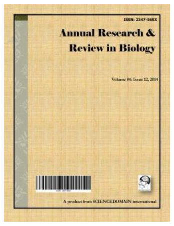

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Effect of Shea Nut Shell Biochar on Root Knot Nematodes (*Meloidogyne* spp.) of Tomato (*Solanum lycopersicum* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Effect of shea nut shell biochar on root knot nematodes and performance of tomato was investigated under nematode inoculated soils. Steam sterilized soil was admixed with biochar, which was later inoculated with 1000 second stage juveniles (J_2) two weeks after transplanting. Tomato variety (Petomech-GH) was planted in potting medium of soil to biochar ratio of one part of biochar (250 g) is to one part of soil (1B1S), one part of biochar is to two parts of soil (1B2S), two parts of biochar is to one part of soil (2B1S), and no biochar application (control). Steam sterilized soil amended with biochar inoculated with 1000 second stage juveniles (J_2). The result indicated that, biochar increased the pH of the soil, lessened the adverse effects of *Meloidogyne* spp., resulting in decline in galling and improvement in growth and yield of tomato. Increased biochar concentration resulted in decreased nematode gall formation on the roots of the tomato plant. Biochar amended soils resulted in lower egg masses. Increased biochar concentration resulted in decreased performance of tomato plant. Tomato plants treated with low biochar concentrations (1B2S and 1B1S) produced higher fruit numbers and weights, and plant biomass.

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Keywords: Biochar; shea nut shell; root knot nematode; tomato.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable consumed in almost every Ghanaian household [1]. It is an important component of balanced diet of most Ghanaians that provide vitamin A and C, lycopene which serves as antioxidant and can help reduce the risk of cardiac diseases and some types of cancer [2]. Tomato production in Ghana has been significantly affected by the incidence of pests and diseases [3] especially the root knot nematodes (RKN) [4,5]. Crop damages more than 27% in tomato [6] and in excess of $100 billion loss globally [7]. At the Bontanga irrigation zone in the northern region of Ghana, total crop loss of tomato occurs and currently, most farmers do not cultivate tomato in this area [1]. Soil fumigants and chemical nematicides are used in controlling nematodes. These are, however, expensive and pose threats to environment and human health resulting in its withdrawal. Several reports indicated the use of botanicals, aqueous and crude plant extracts for nematodes management, which contain minimum bioactive concentration against RKN [8, 9,10]. Biochar, most agro byproducts has now been directed to manage nematodes. It was found that the admixing of biochar into the soil increases the soil pH to become alkaline [11]. Decomposition of organic matter releases toxic components like NH_3^+ that can be nematicidal to plant parasitic nematodes [12]. There is one published report that biochar soil amendment at the concentration of 1.2% delays the development of root knot nematode [13].

Therefore, the present investigation aimed to evaluate the impact of biochar on the root knot nematodes development and the growth performance of tomato plants.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was carried out at the plant house of the University for Development Studies (UDS), Nyankpala campus which lies within latitude 9° 25°41° and longitude 0°58°42°W. The soil is an Alfisol under USDA classification, and Savanna Ochrosol under the Ghanaian system of classification [14]. The entire experiment was conducted from September to December, 2017.

2.2 Source of Study Materials

Tomato (Petomech GH) seeds were obtained from the local farmers in Nyankpala. The shea nut shell used to make the biochar was sourced from Cheyohi, a superb of UDS Nyankpala campus. Nematode infested soil sample was collected from Bontanga irrigation farm in the Kumbungu district of the northern region of Ghana.

2.3 Biochar Preparation

Shea nut shells were placed in a barrel with holes under and a chimney on top which served as a pyrolizer. Dried leaves were lighted on top of the shea nut shell for a few minutes and covered with a chimney to allow charring or incomplete burning of the shells which will eventually form biochar. It is a slow process which took about 3-6 hours but very efficient when done in small quantities [15].

2.4 Experimental Approach

Steam sterilized soil was admixed with biochar, which was later inoculated with 1000 second stage juveniles (J_2) two weeks after transplanting. Soil was sterilized using the steam barrel sterilization method. Gravels were removed from sandy loam soil by sieving, which was then packed into a jute sack. Three stones were laid in a triangular form above the ground level to provide space for fire wood. Water was poured into a tank about one quarter. Tripod wooden slaps were placed little above the water surface to provide room for vapor to form. The soil was then placed on this wooding slaps and the tank covered with polythene. Fire was set under the tank and the heat produced was used to generate steam below the soil in the tank which was then allowed to stand for 6 hours.

2.5 Soil Sampling, Extraction and Identification of Nematodes

Twenty core soil samples were taken from each plot and thoroughly mixed to form a composite sample. The root knot nematode juveniles (J_2) were extracted from 200 cm^3 of soil samples using a series of sieves (850, 250, 75 and 38 µm) and a 48 h decanting period using the modified Baermann tray method [16]. Counting of J_2 was carried out with stereoscopic microscope.

2.6 Nursing of Seeds and Transplanting

Tomato seeds were sown in steam sterilized soil placed in a wooden box measuring 1.0 m by 0.6 m. Cultural practices such as watering and shading was done to ensure proper germination. The most uniform seedlings were transplanted three weeks after emergence.

2.7 Application of *Meloidogyne* spp. Inoculum Level to Potted Tomato Seedlings

In the inoculated soil experiment, the potted seedlings were inoculated with 3 ml of the *Meloidogyne* spp. solution per pot two weeks after transplanting [approximately 1000 second stage juveniles (J_2)]. Three holes were made in a triangular form 2 cm equidistant from the base of each plant.

2.8 Experimental Design and Treatments

The two experiments were laid out in completely randomized design with five replications. Soil-biochar treatment was prepared into a 2 L size pot. In the naturally infested soil experiment, the 20 pots were filled with 1.6 L of the naturally infested soil-biochar combination, whilst in the inoculated soil experiment, the 20 pots were filled with 1.6 L of steam sterilized soil-biochar combination in different proportions (v/v). The control was without biochar. Watering was done early mornings or evenings. Too much watering was avoided to prevent water logging. Detailed treatment descriptions (v/v) were as follows: one part of biochar (250 g) is to one part of soil (1B1S); one part of biochar (150 g) is to two parts of soil (1B2S); two parts of biochar (350 g) is to one part of soil (2B1S); no biochar application (control).

2.9 Data Collection and Statistical Analysis

The pH of the various treatments was determined using a pH meter. The Plant growth parameters such as plant height, number of leaves and root weight were taken at two weeks interval after (2WAP) transplanting. Similarly, yield characteristics such as shoot weight and plant biomass were taken at two weeks interval after planting. At 4 and 6WAP after planting, the sampled plants were then dried separately at 80°C in an oven for 48 h to constant weights and the root and shoot dry weights were recorded. The various organs were thoroughly dried to obtain the biomass comprising of the fruits, roots, stems, and the leaves. Number of fruits and fruit weight were taken at ten weeks after planting (10WAP). The weight measurements were done using an electronic digital balance. Nematode induced parameter such as root galling was scored using the Bridge and Page [17] rating chart. Root systems were also rated for number of egg masses produced [18]. The egg mass index consisted of a 0–to-5 scale, with 0 = no egg masses, 1 = 1 to 2 egg masses, 2 = 3 to10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses, and 5 = >100 egg masses. Final nematode population was also taken at ten weeks after planting (10WAP). Reproductive factor (Rf) was also calculated.

Data collected were subjected to analysis of variance (ANOVA) using GenStat (18th Edition) statistical package. Treatment means was separated using least significant difference (LSD) at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 The Power of Hydrogen (pH) of the Treatments at the End of the Experiment

The pH of the various treatments is shown in Table 1. There were significant differences in pH among the treatments. 2B1S recorded the highest pH followed by 1B:1S and 1B2S recording the lowest alkaline pH. The control however had a pH that is acidic.

Table 1. The pH of the treatments at the end of experiment

Treatment	pH	Interpretation
1B1S	7.46^c	Alkaline
1B2S	7.12^b	Alkaline
2B1S	8.62^d	Alkaline
Control	6.20^a	Acidic
LSD(α= 0.05)	0.10	
P value	<0.001	

Means followed by the same letter(s) in a column are not significantly different (P > .05)

3.2 Growth Characteristics

No significant difference on the plant height of tomato was observed among the biochar treatments which were significantly different from the control (Table 2). However, it was observed that, as concentration of the biochar increased, the height of tomato plant decreased. Lower mean height was observed in the highest biochar concentration (2B1S). It might be attributed to increase in alkalinity as 2B1S recorded the highest alkaline pH of 8.62, followed by 1B1S with a pH of 7.46 and 1B2S with a pH of 7.12 at the end of the experiment. Similar observation was made by Howard [19] in corn and soybean, where he reported reduced growth in higher biochar weights investigated and suggested that, increment in alkalinity of the soil, the holding of too many nutrients, potential toxic ions and microbes upon too much biochar addition may have negative effect on plant growth. Grabber et al. [20] similarly reported enhanced plant height of tomato following biochar application.

The reduction of plant height under control condition was due to root knot nematode infection. Sharma and Sharma [21] reported significant reduction in plant height of tomato due to root knot nematode (RKN) infection (1000 J_2).

The effect of biochar on the number of leaves was only significant at two weeks after planting (2WAP) and four weeks after planting (4WAP) (Table 2). At two weeks after planting (2WAP), 1B2S treatment recorded the highest average leaf number while 2B1S treatment recorded the lowest. This might be attributed to the fact that, at 2WAP, root knot nematode may have penetrated the roots of tomato but may have not caused significant infection. At 4WAP, similar observation was made but in this case, the average leave number for 1B1S treatment was higher than the control whereas 2B1S treatment recorded the lowest. It was observed that, as the concentration of the biochar increased, leave number decreased.

Root weight generally differed based on the concentration of biochar with 1B2S treatment recording higher significant mean values followed by 1B1S, 2B1S and the control, respectively (Table 2). The root weight of the control plant was significantly low because of the lack of formation of lateral roots due to root knot nematode infection. This agree with the findings of Sharma and Sharma [21], whose report indicated significant reduction in root weight and root length of tomato as a result of root knot nematode infection.

3.3 Yield and Yield Parameters

There was significant effect of biochar on the number of fruits, fruit weight and plant biomass of tomato (Table 3). This varied according to the biochar treated with 1B2S recording the highest average mean value followed by 1B1S and control, respectively. 2B1S treatment produced no fruits and at the same time recorded the lowest dry plant biomass which may be due to the higher biochar concentration. This agree with the findings of Grabber et al. [20] whose reports indicated that, biochar contains chemicals most of which are phytotoxic or biocidal at high concentration and therefore may affect plant growth. 1B2S recorded the highest increment in plant biomass and fruit weight followed by 1B1S with control recording the least. Grabber et al. [20] reported significant improvement in plant growth at low biochar concentration. Hossain et al. [22] also reported improved growth and productivity of cherry tomato at 10t/ha biochar application. The observed low biomass of control was due to *Meloidogyne* spp. infection. Sharma and Sharma [21] reported reduced growth as a result of root knot nematode infection in tomato. Similarly, Maleita et al. [23] reported stunted growth and reduction in yield on root knot nematode heavily infested fields. Moreover, application of 1000 J_2 per plant significantly reduced growth and yield in a trial by Haider et al. [24] using French bean and pea.

Table 2. Effect of biochar concentrations on growth characteristics of tomato

Treatment	Plant height			Number of leaves			Root weight		
	2WAP	**4WAP**	**6WAP**	**2WAP**	**4WAP**	**6WAP**	**2WAP**	**4WAP**	**6WAP**
1B1S	23.80^{a}	33.52^{a}	48.30^{a}	5.20^{b}	8.20^{bc}	13.80^{a}	0.96^{a}	1.18^{a}	1.98^{ab}
1B2S	25.12^{a}	36.10^{a}	52.34^{a}	6.00^{bc}	10.00^{bc}	17.80^{a}	1.27a	1.88^{a}	2.47^{a}
2B1S	22.94^{a}	26.58^{a}	39.50^{a}	4.40^{a}	4.80^{a}	7.50^{a}	0.92a	1.26^{a}	1.60^{ab}
Control	25.54^{a}	31.54^{a}	39.25^{a}	5.60^{bc}	7.60^{b}	8.00^{a}	1.26a	1.18^{a}	1.03^{b}
LSD $\alpha_{=0.05}$	7.11	10.05	18.86	0.43	2.11	11.69	0.85	0.86	1.32
P values	0.56	0.05	0.14	< 0.01	< 0.01	0.05	0.51	0.05	0.06

Means followed by the same letter(s) in a column are not significantly different (P > .05)

Table 3. Effect of biochar concentrations on yield and yield parameters of tomato

Treatment	Shoot weight (g)			Mean plant biomass (g)		Fruit number	Fruit weight (g)
	2WAP	4WAP	6WAP	4WAP	6WAP	10WAP	
1B1S	1.71[a]	4.07[ab]	6.63[a]	0.86[ab]	2.96[a]	3.00[a]	37.60[b]
1B2S	2.27[a]	4.50[ab]	9.20[a]	1.19[ab]	5.28[b]	7.00[b]	170.00[c]
2B1S	1.88[a]	2.79[a]	4.43[a]	0.81[a]	1.91[a]	0.00[a]	0.00[a]
Control	1.60[a]	3.10[ab]	3.76[a]	1.46[ab]	1.92[a]	2.00[a]	25.00[b]
LSD $\alpha_{=0.05}$	1.39	1.71	7.95	0.65	2.70	4.07	17.78
P values	0.44	0.02	0.20	0.02	<0.01	<0.01	<0.01

Means followed by the same letter(s) in a column are not significantly different (P > .05)

3.4 Root Knot Nematode Population and Reproductive Factor

Final nematodes population and reproductive factor is an indication of nematode multiplication. Biochar treatment resulted in significant reduction in final nematode population over the control at (Table 4). 2B1S recorded the highest reduction in final nematode population which is significantly different from 1B1S and 1B2S. The control, however, showed a significant increase in final nematode population ($P < .05$).

Nematode reproductive factor, as indicated in Table 4, also showed significant differences among the treatments with 2B1S recording the lowest reproduction factor less than 1, followed by 1B1S and 1B2S, respectively. The control recorded the highest reproductive factor which was greater than 1. This suggested that, root knot nematode may not multiply in biochar amended soils. It is generally observed that, nematode population and reproduction factor decreased as the concentration of biochar in the medium increased showing the nematicidal potential of biochar against RKN. Biochar soil amendments was targeted to highly weathered and acidic soil because biochar has been reported to increase soil pH and moisture content [25,11]. Aduke [12] reported a sharp decrease in *M. incognita* population when the pH of the soil became alkaline. 2B1S recorded the highest alkaline pH of 8.62, followed by 1B1S with a pH of 7.46 and 1B2S with a pH of 7.12 at the end of the experiment. The control soil was, however, acidic with a pH of 6.20. Since biochar amended soil becomes alkaline at the end of experiment, the reduction in final nematode population and decreased reproduction factor in biochar amended soil may be attributed to increased pH of the medium.

3.5 Root Galling

Root knot nematode infection is manifested by the development of galls or giant cells on the root accompanied by stunted growth, chlorosis and loss of energy by the plant [26]. Biochar lessened the adverse effects of nematodes, resulting in decline in galling and an improvement in the growth and yield of the tomato, but the effect differed based on the treatment applied and parameters measured. Application of biochar treatment significantly reduced the formation of galls on the roots of tomato as shown in Table 5. The number of galls or knots varied with the concentration of the biochar treatment. Results revealed that, extent of gall formation on the roots was significantly lower in higher biochar treated medium with 2B1S recording the lowest root galling followed by 1B1S and 1B2S, respectively. It may be observed that, as the biochar concentration increased, the extent of gall formation on the roots of tomato decreased. At 4WAP, significant galling occurred on the roots which increased at 6WAP. The absence of galls during the first 2WAP may be due to the fact that, most of the RKN has a life cycle of at least three (3) weeks [27]. The root knot nematodes may have penetrated the roots but may have not reproduced to establish permanent feeding sites in the roots which lead to the formation of galls.

Moreover, the control recorded higher number of root galls, where most of the plants showed symptoms of wilting during the day and most died before maturity. This agrees with the findings of Mitkowski and Abawi [28] who reported wilting and stunted growth in lettuce as a result of root knot nematode infection. It is observed that, the extent of gall formation on the roots positively correlated with egg mass indices analyzed. Treatments that recorded higher root gall indices had higher egg masses (Table 5). Biochar amended soils had lower egg masses in which no significant differences occur among the three biochar concentrations, but all were significantly different from the control. Hence, biochar may have the potential to manage gall formation on the roots of tomato.

Table 4. Effect of biochar concentration on final *M. incognita* population and reproductive factor at ten weeks after planting

Treatment	Final *M. incognita* population per ml	Reproductive factor (Pf/Pi)
1B1S	23.00[b]	0.72[ab]
1B2S	32.00[c]	1.00[b]
2B1S	13.00[a]	0.41[a]
Control	61.00[d]	1.91[c]
LSD$\alpha_{=0.05}$	2.33	0.56
P values	<0.001	<0.01

Means followed by the same letter(s) in a column are not significantly different (P > .05)

Table 5. Effect of biochar concentration on root gall formation and egg masses

Treatment	Root gall index		Egg mass index	
	4WAP	6WAP	Infested soil	Inoculated soil
1B1S	2.10[a]	2.60[b]	1.10[a]	1.60[a]
1B2S	3.00[a]	3.10[b]	1.62[a]	1.81[a]
2B1S	1.20[a]	1.30[a]	0.00[a]	0.60[a]
Control	6.40[b]	8.10[c]	3.67[b]	3.50[b]
LSD $\alpha_{=0.05}$	2.17	1.57	1.65	1.29
P value	<0.01	<0.01	<0.01	<0.01

Means followed by the same letter(s) in a column are not significantly different (P > .05)

4. CONCLUSION

The effectiveness of biochar against root knot nematodes may be confirmed by an increment in shoot growth, plant biomass, fruit numbers and weight which are due to decline in nematode attack as indicated by decreased final nematode populations in biochar treated soils. The study demonstrated that, root knot nematode densities decreased, whilst plant growth parameters were enhanced significantly due to biochar application. Biochar increased the pH of the soil to become alkaline at the end of the experiments. Soil pH control should be carried out after biochar application to a range that is suitable for the growth of tomato.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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