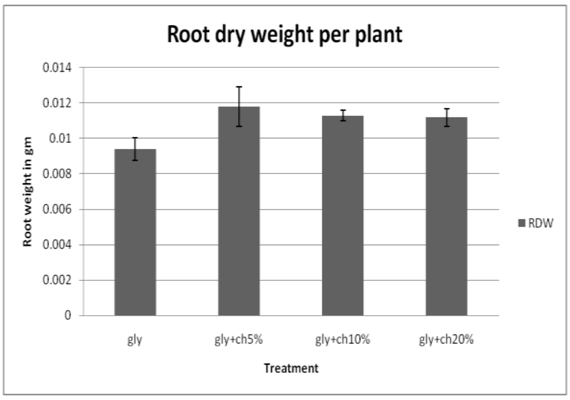

Fig. 7. Root dry weight values of winter wheat of different treatments 7th days after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont= soil with glyphosate, gly+ch5% =glyphosate with biochar amendment of 5%, gly+ch10%=glyphosate with biochar amendment of 10%, gly+ch20%=glyphosate with biochar amendment of 20%. Error bars indicating standard error. Different letters above the bars indicating significant differences (α = 0.05).

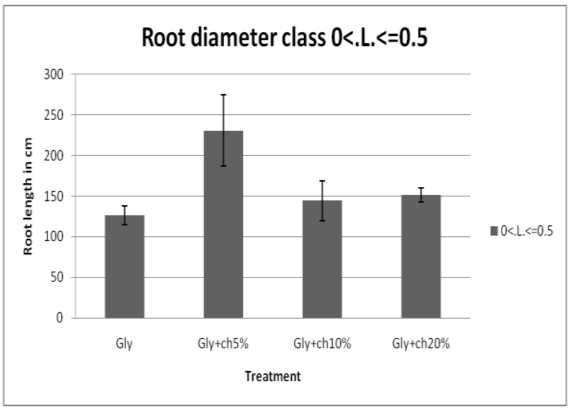

Fig. 8. Root length of winter wheat seedlings (cv. Isengrain) of different treatments 7th days after seeding. Showing fig.: Cont= soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, Gly+ch10%= glyphosate with biochar amendment of 10%, Gly+ch20%= glyphosate with biochar amendment of 20%. Data show average treatment values of four independent replicates. Error bars indicating standard error.

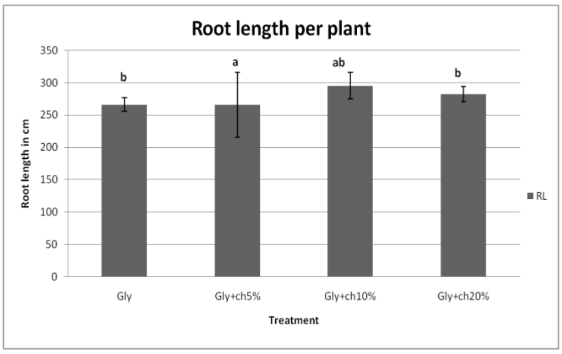

Fig. 9. Total root length of winter wheat of different treatments 7th days after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont= soil with glyphosate, gly+ch5%=glyphosate with biochar amendment of 5%, gly+ch10%=glyphosate with biochar amendment of 10%, gly+ch20%= glyphosate with biochar amendment of 20%. Error bars indicating standard error. Different letters above the bars indicating significant differences (α = 0.05).

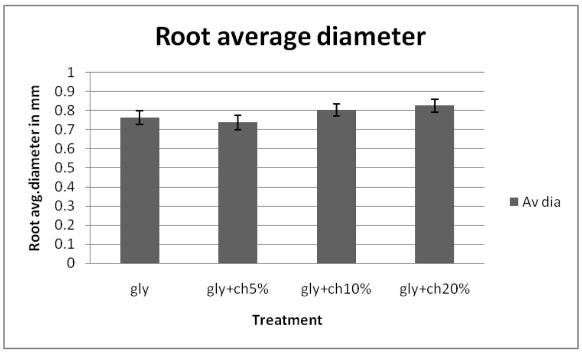

Fig. 10. Root average diameter of wheat seedlings of different treatments 7 days after seeding. Data show average treatment values of four independent replicates. Treatment letters were as followed: Cont = soil with glyphosate, gly+ch5% = glyphosate with biochar amendment of 5%, Gly+ch10% = glyphosate with biochar amendment of 10%, Gly+ch20% = glyphosate with biochar amendment of 20%. Error bars indicating standard error.

Comparing with glyphosate control and Gly+ch20%, total root length per plant was significantly increased invariant with addition of biochar5%. The biochar application had a positive effect on the root length compared to glyphosate control treatment. In addition, Gly+ch5% treatment performed higher in root length among all the treatments.

Similarly, the worse root length was observed in case of the glyphosate control treatment. In comparison to all other biochar treatments Gly+ch5% showed higher in root length than all other biochar treatments. There was no significant difference found in case of root average diameter.

Discussion

Biochar is so beneficial to adsorb organic contaminants, it is important to examine the mechanism of biochar sorption. As per recommendation glyphosate is applied pre-sowing and it must be degraded or bind before seeding. The treatments among different biochar content performed better in emergence percentage of seeds per unit of time. Gly+ch10% treatment showed a better emergence rate than all other treatments. Among different treatments, glyphosate control treatment showed lower emergence percentage which was according to the expectation that long term glyphosate application has a negative effect on both seed germination and plant growth by glyphosate (Neumann *et al.,* 2012). Among biochar amendment treatments, slower emergence percentage was found for gly+ch5% treatment in the beginning of emergence, whereas the highest value was revealed for gly+ch10% that was higher than 97% in emergence. Many studies revealed that addition of biochar decreases the leaching of glyphosate(Jones et at. 2011). As it was that biochar can do amendment in soil with high residues of glyphosate. The relationship between the biochar amendment and chlorophyll content is not completely clear yet. Glyphosate control treatment showed significantly better performance than variant with additional biochar 5%. Some studies showed that biochar can bind NO_3^- and NH_4^+ (Clough & Condron, 2010). This could be a possible explanation of leaf chlorophyll concentration being lower in the biochar amendment treatment than glyphosate control treatment. Among glyphosate with biochar amendment treatments, gly+ch10% and gly+ch20% performed significantly similar than gly+ch5% on the sampling date. Gly+ch5% treatment performed significantly worse than gly+ch10% and gly+ch20% treatments respectively. Shoot fresh and dry biomass stands in a close relationship with the nutrient status of the plants. Shoot fresh weight showed a significant difference among Gly+ch5%, Gly+10% and Gly+ch20% treatment however, in the case of Gly+ch10% showed a slightly higher value of shoot fresh biomass than all the other biochar treatments. On the other hand, Gly+ch20% treatment was performed worse than all other treatments. Probably the reason behind it was the higher rate of biochar application could suppress shoot and root growth (Solaiman *et al.,* 2012). Shoot dry weight showed the similar result as like as shoot fresh weight. A significant difference was observed in the case of root fresh weight. Gly+ch5% treatment showed significant differences in root fresh weight with control treatments and had higher biomass production than other three treatments. The possible explanation could be negative long term effects of glyphosate application reducing nutrient uptake and a mitigation effect by biochar are visible when comparing shoot and root weights of glyphosate control and biochar amendment treatment. Root dry weight showed similar the result as compared to fresh weight (Fig7). Compared with the glyphosate-treated control, total root length was significantly increased in the variant with additional application of 5% biochar. As mentioned before, biochar has a binding capacity for herbicide molecules and nutrient retention to a great extent (Lehmann, 2006) and in consequence could have led to enhanced root growth of biochar treatments in comparison to glyphosate control treatments. In this prospect, Gly+ch5% treatment performed higher in root length and on the other hand the worse root length was observed in the case of Glyphosate control treatment. These findings are suggesting that glyphosate mediated root damage can be mitigated by biochar amendment.

Conclusion

Biochar has been proved as an efficient sorbent of various contaminants, organic and inorganic compound. It offers an opportunity to bind binding organic pollutants in the environment due to its high sorption affinity and resistance to microbial decomposition.

In this experiment, the treatments among different biochar content were performed better in emergence percentage of seeds per unit of time. As glyphosate control treatment showed lower germination percentage of emergence compared to other treatment. So, the results show that long term application of glyphosate application has a negative effect on both seed germination and plant growth. The relationship between the biochar amendment and chlorophyll content is not completely clear yet. Shoot fresh and dry biomass stands in a close relationship with the nutrient status of the plants. In the comparison of control, biochar treatment seemed to increase shoot fresh weight compared to without biochar treatment. The possible explanation could be negative long term effects of glyphosate application reducing nutrient uptake and a mitigation effect by biochar is visible when comparing shoot and root weights of control and biochar amendment treatments. Shoot dry weight showed significantly similar result as like as shoot fresh weight. Root dry weight showed similar result as compared to fresh weight. Root morphology results of the biochar amendment treatments and glyphosate treatment did not show significant difference in fine roots production. These findings are suggesting that biochar amendments (5-10% v/v) can mitigate absorbs effects of herbicidal residues and there is no toxic effect of glyphosate resides. For a successful introduction of biochar application in agriculture field acts as a huge amount of carbon sink and increased crop production as well as positive effect to mitigate climate change.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this study.

Competing interests

The authors declare that they have no competing interests.

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There are currently no funding sources in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript

Authors' contributions

Conceptualization, M.A.S.J.; data curation, M.A.S.J.; writing—original draft preparation, M.A.S.J, A.S, S.R.S., M.A.R.; writing—review and editing, M.A.S.J. and A.S, M.A.R; supervision, M.A.S.J.; data analysis and visualization, S.R.S., M.A.R.; project administration, A.S.; funding acquisition, All are equally contributed. All authors revised, read, and approved the final manuscript.

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