Incorporation of Engineered Nanoparticles of Biochar and Fly Ash Against Bacterial Leaf Spot of Pepper

Muhammad Danish Ali (✉ danishmsc15@gmail.com)
University of the Punjab https://orcid.org/0000-0002-4717-592X
Zill-e-Huma Aftab
University of the Punjab
Adnan Akhter
University of the Punjab
Farzana Majid
University of the Punjab
Iffat Siddiqui
Eastern Cereal and Oil Seed Research Centre
Arusa Aftab
Lahore College for Women University
Waqar Aslam
University of the Punjab
Muzammil Aftab
Government College University Lahore

Research

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Abstract

In agriculture, the search for higher net profit is the main challenge in the economy of the producers and nano biochar attracts increasing interest in recent years due to its unique environmental behaviour and increasing the productivity of plants by inducing resistance against phyto-pathogens. The effect of rice straw biochar and fly ash nanoparticles (RSBNPs and FNPs, respectively) in combination with compost soil on bacterial leaf spot of pepper caused by *Xanthomonas campestris* pv. *vesicatoria* was investigated both *in vitro* and *in vivo*. The application of nanoparticles as soil amendment significantly improved the chili pepper plant growth. However, RSBNPs were more effective in enhancing the above and belowground plant biomass production. Moreover, both RSBNPs and FNPs, significantly reduced (30.5 and 22.5 %, respectively), while RSBNPs had shown *in vitro* growth inhibition of *X. campestris* pv. *vesicatoria* by more than 50%. The X-ray diffractometry of RSBNPs and FNPs highlighted the unique composition of nano forms which possibly contributed in enhancing the plant defence against invading *X. campestris* pv. *vesicatoria*.

On the basis of our findings, it is suggested that biochar and fly ash nanoparticles can be used for reclaiming the problem soil and enhance the crop productivity depending upon the nature of soil and the pathosystem under investigation.

1 Introduction

Capsicum or bell pepper or sweet pepper (*Capsicum annum* L.) is a crop of solanaceae family and genus ‘capsicum’. Basically characterized by their green color and bell shape. These medium-sized fruit pods have wonderful colors (green, red, orange and yellow) thick and brittle skin with a glossy outer cover, and a fleshy texture. It is highly appreciated crop being good source of vitamin A, C, E, thiamine, beta carotene, folic acid and vitamin B6 and great therapeutic values (El-Ghorab, et al., 2013). Pepper is a globally produced except Antarctica and economically very important and valuable crop throughout the world. Production of capsicum was 11 million metric tons in 1990 and became more than 30 million metric tons globally in two decades and further increases are still expected (Parisi et al., 2020). In Pakistan, the area under pepper has been 62,742 hectares in 2018-19 with a total production of 145,856 tonnes and comes at 5th position worldwide. Bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv. *vesicatoria* result in severe damage to sweet pepper. The bacterium attacks leaves, fruits, and stems causing blemishes on these plant parts. It is a gram-negative, rod-shaped bacterium that can survive in seeds and plant debris from one season to another (Frank et al. 2005; Osdaghi et al., 2016; Ramzan et al., 2021). Pathogen can devastate a pepper crop by early defoliation of infected leaves and disfiguring fruit. In severe cases, complete crop failure has occurred due to this disease. Marketable yield is reduced both by defoliation and damaged fruits (Stall et al., 2009; Parisi et al., 2020). For the management of BLS different techniques have been under application such as chemical control (Fayette et al., 2012), cultural methods (Roberts et al., 2004, Sevic et al., 2019), bio control strategies (Le et al., 2020), and use of resistant plant genome (Potnis et al., 2015).

In the recent years, organic amendment, including crop residues, compost, organic waste and biochar application has become auspicious strategy for the control of soil-borne diseases because of its strengths as, cost-effectiveness, resource utilization and environmental protection (Scotti et al. 2015; Bonanomi et al. 2018, Zahra et al., 2021). Biochar (BC) or black gold is noval organic soil amendment with some special physical and chemical properties has been increasingly discussed in agriculture as a strategy for the sequestration of recalcitrant carbon into soils to increase soil fertility, (Zahar et al., 2021) improve plant growth and suppression of soil borne diseases (Wang et al., 2019; Alharbi et al., 2020; Rasool et al., 2021). Fly ash has been defined as the fine particulate by-product released into the atmosphere together with gases as a result of combustion processes (Rose, 2018). The dynamic physic chemical properties (low bulk density (1.01–1.43 g cm − 3), hydraulic conductivity and specific gravity (1.6–3.1 g cm − 3), while the moisture retention ranging from 6.1% at 15 bar to 13.4% at 1/3 bar and being rich in P, K, Ca, Mg and S and micronutrients like Fe, Mn, Zn, Cu, Co, Ba, Mo, Cd and Ni) (Basu et al., 2009; Sharma and Sharma, 2017) of fly ash make it a potential source in agricultural applications, as improving biological and physic-chemical properties of soil (Sahu et al., 2017; Singh and Sukkul, 2019) as competent as the compost and Biochar are.

Incorporation of engineered nanoparticles has gained an undeniable importance in our daily life from electronics to medicine and agriculture. In agriculture, for instance, nano-pesticides, nano-fertilizers and nano-sensors are in direct applications to agricultural soils to get an enhanced crop productivity and reduce cost (Servin et al., 2015), or control plant pathogens (Sevin et al., 2017; Ashraf et al., 2021). Characterized nanoparticles of Fly ash (Liang et al., 2020; Uda et al., 2021) and Biochar (Das et al., 2021) have been extensively used in agriculture sector not only to reduce the hazards of deposited chemical pesticides and fertilizers but also to control infectious pathogen and to improve crop yields (Narayanasamy, 2019; Ramezani et al., 2019; Salama et al., 2021).

In this study we explored the synthesis of nanoparticles form rice straw biochar (RSBNPs) and fly ash (FNPs) and secondly to access the prepared nanoparticles potential against bacterial leaf spot of pepper caused by *Xanthomonas campestris* pv. *vesicatoria*.

2 Materials And Methods

2.1 Experimental site:

The experiment was carried out at the experimental station of Department of Plant Pathology, (31º29’42.2664”N, 74º17’49.1316”E, 217 m altitude) Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan, from March 2019 to April 2021. The local climate is semi-arid (Köppen climate classification BSh) with an average temperature of 40∘C and 350 mm annual rainfall and rainy season July-September.

2.2 Plant material and soil substrate:

Capsicum annum L. seeds (Yolo Wonder) were purchased from local seed market (Ghula Mandi, Lahore) and surface sterilised with 70% ethanol followed by 50% NaOHCl solution (100 mL of NaOHCl + 100 mL of distilled + 50 μL tween-20 detergent) and thrice washing with distilled deionized water. These seeds were sown in clay pots ¾ filled with sterilized 20% leaf compost soil (The soil texture was a sandy loam (82.88% sand, 13.04% silt, and 4.08% clay) with a pH of 7.88 and an electrical conductivity (EC) of 1.55 dS/m (measured using a pH meter and an EC meter); organic matter content (OM) of 0.54%; containing 3% total N and 1.5% total C; having a C/N ratio of 0.5; and containing 12, 68, and 100 mg·kg−1 of Ca, P, and K, respectively). Fully developed plants at 4-5 leaf stage were transplanted, into clay pots of bigger size (Volume: 2 L, 15.5 cm height × 14 cm width) (Joshi et al., 2019; Dhaliwal et al., 2017) with the same soil composition up to 1-2 inches depth with 2-3 plants per pot. Lighter irrigations were applied on day-to-day basis to keep water level as desired. Then established young plants in pots were transferred to open area where seedlings were exposed to light so that they can carry their photosynthetic activity (Al-Herbi et al., 2020).

2.3 *Xanthomonas campestris* pv. *vesicatoria* culture acquisition:

Pure culture of *Xanthomonas campestris* pv. *vesicatoria* (FCBP-DNA B0003) was acquired from First Fungal culture Bank of Pakistan (FCBP), Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The inoculum was prepared by re-culturing in LB broth (MERCK, USA) based on Lennox formulation and incubating on shaker at 120 rpm for 36 h at 28 ± 2 °C. Bacterial culture was then centrifuged at 5,000 rpm for 10 min at 4 °C. The suspension was diluted through serial dilution process to obtain bacterial concentration of 10^8 at 600 nm wavelength having optical density of 0.3 in spectrophotometer (Ibrahim and Al-Saleh, 2012).

2.4 Biochar and nano particles production:

TLUD portable kiln method (McLaughlin, 2010) on farm biochar production was used, with minor adjustments, to prepare biochar from Rice Straws collected from field area of University of the Punjab, new campus, Lahore, at pyrolysis temperature of 500 °C to be used in this experiment. Fly ash was procured from textile industry as left over after burning of corn cobs and coal as fuel. Physico-chemical properties of rice straw biochar and fly ash used for further nanoparticles production are summarized in table 1 and 2.

Nanocomposite of rice starw biochar (RSBNPs) and fly ash (FNPs) were isolated from their bulk materials following protocols of Yeu et al., 2019; Guo et al., 2020, by grinding bulk-biochar into a commercial blender to produce fine biochar powder. Fly ash obtained was already packed in sealed polythene bags. Fly ash (30 g) and fine biochar powder were mixed in 800 mL of sterile water, separately. Both the solutions were shaken vigorously and autoclaved to physically and thermally disperse the bulk forms of biochar fine powder and fly ash. After dispersion of bulk material, prepared solutions were passed through 500 μm filter membrane to remove large particles. Filtrates were centrifuged twice at 3500 rpm for 25 minutes to isolate the nanoparticles in supernatant based on density gradient. XRD analysis of Biochar and fly ash nanoparticles was done by following Du et al., 2020, from the Department of Physics, Lahore College for Women University, Lahore, Pakistan.

Biochar and fly ash nanoparticles were applied through drenching (Imada *et al.,* 2016) to chilli plants by applying 50 mL of solution, containing nanoparticles (RSBNPs and FNPs), in the root zone by injecting with the help of disposable syringe (Telemart: 10cc, Bd).

Table 1: Physico-chemical characterization of rice straw biochar.

Parameter measured	Value
pH	9.3
Basic gps (meq/g)	7.8
Acidic gps (meq/g)	1.8
Ash%	50
Density (g/cm^3)	0.28
Surface area (S_{BET}) (m^2/g)	100
C wt%	53
H wt%	3.0
N wt%	1.5
O	42.4
C/N	25
H/C	0.08
O/C	0.79
Alkaline elements (ppm (ug/g) by dry weight)	
K	14,000
Mg	3500
Na	2190
Ca	7354
Other Essential Elements	
Fe	5754
P	2765
Heavy Toxic Elements	
Zn	0.01
Mn	575
Al	4231
Cu	4

Table 2: Physico-chemical characterization of fly ash.

Parameter measured	Value
Ash %	46
pH	9.75
EC dSm^{-1}	2.43
C%	39.3
N (g Kg-1)	6.71
P (g Kg-1)	2.97
K (g Kg-1)	0.31
Ca (g Kg-1)	2.51
Mg (g Kg-1)	1.37
S (g Kg-1)	7.52

2.5 Chili pepper plant inoculation and disease assessment:

The plants were grown for 7-8 days before the inoculation of the pathogen (*X. campestris* pv. *vesicatoria*). Leaves of chilli plant were injured by needle prick method of bacterial inoculation (Gao et al., 2021). In this method 8-10 clean needles were tightly held by rubber band at equal heights. These needles were used to damage the leaves. Slight gentle injuries were done to leaves to provide entry sites to bacteria. Then bacterial suspension was sprayed with the help of atomizer. Inoculated plants were covered again with polythene bags and water was sprinkled on inner bag surface to maintain high relative humidity.

The whole research trail was comprised of two groups. Each group was further divided into three treatments, having five replicates. Group I: Inoculated set: (1. Fly-ash nanoparticles + Xnth, 2. Rice Straw Biochar Nanoparticles + Xnth 3. Only Soil + Xnth). Group II: Un-inoculated set/Control group: (1. Fly-ash nanoparticles, 2. Rice Straw Biochar Nanoparticles, 3. Only Soil).

After application of nanoparticles and inoculation of pathogen, agronomic data were recorded as shoot and root length, weight (Sharma, 2018). Disease incidence was calculated by following formula

$$\textbf{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Disease severity was calculated by formulating a disease grading scale in which severity was rated from 0 to 4 grades with zero indicating minimal or no disease symptoms to grade four showing 76% or above leaf area infected (Chiang et al., 2017).

***2.6 In vitro X. campestris* pv. *vesicatoria* and other isolated potential bacterial and fungal pathogens growth inhibition assay:**

Antimicrobial activity of RSBNPs and FNPs was investigated against *X. campestris* pv. *vesicatoria* used in this experiment. Agar well diffusion method (Irshad et al., 2018) was employed for the estimation of antimicrobial potential of RSBNPs and FNPs.

Antimicrobial activity of RSBNPs and fly FNPs was also investigated against microflora isolated from soil used in this experiment. Fungi and bacteria were isolated through serial dilution method on Malt Extract Agar (MEA) and Luria Bertani Agar (LBA), respectively. Agar well diffusion method (Irshad et al., 2018) was employed for estimation of antimicrobial potential of RSBNPs and FNPs against isolated fungal and bacterial isolates including *Escherichia coli*, *Erwinia spp*, *Pseudomonas syringae, Xanthomonas campestris* pv. *citri*, *X. campestris* pv. v*esicatoria, Fusarium solani*, *F. oxysporum, Alternaria alternata* and *Alternaria solani*,.

2.7 Statistical analysis:

The experimental data were analysed by 'Statistix version 8.1' analytical software by analysis of variance (ANOVA), while the means were differentiated by Tuckey's HSD test at P = 0.05. Additionally, the percentage data were transformed for disease incidence, severity and *in vitro* bacterial growth inhibition before analysis.

4 Results

4.1 Effect of rice straw biochar nanoparticles and fly ash nanoparticles on plant growth:

Maximum shoot length (28 cm) was observed in un-inoculated rice straw biochar nanoparticles (RSBNPs) treated plants. In pathogen inoculated set of treatments, maximum shoot length was observed in both fly ash and biochar-based nanoparticles treated plants (Table 3). While, minimum shoot length of 8 cm was observed in pathogen inoculated control plants grown in soil only. RSBNPs had significantly enhanced root length as suggested by the results because maximum root length i.e. 27 cm was observed in uninoculated plants treated with RSBNPs. Pathogen inoculated plants of grown in soil, RSBNPs + Soil and FNPs + soil had root length of 6.1 cm, 13.4 cm, and 11 cm, respectively. RSBNPs treated plants resisted the pathogen stress and had 101%, while, FNPs treated plants had shown 65.1% increase in root length as compared to plants grown in only soil.

With the addition of composite nanoforms derived from rice straw biochar and fly ash a significant increase in chilli shoots weight was observed as compared to plants grown in only soil in both inoculated and un-inoculated set of treatments (Table 3). Shoot weight was significantly increased in RSBNPs treated plants in both pathogen-inoculated and uninoculated chilli plants. But highest average shoot weight was recorded in un-inoculated, RSBNPs treated plants as 2.039 g. In pathogen inoculated plants, average shoot weight in only soil grown plants was 0.219 g as compare to 1.067 g of RSBNPs treated and 0.748 g of FNPs treated plants.

An increase in root weight was observed in RSBNPs and FNPs treated plants. Very robust root hair growth was found in nanoparticles treated plants. In pathogen inoculated plants, average root weights were 0.154 g in soil grown plants, 0.714 g in RSBNPs treated plants, and 0.456 g in FNPs treated plants. RSBNPs treated, pathogen inoculated plants had 150.7% more root weight as compare to un-inoculated, only soil grown plants and 363.3% more average root weight as compare to pathogen inoculated plants grown in only soil. On the other hand, FNPs treated, pathogen inoculated plants had 60% enhanced root weight as compare to un-inoculated only soil grown plants and 195.7% more root weight in comparison with pathogen inoculated plants grown in only soil.

Table 3
Effect of rice straw biochar nanoparticles (RSBNPs) and fly ash nanoparticles (FNPs) and *Xanthomonas campestris* pv. *vesicatoria* inoculation on chilli plant growth parameters including shoot length, root length as well as root and shoot weights.

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Only Soil	13.2 ± 1.20^{d}	0.47 ± 0.01^{e}	6.66 ± 0.53^{e}	0.285 ± 0.05^{f}
Soil + Xnth	11 ± 1.00^{de}	0.22 ± 0.03^{f}	6.1 ± 0.74^{e}	0.1542 ± 0.03^{e}
Soil + RSBNPs	25 ± 1.14^{a}	2.09 ± 0.09^{a}	22.8 ± 1.32^{a}	1.9852 ± 0.33^{a}
Soil + Xnth + RSBNPs	19.6 ± 0.51^{c}	1.07 ± 0.15^{c}	13.4 ± 1.21^{c}	0.7144 ± 0.10^{c}
Soil + FNPs	23.2 ± 1.07^{ab}	1.73 ± 0.12^{b}	21 ± 0.55^{ab}	1.4172 ± 0.14^{b}
Soil + Xnth + FNPs	19 ± 0.65^{c}	0.75 ± 0.11^{d}	11 ± 1.00^{cd}	0.456 ± 0.08^{d}

Data represents mean vlaues ± standard error.

4.2 Disease incidence and severity

Among the inoculated set of the treatments, RSBNPs treated plants showed a different response to pathogen inoculation by showing significantly reduced disease incidence and disease severity (50 and 22.5%, respectively) as shown in Table 4. While, there was disease incidence of 100% in plants grown in only soil. Disease severity of FNPs treated plants was (30.5%) followed by the highest (94.5%) in plants grown in untreated soil. Severity of the disease symptoms on chilli plant leaves treated with nanoparticles (RSBNPs, FNPs) and without any nanoparticles are shown in Fig. 1.

Table 4

Effect of rice straw biochar nanoparticles (RSBNPs) and fly ash nanoparticles (FNPs) on the incidence and severity of *Xanthomonas campestris* pv. *vesicatoria* in chilli plants.

Treatments	**Disease Incidence (%)**	**Disease Severity (%)**	**Disease Rating Category**
RSBNPs + Soil	50^{c}	22.5 ± 2.84^{c}	1
FNPs + Soil	60^{b}	30.5 ± 3.75^{b}	1
Only Soil	100^{a}	94.5 ± 10.58^{a}	4

In vitro inhibitory effect of RSBNPs and fly ash nanoparticles was evaluated against bacterial leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria*. Zone of inhibition was calculated and shown in Table 4. RSBNPs have shown 51.2% growth inhibition of *Xanthomonas campestris* pv. *vesicatoria.* However, FNPs had shown inhibition of only 42.4% as compared to un-amended control. In addition to that both RSBNPs and FNPs had shown significant growth inhibition of isolated bacterial and fungal pathogens as summarized in Table 4.

Table 4

In vitro percentage (%) growth inhibition of five different phyto-pathogenic bacteria and four fungal isolates by RSBNPs and FNPs.

Bacterial Pathogens	**Percentage (%) Growth Inhibition**	
	RSBNPs	**FNPs**
Escherichia coli	56.25 ± 8.45^{cd}	53.1 ± 5.21^{cd}
Erwinia spp.	52.5 ± 4.89^{cd}	65 ± 7.29^{ab}
Pseudomonas syringae	73.1 ± 8.60^{a}	64.6 ± 5.55^{ab}
Xanthomonas campestris **pv.** citri	75 ± 7.84^{a}	62.4 ± 6.74^{ab}
X. campestris **pv.** vesicatoria	51.2 ± 6.67^{cd}	42.4 ± 3.94^{e}
Fungal Pathogens		
Fusarium solani	62 ± 6.83^{ab}	60.2 ± 4.77^{cd}
Fusarium oxysporum	47.5 ± 3.99^{e}	69.3 ± 3.01^{ab}
Alternaria alternata	70 ± 5.50^{ab}	58 ± 4.85^{bc}
Alternaria solani	59.7 ± 6.36^{ab}	52.5 ± 5.01^{bcd}

4.3 X-ray diffractometry of rice straw biochar nanoparticles and fly ash:

XRD data of biochar is shown in Fig. 1. The range of XRD spectrum is 2θ = 10–80^o. In Fig. 1 different peaks are observed at various angles due to different elemental composition. In the region of 20 to 30 a hump is observed due to C(002). Around 42–46^o another hump is observed due to C(100) which is attributed to condensed carbonized planes. In the XRD spectra there are three peaks which are observed around at 28, 68, and 73 due to the concentration of SiO_2 and well matched with (JCPDS card no. 46-1045). A peak is detected around 39 due to CaO presence (JCPDS card no. 011-1160). A detected peak of $Ca(OH)_2$ is well matched with (JCPDS card no. 01-073-5492) around 51^o. The presence of $CaCO_3$ is detected at around 45 and 79^o and confirmed through (JCPDS card no. 05-0586). Whereas a peak of MnO_2 is well matched with (JCPDS card no. 44–0141) and detected around 65^o.

By using XRD the peak identification and material confirmation of the fly ash have been characterized and demonstrated, in range 10–80 as shown in Fig. 2. The following graph showed that the material contained an appropriate amount of SiO_2, Al_2O_3, TiO_2, Na_2O_3, magnetite, K_2O, MgO,and CaO. It can be observed that calcium, silica, and aluminium are the main elements of the fly ash and comprise of 72 % the total mass of the fly ash. In XRD section the magnetite peaks are observed at 35.61, 42.5, 60.33 and 72.22 o, which are well matched with JCPDS card no. 73-2143, while the Al_2O_3 peaks were observed at 11.9,16.20, 35.61, 40.62, 46.02 and 65.73^o and according to JCPDS card no. 51–0769. The JCPDS card no. 80-2157 is matched with SiO_2 and peaks are observed at 35.61, 42.5, 46.02, 50.4 and 70.65 ^{o} while the Na_2O_3 observed at the peaks of 26.71, 31.28, 46.02 ^{o}. Due to the presence of K_2O the JCPDS card no. 23–0493 is well matched at 29.52, 40.62, 50.4, 65.73 and 72.22 ^{o}. The presence of MgO is detected on 29.52, 40.62, 60.33, 65.73, 75.66 ^{o} and matched with JCPDS card no. 30–0794. The peaks of CaO found fit with JCPDS Card no. 28–0775 at peaks of 24.28, 29.52, 31.28, 35.61^o. The other component TiO_2 is well matched with JCPDS card no. 33-1381 and the peaks were recorded at 35.61, 40.62, 53.9, 65.73^o.

5 Discussion

The rise in unprecedented climatic changes like temperature and changing weather patterns, had worsened the situation over the past few decades. While annual crop losses due to insect pests and diseases is estimated to range from 20 to 40% of total agricultural produce worldwide further escalating the hostility to existing food insecurity (Savary et al., 2012). Discovery of innovative technological advancements in agriculture sector is mandatory, to supersede an otherwise deteriorating global food scenario, in a sustainable manner. The recent innovations in scientific research, particularly, the advent of molecular nanotechnology have provided a ray of hope against all the odds through its effective role in drug delivery, target specificity, diagnostics, anti-microbial activity in pharmacology and medicine industry (Prasad et al., 2017). Nanotechnology have marked its foot prints in the field of agricultural research byits utility in establishing disease and pest diagnostic systems, phytohormonal delivery systems, nanobarcoding, enhancing germination of seeds, providing nano-vector for successful transfer of genes, establishing efficient and targeted slow releasing chemical pesticides (Khandelwal et al., 2016).

Rangaraj et al., (2014) has reported that silica NPs as effective agents for building resistance against *Fusarium oxysporum* and *Aspergillus niger* in maize. Nanotechnology is being widely used in plant pathological studies (Abd-Elsalam & Prasad 2018). There exist thorough studies on effect of biochar in controlling plant diseases (Akhter *et al.*, 2016). But major portion of studies on biochar involves macro fraction of biochar and material behaves differently when used in nano (10^{-9}) size in contrast to their bulk/macro forms. The present study was designed to fulfil the research needs on nano fractions of biochar and their role in controlling plant disease. Yue et al. (2019) attributed the increase in plant growth in response to biochar NPs in negating the effect of allelopathic materials in soil (Yue et al., 2019). In accordance with our results, Xu et al. (2017) demonstrated that nano biochar possessed unique set of physical and chemical traits other than in their bulk forms which enhanced root lengths. High, surface reactive tendencies and capacities to disperse allow them to attach and interact easily to root surfaces that is quite beneficial for protection of roots by physical means against heavy metal adversities.

Moreover, the nano biochar due to its smaller particle size have high mobility in soils and helps to transport water (Chen *et al.*, 2017; Lahiani *et al.*, 2016). Bashir *et al.* (2020) used composts and ZnO-nanoparticles to evaluate their effect on growth parameters like dry weight of roots, shoots, husk, and kernels, plant height and spike length of the plant concerned. Results obtained suggested strong effect of used nanoparticles and compost material on the growth promotion. Furthermore, increased photosynthetic activity owing to nanoparticles inoculation, which reduce the effects of osmotic and oxidative stress, is well documented as the process increase the plant biomass (Haghighi & Pessarakli 2013; Siddiqui *et al.*, 2014).

The decrease in disease incidence and severity can be attributed to, up regulation of innate immune response of plants against pathogens, due to induction of nanoparticles (Sathiyabama and Manikandan, 2016). Chandra *et al.* (2015) reported sufficient enhancement in plant's response though activation of innate immunity by induction of chitosan nanoparticles which, in return, increased the activity of defense related enzymes. Enhancement in total phenolic compounds, anti-oxidative enzymes and genes involved in defense mechanism was also reported due to treatment of carbon base chitosan nanoparticles. Studies indicate that induction of carbon-based nanoparticles stimulate the production of enzymes related to defense mechanisms, like Peroxidase (PO), Phenyl Alanine amonia Lyase (PAL), Poly Phenol Oxidase (PPO), and plant defense regulating molecules such as beta 1–3 glucanase, nitric oxide (NO) and etc. Nitric oxide is involved in many physiological processes (Baudouin, 2011) including regulation of defense process in plants (Chandra *et al.*, 2014; Acharya *et al.*, 2011).

Disease incidence and severity percentages of bacterial pathogen were decreased that can be due to direct destructive effects of nanoparticles on the bacterial membrane as nano forms of materials are electro-statically active and interact with lipo-polysaccarhide structure of bacterial membrane. As, XRD data revealed novel characteristics of BNPs including azimuthal and parallel orientation of aromaticity, partly carbonized lamellae (Huang et al., 2009). The hump around 42–46° due to C(100) proves a large amount of carbon present in sample and due to this carbon presence, a crystalline orientation is appeared in simple in the form of peaks (Huang et al., 2016; Chen et al., 2016; Mohan et al., 2018), While in case of fly ash NPs, the XRD data is the evidence of presence of different phases of Al and fly ash particles (Sobiecka 2013).

Secondly, nanoparticles constituting, mostly, heavy metals bind with DNA/RNA molecules of bacteria by passing through cell membrane and hinder transcription- translation process thus inhibiting bacterial proliferation (Xing et al., 2015). Nanoparticles trigger the production of salicylic acid (SA), a phytohormone that activates SAR mechanism in plants (Bari & Jones, 2009). Carbon material based nanoparticles triggered systemic acquired resistance mechanism that provides resistance to infection to remote plant tissue from the site of its production (García-sánchez et al., 2015).

Fly ash is known previously (Eswaran and Manivannan, 2007), to limit the papaya leaf curl disease spread along with the regulation of vector population (*Bemisia tabaci*). However, there are also risks associated with fly ash use including leaching of heavy metals or changes in microbial composition of the soil. So, caution should be practiced while using fly ash for agricultural purposes (Singh et al., 2010).

Owing to increasing food demand, rapidly changing climate, high pathogens adaptability to climatic changes and hazardous effects of least efficient chemical control measures, the need of natural, effective, climate friendly way of disease control is inevitable.

The NPs induced changes were significant regarding chilies growth and bacterial leaf spot suppression. However, the plant response to NPs was dependent on the source or material used for their production. RSBNPs could provide a better alternative to unchecked bulk use of pesticides. There is need to check the possible hazards like dose, toxicological issues and eco-acceptability of these nano forms. Further exploration NPs utility obtained from fly ash and biochar would certainly help in managing plant diseases and addressing environmental concerns associated with toxic pesticides.

6 Conclusion

In agriculture, the search for higher net profit is the main challenge in the economy of the producers and nano biochar attracts increasing interest in recent years due to its unique environmental behaviour and increasing the productivity of plants by inducing resistance against phyto-pathogens. The effect of rice straw biochar and fly ash nanoparticles (RSBNPs and FNPs, respectively) in combination with compost soil on bacterial leaf spot of pepper caused by *Xanthomonas campestris* pv. *vesicatoria* was investigated both *in vitro* and *in vivo*. The application of nanoparticles as soil amendment significantly improved the chili pepper plant growth. However, RSBNPs were more effective in enhancing the above and belowground plant biomass production. Moreover, both RSBNPs and FNPs, significantly reduced (30.5 and 22.5 %, respectively), while RSBNPs had shown *in vitro* growth inhibition of *X. campestris* pv. *vesicatoria* by more than 50%. The X-ray diffractometry of RSBNPs and FNPs highlighted the unique composition of nano forms which possibly contributed in enhancing the plant defence against invading *X. campestris* pv. *vesicatoria*.

On the basis of our findings, it is suggested that biochar and fly ash nanoparticles can be used for reclaiming the problem soil and enhance the crop productivity depending upon the nature of soil and the pathosystem under investigation.

Declarations

Availability of data and materials:

Not Applicable

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Figures

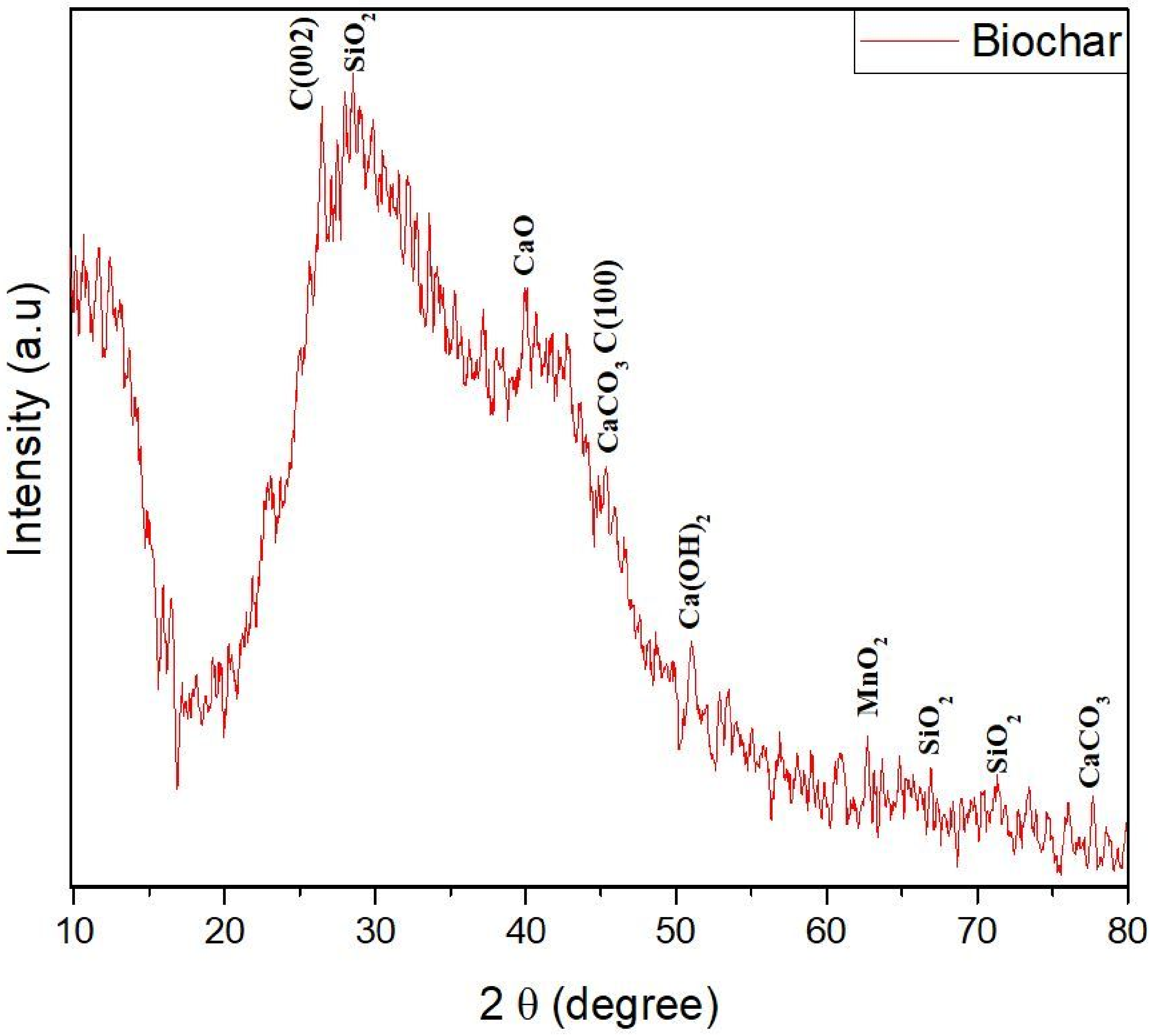

Figure 1

XRD spectrum of rice straw biochar.

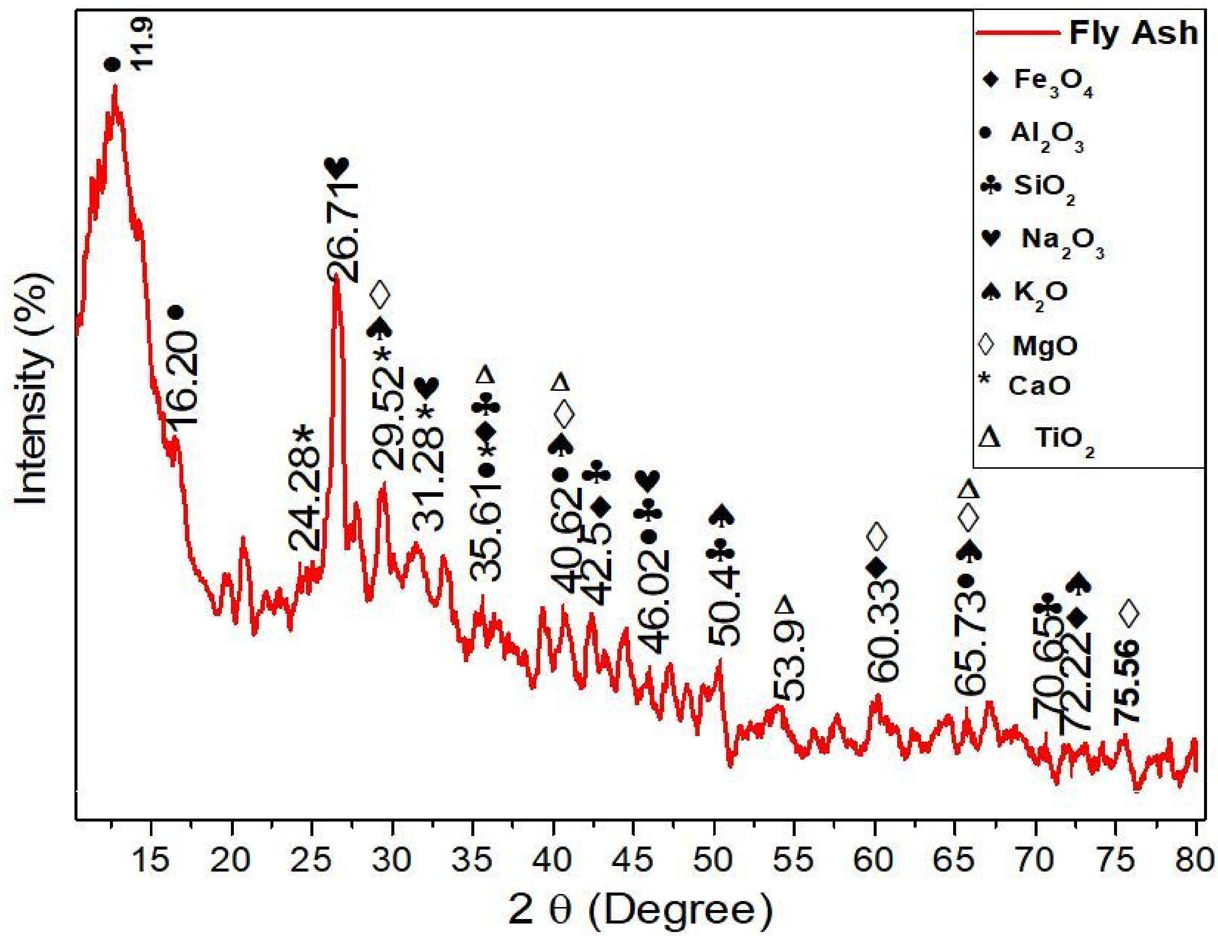

Figure 2

XRD spectrum of fly ash.

Figure 3

Leaves of chilli plants showing disease symptoms in RSBNPs treated plants (A), FNPs treated plants (B) and only soil grown plants (C). In vitro inhibitory effect of rice straw biochar nanoparticles (RSBNPs) and fly ash nanoparticles (FNPs) on Xanthomonas campestris pv. vesicatoria