BIOCHEMICAL AND PHYSIOLOGICAL ELUCIDATION OF BIOCHAR INDUCED DEFENSE RESPONSE ACTIVATION AGAINST BACTERIAL LEAF SPOT IN CHILIES

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Abstract. Biochar plays an important role in improving the plant growth and activating defense mechanisms against biotic and abiotic stresses. In our study, the focus was on the effect of biochar made from rice straw and flyash on plant growth and development of *Xanthomonas campestris* pv. *vesicatoria* causing bacterial leaf spot in chilies. The chilli plants were grown in different substrate compositions containing either only soil or amended with Rice straw biochar (RSB)/and flyash at the rate of 3%, each. Rice straw biochar had a positive effect on plant growth, in the form of increased root and shoot weight. In comparison to flyash and soil only treatment, the application of biochar has enhanced the production of phenols, catalase, peroxidase and flavonoids. The rise in level of defense related biochemical resulted in significant reduction of bacterial leaf spot development in chilies grown in biochar amended soil. Moreover, RSB had also shown a significant *in vitro* inhibitory effect (up to 50% or more) on different pathogenic fungi and bacteria. Disease severity and incidence was significantly minimized among the plants grown in RSB containing substrate as compared to other substrate compositions. Overall, our findings indicated a positive effect of RSB amendment on plant health, as well as by enhanced protection against bacterial leaf spot in chilli plants. Biochar addition in soil not only improve fertility but also helps in achieving long-lasting Carbon sequestration goals. The ability of biochar to influence plant growth and defense pathways apparently contributes towards its ability of disease suppression.
Keywords: *environment protection, disease management, soil amendment, Xanthomonas campestris pv. Vesicatoria, resistance activation*

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Introduction

Biochar is a pyrolyzed product of organic waste (e.g., domestic organic waste, animal manure and plant residues). It is produced by burning the biomass in the absence or low concentration of Oxygen. During this process, half of the biomass is converted to char with increased surface area and stability in the soil (Laird et al., 2009). Biochar does not decompose easily in the soil, thus can serve as a long-term carbon sequestration organic source. In routine agriculture practices application of biochar contributes to increasing productivity and decreasing emission of harmful carbon in atmosphere. Soil biochar amendments are known to increase plant growth by enhancing nutrients availability as well as reduction in nutrient leaching losses (Bera et al., 2018), and by improving the biological and physico-chemical properties of soil (Mandal et al., 2016; Purakayastha et al., 2016).

In soil biochar acts as a remediate to remove pollutants. Due to its large surface area biochar has great sorption ability for of recalcitrant contaminants (Rasool et al., 2021). Biochar application modifies soil structures by influencing the nutrient flow patterns, along with soil moisture retention and pore size distribution (Olmo et al., 2016).

Disease suppressing ability of biochar was described many years ago (Allen, 1847), while studying the role of biochar in diseases like rust and mildews of wheat. Recent, (Elad et al., 2010) studies reported that the biochar had the ability to induce systemic defense response in tomatoes and pepper against *Botrytis cinerea* and *Leveillula taurica*, respectively. In strawberry plants defense response was studied against *B. cinerea*, *Colletotrichum acutatum* and *Podosphaera aphanis* (Harel et al., 2012). Jaiswal et al. (2015) explained the fact of biochar concentration in soil substrate and its impact on plant disease development.

Usually, lower application rates of biochar help in suppressing the disease, so thatplants can withstand or tolerate the infections. There are also reports that higher concentration of biochar in the soil substrate negatively influenced the plant productivity and growth (Spokas et al., 2012). Akhter et al. (2015) also found that the 3% application level of biochar was effective in suppressing the tomato wilt caused by *Fusarium oxysporum* f. sp. *lycoperisici*. However, data is scarce regarding the impact of biochar on chilli plants and bacterial leaf spot pathosystem.

In addition to biochar, flyash (amorphous mixture of ferro-aluminosilicate minerals produced at 400-1500°C) or powdered carbon utility needs to be explored for agriculture purposes (Mattigod et al., 1990). Flyash, which can be acidic or alkaline depending on the source, used to regulate soil pH (Elseewi et al., 1978; Phung, 1978). Lime in flyash reacts easily with acidic substances in the soil and release nutrients such as S, B and Mo in the quantity and form beneficial for crop plants. The use of flyash to increase the pH of acidic soil (Phung et al., 1979) and to improve the soil texture (Chang et al., 1977) has been studied for agricultural benefits (Adriano et al., 1980; Doran and Martens, 1972), previously. The presence of almost all basic nutrients in ionic form and the attenuating effect on the physical, chemical, and microbial nature of the soil make flying ash a useful tool for crop production, particularly in degraded or contaminated soils.

Chilies (*Capsicum annuum* L). are native to South America, while prevalence of wild cultivars reporrted from southern America, Africa, Australia to central Argentina (Utami et al., 2022). *Xanthomonas campestris* pv. *vesicatoria* is the gram-negative bacterium that causes bacterial leaf spot, a debilitating disease that affects chilies worldwide. Bacterial leaf spot develops in warm (30 to 34°C) and humid (relative

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humidity; 80%) cultivated areas globally (Scaldaferro et al., 2018). The pathogenic bacterium has limited survival duration ranging from few days to weeks in plant debris, infected seeds or soil. Rain splashes and mechanized irrigation or pesticide application are the main cause of pathogen spread (Larrahondo-Rodríguez et al., 2022). Control of the bacterial leaf spot by chemicals was discouraged globally because of harmful and residual effects on all life forms on earth. So far seven Bacterial spot resistance genes (R genes) have been identified in peppers including “VI037601” a resistant genotype of chilli pepper (Wang et al., 2022). On the basis of whole genome analysis, a total of 89 pathogenic strains of Xanthomonas are known to infect chilli pepper worldwide (Tambong et al., 2022). Amongst the alternative methods for controlling diseases are the use of organic amendments like compost and biochar (Akhter et al., 2015).

The purpose of this study was to determine (i) the effect of biochar (rice straw biochar) on the development of Xanthomonas campestris pv. vesicatoria, ii) the direct toxicity of rice straw biochar on selected phyto-pathogens, and iii) the potential of biochar and flyash on chilies growth in an environmentally friendly manner.

Materials and methods

Xanthomonas campestris pv. vesicatoria culture multiplication

The *Xanthomonas campestris* pv. *vesicatoria* (Accession no: 003) culture was kindly provided by the First Fungal Culture Bank of Pakistan (FCBP), University of the Punjab Lahore. *Xanthomonas campestris* pv. *vesicatoria* was multiplied on LB broth media as suggested by Rayner et al. (1990). Flasks (250 mL) containing luria-bartania (LB) broth were inoculated with *X. campestris* pv. *vesicatoria* and placed on a shaker for vigorous and continuous shaking. The bacterial growth was checked by determining the absorbance with the help of spectrophotometer.

Production of biochar

The biochar was prepared by taking the Rice Straw as a raw material of feed stock. The biomass was air dried and burnt in the oil barrels according to Steiner et al. (2018). After complete burning and cooling final product was packed in plastic bags for further use. *Table 1* shows the physical and chemical characters of rice star biochar as compared to flyash and soil.

Table 1. *Chemical and physical characteristics of rice straw biochar (RSB), flyash and soil*

Parameters assessed	RSB	Flyash	Soil
Ash (%)	35	46	---
pH	8.54	9.75	5.6
Electrical conductivity dsm^{-1}	3.0	2.43	0.23
Carbon (%)	45.5	39.3	30.2
Nitrogen (g Kg^{-1})	9.80	6.71	10.5
Phosphorous (g Kg^{-1})	3.31	2.97	2.31
Potassium (g Kg^{-1})	0.29	0.31	0.71
Calcium (g Kg^{-1})	3.51	2.51	3.32
Magnesium (g Kg^{-1})	2.37	1.37	1.92
Sulfur (g Kg^{-1})	9.51	7.52	4.71

Soil and chilies nursery preparation

The soil substrate was used for this experiment was autoclaved for 20 min at 121°C. Biochar was made from Rice Straws taken from local fields from University of the Punjab, Lahore and named as Rice Straw Biochar (RSB). Chilli pepper (Yolo Wonder: cv) seeds were surface sterilized before sowing. Chilli pepper seeds were soaked in NaOCl solution (2%) for 10 min. Afterwards, seeds were washed thrice with distilled water to remove residues of NaOCl from the seeds (Steinkellner et al., 2005). The seeds were cultivated in autoclaved soil substrate and placed in glass house to maintain optimum growing conditions (24-26°C). After 8-12 days, chilli seedlings start emerging. After 3 weeks, the seedlings were uprooted gently for transplanting into the separate pots according to experimental design as described below.

Soil served as a basic plant growth medium and further amended with compost (20%; v/v), while biochar and flyash were mixed at the rate of 3% each, according to treatment set-up (Akhter et al., 2016). The experiment comprised of the following treatments:

i. Rice straw biochar with *Xanthomonas campestris* pv. *vesicatoria* (RSB + XCV)
ii. Rice straw biochar without *Xanthomonas campestris* pv. *vesicatoria* (RSB - XCV)
iii. Flyash with *Xanthomonas campestris* pv. *vesicatoria* (Flyash + XCV)
iv. Flyash without *Xanthomonas campestris* pv. *vesicatoria* (Flyash - XCV)
v. Only Soil
vi. Soil with *Xanthomonas campestris* pv. *vesicatoria* (Soil + XCV)

The experiment was repeated twice with 5 replicates for each treatment. Each pot with one plant represent one replicate. The experiment followed a randomized complete block design in the greenhouse under long day conditions (Yadav et al., 2020).

Plant inoculation with Xanthomonas campestris pv. vesicatoria

A week after transplantation, the ‘XCV’ was inoculated with a hypodermic syringe. Plant leaves were washed with double distilled water to eradicate any surface grime. Using an atomizer, broth bacterial culture medium (approximately 107 CFU/mL) was then applied on the injured/prickled leaf surface. For 36 h, plants were placed under polythene bags to regulate optimal humidity for bacterial infection to establish (Bashan et al., 1985). Daily irrigation was performed to maintain optimal moisture conditions.

Agronomic traits

Agronomic traits of plants including shoot height, root length, root and shoot weights were calculated by using standard procedure (CIMMYT, 1988), 20 days after transplanting.

Disease incidence and severity assessment

Disease incidence was calculated by dividing number of plants infected by total number of plants grown in a specific treatment according to the formula as given below (Akhter et al., 2015):

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$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

For disease severity assessment, a disease rating scale based on the number of spots and lesions on the leaf surface (%) covered by spots was developed. According to the scale, plants were rated from 0 to 4 (0 = not infected, 1 = 0-25% leaf surface area covered with spots, 2 = 26-50%, 3 = 51-75%, and 4 = greater than 75% of leaf area covered with spots) (Patil and Bodhe, 2011). The disease severity index was calculated using the following formula (Chiang et al., 2017):

$$\text{Disease severity index (DSI)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total numbers of leaves assessed}} \times \text{Maximum rating} \times 100$$

Chilli plant samples were taken 3 weeks after inoculation with *Xanthomonas campestris pv. vesicatoria* for the biochemical analysis (Awan et al., 2018).

Determination of the level of total phenols, catalase, peroxidase and flavonoids determination

Chilli leaf samples were taken 5 days after inoculation for the biochemical analysis. Leaf sample of 250 mg was collected and processed according to the Zieslin and Ben Zaken (1993) protocol for the estimations of total phenols by utilizing the gallic acid’s standard curve (Bijali et al., 2021). Similarly for the catalase, peroxidase and flavonoid contents determination samples were prepared separately as described by Bijali et al. (2021), for chilli plants. To quantify the peroxidases, the processed sample absorbance was recorded at 470 nm, while for catalase and flavonoid contents at 240 and 495 nm, respectively.

In vitro effect of biochar on different plant pathogenic microorganism

The effect of biochar on the growth of different plant pathogenic bacteria and fungi was carried out on PDA plates. All the pathogenic isolates were taken from FCBP (1st Fungal Culture Bank of Pakistan) University of the Punjab, Lahore. Phyto-pathogenic bacteria are used in this test: *Pseudomonas syringae* (Bacterial brown blotches), *Escherichia coli* (Vegetable maladies), and *Erwinia spp* (Soft rot of fruits). *Fusarium solani* (Root and stem rot), *Fusarium oxysporum* (Fusarium Wilt), *Alternaria solani* (Early blight), *Alternaria alternata* (Leaf blotches), and *Penicillium spp*. were used to investigate the antifungal activity of RSB (Ear rot). Agar disk diffusion was used to measure the RSB’s direct toxicity (Heatley, 1944). The diameters of the inhibition growth zones were measured after the antimicrobial agent (Rice Straw Biochar) diffused into the agar and inhibited the germination and growth of the microbial colonies (Swenson et al., 2014).

Statistical analysis

The data was analyzed by Statistix 8.1 software. The experimental data was pooled before the analysis. The two-way analysis of variance (ANOVA) was used for data analysis at $P < 0.05\%$. The means were separated by Tuckey’s HSD test.

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Results

Agronomic parameters estimation

The data analysis revealed significant influence of individual factors namely soil substrate (SS) and 'XCV' on the shoot weight, height, root weight, except of root length (*Table 2*). chilli plant biomass production in biochar and flyash amended substrate are shown in *Figures 1* and *2*, respectively. The root dry weigh was maximum (0.45 g) for the plants grown in soil amended with 'RSB', followed by the root dry weight of plants grown in flyash amended substrate in the absence of inoculation with leaf spot inducing bacterium (-XCV). However, a significant reduction in root biomass was recorded among all the treatments under disease stress (+XCV) (*Table 2*). Chilli plants in RSB containing soil sustained minimum reduction in root dry weight as compared to the soil or un-amended control. The root dry weight was significantly reduced in soil only substrate in the presence of XCV. Chilli shoot weight was highest (1.62 g) in "RSB-XCV", while the lowest (0.24 g) was recorded for "Soil + XCV" treatment.

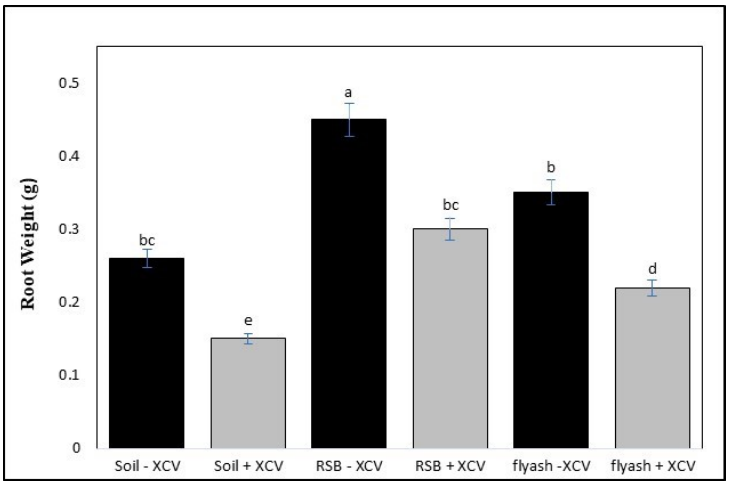

Figure 1. *Effect of rice straw biochar and flyash on the root weight of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. Vesicatoria*

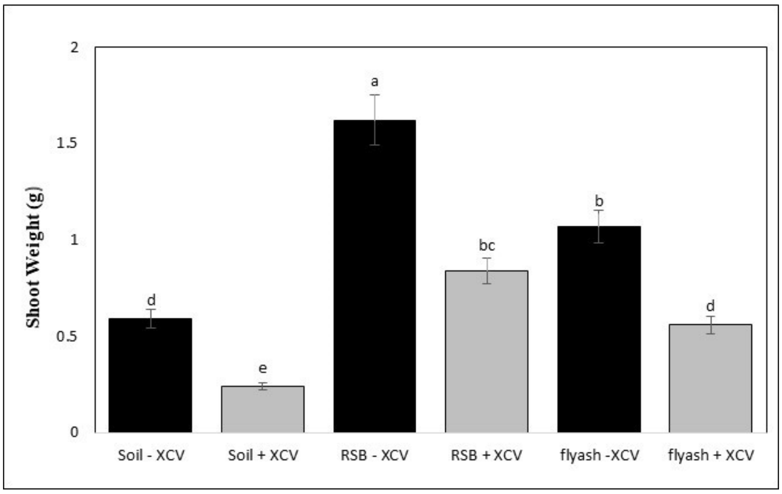

Figure 2. *Effect of rice straw biochar and flyash on the shoot weight of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. vesicatoria*

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Our results revealed that height of plants were increased by the biochar addition in ‘SS’ (*Fig. 3*). The plant height was approx. 18 and 14 cm, for the plants received biochar both in the presence and absence of ‘XCV’, respectively.

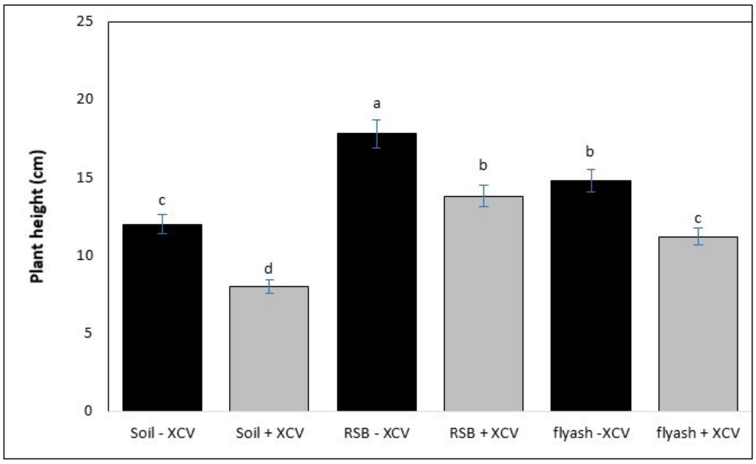

Figure 3. *Effect of rice straw biochar and flyash on the plant height of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. vesicatoria*

Table 2. *Two-way analysis of variance (ANOVA) with the factors of soil substrate composition (SSC) and Xanthomonas campestris pv. vesicatoria (XCV) to determine their lone and interactive effect on chilies growth and biochemical alterations*

	Total phenols	**Peroxidase**	**Catalase**	**Flavonoids**	**Root length**	**Plant height**	**Dry root weight**	**Dry shoot weight**
SS	*	**	*	**	*	**	**	**
XCV	**	**	**	**	N-S	**	**	**
SS × XCV	*	N-S	N-S	**	**	N-S	N-S	N-S

*$P \leq 0.05$; **$P < 0.001$; and N-S = non-significant

However, a highly significant interactive effect (SS × XCV) was observed for maximum root length (*Table 2*). Biochar amended ‘SS” has significantly higher root length than flyash and soil. There was no significant difference in mean root length of inoculated chilli plants grown in flyash or rice straw biochar in comparison to their un-inoculated counterparts. Treatment with ‘only soil’ had minimum root length of 7.62 and 5.5 cm in un-inoculated and inoculated plants, respectively (*Fig. 4*).

Assessment of disease incidence and severity

The bacterial leaf spot disease assessment on chilli plants was performed 21 days after nursery transplantation (*Table 3*). There was a reduction in disease incidence when plants were grown in biochar in comparison to soil only and flyash containing treatments. The minimum (40%) disease incidence was in inoculated treatment containing biochar (RSB + XCV), followed by treatment containing “flyash + XCV” (60%). On the other hand, treatments without any amendment (Soil + XCV) had shown incidence of 100%. Reduction in disease severity was recorded in treatments having RSB and flyash. Plants

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grown in "RSB + XCV" treatments were resistant against 'XCV' with the disease severity of 21.25%, while highly susceptible (95%; disease severity) response was recorded in plants growing without biochar amendment (*Figs. 5* and *6*).

Biochemical analysis of chilli plants

The statistical analysis revealed significant interactive effect (SS × XCV) for the total phenols and flavonoid contents of chilli leaf samples, however influence of individual factors 'SS' and 'XCV' was significant for peroxidase and catalase contents of leaf samples (*Table 2*).

Table 3. *Effect of soil substrate composition on the disease parameters of Xanthomonas campestris pv. vesicatoria on chillies*

Treatment	RSB + XCV	Flyash + XCV	Soil + XCV
Disease incidence (%)	40[c]	60[b]	100[a]
Disease Severity (%)	21.25[c]	32.5[b]	95[a]

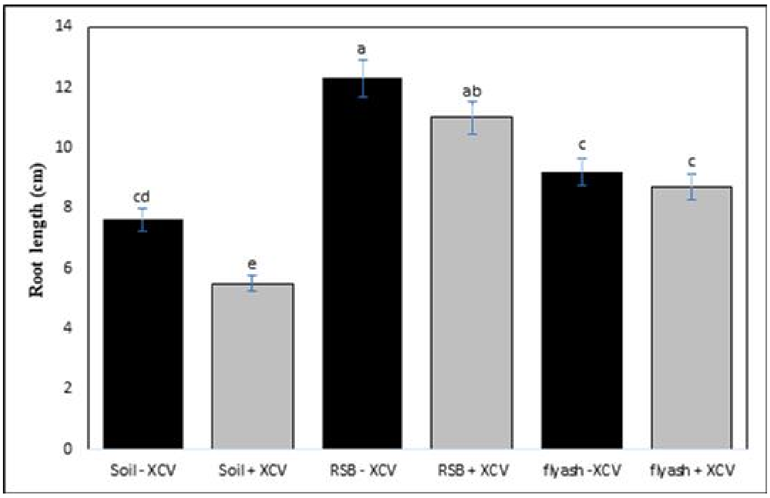

Figure 4. *Effect of RSB and flyash on the root length of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. Vesicatoria*

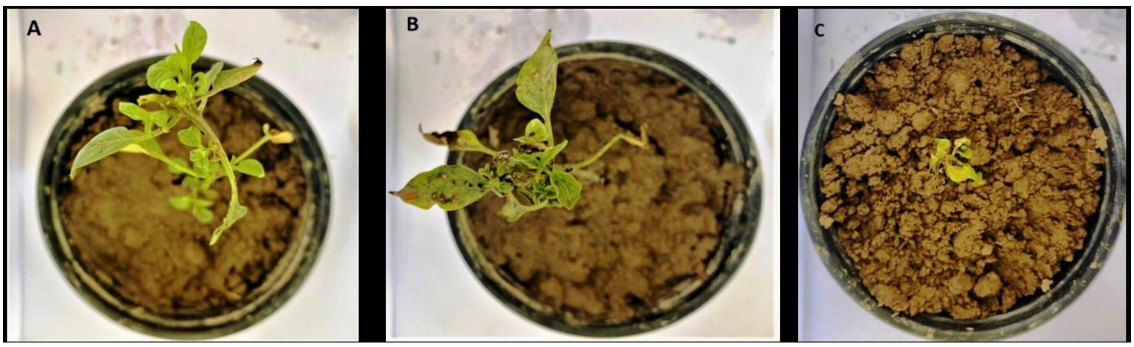

Figure 5. *Disease symptoms intensity on chilli plants grown in in (A), biochar (RSB), (B) flyash, and (C) in only soil, inoculated with Xanthomonas campestris pv. vesicatoria (top view)*

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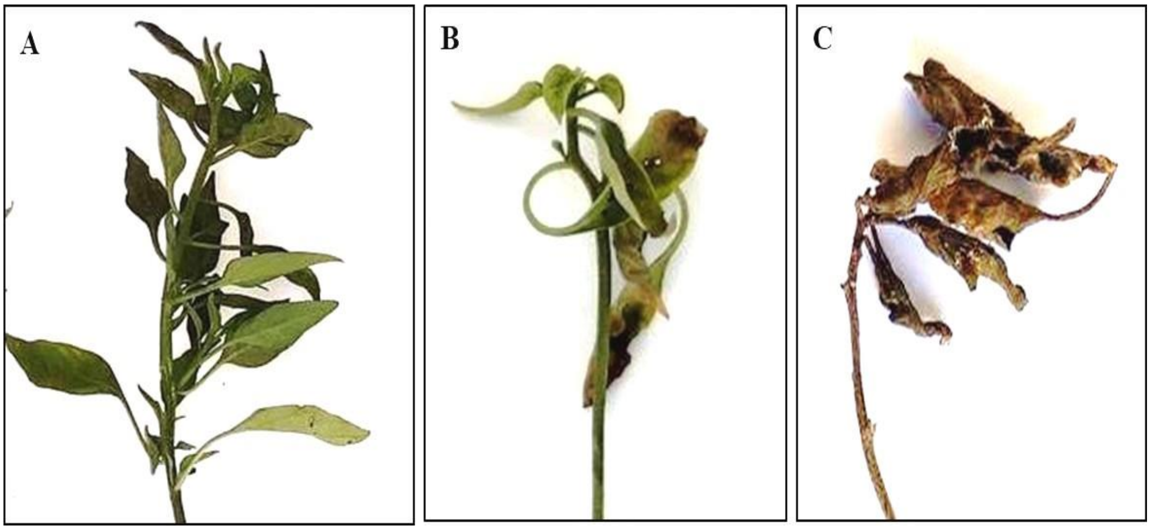

***Figure 6.** Bacterial leaf spot symptoms expression on the foliage of chilli plants grown in (A), biochar (RSB), (B) flyash, and (C) in only soil, inoculated with Xanthomonas campestris pv. vesicatoria*

(a) Total phenols

The phenol contents were significantly higher in chilli plants both with and free from pathogen grown in rice straw biochar (*Table 2*). The presence of biochar in soil substrate resulted in 30.81% increase in phenol contents of chilies inoculated with ‘XCV’ as compared to their un-inoculated (–XCV) counterparts. However, no such increase was observed for diseased chilli plants grown in flyash or soil only treatment. The lowest (2.23) phenol contents were recorded for ‘Soil + XCV’ treatment (*Fig. 7*).

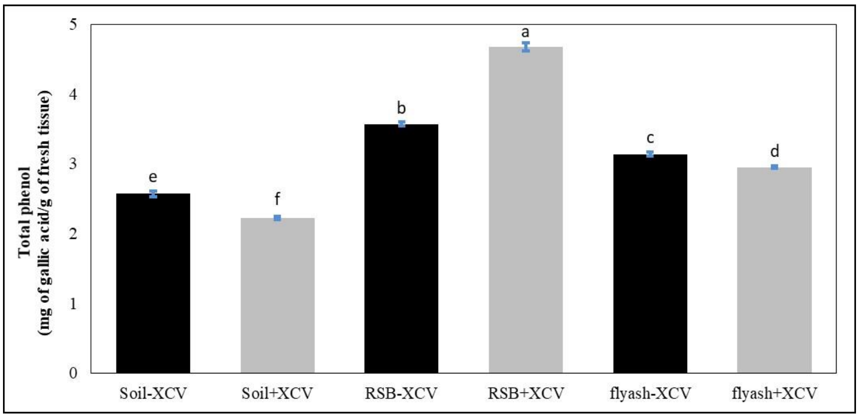

***Figure 7.** Effect of RSB and flyash on the total phenols of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. vesicatoria*

(b) Peroxidase

The peroxidase activity was maximum for the ‘+XCV’ chilli plants grown in biochar amended soil, while in case of flyash amended soil substrate the plants had sustained the level of peroxidase irrespective to the disease stress on chilli plants (*Fig. 8*). Overall, the increase in peroxidase activity was greater both for biochar and

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flyash, in the presence of disease stress, while the lowest (1.73) activity was recorded in soil only treatment.

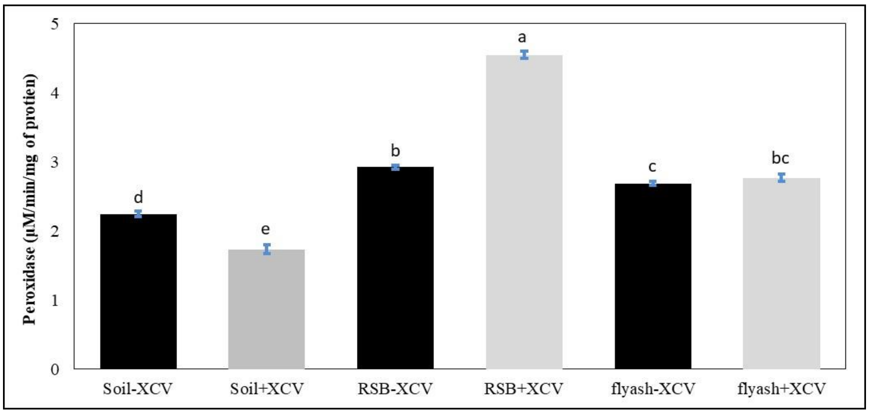

Figure 8. *Effect of RSB and flyash on the peroxidase level of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. vesicatoria*

(c) Catalase

The catalase levels were significantly up-regulated by the inoculation of *Xanthomonas campestris* pv. v*esicatoria*, in all of the treatments either with or without biochar (*Table 2*). However, the maximum (4.72) value was recorded for ‘RSB + XCV’ followed by ‘flyash + XCV’ (3.90). Although, catalase production was slightly higher (2.89) in ‘Soil + XCV’ treatment, but the difference was non-significant when compared to its respective un-inoculated control (2.64).

Overall, the presence of biochars and chilies inoculation with ‘XCV’ significantly increased the catalase level in leaves (*Fig. 9*).

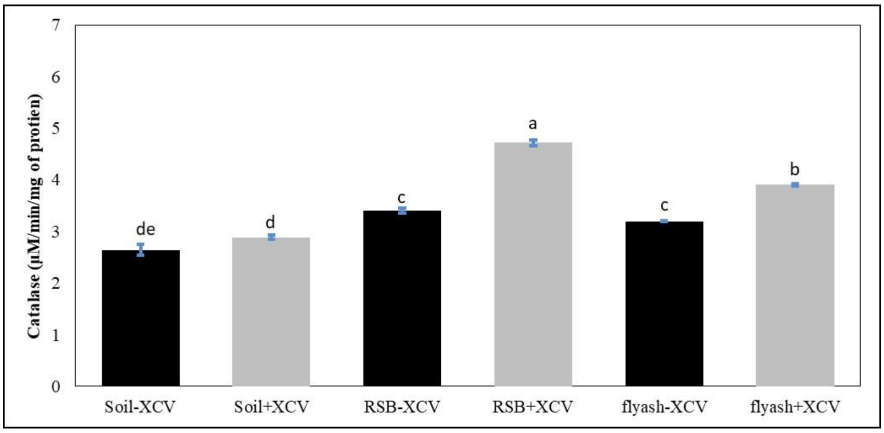

Figure 9. *Effect of RSB and flyash on the catalase contents of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. vesicatoria*

(d) Flavonoids estimation

The increase of 26% and 12.26% was calculated for pathogen inoculated chilli plants grown in biochar and flyash containing soil substrate, respectively (*Fig. 10*). However,

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in soil control, the flavonoids estimates of inoculated (+XCV) and un-inoculated plants were non-significant. The flavonoids were ranked according to maximum to lowest concentration in chilli leaves among the 'XCV' inoculated treatments in the following order 'RSB > flyash and > and Soil only.

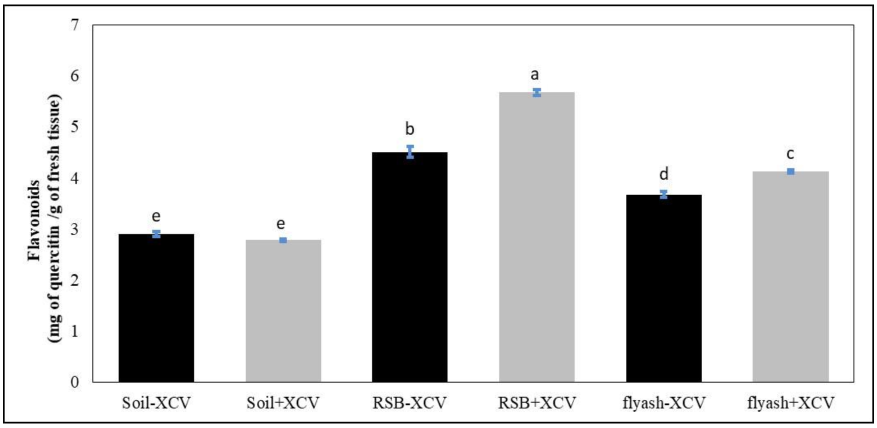

***Figure 10.** Effect of RSB and flyash on the concentration of flavonoids of chilli plants either inoculated (+XCV) or un-inoculated (-XCV) with Xanthomonas campestris pv. Vesicatoria*

Toxicity assay of rice straw biochar

In vitro antimicrobial effect of RSB was carried out on PDA plates. RSB amended media has shown growth suppression of 65.7% against *X. campestris* pv. *vesicatoria* in comparison to un-amended control. Whereas colony diameter suppression (62%) was recorded for *X. campestris* pv *citri* followed by *Erwinia spp* (65%). Moreover, a significant toxic effect of RSB was also recorded for fungal phytopathogens. Highest fungal growth suppression (78.8%) was recorded for *penicillium spp*, while the lowest %58%) for *Alternaria alternata* (*Table 4*).

***Table 4.** Percentage inhibitory effect of Rice straw biochar (RSB) against fungal and bacterial phyto-pathogens*

Pathogens	Percentage inhibition
Alternaria solani	59.7^{d}
Fusarium solani	60.2^{cd}
Fusarium oxysporum	69.3^{b}
Alternaria alternate	58^{d}
Penicillium spp	78.8^{a}
Escherichia coli	53.1^{e}
Erwinia spp	65^{c}
Xanthomonas campestris pv. *citri*	62.4^{cd}
Pseudomonas syringae	64.6^{c}
Xanthomonas campestris pv. *Vesicatoria*	65.7^{bc}

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Discussion

The negative impact of pesticides like fungicides, weedicides and etc. on crop plants pose a serious threat to human and environmental health. Agricultural commodities are continuously exposed to the toxic chemicals and multiple abiotic stresses. Heavy and non-regulated use of pesticides is a constant threat for all kind of life forms on earth, so to create an eco-friendly atmosphere, the adoption of sustainable agriculture practices is the key to success (Yadav and Devi, 2017).

Agro-chemicals usage shifts the normal environmental balance such as by disruption of ozone layer contributing to climate change (Singh and Mandal, 2013). In addition to that, burning of plant derived biomass is a major source of pollution releasing gases like carbon dioxide (CO_2), carbon monoxide (CO), nitrous oxides (NO_x), methane (CH_4), and other various hydrocarbons in the troposphere (Crutzen and Andreae, 1990). Open burning of wheat straw had a total particulate matter (PM) of 8.8 g/kg and 0.1 g/kg and 3.5 g/kg of total emission of elemental carbon of organic waste and organic carbon, respectively, was recorded (Guoliang et al., 2008). Carbon dioxide emission has increased by more than 3% annually for last 20 years (Raupach et al., 2007), thus adding into existing volume of greenhouse gases (GHGs). Preparation of pyrlytic product (biochar) has been suggested as one of the possible ways to reduce CO_2 levels in the atmosphere (Lehmann et al., 2006). Converting the organic biomass into biochar by pyrolysis avoid the emission of GHGs. Biochar’s ability to store carbon enables it to use as reclaimant which helps the plants to grow faster and healthier (Woolf et al., 2010).

Biochar proves to be an alternative to chemical inputs, without any damaging consequences to the environment. Biochar incorporation in the soil to maintain carbon cycle balance is a burning issue and a popular matter frequently discussed by policy makers globally (Gaunt and Lehmann, 2008).

The popularity of biochar is mainly because of its ability to carry out functions of novel green sorbent organic soil fertilizers (Lehmann et al., 2003) and carbon sequestering agent. Biochar is being widely used among farmers to increase the soil organic carbon content (Villamil et al., 2015). Carbon sequestration helps in climate change mitigation and is most important soil quality parameter (Lal, 2009). The aromatic structure of biochar makes it less degradable, thus has higher potential for carbon sequestration as compared to other soil amendments (Brewer et al., 2011; Hansen et al., 2015). Leppäkoski et al. (2021) reported the negative carbon foot print of biochar made from willow which offers its useful application in limiting the emission of greenhouse gases.

The strong correlation between pH correction and soil nutrient availability suggests that most of the flyash elements are related to the mineral phase. We can therefore expect that the interaction between inorganic flyash and organic matter may further intensify the beneficial effects on plant growth in problem soils (Page et al., 1979; Molliner and Street, 1982). Boron toxicity is the main limiting factor in the agricultural use of flyash. Inhibition of boron-induced microbial respiration can be avoided by the concomitant use of an easily oxidizable organic substrate (Page et al., 1979). Adriano et al. (1980) recommended mixing alkaline fly ash with highly carbonated acidic material to make fertilizers for soil treatment.

In this study, RSB has shown its potential against bacterial induced chilies leaf spot (Kloss et al., 2014). Bacterial leaf spot severity and incidence was notably decreased in

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biochar amended soil. As Harel et al. (2012) also reported that the biochar application can activate defense response of plants.

Biochar addition has increased plant growth measurements, while also suppressed the disease severity and incidence of *X. campestris* pv. *vesicatoria*. Biochar and compost association had significant plant growth promotion effect, when compared with no biochar soils (Schulz et al., 2012).

The biochar ability to hold nutrient positively influence plant growth (Glaser et al., 2002). Moreover, higher carbon contents of soil improves the micronutrient sorption capacity as previously reported by Hille et al. (2005). Biochar manufacturing at high temperatures (above 600°C) usually generate stable carbon compounds, which were reported to interfere with microbial organic decomposition. Thus, significantly contributes toward the sustainable carbon sequestration (Ippolito et al., 2020)

While, the increase of plant height plausibly related to the sorption of organic allelopathic substances by the biochar (Lair et al., 2006). Moreover, rice straw based biochars have plant growth enhancement capacity for considerably longer period of times (Ogawa and Okimori, 2010).

Xiang et al. (2017) found that the biochar amendments modify the roots to enhance the absorption efficiency of water and nutrients from soil. Thus, greater availability of solutes and solvents required for plant growth had a positive effect on both root and shoot length and overall dry matter production (Jeffery et al., 2015; Liu et al., 2016). The increase in maximum plant root length due to biochar amendment is helpful for plants because of expansion in rhizosphere to facilitate absorption of essential nutrients and water which otherwise becomes difficult for the plants grown in only soil (Prendergast-Miller et al., 2014). Soil-biochar interaction improves root growth of chilies. Makoto et al. (2010) documented the increase in root biomass as well as development of number of root tips (64%) in biochar containing soil.

In this study, the addition of biochar significantly decreased the incidence and severity of disease. When grown in soil-biochar (RSB) combination, disease severity was reduced compared to other treatments. In general, biochar improved the resistance of chili plants to X. campestris pv. vesicatoria infection. The mechanisms behind biochar’s disease suppressing ability are similar to those of other soil amendments, such as compost (Noble and Coventry, 2005). These mechanisms include (a) release of organic inhibitors, (b) increase in the population and diversity of beneficial microflora, (c) increased nutrients availability to the plants, and (d) activation of plant resistance inducing pathways including intercellular adhesion molecules (Jasmonic acid pathway) (Rasool et al., 2021). In our study, the increase in level of phenols, catalase, peroxidases and flavonoids also played a significant role by inhibiting the establishment of *X. campestris* pv. *vesicatoria* in chilies. Enhanced lignification and expression of SAR pathways due to phenols plausibly contributed towards plant’s resistance response to the invading pathogen (Bijali et al., 2021). Additionally, enhanced levels of catalase and peroxidases due to rice straw biochar amendment plausibly contributed in protecting chilies from bacterial leaf spot development (Dey et al., 2019). The suppression of disease severity in chili plants may be linked to biochar’s activation of defense-related genes. In other research, the addition of biochar made from wood and municipal waste reduced the incidence of bacterial wilt (*Ralstonia solanacearum*) in tomato (Nerome et al., 2005). However, biochar role in disease prevention cannot be solely attributed to the defense genes activation. The improvement in plant growing conditions and soil properties also among the majour contributors in modulating chilies response to the

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'XCV' (Hou et al., 2022). Thus, our study agreed with previous findings that biochar application has a stimulating effect on plant's physiological growth parameters. Moreover, biochar is also responsible for the induction of defense associated compounds production, thus moderating the infection of bacterial leaf spot of chilies.

Conclusion

Biochar, a low-cost carbonaceous material, is widely being used as a substitute for several pesticides and fertilizers because of its ability to retain and provides essential nutrients to plants as well as protection from pests and disease, while preserving its eco-friendly nature. In our study we evaluated the effect of biochar on chilli plants growth and its ability to suppress leaf spot disease on chilies. Flyash as well as biochar had shown a positive impact on disease suppression and plant growth, but biochar was most effective in activating defense response of chilies against *Xanthomonas campestris* pv. *vesicatoria*. Crop residues, open burning of fossil fuels, and production of organic wastes result in global emission of greenhouse gases which contributes to climate change. In this scenario, biochar provides an effective remedy and can be used as a carbon sequester agent to draw excess carbon from the atmosphere, improving water and soil quality and maintaining carbon sink to terrestrial ecosystem. Biochar application is an innovative strategy which helps in reduction of pesticide usage, thus contributing to climate change mitigation and improving soil and plant health.

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REFERENCES

[1] Adriano, D. C., Page, A. L., Elseewi, A. A., Chang, A. C., Straughan, I. (1980): Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: a review. – Journal of Environmental Quality 9(3): 333-344.
[2] Akhter, A., Hage-Ahmed, K., Soja, G., Steinkellner, S. (2015): Compost and biochar alter mycorrhization, tomato root exudation, and development of Fusarium oxysporum f. sp. lycopersici. – Frontiers in Plant Science 6: 529.
[3] Akhter, A., Hage-Ahmed, K., Soja, G., Steinkellner, S. (2016): Potential of Fusarium wilt-inducing chlamydospores, in vitro behaviour in root exudates and physiology of tomato in biochar and compost amended soil. – Plant and Soil 406(1): 425-440.
[4] Allen, R. L. (1847): A Brief Compend of American Agriculture. – CM Saxton, New York.
[5] Awan, Z. A., Shoaib, A., Khan, K. A. (2018): Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease. – Scientia Horticulturae 239: 216-223.
[6] Bashan, Y., Azaizeh, M., Diab, S., Yunis, H., Okon, Y. (1985): Crop loss of pepper plants artificially infected with Xanthomonas campestris pv. vesicatoria in relation to symptom expression. – Crop Protection 4(1): 77-84.
[7] Bera, T., Purakayastha, T. J., Patra, A. K., Datta, S. C. (2018): Comparative analysis of physicochemical, nutrient, and spectral properties of agricultural residue biochars as influenced by pyrolysis temperatures. – Journal of Material Cycles and Waste Management 20(2): 1115-1127.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2104_30573074

[8] Bijali, J., Halder, T., Acharya, K. (2021): Elucidation of the biochemical and molecular basis of the differential disease expression in two cultivars of chili (Capsicum annuum) in response to Colletotrichum capsici infection. – Acta physiologiae plantarum 43(12): 1-16.
[9] Brewer, C. E., Unger, R., Schmidt-Rohr, K., Brown, R. C. (2011): Criteria to select biochars for field studies based on biochar chemical properties. – Bioenergy Research 4(4): 312-323.
[10] Chang, A. C., Lund, L. J., Page, A. L., Warneke, J. E. (1977): Physical properties of fly ash-amended soils. – Journal of Environmental Quality 6(3): 267-270).
[11] Chiang, K. S., Liu, H. I., Bock, C. H. (2017): A discussion on disease severity index values. Part I: warning on inherent errors and suggestions to maximise accuracy. – Annals of Applied Biology 171(2): 139-154.
[12] CIMMYT Economics Program, International Maize, & Wheat Improvement Center (1988): From agronomic data to farmer recommendations: an economics training manual (No. 27). – CIMMYT, Mexico.
[13] Crutzen, P. J., Andreae, M. O. (1990): Biomass burning in the tropics: impact on atmospheric chemistry and biogeochemical cycles. – Science 250(4988): 1669-78.
[14] Dey, P., Ramanujam, R., Venkatesan, G., Nagarathnam, R. (2019): Sodium alginate potentiates antioxidant defense and PR proteins against early blight disease caused by Alternaria solani in Solanum lycopersicum Linn. – PLoS ONE 14: e0223216. DOI: 10.1371/journal.pone.0223216.
[15] Doran, J. W., Martens, D. C. (1972): Molybdenum availability as influenced by application of fly ash to soil. – Journal of Environmental Quality 1(2): 186-189).
[16] Elad, Y., David, D. R., Harel, Y. M., Borenshtein, M., Kalifa, H. B., Silber, A., Graber, E. R. (2010): Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. – Phytopathology 100(9): 913-921.
[17] Elseewi, A. A., Bingham, F. T., Page, A. L. (1978): Availability of sulfur in fly ash to plants. – Journal of Environmental Quality 7(1): 69-73).
[18] Gaunt, J. L., Lehmann, J. (2008): Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. – Environmental Science & Technology 42(11): 4152-4158.
[19] Glaser, B., Lehmann, J., Zech, W. (2002): Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal–a review. – Biology and Fertility of Soils 35(4): 219-230.
[20] Guoliang, C. A. O., Zhang, X., Sunling, G. O. N. G., Zheng, F. (2008): Investigation on emission factors of particulate matter and gaseous pollutants from crop residue burning. – Journal of Environmental Sciences 20(1): 50-55.
[21] Hansen, V., Müller-Stöver, D., Ahrenfeldt, J., Holm, J. K., Henriksen, U. B., Hauggaard-Nielsen, H. (2015): Gasification biochar as a valuable by-product for carbon sequestration and soil amendment. – Biomass and Bioenergy 72: 300-308.
[22] Harel, Y. M., Elad, Y., Rav-David, D., Borenstein, M., Shulchani, R., Lew, B., Graber, E. R. (2012): Biochar mediates systemic response of strawberry to foliar fungal pathogens. – Plant and Soil 357(1): 245-257.
[23] Heatley, N. G. (1944): A method for the assay of penicillin. – Biochemical Journal 38(1): 61-65.
[24] Hille, M., Den Ouden, J. (2005): Charcoal and activated carbon as adsorbate of phytotoxic compounds—a comparative study. – Oikos 108(1): 202-207.
[25] Hou, J., Pugazhendhi, A., Phuong, T. N., Thanh, N. C., Brindhadevi, K., Velu, G., ... Yuan, D. (2022): Plant resistance to disease: using biochar to inhibit harmful microbes and absorb nutrients. – Environmental Research 214: 113883.
[26] Ippolito, J. A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J. M., Fuertes-Mendizabal, T., ... Borchard, N. (2020): Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. – Biochar 2: 421-438.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2104_30573074

[27] Jaiswal, A. K., Frenkel, O., Elad, Y., Lew, B., Graber, E. R. (2015): Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to Rhizoctonia solani: the “Shifted R max-Effect”. – Plant and Soil 395(1): 125-140.
[28] Jeffery, S., Bezemer, T. M., Cornelissen, G., Kuyper, T. W., Lehmann, J., Mommer, L., ... van Groenigen, J. W. (2015): The way forward in biochar research: targeting trade-offs between the potential wins. – GCB Bioenergy 7(1): 1-13.
[29] Kloss, S., Zehetner, F., Wimmer, B., Buecker, J., Rempt, F., Soja, G. (2014): Biochar application to temperate soils: effects on soil fertility and crop growth under greenhouse conditions. – Journal of Plant Nutrition and Soil Science 177(1): 3-15.
[30] Lair, G. J., Gerzabek, M. H., Haberhauer, G., Jakusch, M., Kirchmann, H. (2006): Response of the sorption behavior of Cu, Cd, and Zn to different soil management. – Journal of Plant Nutrition and Soil Science 169(1): 60-68.
[31] Laird, D. A., Brown, R. C., Amonette, J. E., Lehmann, J. (2009): Review of the pyrolysis platform for coproducing bio-oil and biochar. – Biofuels, Bioproducts and Biorefining 3(5): 547-562.
[32] Lal, R. (2009): Challenges and opportunities in soil organic matter research. – European Journal of Soil Science 60(2): 158-169.
[33] Larrahondo-Rodríguez, E., Liao, Y. Y., Huerta, A. I. (2022): Diagnostic guide for bacterial spot of tomato and pepper. – Plant Health Progress 23(3): 355-361.
[34] Lehmann, J., da Silva, J. P., Steiner, C., Nehls, T., Zech, W., Glaser, B. (2003): Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. – Plant and Soil 249(2): 343-357.
[35] Lehmann, J., Gaunt, J., Rondon, M. (2006): Bio-char sequestration in terrestrial ecosystems–a review. – Mitigation and Adaptation Strategies for Global Change 11(2): 403-427.
[36] Leppäkoski, L., Marttila, M. P., Uusitalo, V., Levänen, J., Halonen, V., Mikkilä, M. H. (2021): Assessing the carbon footprint of biochar from willow grown on marginal lands in Finland. – Sustainability 13(18): 10097.
[37] Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., ... Zou, J. (2016): Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. – GCB Bioenergy 8(2): 392-406.
[38] Makoto, K., Tamai, Y., Kim, Y. S., Koike, T. (2010): Buried charcoal layer and ectomycorrhizae cooperatively promote the growth of Larix gmelinii seedlings. – Plant and Soil 327(1): 143-152.
[39] Mandal, S., Thangarajan, R., Bolan, N. S., Sarkar, B., Khan, N., Ok, Y. S., Naidu, R. (2016): Biochar-induced concomitant decrease in ammonia volatilization and increase in nitrogen use efficiency by wheat. – Chemosphere 142: 120-127.
[40] Mattigod, S. V., Rai, D., Eary, L. E., Ainsworth, C. C. (1990): Geochemical factors controlling the mobilization of inorganic constituents from fossil fuel combustion residues: I. Review of the major elements. – Journal of Environmental Quality 19(2): 188-201.
[41] Molliner, A. M., Street, J. J. (1982): Effect of fly ash and lime on growth and composition of corn (Zea mays L.) on acid sandy soils. – Proc. Soil Crop Sci. Soc 41: 217-220.
[42] Nerome, M., Toyota, K., Islam, T. M., Nishijima, T., Matsuoka, T., Sato, K., Yamaguchi, Y. (2005): Suppression of bacterial wilt of tomato by incorporation of municipal biowaste charcoal into soil. – Soil Microorganisms (Japan).
[43] Noble, R., Coventry, E. (2005): Suppression of soil-borne plant diseases with composts: a review. – Biocontrol Science and Technology 15(1): 3-20.
[44] Ogawa, M., Okimori, Y. (2010): Pioneering works in biochar research, Japan. – Soil Research 48(7): 489-500.

[45] Olmo, M., Villar, R., Salazar, P., Alburquerque, J. – A. (2016): Changes in soil nutrient availability explain biochar's impact on wheat root development. – Plant and Soil 399(1-2): 333-343.
[46] Page, A. L., Elseewi, A. A., Straughan, I. R. (1979): Physical and Chemical Properties of Fly Ash from Coal-Fired Power Plants with Reference to Environmental Impacts. – In: Gunther, F. A., Gunther, J. D. (eds.) Residue Reviews. Conference Proceedings. Springer, New York, pp. 83-120.
[47] Patil, S. B., Bodhe, S. K. (2011): Leaf disease severity measurement using image processing. – International Journal of Engineering and Technology 3(5): 297-301.
[48] Phung, H. T. (1978): Potential Use of Fly-Ash as a Liming Material in Environmental Chemistry and Cycling Processes. – In: Adriano, D. C., Brisbin, I. L. (eds.) Environmental Chemistry and Cycling Processes. Mineral Cycling Symposium. US Dept Energy Conf-760427, Washington, DC.
[49] Phung, H. T., Lam, H. V., Page, A. L., Lund, L. J. (1979): The practice of leaching boron and soluble salts from fly ash-amended soils. – Water, Air, and Soil Pollution 12(2): 247-254.
[50] Prendergast-Miller, M. T., Duvall, M., Sohi, S. P. (2014): Biochar–root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. – European Journal of Soil Science 65(1): 173-185.
[51] Purakayastha, T. J., Das, K. C., Gaskin, J., Harris, K., Smith, J. L., Kumari, S. (2016): Effect of pyrolysis temperatures on stability and priming effects of C3 and C4 biochars applied to two different soils. – Soil and Tillage Research 155: 107-115.
[52] Rasool, M., Akhter, A., Soja, G., Haider, M. S. (2021): Role of biochar, compost and plant growth promoting rhizobacteria in the management of tomato early blight disease. – Scientific Reports 11(1): 6092.
[53] Raupach, M. R., Marland, G., Ciais, P., Le Quéré, C., Canadell, J. G., Klepper, G., Field, C. B. (2007): Global and regional drivers of accelerating CO2 emissions. – Proceedings of the National Academy of Sciences 104(24): 10288-10293.
[54] Rayner, M. H., Sadler, P. J., Scawen, M. D. (1990): NMR studies of a bacterial cell culture medium (LB Broth): cyclic nucleosides in yeast extracts. – FEMS Microbiology Letters 68(1-2): 217-221.
[55] Scaldaferro, M. A., Barboza, G. E., Acosta, M. C. (2018): Evolutionary history of the chili pepper Capsicum baccatum L. (Solanaceae): domestication in South America and natural diversification in the seasonally dry tropical forests. – Biological Journal of the Linnean Society 124(3): 466-478.
[56] Schulz, H., Glaser, B. (2012): Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. – Journal of Plant Nutrition and Soil Science 175(3): 410-422.
[57] Singh, B., Mandal, K. (2013): Environmental impact of pesticides belonging to newer chemistry. – Integrated Pest Management 152-190.
[58] Spokas, K. A., Cantrell, K. B., Novak, J. M., Archer, D. W., Ippolito, J. A., Collins, H. P., ... Nichols, K. A. (2012): Biochar: a synthesis of its agronomic impact beyond carbon sequestration. – Journal of Environmental Quality 41(4): 973-989.
[59] Steiner, C., Bellwood-Howard, I., Häring, V., Tonkudor, K., Addai, F., Atiah, K., ... Buerkert, A. (2018): Participatory trials of on-farm biochar production and use in Tamale, Ghana. – Agronomy for Sustainable Development 38(1): 1-10.
[60] Steinkellner, S., Mammerler, R., Vierheilig, H. (2005): Microconidia germination of the tomato pathogen Fusarium oxysporum in the presence of root exudates. – Journal of Plant Interactions 1(1): 23-30.
[61] Swenson, J. M., Killgore, G. E., Tenover, F. C. (2004): Antimicrobial susceptibility testing of Acinetobacter spp. by NCCLS broth microdilution and disk diffusion methods. – Journal of Clinical Microbiology 42(11): 5102-5108.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2104_30573074

[62] Tambong, J. T., Xu, R., Cuppels, D., Chapados, J., Gerdis, S., Eyres, J., ... Dettman, J. (2022): Whole-genome resources and species-level taxonomic validation of 89 plant-pathogenic Xanthomonas strains isolated from various host plants. – Plant Disease 106(6): 1558-1565.
[63] Utami, D., Meale, S. J., Young, A. J. (2022): A Pan-Global Study of Bacterial Leaf Spot of Chilli Caused by Xanthomonas spp. – Plants 11(17): 2291.
[64] Villamil, M. B., Little, J., Nafziger, E. D. (2015): Corn residue, tillage, and nitrogen rate effects on soil properties. – Soil and Tillage Research 151: 61-66.
[65] Wang, F., Gao, S., Niran, J., Li, N., Yin, Y., Yu, C., ... Yao, M. (2022): iTRAQ proteomics reveals the regulatory response to Xanthomonas campestris pv. vesicatoria in resistant vs. susceptible pepper genotypes. – Horticultural Plant Journal 8(6): 747-756.
[66] Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., Joseph, S. (2010): Sustainable biochar to mitigate global climate change. – Nature Communications 1(1): 1-9.
[67] Xiang, Y., Deng, Q., Duan, H., Guo, Y. (2017): Effects of biochar application on root traits: a meta-analysis. – GCB Bioenergy 9(10): 1563-1572.
[68] Yadav, A., Ravindran, N., Singh, D., Rahul, P. V., Datta, S. (2020): Role of Arabidopsis BBX proteins in light signaling. – Journal of Plant Biochemistry and Biotechnology 29: 623-635.
[69] Yadav, I. C., Devi, N. L. (2017): Pesticides classification and its impact on human and environment. – Environmental Science and Engineering 6: 140-158.
[70] Zieslin, N., Ben-Zaken, R. (1993): Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. – Plant Physiology and Biochemistry (Paris) 31(3): 333-339.

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DOI: http://dx.doi.org/10.15666/aeer/2104_30573074