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The influence of bio-char on Common scab disease of potatoes

Omnia A. Abd El-Hafez [1] and Mariam A. Amer[2*]

[1] Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.
[2] Bioengineering Research Department, Agricultural Engineering Research Institute, Agricultural Research Center, Giza, Egypt.

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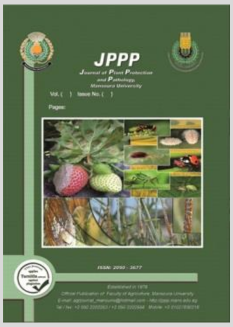

ABSTRACT

The study was carried out in a greenhouse at Plant Pathology Research Institute, "Agricultural Research Center, Giza, Egypt" to evaluate bio-char applications of potato scab controlling caused by *Streptomyces scabies* in sandy and clayey textured soil. Water dressing of potting soils was discussed with the influence of bio-char application to both kinds of infested and non-infested soils. The results showed that the application of bio-char for potato seed bed and the regular irrigation (every 3 days) caused an increase in organic carbon in both sterilized soils. While, the bio-char application in state under seedbed for infested sandy soil caused low nitrogen content at both watering treatments. In this preliminary study similar results were shown in clayey infested soil. Which soil porosity that is being inversely related to disease and it is positively correlated to soil bulk density (bd). Also, the results showed that bio-char application decreased the interspaces between particles consequentially minimizing soil bulk density for watering treatments in concern. The soil reaction (pH) was increased with the application of bio-char to infested soil under both watering system. Bio-char amended sandy and clayey soils expressed decreased incidence of scabby tubers especially at higher dose regular watering. This preliminary study reveals that the necessity of bio-char field treatments, to explain in depth the physiological, histological and changes in soil edaphic factors as related to *Streptomyces scabies* infections.

Keywords; Potato, potato common scab, bio-char

INTRODUCTION

Potato (*Solanum tuberosum* L.) crop is subject to numerous diseases of different microbial origins especially bacterial pathogens (Kirk, 2015). Potato common scab is one of the main concerns for farmers worldwide, since it decreases the marketing quality of tubers via in skin blemishes that render them unacceptable for seed potato production, fresh consumption and processing (Loria *et al*., 1997; Wanner and Kirk, 2015). The disease also can cause considerable economic loss to other root crops including radish, beet, sweet potato, turnip and carrot (Wanner 2004).

Common scab of potato is caused by Gram-positive filamentous actinomycetes belonging to the genus Streptomyces (Thaxter, 1891), and the major common scab inducing organism was nominated Streptomyces scabies. Furthermore, Streptomyces scabies isolates causing symptoms with aggressiveness of strains varying from mild to moderately or severe ones (DSMZ, 2015). The disease induces a variety of symptoms such as raised lesions, deep pitted, or sunken lesions and superficially netted skin on tuber surface (Kers *et al*., 2005). The disease worldwide has been reported in many countries as China (Zhang, *et al.*2013), South Africa (Slabbert, 1994), Pakistan (Ahmad *et al*., 1995), Iran (Zadeh *et al*., 2006), Russia (Lysenko *et al*., 2005), United States (Pasiecznik *et al*., 2005).

Bio-char is resulting from the thermal-chemical change for the organic biomass that contains of carbon richness by pyrolysis process wherefore considered the black gold for agriculture (Masiello et al., 2015). The bio-char applications are used in augmenting both plant-available water, agriculture to improve soil properties, enhancing crop yield and avoiding short and long-term detrimental effects to the environment (Lehmann, 2007; Sohi *et al*., 2010; Cheng *et al*., 2008 Verheijen et al., 2010: Verheijen et al., 2019). One of the most important applications of bio-char is can be used as different quantities in soil amendment on the top, or mixed into deeper soil layers. It was noted that, mixing bio-char into deeper soil layers alters soil physical properties as a function of bio-char characteristics (particle size, shape and material) (Lim and Spokas, 2018 and Fischer *et al*., 2019). On the contrary added bio-char on the Surface has only a minor effect on the soil physical properties (Blanco-Canqui, 2017).

Soil organic amendments and are an increasingly popular practice used to enhance soil health quality in managed agroecosystems (Gómez-Sagasti *et.al*., 2018). Green manures, crop residues and composts have been used broadly as means to increase soil organic matter content and carbon availability, and consequently, stimulate microbial biomass and activity in the soil (Larkin, 2015; Francioli *et al*., 2016). Addition of organic amendments to soil has been associated with natural suppression of soilborne plant pathogens (Larkin, 2015; Liu *et al*., 2015; Jaiswal *et al*., 2017). The Possible mechanisms of suppression include the increase in microbial antagonism, as well as plant growth promotion, activation of systemic induced resistance, and accumulation of plant-derived biocidal compounds (Zhang et al., 1998; Wiggins and Kinkel, 2005; Bressan *et al*., 2009).

170 years ago (Allen 1847) attracted attention in the last decade appositive influence of bio-char on limiting plant diseases as rust and mildews in other crops. in addition, several pathosystems were studied by different groups worldwide.

* **Corresponding author.**
E-mail address: mariam.amer@arc.sci.eg
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(Elad *et al*., 2010; Elmer, Pignatello 2011; Jaiswal *et al*., 2014; Copley *et al*., 2015; Jaiswal *et al*., 2015). Whereas, Pathosystems included both foliar pathogens and soil borne pathogens (Elad *et al.*, 2011; Graber *et al.*, 2014).

Newly, Bonanomi *et al.,* (2015) focused on the tested the effect of bio-char on the 13 pathosystems on plant disease. They reported that a positive influence of bio-char in reducing plant disease severity were very high compared to no effect on plant disease severity. while, they reported only 3% from bio-char additions were conducive to plant disease. Moreover, several studies on bio-char in detached growing media reported that while relatively low concentrations (≤1%) of bio-char suppressed the diseases (Jaiswal *et al.* 2014, 2015; Huang *et al.* 2015), higher concentrations (3%) were mostly ineffective or even accelerated plant disease (Jaiswal *et al.*, 2014, 2015; Copley *et al.*, 2015; Huang *et al.*, 2015 and Frenkel *et al*., 2017.

Overall, the application of bio-char to the clayey nutrient rich paddy soil may be sustain an increase in soil organic carbon (SOC), soil aggregation and soil health functioning with positive changes in microbial community over years. Thus, carbon increase as required could be a mechanistic driver to enhance soil fertility and improve soil-(plant) health for ensuring food production in rice agriculture facing land degradation and climate change impacts, which could be assisted by bio-char from crop residue in an approach of circular economy (Lua *et al*., 2020).

In addition, bio-char application in soils caused a significant increase microbial activity, functional diversity and microbial taxonomicas well as an overall shift in carbon-source utilization. All of these bio-char amendments in soils in the rhizosphere related with suppression of diseases that occur because plant growth promotion or soilborne pathogens. And therefore, these give an explanation of improvement in plant performance and reduction of disease in the presence of bio-char through changing soil chemical properties and microbial composition. (Jaiswal *et al*., 2017). The influence of bio-char on soil properties, incidence of bacterial wilt and microbial community were characterized. In general, bio-char application efficaciously monitored through also moving specific beneficial bacteria, sequestering more nitrogen and carbon as well as decreasing pathogen abundance. The potential of bio-char in control of bacterial wilt bio-char still seems to be an attractive filler for removing pathogens considering that it can be produced economically and easily using locally available biowastes and can be recycled as a soil amendment (Chen et al., 2020 and Deng et al., 2021). Therefore, the purpose of this study is to determine the possible use of bio-char application at different levels of the soil to overcome common scab disease in potatoes.

MATERIALS AND METHODS

1- Potato tubers

Potato seed tubers (CV. Spunta) were kindly obtained from the potato brown rot project ("PBRP"), Agricultural Research Center (ARC), Egypt.

2- Soils

Sandy and clayey soils are used in this experiment. The chemical properties of the soils are shown in the table (1). Chemical soil analysis was conducted by the standard methods described by (Tan, 1996).

Table 1. The chemical properties of the soils

	Organic C (%)	Total N (%)	Bulk density (g/cm^3)
Sandy soil	0.107	0.036	1.920
Clayey soil	0.829	0.035	1.225

3- Bio-char

Bio-char is produced by isolated furnace (El-Raie et al., 2016) from different agricultural wastes at 200 °C for 2 hours using the stable gas of nitrogen which is pumped with a flow rate of 100 cm^3/h. The chemical properties of the bio-char are shown in table (2). The bio-char particle size distribution was determined using sieves of < 1 mm using sieve shaking machine, model number 62,020.

Table 2. physical and chemical properties of the bio-char

Organic C (%)	Total N (%)	Moisture content (%)	Bulk density (g/cm^3)
2.985	0.664	6	0.5558

4- Pathogen

Isolation of potato common scab pathogen were carried out from naturally infected potato tubers with well-developed superficial scab symptoms (Wanner, 2004). Isolate of *Streptomyces spp.* was maintained and routinely sub-cultured on Nutrient - glucose agar medium unless otherwise stated (Wanner, 2006).

Pathogenicity tests:

Pathogenicity of Streptomyces isolated was evaluated by radish seedling assay according to Leiner *et al.* (1996) .And also, by mini tubers assay (leaf bud cutting method) as explained by (Lauer, 1977).

Identification of the causal pathogen:

Morphological and physiological characters of *Streptomyces* spp. isolated were detected using the methods of (Krieg & Holt 1984). The morphology of the sporophores was examined microscopically at 400x magnification, and the color of spores and colonies were observed on the medium after 14 days of incubation at 28 °C (Holt *et al*. 1994).

Greenhouse experiment

A pot experiment was performed during the summer season 2020 at greenhouse in PPRI, ARC Giza, Cairo, Egypt to study the effect of bio-char upon development of potato tubers in different soils treating with potato common scab. The experiment was arranged in a complete randomized block design with three replicates. The 32 pots (20 cm in dim) were divided into 2 groups sandy and clayey soil, each group splits to soil treated by pathogen (infected soil) and non- pathogen (sterilized soil). Two irrigation treatments were scheduled into the regular irrigation (every 3 days) and irregular irrigation (weekly). In addition, bio-char particles were added with rate of 1.25 ton/feddan and were incorporated into the soil at 3 treatments being of; at surface area of pots, with the potato tuber and 2.45 cm under potato tuber Fig (1).

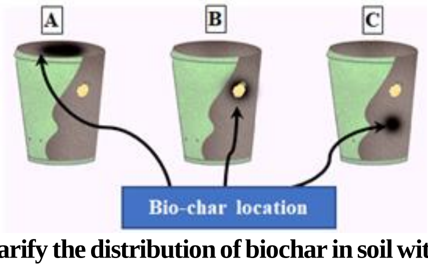

Fig .1. clarify the distribution of biochar in soil with potato tubers where (a) at surface area of pots, (b) with the potato tuber and (c) 2.45 cm under potato tuber.

The potatoes were collected at the Mid age of the production season, and samples were taken from the potatoes and soils to be subjected to laboratory analysis.

Recorded data

1.Soil organic carbon content (%) and Soil Total nitrogen

Soil organic carbon and Soil Total nitrogen are determined according to Bakr and El-Ashry, 2018.

2.Soil bulk density

Samples of the different soils after sun drying and before pyrolysis for each treatment were taken to determine mass and volume for measuring the bulk density. The volume before pyrolysis measured by cup 500 cm^3 and after pyrolysis measured by a cylindrical container (115cm^3). The cup and container were filled by the sample above the top edge of the container. The cup and container dropped five times from a height of 15cm onto a non-resilient surface to allow setting and struck off level with the top surface. Then samples weighted (g) and the following equation was used for determination of bulk density.

$$\textit{Bulk density} = \frac{Mass}{Volume}, (g/cm^3).$$

3.The soil pH

The pH meter was used to determine the pH range of soil after tuber harvest.

4.Disease decrease % = [(scab index in control-scab index in treatment)/scab index in control) X 100.

5.Scab index %

Scab index in 1 cm² of tuber surface was determined using a 0 to 5 scale as described by (Shihata, 1974) of tuber surface in different treatments according to the scab proposed by (Shihata, 1974.

$$\textit{Potato}\ \text{Disease incidence (DI)}\% = \frac{0A + 1B + 2C + 3D + 4E + 5F}{5T} * 100$$

Where:

0= symptomless tubers, 1 = trace – 10% tuber surface is scabbed,
2= 11 – 20 % tuber surface is scabbed, 3= 21 –30 % tuber surface is scabbed,
4 =31 – 40 % tuber surface is scabbed, 5 = more than 40 % tuber surface is scabbed
A, B, C, D, E and F are the number of tubers corresponding to the numerical grades respectively. T = is the total number of tubers, i.e. T = A+ B+ C+ D+ E+ F.

RESULTS AND DISCUSSION

Results

1.Isolation and identification of the pathogen

The obtained isolate was filamentous shape, non-motile, sporing, gram positive, gelatin liquefaction positive, starch hydrolysis positive, urease negative, catalase test positive, there was no growth at both 4 and 40 °C, H_2S production negative, levan production negative, methyl-red test negative and Casein hydrolysis positive. The tested isolate produce acid and does not produce gas from glucose, fructose, sucrose, lactose, maltose, manitol, dextrin and anhydrous dextrose.

Pathogenicity tests

Streptomyces spp. isolate was tested for pathogenicity on radish seedlings and mini tubers assay it showed the ability to inhibit radish seed germination and formed lesions of scab on progeny tubers. Pathogen reisolated from inoculated progeny tubers had spore colors and morphologies identical to those of the original pathogen.

On the basis of morphological, cultural, physiological and pathological characteristics of the isolated bacteria and according to the report made by Krieg and Holt (1984), it was concluded that the isolate could be identified as *S. scabies.*

2.Effect of bio-char application on organic carbon

Results in Fig. 2 (a & b) showed that, at adding bio-char at 2.45cm under potato tuber and regular irrigation, the highest organic carbon in sterilized soil treatments in sandy soils (0.210% $^{\pm 0.010}$) and in clayey soil (0.897% $^{\pm 0.007}$) were recorded. While, at irregular irrigation, the highest organic carbon recorded 0.206% $^{\pm 0.00}$ and 0.895% $^{\pm 0.002}$ for sandy and clayey soils, respectively. However, in infected soil, the highest organic carbon were acquired of 0.278% $^{\pm 0.001}$ and 1.252% $^{\pm 0.009}$ in sandy and clayey soils, respectively at adding bio-char on 2.45cm under potato tuber and regular irrigation. Moreover, at irregular irrigation, the highest organic carbon were recorded 0.248% $^{\pm 0.018}$ and 1.017% $^{\pm 0.015}$ in sandy and clayey soils, respectively.

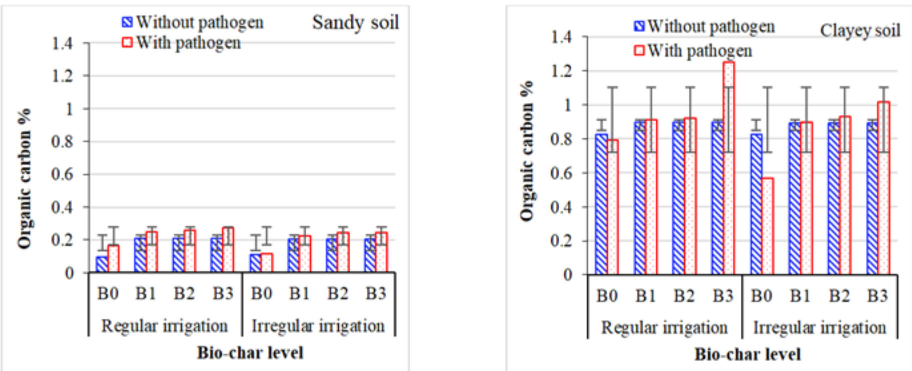

Figure 2 a　　Figure 2 b

Fig.2 (a&b) Effect bio-char level on organic carbon under water requirements and soils.

3.Effects of bio-char application on total nitrogen

In the infected sandy soil, the lowest total nitrogen was estimated at added bio-char under 2.45cm from potato tubers regular and irregular irrigation being, which recorded 0.028% ± 0.001and 0.30% $^{\pm 0.006}$, for both watering treatments, and 0.024 % $^{\pm 0.001}$ and 0.029% $^{\pm 0.001}$ in infested clayey soil under the same condition of infected sandy one (figure 3).

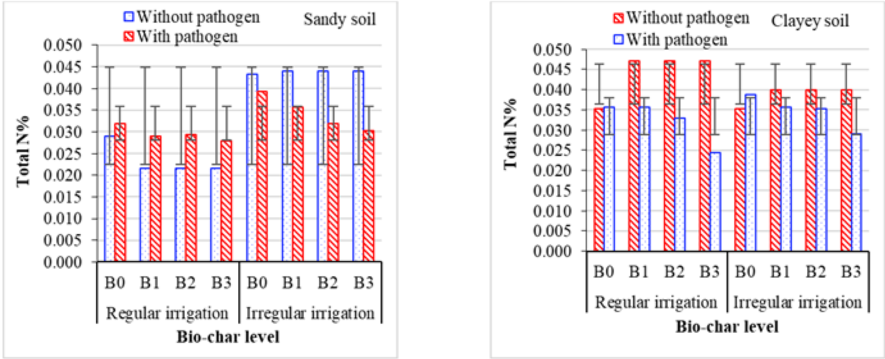

Figure 3 a Figure 3 b

Fig 3 (a&b) Effect bio-char level on total nitrogen under water requirements and soils.

4.Effect of bio-char application on soil bulk density

Soil porosity is being inversely related to disease, and is positively contributed to soil bulk density. In figure 4 the results indicated that the bio-char application, decreases the size of the pores and therefore minimize the soil bulk density. In sandy soil, the bulk density was decreased when bio-char added to soil at 2.45cm depth under potato tubers where 1.816 g/ $cm^{3\ \pm 0.024}$ and 1.694 g/ $cm^{3\ \pm 0.007}$ under both irrigation treatments; regular and irregular irrigation, respectively for sterilized soil. This also happened in infested soil where the soil bulk density were 1.403 g/ $cm^{3\pm\ 0.016}$ under regular irrigation and 1.588 g/ $cm^{3\ \pm 0.011}$ under irregular irrigation.

In addition, the soil bulk density were measured under; regular irrigation (1.251 g/ cm^3 ± 0.046) and (1.005 g/ $cm^{3\ \pm 0.013}$) in sterilized and infected clayey soils, respectively. Whilst, the soil bulk density under irregular irrigation was (1.034 g/ $cm^{3\ \pm 0.020}$) and (0.928 g/ $cm^{3\ \pm 0.014}$) in sterilized and infected clayey soils, respectively.

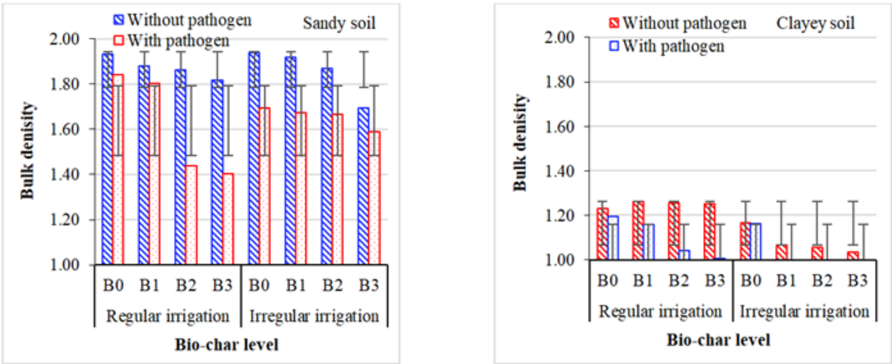

Figure 4 a Figure 4 b

Fig. 4 (a&b) Effect bio-char level on soil bulk density under water requirements and soils.

5. Effect of bio-char application on soil reaction (pH).

In figure 5 the results showed that the soil pH was increased with the application of bio-char in infested soil compared to sterilized one, where the highest soil pH in infested soil was 7.877 $^{\pm 0.180}$ regular irrigation and 7.663 $^{\pm 0.101}$ with irregular irrigation in sandy soil.

As well in clayey soil, however the highest soil pH level with added regular irrigation was 8.507 $^{\pm 0.060}$ down added bio-char under 2.45 cm the potato tuber. Also, that was happened under added irregular irrigation whereas the highest soil pH was 8.$^{410 \pm 0.130}$ in infested soil.

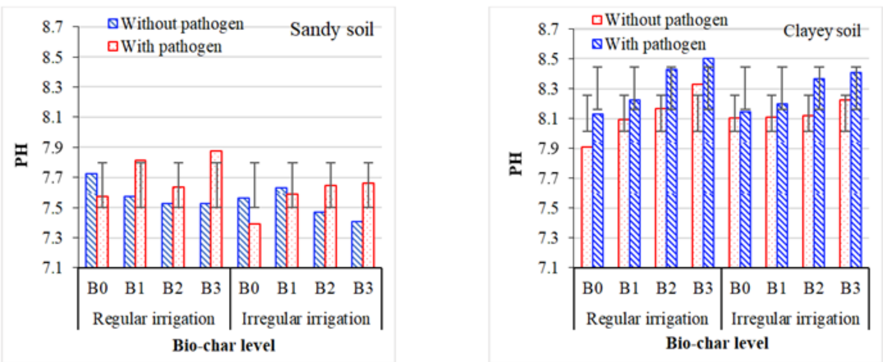

Figure 5 a Figure 5 b

Fig. 5 (a&b) Effect bio-char level on soil reaction (pH) under water requirements and soils.

6. Effect of bio-char application on disease severity.

The outcomes in figure 6 appeared that the effect of common scab infection(s) to potato tuber was decreased with the application of bio-char in both soils (sandy and clayey) that were being in infested soil. On the other hand, in clayey soil infestation, Infection showed 37.5 % $^{\pm 0.50}$ and 72.00 % ±

$^{2.00}$ in regular and irregular irrigation treatments, respectively. Whereas, the infection increasing to 90% and 100% in in regular and irregular irrigation treatments, respectively without bio-char application.

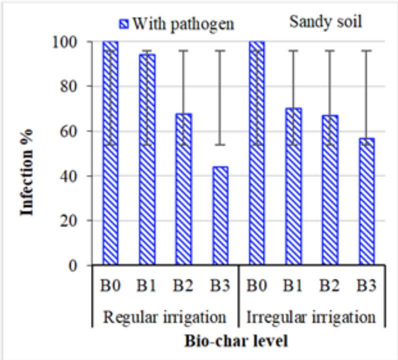

Figure 6 a

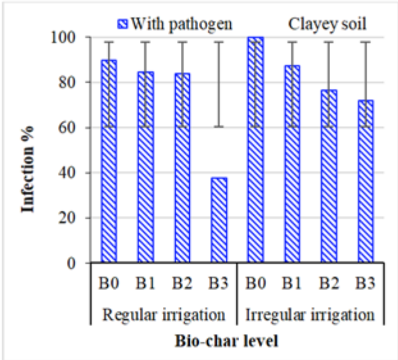

Figure 6 b

Fig. 6 (a&b) Effect bio-char level on common scab infection under water requirements and soils.

On the other side, when calculating disease decrease percentage, it was found that the percentage of disease decrease was zero in case of bio-char was not amended in both soils and both water regimes. Moreover, the addition of bio-char, increased the disease decrease, also in both soils and both water treatments in fig 7. The highest percentage of disease decrease was recorded for bio-char added at 2.45 cm of the potato tuber treatment where 73.3% $^{\pm 2.43}$ at regular irrigation and 51.7% $^{\pm 2.86}$ at irregular irrigation in sandy soil. While, in clayey soil the disease decrease was 60.1% $^{\pm 0.65}$ at regular irrigation and 42.8% $^{\pm 2.17}$ at irregular irrigation treatments.

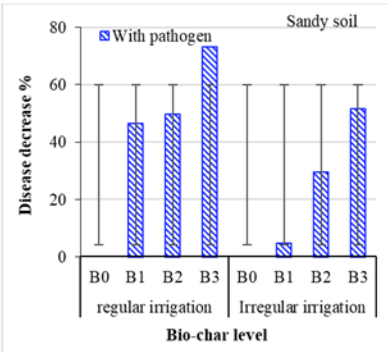

Figure 7 a

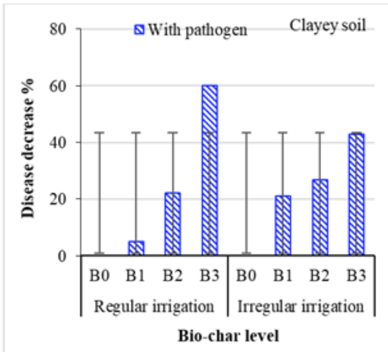

Figure 7 b

Fig. 7 (a&b) Effect bio-char level on disease decrease under water requirements and soils.

Discussion

In this study, soil amended-bio-char, resulted in increasing organic carbon and this is maybe correlated to decrease the diseases. This finding is similar to Dundore-Arias *et al*., 2020, who confirmed that soil carbon amendments can impose strong selection on indigenous soil microbiomes, and this outcome is the result of a series of biotic and abiotic interactions, and not exclusively due to the direct addition of carbon to soil

From the afore-mentioned results relation between pH and bio-char treated soil may be recognized and pH. Therefore, the increase in soil pH with application of bio-char was proportional to their alkaline range. Bio-char's highly alkaline implies high pH and basic cation content as well, even with bio-char prepared from wood material that possess considerable alkaline reaction due to its greater uptake of basic cations (Yuan *et al*. 2011). There was little change in total tuber yields over this pH range, but the incidence and severity of scab lesions declined as the pH being elevated, the small sized particles of sieved bio-char have had the largest liming effect, and increasing substrate pH values by an additional ~0.3 pH units.

Furthermore, the inconsistent initial increase in soil pH to eight or above by the addition of organic materials resulted in an increase in free ammonium levels that might be toxic to populations of *S. scabies* (Conn and Lazarovits 1999, Sagova-Mareckov , *et al*., 2015, Roy, *et al*., 2006 and Kopecky *et al*., 2021). Soil amended-bio-char, in this study, resulted in significant decrease of potato common scab infection, similar findings is being reported elsewhere (Siddiqui et al. 2008; Ghorbani et al. 2008; Siddiqui *et al*., 2009; Siddiqui *et al*., 2013, On *et al*., 2015 and Abdel-Hamid *et.al*., 2016. The observed decrease in bulk density (Bd) could be created by several reasons, that may co-inside with the bio-char properties such as particle size, active surface area, porosity as well as properties of the soil (Toková *et al*., 2020).

CONCLUSION

It can conclude that field studies at different edaphic field conditions are urgently requested.

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تأثير الفحم الحيوي على مرض الجرب البكتيري في البطاطس

امنيه احمد عبد الحافظ [1] و مريم احمد عبد العزيز عامر [2]

[1] معهد بحوث امراض النبات، مركز البحوث الزراعية – الجيزة - مصر

[2] معهد بحوث الهندسه الزراعية، مركز البحوث الزراعية – الجيزة - مصر

أجريت الدراسة المقدمة هنا في صوبه معهد بحوث أمراض النبات، مركز البحوث الزراعية، الجيزة، مصر، بهدف تقييم تطبيقات الفحم الحيوي في السيطرة على مرض جرب البطاطس في التربة الرملية والطينية. تمت مناقشة معاملتين للري للتربة المزروعة مع تأثير اضافة الفحم الحيوي على كلا النوعين من التربة المصابة وغير المصابة. أظهرت النتائج أن إضافة الفحم الحيوي تحت طبقة بذور البطاطس المنتظم كل ٣ ايام تسبب في زيادة الكربون العضوي في كل من التربة المعقمة. بينما، في التربة الرملية المصابة، تسبب اضافة الفحم الحيوي تحت مستوي البذور مباشرة في انخفاض محتوى النيتروجين في كلا معاملتي الري. وأظهرت نتائج مماثلة في التربة الطينية المصابة. تم النظر في مسامية التربة المرتبطة عكسيًا بالمرض والتي ترتبط ارتباطًا إيجابيًا بكثافة التربة (BD) في هذه الدراسة الأولية. أظهرت النتائج أن استخدام الفحم الحيوي قلل من المسافات البينية بين الجزيئات والمسام وبالتالي قلل من الكثافة الظاهرية للتربة تحت معاملات الري المعنية. تمت زيادة تفاعل التربة (الرقم الهيدروجيني) مع اضافة الفحم الحيوي على التربة المصابة تحت نظام الري. الفحم الحيوي - أعربت التربة الرملية والطينية المعدلة عن انخفاض معدل حدوث درنات الجرب خاصةً في الجرعات العالية من الري المنتظم. اكدت هذه الدراسة الأولية أهمية اضافة الفحم الحيوي للتربة الزراعية في تحسين العوامل الفسيولوجية والنسيجية والتغيرات في عوامل تكوّن التربة خاصة فيما يتعلق بعدوى الجرب *Streptomyces scabies*.