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Perspectives usages of biochar to minimise the herbicide residues in agricultural soil

Md Arifur Rahaman^{*1}, Imran Wasiq², Md. Abu Sayem Jiku³, Ashutus Singha^{4,5}, Muhiuddin Faruquee⁶

¹Department of Farm Structure and Environmental Engineering, Faculty of Agricultural Engineering and Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh

²Department of Agronomy, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

³Department of Agricultural Chemistry, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

⁴Department of Irrigation and Water Management, Faculty of Agricultural Engineering and Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh

⁵Department of Irrigation and Water Management, Faculty of Agricultural Engineering and Technology, Sylhet Agricultural University, Sylhet, Bangladesh

⁶Department of Genetics and Plant Breeding, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

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Abstract

Biochar is a solid product from the pyrolysis process, a thermochemical conversion where biomass is heated with limited oxygen. Biochar offers an opportunity to binding organic pollutants in the environment due to its high sorption affinity. So biochar might be possible to absorb the strong polar glyphosate molecule and its metabolite amino methyl propionic acid, AMPA. As biochar is being used to remove herbicide, it can be used to bind herbicide residues in soil at time of seeding. Therefore, the experiments were carried out in order to test perspectives for detoxification of glyphosate soil residues by biochar amendments, assessment of various application methods to find practically suitable way of application of biochar. Total five treatments were used in this experiment included control, glyphosate (gly), gly+ch5%, gly+ch10%, and gly+ch20%. The results showed that seedling emergence was slightly affected by the glyphosate treatments and this effect was mitigated by 5% biochar application. For Leaf chlorophyll content, significant results were observed on 8th day. In contrast, significant results were not observed on 10th days. Root fresh weight did not reveal any significant difference. However, a significant difference was observed in root dry weight. Root morphological analysis, there was a trend for mitigation effect was observed in the biochar treatments. However, the difference was not significant. Finally, we can be concluded that plant damage symptoms were mitigated by additions of biochar (5% w/w) with known potential to adsorb toxic compounds such as herbicide residues in soils.

* Corresponding Author: Md Arifur Rahaman ✉ arifur.bau00@yahoo.com

Introduction

Glyphosate is a non-selective, post emergence systemic herbicide, applied through the leaves to desiccate all annual and perennial weed species and can be effectively control the world's 78 worst weeds (Franz, 1985). Glyphosate is a Roundup's active ingredient and the world's biggest-selling chemical used for weed control in agricultural and urban environments (Baylis, 2000). Glyphosate was first introduced to the market by Monsanto in 1974 (Monsanto, 2005). Presently, different types of glyphosates are produced by Monsanto which are registered in more than 130 countries and approved for weed control for more than 100 crop species (Monsanto, 2005). Chemically glyphosate is an organic acid derivate of the amino acid glycine and phosphonic acid. Glyphosate is practically insoluble in organic solvents due to its high polarity for example benzene, ethanol and acetone (Franz, 1985). (Giesy *et al.*, 2000) reported that half-life times of glyphosate range from 1-197 days but are typically less than 60 days under agricultural soil conditions. Glyphosate has unique sorption characteristics in soil (Borggaard and Elberling, 2004) and has three polar functional groups (carboxyl, amino and phosphonate groups) which have strongly absorbed in soil and minerals. (Gimsing *et al.*, 2007) suggested that glyphosate is absorbed to soil and minerals by ligand exchange through its phosphonic acid group. (Gerritse *et al.*, 1996) reported that glyphosate absorption is increased by presence of Al and Fe contents in soils and decreased with soil organic matter. In contrast, soils with high iron and aluminium content, soils dominated by less adsorbed glyphosate (Gimsing and Borggaard, 2007). Several studies have been found that glyphosate adsorption also depends on soil pH (Barja *et al.*, 2007). Glyphosate degradation in soils are a pure microbiological process. Several investigations show soils can exhibit great variability in their ability to degrade glyphosate. (Franz *et al.*, 1997; Mamy *et al.*, 2005) reported that glyphosate degradation is correlated with the general microbial activity. Glyphosate is applied before sowing to reduce soil erosion by leaving dead weeds or catch crops on the field. About 68% of all glyphosate treatments are

done on the stubble (Schmitz *et al.*, 2012). Recent studies suggest a relationship between long term glyphosate application has adverse effects on various non-target organisms in agro-ecosystems. According to (Huber *et al.*, 1993; King *et al.*, 2001) the adverse effects are increased sensitivity to diseases, inhibition of root growth. Potential risks of glyphosate toxicity to non-target plants in soils are generally considered as marginal, as glyphosate in the soil solution is prone to rapid microbial degradation (Giesy *et al.*, 2000) or instantaneous inactivation by sorption to the soil mineral matrix (Giesy *et al.*, 2000; Piccolo *et al.*, 1992). Plant damages associated with glyphosate toxicity is influenced by several factors such as weed density, cropping system pattern, fertilizer management and genotypic patterns within crop species. In long term no tillage soils, the background of these damages is not fully clear until now and need to further investigation. The accumulation of glyphosate and its metabolite AMPA in soil and plant residues could be a potential reason behind it (Bott, 2010). Glyphosate is rapidly translocated to stems, leaves and roots of the entire plant and finally accumulating in young growing tissues (Franz *et al.*, 1997). According to the results of (Bingham *et al.*, 1980) the accumulation of glyphosate occurs in meristematic regions of the roots, shoots, rhizomes, tubers, stolons of plants etc. However, most studies suggested negative side effects on non-target plants supposed to intensive use of glyphosate herbicides in mulch tillage or direct seeding system. In the investigated area farmers reported that high organic carbon content did not show severe glyphosate damage or visible injuries at all (Afzal, personal communication).

A high organic carbon content performs better crop growth in long term non-tillage farming which lead to the idea that soil amelioration with biochar would be an appropriate approach to better plant performance. Plant damage symptoms can be mitigated by additions of different application rate of biochar with known potential to adsorb toxic compounds. Additionally, biochar offers an opportunity to binding organic pollutants due to its high sorption affinity. The sorption capacity depends on biochar carbon fraction composition.

The biochar can be divided into the carbonized organic matter and the non-carbonized organic matter. Sorption capacity to biochar is determined by the relative carbonized and non-carbonized fractions and their surface and bulk properties (Woolf *et al.*, 2010). Biochar sorption capacity increased when produced at higher temperature due to the presence of micropores and its higher specific surface area (Xu *et al.*, 2012). (Zhang *et al.*, 2010) reported that *Pinus radiata* derived biochars influenced sorption and desorption of phenanthrene in soil. Biodegradation and leaching of simazine was reduced by biochar application. According to (Jones *et al.*, 2011) investigated that the availability of simazine is limited to microbial communities due to a fast and strong sorption by biochar. (Cao *et al.*, 2009) reported that dairy manure derived biochar absorbed Pb and atrazine as high as 100% and 77% respectively from aqueous solution. The results indicated that dairy manure are converted into biochar as an efficient adsorbent for using in potential environmental remediation. (Sun *et al.*, 2012) reported that two herbicide fluridone (FLUN) and norflurazon (NORO) can be efficiently sorbed by biochars. Moreover, heavy metals and non-pesticide organic contaminants are might be absorbed by biochars (Cao *et al.*, 2009). (Méndez *et al.*, 2012) investigated the effects of sewage sludge biochar on solubility and bioavailability of several heavy metals and found biochar amended soil in a lower leaching of Cu, Ni and Zn and a lower plant availability of Pb, Ni, Zn and Cd. Biochar had shown important adsorptivity for organic contamination e.g., POPs. Researchers found that application of biochar into soil assist to mitigating the PAHs-contaminated soils through transferring PAHs from soil to biochar (Chen and Yuan, 2010).

For this reason, this study revealed that both aliphatic moieties and aromatic moieties of biochar respectively were possibly responsible for herbicide sorption. So it is possible to absorb the strong polar glyphosate molecule and its metabolite AMPA by biochar has to be studied in this experiments. As per recommendation glyphosate is applied pre sowing and it must be degraded or bind before seeding.

Biochar was used to binding the glyphosate effects on plant growth. Therefore, this study investigated how these phytotoxic effects could be overcome through the application of biochar. For this reason, the following specific objectives were developed to be addressed in the study such as (1) to find out optimum doses of biochar ratio with soil and (2) Assessment of suitable application methods to find practically sustainable way of application of biochar. To reach the aims the following hypothesis of the study are considered: Biochar amendments would be eliminated plant damage induced by glyphosate residues.

Materials and methods

Experimental approach and designs

A greenhouse pot experiment was conducted in a completely randomized design (CRD) with five replicates. The experiment was carried out at the climate chamber, Irrigation and water management under the faculty of Agricultural Engineering and Technology, Sylhet Agricultural University from beginning on the 10th of April 2013. In this study, Roundup Ultramax® was applied in soil with different doses of biochar addition and Winter wheat (*Triticum aestivum* cv. Isengrain) was used as a model plant. The soil was collected from Tahirpur Upazilla, Sunamganj district, Bangladesh. Farmers treated the neighbored field sites with short-term (2 years) and long-term (4 years) no tillage management including pre-emergence weed control with glyphosate formulation. To get a homogeneous substrate, the soil was sieved by a 2 mm sieve.

Biochar and glyphosate application

Biochar was applied in soil as 5%, 10% and 20% v/v, respectively. Soil and biochar volume was compared and converted to weight and final dose was calculated for 5%, which was further used to get 10% and 20%. Glyphosate solution was applied directly to the soil and homogenously mixed to the soil. Depending on the aim and approach of the experiment, we planted the winter wheat seeds into the pot after 24 hours of glyphosate application.

Pot Experiment

Each pot was filled with 200g pure soil on bottom and top layer was different according to treatment.

In control top 200g soil was also pure soil. In Roundup treatment, each pot was filled with 200g top soil mixed homogeneously with Roundup Ultramax® solution. In case of combined treatments of roundup and biochar, 200g soil was mixed with Roundup Ultramax® and biochar (5%, 10% and 20%). In this way two soil layers were maintained, bottom layer of pure soil and top soil with glyphosate and biochar. Finally, each pot had total 400g soil. Total 10 seeds were sown in each pot and each pot was topped with layer of fine sand to reduce evaporation. Every day the pots were randomized and watering. The data were recorded and photos taken every 48 hours till 2 weeks.

Evaluation of plant growth and performance in pot experiment

Each plant was harvested and shoots were cut above the top soil level at the end of each experiment. Washed the roots and measured the fresh weight of shoots and roots. Shoots and roots were dried at 60°C until constant weight and measured shoot and root dry weight. SPAD value of wheat leaves were collected from each plant and measured to determine 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. Before oven dry, roots were preserved in 20% ethanol solution. The root system was distributed on the scanner plate and scanned with scanner (Epson Perfection V700 Photo, Epson, USA) of each treatment. The image was analyzed with WinRHIZO software (Regent Instruments Inc., Canada) 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

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

In this experiment, determining the suitable application techniques for farmers practice to mitigate the phytotoxic effects on top soil with addition of biochar.

Emergence of seedlings

The emergence of seedlings was occurred after 3rd days of seeding. There was no significant difference observed among all the treatment in different days after emergence except 3rd days (Fig. 1). Among the biochar treatments the highest emergence percentage was observed in Gly6L+ch5% treatment and the lowest germination percentage was revealed in Gly6L+ch10% treatment in 3rd day after emergence.

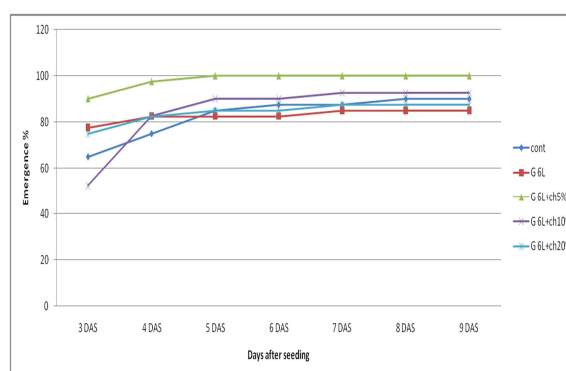


Fig. 1. Percentage of emerged winter wheat seeds among different biochar amendment treatment per day after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont: pure soil, gly: glyphosate control, gly+ch5%: glyphosate with biochar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

Leaf chlorophyll content

In this experiment SPAD value was taken after 8 days and 10 days of seedling emergence (Fig. 2). No significant difference observed among all the treatments on 10th day. In contrast, significant results were observed on 8th day. In addition, higher value was found 8th day of seedling emergence. Particularly, Gly6L+ch5% treatment was significantly different with gly6L+ch20% and control treatments.

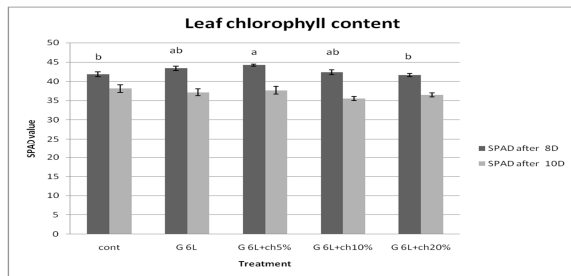


Fig. 2. Soil Plant Analysis (SPAD) values showing leaf chlorophyll content of winter wheat seeds (cv. Isengrain) after 8th and 10th days of seeding. Every data point show average treatment values of 4 independent replications. Treatment letters were as followed: Cont: pure soil, gly: glyphosate, gly+ch5%: 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 ($\alpha=0.05$).

Shoot-root fresh and dry biomass

In the experiment fresh and dry shoot weight did not significantly different among all the treatments (Fig.

3a and 3b). In comparison different, gly6L performed slightly higher shoot fresh biomass production than other treatment as well as shoot dry weight. Root fresh weight did not reveal any significant difference among the treatments (Fig. 3c). However, a significant difference was observed in root dry weight. Compared with the G6L+ch10%, root dry weight was significantly increased in the variant with additional application of 20% biochar (Fig. 3d).

Root morphology

Root morphological analysis showed significant differences in length of the fine root diameter classes from 0.0 to 0.4mm in this experiment. Only (Fig. 4a) soil control treatments performed significantly better fine root in length compared to glyphosate control treatments. There was a trend for mitigation effect was observed in the biochar treatments in diameter range 0.0 to 0.4mm compared to glyphosate control treatment, however the difference was not significant. For the remaining results, top soil application of glyphosate to the upper 2-3cm did not significantly affect plant growth for most growth parameters.

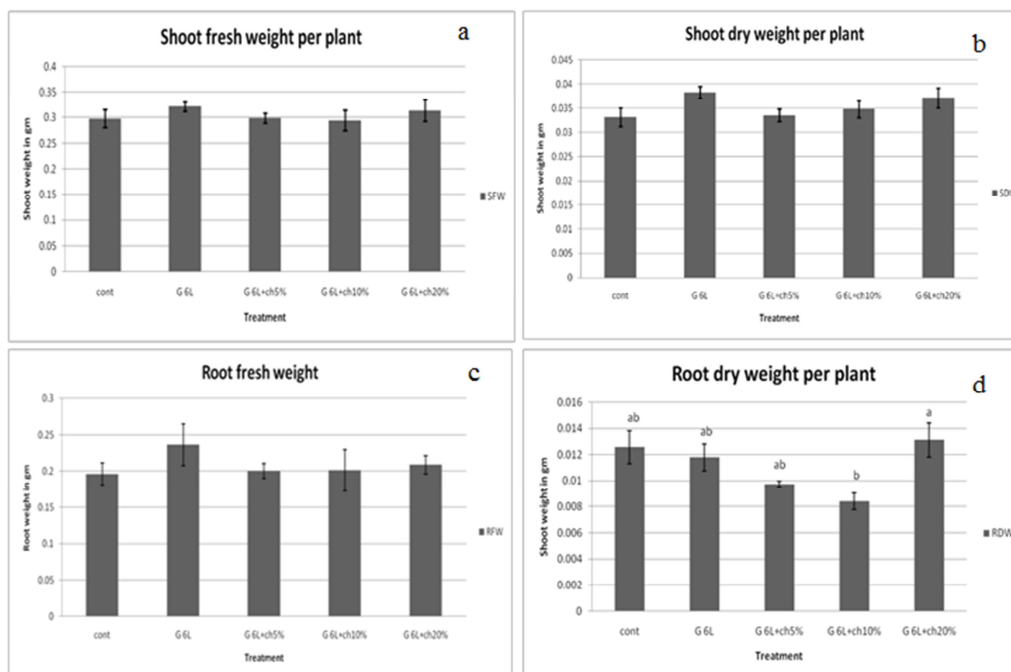


Fig. 3. Shoot and root fresh and dry weight values of winter wheat seedlings (cv. Isengrain) of different treatments 11th days after seeding. Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont: pure soil, gly: glyphosate, gly+ch5%: glyphosate 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 ($\alpha=0.05$).

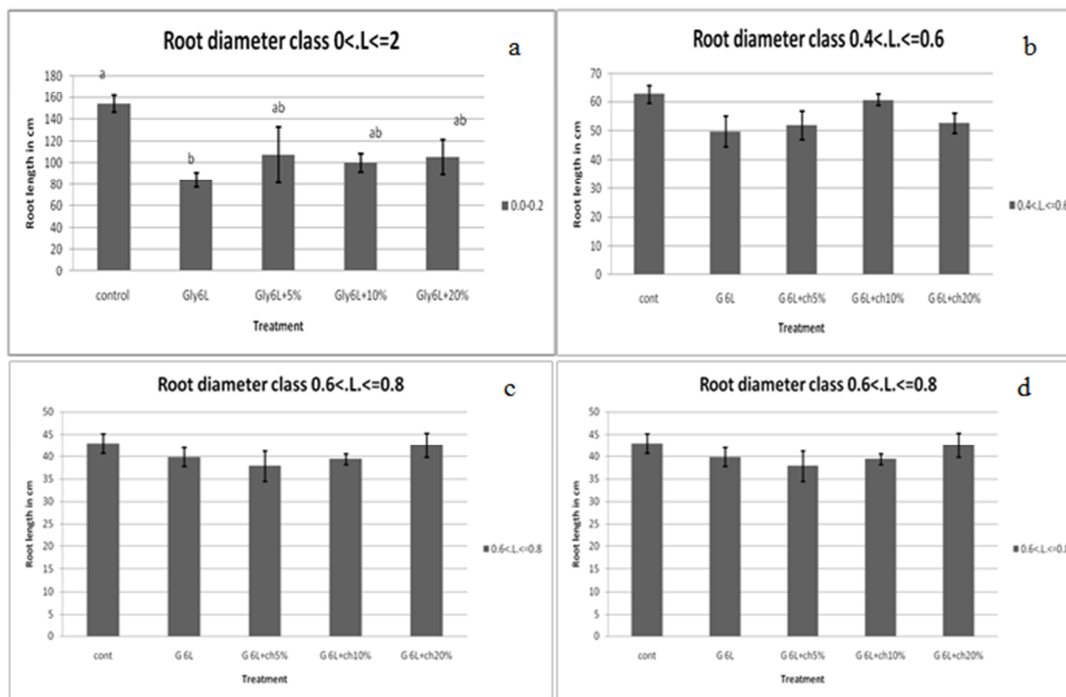


Fig. 4. Root length of winter wheat seedlings (cv. Isengrain) of different treatments 11th days after seeding in the diameter range 0.2-0.4mm (top left), 0.4-0.6mm (top right), 0.6-0.8mm (bottom left) and 0.8-1mm (bottom right). Every data point show average treatment values of 4 independent replicates. Treatment letters were as followed: Cont: pure soil, gly: 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 ($\alpha=0.05$).

There was no significant different observed in total length per plant in different treatment. Biochar amendment treatments performed better fine root length compared to glyphosate 6L treatments (Fig. 5a). In case of average diameter, Gly6L+ch20%

treatment showed significant difference compare with Gly6L+ch10% and soil control treatment. Whereas Gly6L+ch20% treatment showed higher root average diameter and soil control treatment showed lower root average diameter (Fig. 5b).

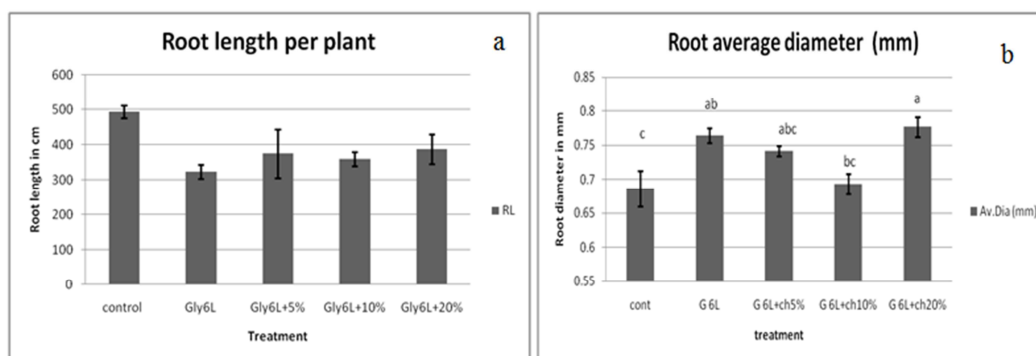


Fig. 5. Total root length (left) and average root diameter (right) of winter wheat seedlings (cv. Isengrain) of different treatments 11 days after seeding. Data show average treatment values of four independent replicates. Treatment letters were as followed: Cont: pure soil, G6L= 6L dose without biochar amendment, gly6L+ch5%: glyphosate with biochar amendment of 5%, Gly6L+ch10%: glyphosate with biochar amendment of 10%, Gly6L+ch20%: glyphosate with biochar amendment of 20%, G6L: 6L dose without biochar amendment, Error bars indicating standard error. Different letters above the bars indicating significant differences ($\alpha=0.05$).

Discussion

The experiment was conducted to find out the suitable application techniques for farmers practice. The results of experiment showed that biochar amendments (5-10% v/v) can mitigate toxic effects of glyphosate residues in soils. Gly6L+ch5% treatment was performed the highest emergence percentage in the beginning of emergence and after 5th days the value reached 100% in emergence. Among different treatments, glyphosate control treatment showed lower germination percentage of emergence which was according to the expectation that long term glyphosate application has negative effect on both seed germination and plant growth by glyphosate or its metabolite AMPA (Neumann *et al.*, 2012). However, some studies suggested that application of lower rate biochar generally increased wheat seed germination and decreased or had no effect at higher rates of application (Solaiman *et al.*, 2012). The relationship between biochar amendment and chlorophyll content is not completely clear. Particularly, there was no significant difference observed between glyphosate control and biochar amendment treatment. So biochar amendment treatment did reveal any mitigation effect on leaf chlorophyll content. Root fresh weight did not reveal any significant difference among the treatments. So the biochar amendments did not show any mitigation effect in case of root fresh weight parameter. However, a significant difference was observed in case of root dry weight.

Root morphological analysis showed significant differences in length of the fine root diameter classes from 0.0 to 0.4mm in this study. Soil control treatment performed significantly better fine root length compared to glyphosate control treatment. There was a trend for mitigation effect was observed in the biochar treatments in diameter range 0.0 to 0.4mm compared to glyphosate control treatment, however the difference was not significant. Since it was hypothesized that biochar around seed in drilling row can be practical approach of biochar application in field to protect seed from herbicide residues, by analyzing the emergence trend and fine root length it can be concluded that biochar could have an

enhancing effect on fine root growth and would be a suitable application technique for farmers practice. For the remaining results we can say that top soil application of glyphosate to the upper 2-3cm had only little affect but not significant on most growth parameters. Consequently, also no mitigation effects of biochar applications could be detected. The only exception was fine root production, which was significantly reduced after glyphosate application. There was a trend for mitigation of this effect in the biochar treatments (increase by approx. 20% in all biochar treatments), however the difference was not significant. There was no significant different observed in total length per plant in different treatment. In case of average diameter, Gly6L+ch20% treatment showed significant difference compare with Gly6L+ch10% and soil control treatment.

Conclusion

The experiment was conducted to find out the suitable application techniques for farmers practice. Emergence of seedlings and plant growth of the biochar treatment cultivated on long term no tillage soil was comparatively increased than the glyphosate control treatments. In the experiment, the relationship between biochar amendment and chlorophyll content is not completely clear. Root fresh weight did not reveal any significant difference among the treatments. However, a significant difference was observed in case of root dry weight. Root morphological analysis showed little differences in length of the fine root diameter classes from 0.0 to 0.4mm in the experiment. Since it was hypothesized that biochar around seed in drilling row can be practical approach of biochar application in field to protect seed from herbicide residues, by analyzing the emergence trend and fine root length it can be concluded that biochar could have an enhancing effect on fine root growth and would be the suitable application techniques for farmers practice. Finally, it can be concluded that glyphosate application has mitigation effect to absorb herbicidal residues. For a successful introduction of biochar application in agriculture field acts as a huge amount of carbon sink and have also a positive effect to mitigate climate change.

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