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Evaluation of Faba Bean (*Vicia faba*) Resistance to *Rhizoctonia solani* Infection in Soil Amended with Nano-Biochar under Controlled Conditions

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Abstract

Nano-biochar is considered an eco-friendly tool used as a soil amendment in suppressing broad range of plant pathogens and promoting plant growth. This study aimed to emphasize the importance of using nano-biochar, with a particular focus on investigating the effectiveness of corncob nano-biochar (CCNB) at a concentration of 0.5% in suppressing plant pathogens, such as *Rhizoctonia solani*, and promoting the growth of faba bean plants. Applying 0.5% CCNB was found to have a significant inhibitory effect on the growth and pathogenicity of *R. solani*, as demonstrated by reduced disease severity and increased total phenolic content in faba bean plants that were infected by Iso1. Nano-biochar not only suppresses disease symptoms but also enhances the physiological processes in plants, such as chlorophyll content and nutrient uptake (N, P, and K), leading to improved plant growth and productivity. These findings support the potential role of biochar as a sustainable soil amendment to manage soil-borne pathogens and improve crop performance and productivity.

Keywords: Disease resistance; Fava Beans; Nano-biochar; Rhizoctonia stem canker.

1. Introduction

Globally, the faba bean (*Vicia faba L.*) is considered one of the most important legumes crops due to its significance in human nutrition, as it is a perfect source of vegetarian protein (28–30%) **[1 , 2].** It also plays a key role in promoting soil fertility, animal feeding, and industrial processes. Faba bean is the most important food crop in Egypt, although the cultivated area has decreased due to several biotic and abiotic factors, impacting yield loss in terms of both quantity and quality. Increasing quantity and quality of faba bean are master targets for meeting the increasing demands of faba bean as the main sustenance of Egyptian diet [2]. Despite all efforts aiming to improve productivity, faba bean cultivation is facing biotic and abiotic threats. Pathogens such as *Rhizoctonia solani*, *Alternaria alternata*, *Botrytis fabae,* and *Uromyces fabae* are among the major abiotic threats. They cause Rhizoctonia stem canker, root rot and damping-off, as well as leaf spot, chocolate spot, and rust [3]. Considering the disease's life cycle and agricultural practices, farmers cannot avoid cultivating host plants in a certain area, as the pathogen could infect a wide range of economically important crops. Furthermore, soil movement and infected seeds make it difficult to manage this disease, as the fungus produces sclerotia that remains in the soil, seeds and plant debris for a long time [4]. *Rhizoctonia solani* was found nearly in all Egyptian Governorates, particularly in the Nile Delta, specifically in Beheira, Monufia, Sharqia, Dakahlia, and Gharbia [5]. Therefore, implementing an integrated management system to control *R. solani* diseases is crucial. This can be achieved by using modern management approaches such as breeding for disease resistance, fungicide treatments, soil amendments (e.g. biochar and biofertilizers), plant defense activators and cultural practices (e.g. crop rotation, mixed cropping, cover cropping, green manuring and bio-fumigation). These measures may reduce soil-borne diseases by creating unfavorable conditions for pathogen growth and survival [6]. Unfortunately, none of these methods could eradicate the pathogen completely due to its lifestyle [7]. In addition, the pathogen could evolve and develop resistant strains, and some unfavorable practices could make it difficult to control the disease. For these reasons, effective control measures must be in place to

reduce these diseases without having an adverse effect on humans or the environment.

Utilization of biochar in agriculture has received much attention and offers a potential approach for enhancing crop productivity and suppressing plant pathogens. Biochar is produced by pyrolysis, burning plant-derived organic materials in a limited oxygen environment, from crop residues such as corncobs, rice husk, and sugarcane bagasse. As a sustainable tool for enhancing soil characteristics, plant productivity and resistance to stressors, researchers have implemented several approaches to enhance its properties. However, there is still limited knowledge on how biochar influences soil properties and plant production [8]. Biochar increases soil quality and reduces the negative effects of excessive pesticides, fertilizers, heavy metals toxicity, and other pollutants [9]. As a result, biochar improves plant growth and sustains crop yields. Also, it reduces soil acidity and improves soil pH, effectively maintain levels of soil organic matter, enhance the base saturation and increase soil cation exchange capacity (CEC), store and supply macro and micronutrients for growing plants, and improves water retention [9, 10]. Greenhouse waste biochar (GHWB), produced at 450 °C and applied at different rates (0.5%, 1% and 3% V/V), may induce systemic resistance in tomato (*Solanum lycopersicum*) plants against the *Botrytis cinerea* fungus, which involves jasmonic acid signaling [11]. It has also been shown to affect *Capsicum annuum* plants at the same concentrations [12, 13]. The application of coconut and commercial Quest biochars at rates of 10–30% (V/V) and 0.5–3% (W/W), respectively, enhanced the suppression of *Fusarium oxysporum* f. sp. *asparagi* in *Asparagus* sp. [14]. The application of olive pomace (0.5%, 1% and 3%), eucalyptus wood (0.5%, 1% and 3%) and citrus wood (3% and 5%) biochars to soil was found to have a significant impact on the resistance of tomato plants to *Botrytis cinerea* (Elad et al., 2011). Therefore, our study aims to investigate the role of nano-biochar on faba bean productivity and resistance to *Rhizoctonia* stem canker.

2. Materials and Methods

2.1 Fungal Isolation and Identification

Samples of faba bean plants that were 5 to 7 weeks old and showed common typical symptoms of damping-off, root rot, and stem canker were collected from various fields to identify the pathogen. Media preparation and isolation procedures were performed according to Tuite method [15]. To identify the obtained isolates, morphological characterization and molecular identification were performed as described by Gillman [16], and Barnett and Hunter [17]. Primarily the nucleic acid (DNA) was extracted from *Rhizoctonia solani* isolates using CTAB procedure according to Doyle and Doyle [18]. Then universal primers ITS1 and ITS4 were used to amplify the internal transcribed spacer region of ribosomal DNA (rDNA ITS) as demonstrated by white et al. [19]. The amplification was performed in PCR thermal cycler (Techne-Progene). The amplified PCR product was loaded in 1.5 % agarose gel stained with ethidium bromide and electrophoresis was run at 80 V. The DNA bands were then visualized and photographed using a special UV camera. DNA ladder (from 100 bp to ≤ 10000 bp) was used as a molecular size standard [20, 21]. The amplified fragments of ITS1-5.8s-ITS2 region (500-700 bp) of the tested isolates were sent for sequencing (Macrogen, Scientific Services Company, Korea). Identification of the isolates were confirmed by applying Basic Local Alignment Search (BLAST search) on National Center for Biotechnology information (NCBI) site (http://www.ncbi.nlm.nih.gov), using the obtained sequences.

2.2 *Nano-biochar preparation and Characterization*

Two types of nano-biochar, corncob nano-biochar (CCNB) and sugarcane bagasse nano-biochar (SCBNB) were prepared as shown in (Fig. 1) according to El-Gamal et al. [22]. The produced black solid materials (biochars) were ground and passed through a 0.5 mm sieve. Then, biochar types were converted into nano size using a high-energy planetary ball mill (Photon Ball Mill Model PH-BML912, Photon Scientific Co., Egypt). To characterize the different types of nano-biochar, the pH and electrical conductivity (EC) were determined according to the method described by Masulili et al. [23]. This involved mixing 1g of solid biomaterial with 100 ml deionized water, heating the mixture to approximately 90°C, and stirring it for 20 minutes to dissolve the soluble components. The suspensions were cooled to room temperature, then the pH and EC were measured by using a conductivity meter (multi-parameter pH-ORP-Conductivity-TDS-TEMP Bench Meter, Adwa, AD8000 model, Hungary). Cation exchange capacity (CEC) was determined using 1 M ammonium acetate at pH 7 (neutral NH_4OAc), as illustrated by Gaskin et al. [24]. The elemental concentrations of experimental nano-biochar were determined using an energy-dispersive X-ray (EDX) system coupled with a scanning electron microscope (SEM; Jeol JSM-6360 LA, Tokyo, Japan). Scanning Electron Microscopy (SEM) was used to examine the physical morphological surface of SCBNB and CCNB. The SEM analysis was carried out using a JEOL JEM6360

LA analytical electron microscope. To avoid the build-up of local electrical charges, the samples were coated with a thin layer of gold using a sputtering coater (model: S150B, Edwards High Vacuum Ltd., UK) before investigation [25]. Fourier Transform Infrared (FT-IR) spectrometry was used to determine the surface functional groups of the SCBNB and CCNB. The FT-IR spectra of the adsorbent were recorded in the range (4000 - 400 cm-1) using SHIMADZU-IR spectrophotometer; model FT/IR-5300, JASCO Corporation Japan [26].

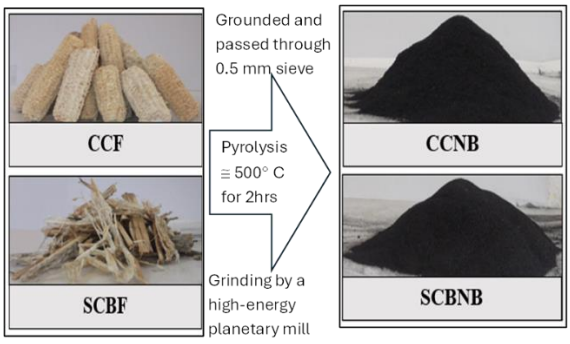


Figure 1. A brief diagram of corncob and sugarcane bagasse nano-biochar (CCNB and SCBNB) preparation.

2.3 Pathogenicity Test

The purified isolates of the identified *Rhizoctonia solani* were tested for pathogenicity on a Faba bean cv. Maryout2 under greenhouse conditions. *R. solani* inoculum was prepared by culturing the fungal isolates on sterilized barley grains to enhance mycelial growth. Three disks of *R. solani* isolate, previously grown on PDA medium, were transferred into conical flasks (500 ml). Each flask contained 200 g of barley grains, which were moistened with water before being autoclaved for 20 minutes. The prepared flasks were inoculated with *R. solani* and incubated at 20 ± 2°C for 14 days until the fungal growth became homogenous. Seeds of faba bean cv. Maryout2 were used for the pathogenicity test. Plastic pots with a diameter of 25 cm containing four Kg autoclaved calcareous soil were prepared. Soil infestation was carried out one week before planting faba bean seeds. Forty grams of the barley grain medium containing *R. solani* inoculum were mixed with soil. Seeds of faba bean were planted in the infested soil at the rate of 6 seeds/pot. Disease severity was determined on the emerged plants according to Khangura et al. [27].

2.4 In vitro Experiments

2.4.1. *In vitro* direct effect of different concentrations of biochar on *R. solani*

The inhibitory effect of the obtained nano-biochar types (SCBNB and CCNB) on the hyphal growth of *R. solani* isolates was studied using an in vitro contact method. Potato Dextrose Agar (PDA) medium was prepared and amended with varying concentrations of SCBNB and CCNB (0.5, 1.0, and 2.0, W/V) as well as control. After autoclaving, PDA medium was supplemented with streptomycin sulphate at the rate of 50 mg/l and dispensed into 9 cm diameter Petri dishes. A 5 mm disk taken from a fresh *R. solani* culture, was placed in the center of petri dishes. Three plates were prepared for each nano-biochar concentration. The dishes were then incubated at 25°C. After 2 days mycelial growth of the fungus was measured as the average of two perpendicular diameters of each culture. The experiment was a factorial design with three main factors (Biochar Types, Biochar levels, and the phytopathogenic fungus *Rhizoctonia solani*) in complete randomized design (CRD) with 16 treatments in three replicates [28].

2.4.2. Effect of different concentrations of nano-biochar on *R. solani* mycelial fresh weight and dry weight.

Potato Dextrose Broth (PDB) was prepared and amended with varying concentrations of SCBNB and CCNB (0.5, 1.0, and 2.0, W/V), and 0.3% Agar (to maintain the biochar in suspension) as well as control. After autoclave, medium was supplemented with streptomycin sulphate. Agar disk was taken from a fresh *R. solani* culture (5mm) and placed in flask (100 ml) containing 50 ml PDB, three flasks were prepared for each nano-biochar concentration. The flasks were incubated for 14 days at 25°C. The mycelial mat was separated from the nutrient solution using a sieve, excess solution was removed and dry on filter paper. Mycelial fresh weight was determined. The mycelial mat was then dried in an air circulation oven at 60°C for 2-3 days until dry weight was unchanged and mycelial dry weight was measured. The experiment was a factorial design with three main factors (Biochar Types, Biochar levels, and *R. solani* isolates) in complete randomized design (CRD) with 16 treatments in three replicates [28].

2.5 Effect of nano-biochar on the development of Rhizoctonia diseases in faba bean

To investigate the effect of CCNB at the concentration of 0.5% on disease development and plant growth parameters of faba bean, a highly susceptible cv. Maryout2 was used. Calcareous soil was collected from the experimental farm of City of Scientific Research and Technological Applications (SRTA-City) located at Borg Al-Arab city, Alexandria, Egypt. The main physicochemical properties of the utilized soil were characterized. The soil texture is loamy sand, pH and EC were recorded as 8.41 (1:2.5, W: V) and 0.61 dS m^{-1} that categorized as non-saline and alkaline soil, respectively. In addition, soil has low levels of total nitrogen 0.14 % and CEC 3.94 meq/100 g soil, medium levels of phosphorus (P) 9.32 mg/Kg, and

extremely high levels of NO^{3-} 183.17 mg/Kg. On the other hand, this calcareous soil has a high potassium (K^{+}) level 324.55 mg/Kg and a moderate level of total carbonate ($CaCO_3$) 21.56 % and organic matter 1.65 % [29, 30]. Plastic pots with a diameter of 25 cm, containing four Kg from the autoclaved, and previously characterized calcareous soil were prepared and mixed with CCNB at concentrations (0 and 0.5 % W/W). Soil infestation was carried out one week before planting faba bean seeds. Forty grams of the barley grain medium containing *R. solani* inoculum of Iso1 and Iso2 were mixed with soil, and seeds were planted in the infested soil at the rate of 4 seeds/pot. The experiment was a factorial design with two main factors (Nano-biochar levels and *R. solani* isolates) in a complete randomized design (CRD) with 6 treatments in three replicates.

2.5.1. Disease Evaluation and Total Phenols determination

A. Damping-off Estimation

Damping-off (percentage of collapsed plants per pot), the percentage of seed decay pre-emergence, post emergence damping-off and survival ratio was calculated after 15, 30, and 45 days of sawing, respectively according to Yehia et al. [31]:

- $\textbf{Pre} - \textbf{emergence}\ (\%) = \frac{\textbf{Number of nongerminated seeds}}{\textbf{Total number of sown seeds}} \textbf{X100}$
- $\textbf{Post} - \textbf{emergence}\ (\%) = \frac{\textbf{Number of dead seedlings}}{\textbf{Total number of seedlings}} \textbf{X100}$
- $\textbf{Survival ratio}\ (\%) = \frac{\textbf{Number of survival plants}}{\textbf{Total number of seedlings}} \textbf{X10}$

B. Disease severity (DS)

To determine disease severity (percentage of the hypocotyls and root tissues area covered by lesions) on the emerged plants, the roots were thoroughly washed and rated for hypocotyl rot on a 0 to 3 scale according to Khangura et al. [27] as follows:

0 = no visible symptoms.

1 = lesions on hypocotyl affecting < 25 % of the length of hypocotyl.

2 = lesions covering 26 to 75% of the length of the hypocotyl.

3 = lesions covering approximately 75% of the length of the hypocotyl.

Percent of disease severity (DS %) was calculated after 60 days as following:

$$\frac{[\Sigma\ (\textbf{no. plants in disease category}) - (\textbf{numerical value of disease category})\ ]}{[(\textbf{no. plants in all categories}) \times (\textbf{maximum value on rating scale})]} \times 100$$

C Total Phenols Quantification

Total phenols were extracted and quantified utilizing Folin-Ciocalteau reagent according to Malik and singh [32]. Leaf samples (one gram) were homogenized with a sterilized pestle and mortar in10 ml of 80% ethanol and agitated for 15 min at 70°C. Using filter paper, the extract was filtrated in a falcon tube (15 ml). Then, 0.2 ml of the ethanolic extract was transferred to a 10 ml glass tube. The volume was made up to a volume of 3 ml by adding 2.8 ml distilled water and then a 500 µl of Folin-Ciocalteau reagent 1N (1:1) was added, the solution was then kept at 25°C for 3 minutes. Next, 2ml of 20 % sodium carbonate was added and mixed well. Then the tubes were placed in boiling water for 1 min and then cooled down. A blank containing all reagents without the extract was also prepared. The absorbance of the developed blue color intensities was measured using a spectrophotometer at 650 nm and catechol solution was used as a source of phenolic compounds to prepare the standard curve. The concentration of phenolics was expressed as µg catechol equivalent g^{-1} fresh weight.

2.5.2. Plant Growth Parameters

2.5.2.1. Shoot and Root Parameters

Leaves, flowers and stem branches of each plant were counted at harvest (after 60 days of sowing). The height of fresh shoots was measured using a ruler at harvest. Shoots and roots were separated from each other and weighed immediately after harvest, but roots were washed under running tap water to remove adhering soil before weighing. Then, each part of plant was washed with distilled water many times, air-dried, and weighed. Parts for each replicate were collected in paper bags and placed in the oven for 48 hours at 70°C and weighed immediately after the drying period ended and grinded to be used for elemental analysis.

Moisture content was measured according to the following formula:

M.C %=(W2-W3)/(W2-W1) X 100

Where: M.C = Moisture Content (%), W1 = Weight of paper bag (g), W2 = Initial weight of bag with the sample (before drying) (g), W3 = Final weight of bag with the sample (after drying) (g).

2.5.2.2. Chlorophyll Quantification

One gram of fresh faba bean leaves was chopped into small pieces, grinded using sterile mortar and pestle in 20 ml acetone (80%) and kept at 4 °C. The mixture was centrifuge at 10000×g for ten minutes, then the supernatant was transferred to a clean test tube. The absorbance was measured by a spectrophotometer at wavelength 663 for chlorophyll a and 644 for chlorophyll b against the solvent blank (acetone 80%). Concentrations of chlorophyll A, chlorophyll B, and the total chlorophyll were estimated as described by Lorenzen [33] according to the following equations:

Conc. of Chlorophyll A (mg/gm. FW) = 12.7(E 663)-2.69(E 644) × V/ (W ×1000)

Conc. of Chlorophyll B (mg/gm. FW) = 22.9(E 644)-4.68(E 663) × V/ (W ×1000)

Total chlorophyll (mg/gm. FW) = (20.2× E 644) + (8.02× E 663)

Where: E= Optical density at the wavelength (663) or (644), V= Total extract volume, W= Leaf fresh weight.

2.5.2.3. Elemental Analysis

The wet digestion method was used to determine the total nitrogen content in seeds. Dry seed (0.25 grams) samples were pre-treated with wet digestion reagent (10 ml of concentrated H_2SO_4, 5 ml H_2O_2 30%, and 2 g of digestive mixture (1 g Se: 10 g $CuSO_4$: 100 g K_2SO_4) in a Kjeldahl digestion tube [34]. Then, contents of the tube were agitated and heated on the block-digester set at 300°C for 2 hours. After the digestion, nitrogen content was determined using the distillation unit of Kjeldahl device. The total P and K in plant parts (shoots, roots, seeds) was determined using the dry digestion method (oven-dried for 48 hours at 70°C). One gram of oven-dried sample was put in a porcelain crucible and burnt in the muffle furnace at 500°C for 6 hours until it reached ash. After cooling, the crucible was removed from the muffle furnace. The ash was dissolved by adding 10 ml of dilute aqua regia (HNO_3: HCl, 1: 3) and left the mixture for 20 min until the digestion is complete. Using filter paper, the mixture was filtrated into a 100-ml measuring flask. The filtrate was completed to 100 ml using distilled water [35]. Total P in the extract was determined by ammonium paramolybdate-vanadate reagent. Five ml of the supernatant were transferred to a 50-ml measuring flask, 10 ml of the reagent were added and then the volume was completed to 50 ml. Measurement was performed on a spectrophotometer (T80 UV/VIS Spectrophotometer, PG Instruments Ltd) after 10 min at a wavelength of 430-nm. A blank containing all reagents without sample was also prepared. Total K was measured by a flame photometer (FP902, PG Instruments).

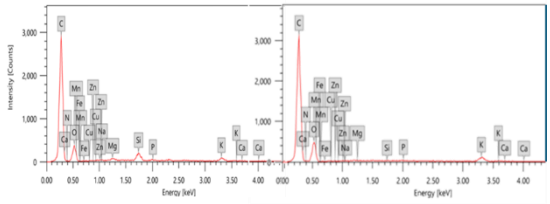
3. Results

3.5 Fungal Isolation and Identification

Two isolates of *Rhizoctonia solani* (*teleomorph*, *Thanatephorus cucumeris*) were recovered from infected faba bean plants showing typical symptoms of Rhizoctonia stem canker, these isolates were named Iso1 and Iso2. The obtained isolates were identified with morphological and molecular characterization. The hyphae of the fungus have unique characteristics of brown color, hyphal branching with a constriction at the point of branching and dolipore septum. On the other hand, the BLAST search using the sequence (over 600 bp) of ITS1-5.8S-ITS2 region indicates that both isolates could be assigned to *Rhizoctonia solani* based on the similarity percentage (99%). The sequences of the two isolates were deposited in Genbank under accession numbers PV972195 and PV972196 for Iso1 and Iso2, respectively.

3.2 Biochar Characterization

Characterization indicated that SCBNB and CCNB possessed alkaline behavior, with a pH value of 8.63 and 8.33, respectively. EC was higher in SCBNB than CCNB, which recorded 0.8 and 1.3 dS m^{-1}, respectively. Moreover, the cation exchange capacity (CEC) of the produced nano-biochar types recorded a higher value (32.35 meq/100 g soil) in CCNB than SCBNB. In general, the total elemental analysis showed that the concentrations of C were higher in both nano-biochar types followed by O concentrations and these results were confirmed by EDX analysis in (Fig. 2).



Element	Line	SCBNB Mass%	SCBNB Atm%	CCNB Mass%	CCNB Atm%
C	K	68.6±0.27	75.2±0.29	72.92±0.2	79.41±0.32
N	K	nd	nd	nd	Nd
O	K	28.7±0.50	24.0±0.41	23.46±0.47	29.18±0.39
NA	K	0.08±0.4	0.04±0.2	0.01±0.04	19.18±0.22
Si	K	nd	Nd	0.38±0.04	0.202±0.02
P	K	0.56±0.02	0.28±0.0	1.33±0.06	0.622±0.03
K	K	1.37±0.06	0.5±0.2	0.25±0.03	0.088±0.01
Ca	K	1.37±0.03	0.21±0.0	1.16±0.05	0.350±0.01
Fe	K	0.05±0.3	0.01±0.0	0.04±0.03	0.010±0.01
Cu	K	0.03±0.6	0.01±0.0	0.04±0.02	0.010±0.01
Zn	K	0.01±0.3	0.01±0.0	0.27±0.06	0.06±0.01
Total		100	100	100	100
Spc_008	Fitting ration 0.0370			Fitting ration	0.0392

Figure 2. Energy dispersive x-ray (EDX) for the SCBNB and CCNB.

Figures (3) show the morphological characteristics and pore structure of SCBNB and CCNB that were described by SEM images. SEM magnified at 500X represents a highly complex network structure with longitudinal pores in varied sizes and different channels in various diameters.

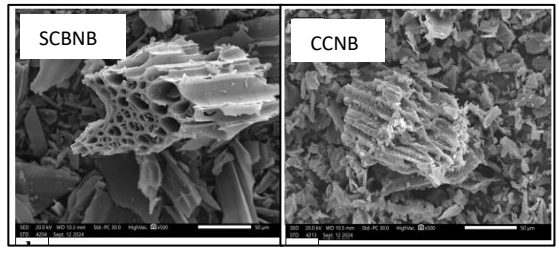


Figure 3. SEM image of SCBNB and CCNB magnified at 500X.

The Fourier Transform infra-red spectroscopy (FT-IR) study illustrates the changes in functional groups between SCBNB and CCNB and results from it depicted presence and absence of functional groups, which revealed the wavelength peak which observed between 500-3500 cm^{-1}. The detected functional groups and characterizing covalent bonding information in SCBNB and CCNB are shown in figure 4. The stretching of hydroxyl group and H-bonds were detected at the band with range from 3438 to 3439 cm^{-1}, which indicates the presence of alcohols, phenols, and organic acids. The peaks in the range 2956 to 2918 cm-1 represent to saturated aliphatic hydrocarbon (C-H, stretching vibration) due to the presence of alkane functional group. Also, the band at 1163 to 1111 cm-1 of alcohol (C-O stretch) and aliphatic amines (C-N) in both nano-biochar. The amines, alkenes and aromatic functional groups were expressed due to the presence of N-H band and C=C stretching vibrations between 1608–1511 cm^{-1} in both biomaterials. In addition, the peak at 873 cm^{-1} in SCBNB indicates the presence of the aromatic CH and carboxyl-carbonate, which disappeared in the CCBN. However, these aromatic carbon structures have been confirmed by aromatic C=C ring stretching between 1608 and 1511 cm-1and the peaks at 1243 cm^{-1}, corresponding to aromatic CO- stretching and aromatic C=C peaks are evidence of hydrocarbon rings (benzene-like). Also, the alkyl halide group (C-Br stretch) was observed at around 670 to 471 cm^{-1} in both types.

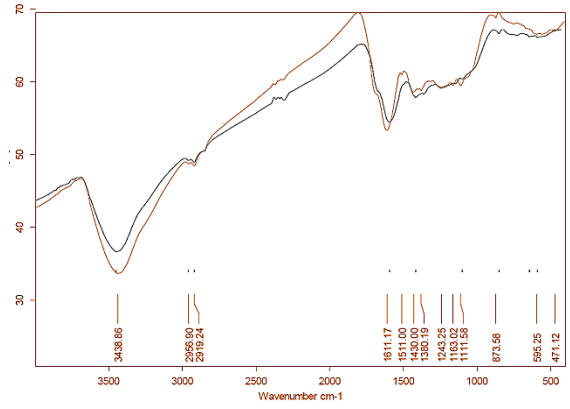


Figure 4. FTIR spectra and expected corresponded functional groups for SCBNB (Red line) and CCNB (blue line).

3.3 Pathogenicity Test

The pathogenic potential of the tested isolates (Iso1 and Iso2) of *Rhizoctonia solani* was tested on faba bean cv. Maryout2. The obtained results showed that the isolates of *R. solani* significantly produced typical symptoms of stem canker on faba bean plants. Iso2 exhibited stronger virulence and disease severity was recorded at 85.55%, while the least severe of symptoms were showed with Iso1 infection with a recorded disease severity of 68.32 % after 60 days of planting. Hence, these results suggest that Iso2 is more aggressive in its interaction with faba bean plants more than Iso1.

3.4 Assessment of Nano-Biochar Efficiency Against Rhizoctonia solani

3.4.1. Effect of different concentrations of Nano-biochar on Mycelial Growth of *Rhizoctonia solani*

As shown in figure 5, examination of the effect of nano-biochar on the mycelium growth of *R. solani* revealed that not all concentrations of either type of biochar could inhibit fungal growth. Notably, *R. solani* growth was significantly slower on CCNB than that on SCBNB. Growth rate in the control treatment was slower than the other concentrations followed by 0.5 % CCNB. This suggests that the lowest CCNB concentration (0.5%) was the most effective to hinder fungal growth.

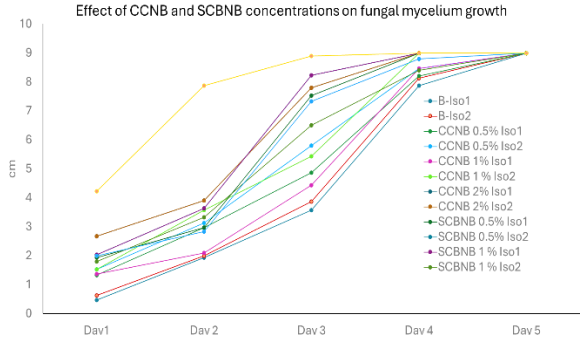


Figure 5. Effect of CCNB and SCBNB concentrations on fungal mycelium growth.

3.4.2. Effect of different concentrations of Nano-biochar on the fresh weight and dry weights of *R. solani* mycelium.

Data in Figure 6 indicates a variable effect of both biochar types on the growth of *R. solani.* The fungal fresh and dry weights were significantly lower with CCNB than with SCBNB. The lowest fresh and dry weights were recorded with CCNB and SCBNB at the concentration 0.5 % and 1%, respectively compared to the other concentrations. At a concentration of 0.5% CCNB decreased the fungal fresh weight by 40.34 % and 31.24 % with Iso1 and Iso2, respectively. However, 1% SCBNB decreased the fungal fresh weight by 9.25 % and 11.99 % with Iso1 and Iso2, respectively. The dry weight decreased to about half compared to the control with 0.5 % CCNB treatment in

the presence of both isolates. Whereas SCBNB decreased the fungal dry weight by 46.20 and 57.94 % with Iso1 and Iso2, respectively.

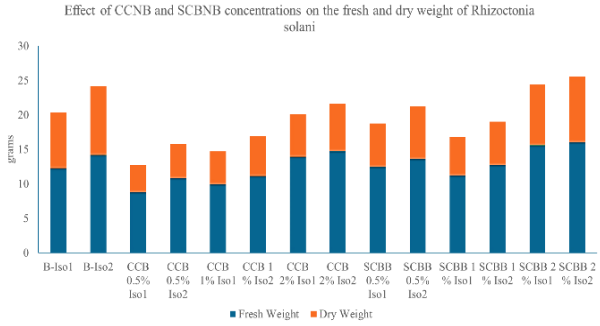


Figure 6. Effect of CCNB and SCBNB concentrations on the fresh (blue) and dry weight (orange) of *Rhizoctonia solani.*

3.5 Effects of Nano-Biochar in Pot Experiments Conducted under Growth Chamber Conditions

3.5.1. Assessment of Nano-biochar Efficiency Against Total Phenolic Contents of Infected Faba bean plants

The obtained results shown in figure 7 illustrated the total phenolics content in the faba bean treated with 0.5% CCNB measured after 60 days of planting and showed a significant increase compared to control plants. This study revealed that CCNB at concentration of 0.5% decreased damping-off and disease severity in Iso1 and Iso2-infected faba bean. In addition, an increase in the survival ratio and total phenolic content were observed in infected plants compared to healthy, non-infected plants. The survival ratio increased by 66.66 % and 50.02 % with Iso1 and Iso2, respectively, compared with the control, nano-biochar-free treatment. Disease severity decreased by 62.43% and 44.61% with Iso1 and Iso2, respectively, compared with treatments without nano-biochar. Total phenolic content increased by 16.41 % and 45.26 % with Iso1 and Iso2, respectively, compared with treatments without nano-biochar.

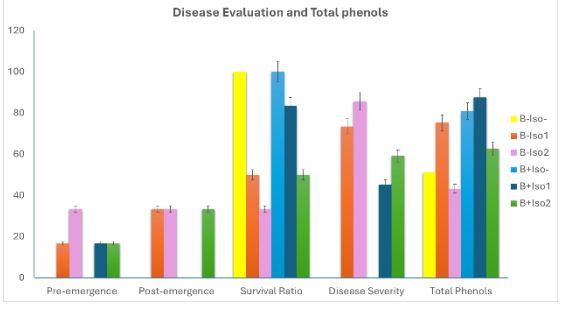


Figure 8. Disease evaluation and total phenolic content (µg catechol equivalent g-1 FW) of the cv. Maryout2 inoculated with *Rhizoctonia solani* isolates grown in soil-amended with 0.5 % CCNB**.**

3.5.2. The Effect of Nano-biochar on Growth Parameters of Infected Plants

The data obtained shown in figure 8 presented the impact of 0.5% CCNB on the shoot growth parameters of non-infected and infected faba bean plants. Generally, the highest values were recorded in the case of pathogen-free treatments. As, the shoot fresh and dry weight recorded at 251.92 g and 81.49 g, with an increase 24.55 % and 67.26 %, respectively. The height of the plants recorded 88.16 cm, with an increase 14.33%. Moreover, 48 leaves, 2.65 branches and 23 flowers were recorded, representing increases of 8.28%, 99.25% and 68.25%, respectively. Additionally, the highest root fresh and dry weights were recorded at 46.67 g and 12.42 g, with increases of 22.08% and 30.19%, respectively, compared to the control.

On the other hand, the plants infected by Iso1 were affected by the 0.5% CCNB application more than the plants infected by Iso2. The highest fresh and dry shoot weight of the Iso1-infected plants was recorded at 171 g and 46.53 g, with increases of 23.06% and 0.7%, respectively. The plant height was 63.30 cm, an increase of 2.80%. Moreover, number of leaves, branches, and flowers recorded 36 leaves, 1 branch, and 6.33 flowers, with an increase by 1.90 %, 0.0 % and 58.25 %, respectively. The highest root fresh and dry weight of the plants infected by Iso1 recorded at 30.91g and 9.52 g, with an increase of 37.07 % and 22.37 %, respectively as compared with nano-biochar-free treatment. While shoot fresh and dry weight of the infected plants by Iso2 recorded at 141.88 g and 37.81 g, with an increase 60.41 % and 73.44 %, respectively. The height of plants recorded 43.44 cm, with an increase of 39.51 %. Moreover, the number of leaves, branches, and flowers recorded 27.67 leaves, 1 branch, and 3 flowers, with an increase of 69.44 %, 0.0 % and 79.61%, respectively. The highest fresh and dry weight of the infected plants by Iso2 recorded at 26.48 g and 8.29 g, with an increase of 32.20 % and 20.85 %, respectively as compared with nano-biochar-free treatment.

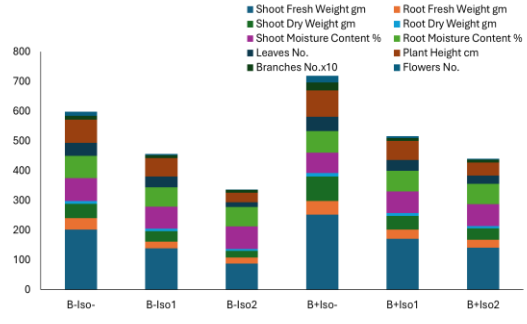


Figure 8. Effect of CCNB and SCBNB on growth parameters of plants infected with different isolates.

3.5.3 The effect of Nano-biochar on Chlorophyll Quantities

The photosynthetic pigment content in faba bean leaves were affected by 0.5% CCNB as shown in Table 1. The highest content of chlorophyll A, B, and total chlorophyll was recorded in case of non-infected plants at 25.88, 15.9, and 30.58 mg/g. Fw, with an increase of 54.32 %, 14.45 %, and 33.01 %, respectively as compared with control. On the other hand, the highest content of chlorophyll A, B, and total chlorophyll of infected plants was recorded in the presence of Iso1 more than Iso2 at 13.44, 10.27, and 17.65 mg/g. Fw, with an increase of 14.77 %, 6.98 %, and 10.59%, respectively as compared with nano-biochar-free treatment.

Table 1: Effect of 0.5% CCNB on Chlorophyll content of faba bean plants

Nano-biochar	*R. solani*	Chlorophyll Content		
		Chlorophyll A	Chlorophyll B	Total Chlorophyll
NB-	Iso-	16.77 b	13.91 b	22.99 b
	Iso1	11.71 cd	9.60 cd	15.96 cd
	Iso2	7.31 e	6.43 e	10.57 e
NB+	Iso-	25.88 a	15.92 a	30.58 a
	Iso1	13.44 bc	10.27 c	17.65 c
	Iso2	9.59 de	8.33 de	13.48 de

3.5.4 Effect of Nano-Biochar on Plant Contents of Elements

The results in figure 9 illustrated that 0.5% CCNB increased the plant content of total phosphorus, potassium, and nitrogen content in infected and non-infected plants of cv. Maryout2. In the case of pathogen-free treatments, the highest N, P, and K content in shoot was recorded at 4.17 %, 0.46 %, and 0.92 %, with an increase 15.19 %, 35.29 %, and 22.67%, respectively. As well as the highest N, P, and K content in root was recorded at 3.22 %, 0.39 %, and 0.71%, with an increase 7.69 %, 25.81 %, and 31.48 %, respectively as comparing with control. On the other hand, the highest concentrations of N, P, and K were achieved in the shoot and roots infected plants with Iso1 and recorded at (3.03 % -2.55%), (0.27 % -0.22%), and (0.55 % -0.40%), with an increase (44.29 % -33.51 %), (50 % -69.23 %), and (31.25 % -41.38 %), respectively as comparing with the nano-biochar-free treatments. Additionally, the content of N and P in the shoot and roots of infected plants with Iso2 was recorded at (2.48 % -0.20%) and (2.05 % -0.16%), with an increase (51.22% -41.38%) and (66.67 % -77.78%), respectively. While K content in shoot and root of plants was 0.55 % -0.40%, and K content approximately 1.5-fold higher than the nano-biochar-free treatments.

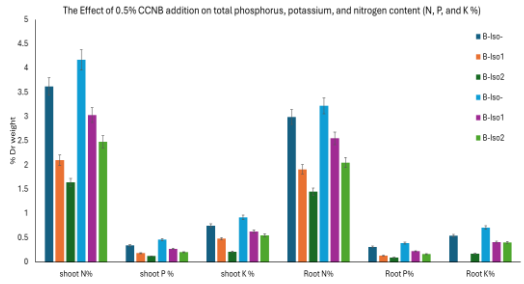


Figure 9. Effect of CCNB and SCBNB on Phosphorus, Potassium, and Nitrogen contents on shoot and root of faba beans.

4. Discussion

In this study, we used the Nano-Biochar CCNB as a soil amendment to manage Rhizoctonia diseases in faba bean. Two *Rhizoctonia solani* isolates, obtained from infected faba bean plants, were suppressed by CCNB at a concentration of 0.5% either directly or indirectly. This fungus resides in the soil and attacks many plant species including faba bean. Due to the infection with *R. solani,* faba bean quantity and quality severely affected. As it could attack the plants pre- and post-emergence causing severe economic losses on faba bean production [36]. In the current study, we have recovered two isolates from several diseased faba bean samples from different fields. These samples showed different symptoms of *R. solani* infections, including damping-off, stem canker, and root rot. As expected, the recovered fungal isolates were assigned to the genus *R. solani* based on its morphological characteristics and ITS1-5.8s-ITS2 sequence [37]. This highlights the significance of monitoring the fields regularly as the phytopathogens could vary in their morphology within the species level [38]. Considering this variability, using molecular markers are a reliable tool for identification, tracking phytopathogens, and detecting variations between, within species and within strains as well. Various regions of ribosomal DNA (rDNA) are widely amplified and sequenced to determine the relationships between organisms using specific primers rDNA [39]. The use of ITS1 and ITS4 primers for detection of *R. solani* obtained from infected faba bean plants produced a specific band of approximately 675 and 682 bp with Iso1 and Iso2, respectively [40]. As this region could be useful for species-level resolution, the blast search for ITS1-5.8s-ITS2 sequence revealed more than 99 % identity with other *R. solani* isolates registered in NCBI [41].

The pathogenicity test of the selected *R. solani* isolates was carried out to confirm its aggressiveness on faba bean plants. In the present study, the obtained isolate of *R. solani* was found pathogenic and produced the typical symptoms on the tested cultivars as those observed on infected plants diagnosed in the field. Rhizoctonia stem canker disease is the main root

disease of faba bean [42]. The incidence of the damage was observed mainly up to 15 days after sowing [41]. These results prove that the isolation, identification and pathogenicity testing of the phytopathogens is crucial for developing control measures and preventing disease outbreak. Therefore, our study aimed to test the efficacy of nano-biochar in management of this disease *in vitro* and *in vivo*. Addition of nano-biochar to the PDA and PDB media could suppress fungal growth. The radial growth, fresh weight, and dry weight were affected at the low concentration at 0.5%. Moreover, the lowest rates of disease severity were observed in faba bean plants grown in a soil amended with 0.5 % of CCNB. On the other hand, nano-biochar amendment had a little impact on damping-off and no symptoms were obtained in pathogen-free treatments. It's well documented that plant derived biochar could suppress plant diseases in many plants such as damping-off caused by *R. solani* in cucumber and common bean [28] grown in a soil amended with GHWB (Green-house pepper plant wastes). This suppression could be attributed to the higher accumulation of phenolic compounds, the enhancement of plant defense, the inhibition of toxin production and the pectinolytic enzymes produced by pathogens [43]. Conclusively, results showed that the soil was amended with 0.5% CCNB increasing the total phenols and enhanced the self-defense mechanism of faba bean plants that affected by Iso1 and Iso2 of *R. solani*.

It is worth noting that the use of 0.5% CCNB has a great impact on plant growth parameters and productivity as well. The general improvements of lengths, fresh and dry weights of plants could be due to the mode of action caused by the nano-biochar such as N2-fixation and general improvement in nutrients and water. Graber et al. [14] explained that biochar application in the soil may profoundly influence the rhizosphere system and disease triangle factors (susceptible host plant, suitable environmental conditions, and virulent pathogen) through its properties which have effects on nutrients content of host, water-holding capacity, adsorption ability of compounds such as allelopathic compounds, soil pH, toxic and hormone-like compounds, and soil microbial communities. In addition, applying biochar improved the leaf photosynthetic rate, which may be due to the improvement of soil physicochemical properties, ultimately increasing nitrogen accumulation and consequently enhancing the photosynthetic rate [44]. In our study, we found that nano-biochar supplementation had a positive effect on plant productivity and chlorophyll content. We observed that biochar at a concentration of 0.5 % significantly increased the content of chlorophyll A, B, and total chlorophyll content in plants compared with other treatments without nano-biochar. Moreover, biochar can increase cation-exchange capacity (CEC), thereby potentially improve plant growth and increase yield [45]. In this study, an increase in the N, P, and K content of biochar-amended soil as compared to control treatment without biochar addition. This reflects the growth-promoting properties and benefits of nano-biochar, which may exist even when its water-wash extracts, which are rich in organic and inorganic compounds, are applied [46].

Overall, the mechanisms by which biochar can protect plants against disease are varied. These mechanisms include enhancing plant growth, providing nutrients to plants, increasing the diversity of soil microbes, absorbing toxins produced by pathogens, stimulating the production of antibiotics or fungitoxic compounds, altering the chemistry of root exudates and inducing systemic plant resistance through chemical compounds that act as elicitors or through microorganisms present in microhabitats [47]. The mechanisms by which nano-biochar increased disease resistant and chlorophyll content, and nutrient availability in calcareous soil is warrant further studies.

5. Conclusion

This study demonstrated the potential of nano-biochar (CCNB) as an effective soil amendment for managing *Rhizoctonia solani* diseases in faba bean. The recovered isolates (Iso1 and Iso2) were successfully identified and confirmed pathogenic through molecular and morphological characterization, emphasizing the importance of accurate pathogen identification in disease management. In vitro assays revealed that CCNB at 0.5% significantly suppressed fungal radial growth, fresh weight, and dry weight. *In vivo* pot experiments further confirmed that nano-biochar reduced disease severity, enhanced total phenolic content, and improved the self-defense mechanisms of faba bean plants.

Beyond disease suppression, CCNB application positively influenced plant growth and productivity by improving fresh and dry weights, nutrient uptake (N, P, K), and chlorophyll content. These findings highlight that nano-biochar is not only a promising tool for controlling soil-borne pathogens like *R. solani* but also a growth-promoting amendment capable of enhancing crop productivity under calcareous soil conditions.

6. Limitations of the study

The present study has some limitations that should be considered. First, the experiments were conducted under controlled growth chamber and pot conditions, which may not fully reflect field-scale variability in soil, climate, and pathogen pressure. The long-term impacts on soil health and microbial communities were not assessed.

7. References

1. Cazzato E, Tufarelli V, Ceci E, Stellacci AM, Laudadio V. Quality, yield and nitrogen fixation of faba bean seeds as affected by sulphur fertilization. Acta Agric Scand B Soil Plant Sci. 2012;62:732-8. doi:10.1080/09064710.2012.698642
2. Abd El-Maged TA, Belal EE, Rady MAO, Abd El-Maged ShA, Mansour E, Awad MF, et al. Acidified biochar as a soil amendment to drought stressed (Vicia faba L.) plants: influences on growth and productivity, nutrient status, and water use efficiency. Agronomy. 2021;11:1-18. doi:10.3390/agronomy11071290
3. Hegedorn DA, Inglis DA. Handbook of bean diseases. Univ. of Wisconsin-Madison: Cooperative Extension Publication; 1986. p.28.
4. Sepulveda R. Identification of Rhizoctonia solani (Kuhn) affecting faba bean (Vicia faba L.) in Chile. Agric Tec Santiago. 1991;51(4):362-3.
5. Abd-Elsatar MA, El-Marzouky H, Elsherif M, Mahmoud MAE. Incidence, anastomosis grouping and genetic variability of Rhizoctonia solani infecting potato in Egypt. Mansoura Univ Agric Sci. 2009;34:6953-66. doi:10.21608/jppp.2009.217179
6. Akber MA, Fang X. Research progress on diseases caused by the soil-borne fungal pathogen Rhizoctonia solani in alfalfa. Agronomy. 2024;14(7):1483. doi:10.3390/agronomy14071483
7. Cubeta MA, Vilgalys R. Rhizoctonia. In: Lederberg J, editor. Encyclopedia of microbiology. 2nd ed. San Diego: Academic Press; 2000. p.109-16.
8. Apori SO, Byalebeka J. Contribution of corncob biochar to the chemical properties of a ferralsol in Uganda. Arab J Geosci. 2021;14:1290. doi:10.1007/s12517-021-07722-y
9. Abdelhafez AA, Abbas MHH, Li J. Biochar: the black diamond for soil sustainability, contamination control and agricultural production. In: Hung W-J, editor. Engineering applications of biochar. InTech; 2017. p.7-27. doi:10.5772/65231
10. Hussain M, Farooq M, Nawaz A, Al-Sadi AM, Soliman ZM, Alghamdi SS, et al. Biochar for crop production: potential benefits and risks. J Soils Sediments. 2017;17:685-716. doi:10.1007/s11368-016-1360-2
11. Mehari ZH, Elad Y, Rav-David D, Graber ER, Harel YM. Induced systemic resistance in tomato (Solanum lycopersicum) against Botrytis cinerea by biochar amendment involves jasmonic acid signaling. Plant Soil. 2015;395:31-44. doi:10.1007/s11104-015-2445-1
12. Elad Y, Cytryn E, Harel YM, Lew B, Graber ER. The biochar effect: plant resistance to biotic stresses. Phytopathol Mediterr. 2011;50:335-49.
13. Frenkel O, Bornestein M, Shulhani R, Sharabani G, Sofer M, Abo-Moch F, et al. Secondary spread of Clavibacter michiganensis subsp. michiganensis in nurseries and the conditions leading to infection of tomato seedlings. Eur J Plant Pathol. 2016;144:569-79. doi:10.1007/s10658-015-0795-4
14. Graber ER, Frenkel O, Jaiswal AK, Elad Y. How may biochar influence severity of diseases caused by soilborne pathogens. Carbon Manag. 2014;5(2):169-83. doi:10.1080/17583004.2014.913360
15. Tuite J. Plant pathological methods: fungi and bacteria. Minneapolis: Burgess Press; 1969. 239 p.
16. Gilman JC. A manual of soil fungi. 2nd ed. Iowa: Iowa State College Press; 1957. 448 p. doi:10.1097/00010694-195708000-00021
17. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 4th ed. Minneapolis: Burgess Publishing; 1998.
18. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987;19:11-5.
19. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, editors. PCR protocols: a guide to methods and applications. Orlando: Academic Press; 1990. p.315-22. doi:10.1016/B978-0-12-372180-8.50042-1
20. Hsiang T, Dean JD. DNA sequencing for anastomosis grouping of Rhizoctonia solani isolates from Poa annua. Int Turfgrass Soc Res J. 2006;9:674-8.
21. Stojsin V, Budakov D, Jacobsen B, Grimme E, Bagi F, Jasni S. Identification of Rhizoctonia solani isolates from sugar beet roots by analyzing the ITS region of ribosomal DNA. Proc Nat Sci Matica Srpska Novi Sad. 2007;113:161-71. doi:10.2298/ZMSPN0713161S
22. El-Gamal EH, Saleh M, Elsokkary I, Rashad M, Abd El-Latif MM. Comparison between properties of biochar produced by traditional and controlled pyrolysis. 2017.
23. Masulili A, Utomo WH, Suyono MS. Rice husk biochar for rice based cropping system in acid soil 1. The characteristics of rice husk biochar and its influence on the properties of acid sulphate soils and rice growth in West Kalimantan, Indonesia. J Agric Sci. 2010;2(1):39-47. doi:10.5539/jas.v2n1p39

24. Gaskin JW, Steiner C, Harris K, Das KC, Bibens B. Effect of low-temperature pyrolysis conditions on biochar for agricultural use. Am Soc Agric Biol Eng. 2008;51(6):2061-9. doi:10.13031/2013.25409

25. Mohamed MAA, El-Maghraby AH, Abd El-Latif MA, Farag HA. Optimum synthesis conditions of nanometric Fe50Ni50 alloy formed by chemical reduction in aqueous solution. Bull Mater Sci. 2013;36(5):845-52. doi:10.1007/s12034-013-0539-z

26. El-Gamal EH, Salem LR, Mahmoud AH, Saleh ME. Evaluation of rice husk biochar as a micronutrient carrier on micronutrient availability in a calcareous sandy soil. J Soil Sci Plant Nutr. 2023;23:1633-47. doi:10.1007/s42729-023-01184-9

27. Khangura RK, Barbetti MJ, Sweetingham MW. Characterization and pathogenicity of Rhizoctonia species on canola. Plant Dis. 1999;83:714-21. doi:10.1094/PDIS.1999.83.8.714

28. Jaiswal AK, Elad Y, Graber ER, Frenkel O. Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to Rhizoctonia solani: the "shifted R max-effect". Plant Soil. 2015;395:125-40. doi:10.1007/s11104-014-2331-2

29. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J. 1978;42(3):421-8. doi:10.2136/sssaj1978.03615995004200030009x

30. Bashour II. Fertility and fertilizer requirements. In: Rural integrated development of the mountains of Northern Lebanon. FAO Report to Ministry of Agriculture. Beirut, Lebanon; 2001.

31. Yehia AG, Mahmoud R, Gaafar M, Mubarak HM. Genetic diversity among Nile delta-isolates of Rhizoctonia solani Kuhn based on pathogenicity, compatibility, isozyme analysis and total protein pattern. Turk J Bot. 2007;31:19-29.

32. Malik EP, Singh MB. Plant enzymology and histoenzymology. 1st ed. New Delhi: Kalyani Publishers; 1980. p.286.

33. Lorenzen CJ. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol Oceanogr. 1967;12:343-6. doi:10.4319/lo.1967.12.2.0343

34. Bremner JM, Mulvaney CS. Nitrogen-total. In: Page AL, editor. Methods of soil analysis, Part 2: chemical and microbiological properties. 2nd ed. Madison (WI): ASA-SSSA; 1982. p.595-624. doi:10.2134/agronmonogr9.2.2ed.c31

35. Olsen SR, Sommers LE. Phosphorus. In: Page AL, editor. Methods of soil analysis, Part 2: chemical and microbiological properties. 2nd ed. Madison (WI): ASA-SSSA; 1982. p.403-30. doi:10.2134/agronmonogr9.2.2ed.c24

36. Trigiano RN, Windham MT, Windham AS. Plant pathology: concepts and laboratory exercises. Washington: CRC Press; 2004.

37. Ferrucho AL. Construction of pathogenic, biologic and genetic bases of the Colombian populations of Rhizoctonia solani AG-3 necessary for the development of management strategies of stem canker and black scurf diseases of potato [PhD thesis]. Bogotá: National University of Colombia; 2011.

38. Xu J. Fundamentals of fungal molecular population genetic analyses. Curr Issues Mol Biol. 2006;8(2):75-90.

39. Bridge PD, Arora DK, Reddy CA. Application of PCR in mycology. New York: CAB International; 1998.

40. Kilicoglu MC, Ozkok I. Molecular characterization of Rhizoctonia solani AG4 using PCR-RFLP of the rDNA-ITS region. Turk J Biol. 2010;34:261-9. doi:10.3906/biy-0812-14

41. Ajayi-Oyetunde OO, Bradley CA. Rhizoctonia solani: taxonomy, population biology and management of Rhizoctonia seedling disease of soybean. Plant Pathol. 2018;67:3-17. doi:10.1111/ppa.12733

42. Infantino A, Kharrat M, Riccioni L, Coyne CJ, McPhee KE, Grunwald NJ. Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica. 2006;147:201-21. doi:10.1007/s10681-006-6963-z

43. Vidhyasekaran P. Fungal pathogenesis in plants and crops. New York: Marcel Dekker; 1997. 537 p.

44. Huang XF, Li SQ, Li SY, Ye GY, Lu LJ, Zhang L. The effects of biochar and dredged sediments on soil structure and fertility promote the growth, photosynthetic and rhizosphere microbial diversity of Phragmites communis (Cav.) Trin. ex Steud. Sci Total Environ. 2019;697:134073. doi:10.1016/j.scitotenv.2019.134073

45. Schulz H, Glaser B. Effect of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. J Environ Qual. 2012;41:1087-95.

46. Lou Y, Joseph S, Li L, Graber ER, Liu X, Pan G. Water extract from straw biochar used for plant growth promotion: an initial test. BioResources. 2016;11(1):249-66. doi:10.15376/biores.11.1.249-266

47. Bonanomi G, Ippolito F, Scala F. A "black" future for plant pathology? Biochar as a new soil

amendment for controlling plant diseases. J Plant Pathol. 2015;97(2):223-34

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