

Original Research Article

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

ABSTRACT

Effect of shea nut shell biochar on root knot nematodes and performance of tomato was investigated under nematode infested and inoculated soils. Tomato variety (Petomech-GH) was planted in potting medium of soil to biochar ratio of one part of biochar 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). Nematode infested soil was amended with biochar as well as steam sterilized soil amended with biochar inoculated with 1000 J2. The result indicated that, biochar increased the pH of the soil, lessened the adverse effects of nematodes, 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.

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 pest and diseases [3] notably among which are root knot nematodes [4, 5]. Crop damages more than 27% take place 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 poses threats to environment and human health resulting in its withdrawal. Several reports indicated the use of botanicals, aqueous and crude plant extracts for nematode control, which contain minimum bioactive concentration against RKN [8, 9, 10]. Biochar of most agro by products has now been directed to manage nematodes. Biochar addition to soil increases the pH of the soil to become alkaline [11]. The

29 increased pH results in a sharp decrease in nematode population when the soil becomes alkaline
30 [12]. There is one published report that biochar soil amendment at the concentration of 1.2% delays
31 the development of root knot nematode [13].

~~32 The objective of the present study, therefore, was to evaluate the effect of biochar on root knot
33 nematode and the performance of tomato.~~

34

35 **2. MATERIALS AND METHODS**

36 **2.1 Experimental Site**

37 The study was carried out at the plant house of the University for Development Studies (UDS),
38 Nyankpala campus which lies within latitude 9° 25' 41" and longitude 0° 58'42" W. The entire
39 experiment was conducted from September to December, 2017.

40 **2.2 Source of Study Materials**

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

45 **2.3 Biochar Preparation**

46 Shea nut shells were placed in a barrel with holes under and a chimney on top which serves as a
47 pyrolyzer. Dried leaves were lighted on top of the shea nut shell for a few minutes and covered with a
48 chimney to allow charring or incomplete burning of the shells which will eventually form biochar. It is a
49 slow process which takes some time but very efficient when done in small quantities.

50 **2.4 Experimental Approach**

51 Two methods of nematode study were employed, using nematode infested and inoculated soils.

52 **2.5 Soil Sampling, Extraction and Identification of Nematodes**

53 Soil was sampled from each plot at the start of the trial and at harvest with a 5 cm diameter soil
54 auger to a depth of 20 cm. Initial soil nematodes were extracted from 200 cm³ of soil before
55 planting using the modified Baermann tray method [14]. Various nematode species were
56 identified under a compound microscope at a magnification of 100x. Nematode species were
57 identified [15] morphologically.

58 2.6 Soil Sterilization

59 Soil for inoculated experiment was sterilized using the steam barrel sterilization method. Gravels were
60 removed from sandy loam soil by sieving, which was then packed into a jute sack. Three stones were
61 laid in a triangular form above the ground level to provide space for fire wood. Water was poured into
62 a tank about one quarter. Tripod wooden slaps were placed little above the water surface to provide
63 room for vapor to form. The soil was then placed on this wooding slaps and the tank covered with
64 polythene. Fire was set under the tank and the heat produced was used to generate steam below the
65 soil in the tank which was then allowed to stand for 6 hours.

66 2.7 Nursing of Seeds and Transplanting

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

70 2.8 Application of Nematode Inoculum Level to Potted Tomato Seedlings

71 The potted seedlings were inoculated with 3 ml of the nematode solution per pot two weeks after
72 transplanting (approximately 1000 J2). Three holes were made in a triangular form 2 cm equidistant
73 from the base of each plant. The nematode solution was homogenized by gentle shaking the test
74 tubes containing the nematode solution and then dispensed into the holes.

75 2.9 Experimental Design and Treatments

76 The experiments were laid out in completely randomized design with five replications. Treatment was
77 prepared into a 2 L size pot. The pots were filled with 1.6 L of the infested soil-biochar combination
78 and sterilized soil-biochar combination in different proportions (v/v). The control was without biochar.

79 Watering was done early mornings or evenings. Too much watering was avoided to prevent water
80 logging. Detailed treatment descriptions (v/v) were as follows: one part of biochar is to one part of soil
81 (1B1S); one part of biochar is to two parts of soil (1B2S); two parts of biochar is to one part of soil
82 (2B1S); no biochar application (control)

83 2.10 Data Collection and Statistical Analysis

84 Plant growth parameters such as plant height, number of leaves and root weight were taken at two
85 weeks interval after transplanting. Yield characteristics such as shoot weight, number of fruits, fruit
86 weight and plant biomass were also taken. Nematode induced parameters such as root galling, egg
87 mass indices and final nematode population were also taken.

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

91 **3. RESULTS AND DISCUSSION**

92 No significant difference on the plant height of tomato was observed among the biochar treatments
93 which were significantly different from the control (Table 1). However, it was observed that, as
94 concentration of the biochar increased, the height of tomato plant decreased. Lower mean height was
95 observed in the highest biochar concentration (2B1S). Similar observation was made by Howard [16]
96 in corn and soybean, where he reported reduced growth in higher biochar weights investigated and
97 suggested that, increment in alkalinity of the soil, the holding of too many nutrients, potential toxic ions
98 and microbes upon too much biochar addition may have negative effect on plant growth. Grabber et
99 al. [17] similarly reported enhanced plant height of tomato following biochar application.

100 The reduction of plant height under control condition was due to root knot nematode infection.
101 Sharma and Sharma [18] reported significant reduction in plant height of tomato due to RKN infection
102 (1000 J2).

103 The effect of biochar on the number of leaves was only significant at 2WAP and 4WAP (Table 1). At
104 2WAP, 1B2S treatment recorded the highest average leaf number while 2B1S treatment recorded the
105 lowest. This might be attributed to the fact that, at 2WAP, root knot nematode may have penetrated
106 the roots of tomato but may have not cause significant infection. At 4WAP, similar observation was
107 made but in this case, the average leave number for 1B1S treatment was higher than the control
108 whereas 2B1S treatment recorded the lowest. It was observed that, as the concentration of the
109 biochar increased, leave number decreased.

110 Root weight generally differs based on the concentration of biochar with 1B2S treatment recording
111 higher significant mean values followed by 1B1S, 2B1S and the control respectively (Table 1). The
112 root weight of the control plant was significantly low because of the lack of formation of lateral roots
113 due to root knot nematode infection. This agree with the findings of Sharma and Sharma [18], whose
114 report indicated significant reduction in root weight and root length of tomato as a result of root knot
115 nematode infection.

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119 **Table 1. 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 | 33.52 | 48.30 | 5.20 ^b | 8.20 ^{bc} | 13.80 ^a | 0.96 | 1.18 | 1.98 |
| 1B2S | 25.12 | 36.10 | 52.34 | 6.00 ^{bc} | 10.00 ^{bc} | 17.80 ^a | 1.27 | 1.88 | 2.47 |
| 2B1S | 22.94 | 26.58 | 39.50 | 4.40 ^a | 4.80 ^a | 7.50 ^a | 0.92 | 1.26 | 1.60 |
| Control | 25.54 | 31.54 | 39.25 | 5.60 ^{bc} | 7.60 ^b | 8.00 ^a | 1.26 | 1.18 | 1.03 |
| LSD $\alpha=0.05$ | 7.11 | 10.05 | 18.86 | 0.43 | 2.11 | 11.69 | 0.85 | 0.86 | 1.32 |
| <i>P</i> values | 0.56 | 0.05 | 0.14 | < 0.01 | < 0.01 | 0.05 | 0.51 | 0.05 | 0.06 |

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

121 **3.2 Yield and Yield Parameters**

122 There was significant effect of biochar on the number of fruit, fruit weight and plant biomass of tomato
 123 (Table 2). This varied according to the biochar treated with 1B2S recording the highest average mean
 124 value followed by 1B1S and control respectively. 2B1S treatment produced no fruits and at the same
 125 time recorded the lowest dry plant biomass which may be due to the high biochar concentration. This
 126 agree with the findings of Grabber et al. [17] whose reports indicated that, biochar contain chemicals
 127 most of which are phytotoxic or biocidal at high concentration and therefore may affect plant growth.
 128 1B2S recorded the highest increment in plant biomass and fruit weight followed by 1B1S with control
 129 recording the least. Grabber et al. [17] reported significant improvement in plant growth in low biochar
 130 concentration. Hossain et al. [19] also reported improved growth and productivity of cherry tomato at
 131 10t/ha biochar application. The observed low biomass of control was due to root knot nematode
 132 infection. Sharma and Sharma [18] reported reduced growth as a result of root knot nematode
 133 infection in tomato. Similarly, Maleita et al. [20] reported stunted growth and reduction in yield on root
 134 knot nematode heavily infested fields. Moreover, application of 1000 J2 per plant significantly reduced
 135 growth and yield in a trial by Haider et al. [21] using French bean and pea.

136 **Table 2. Effect of biochar concentrations on yield and yield parameters of tomato**
 137

| Treatment | Shoot weight (g) | | | Mean plant biomass (g) | | | Fruit number | Fruit weight (g) |
|-----------|-------------------|--------------------|-------------------|------------------------|--------------------|-------------------|-------------------|---------------------|
| | 2WAP | 4WAP | 6WAP | 2WAP | 4WAP | 6WAP | | |
| 1B1S | 1.71 ^a | 4.07 ^{ab} | 6.63 ^a | 0.34 ^a | 0.86 ^{ab} | 2.96 ^a | 3.00 ^a | 37.60 ^b |
| 1B2S | 2.27 ^a | 4.50 ^{ab} | 9.20 ^a | 0.42 ^a | 1.19 ^{ab} | 5.28 ^b | 7.00 ^b | 170.00 ^c |

| | | | | | | | | |
|-------------------|-------------------|--------------------|-------------------|-------------------|--------------------|-------------------|-------------------|--------------------|
| 2B1S | 1.88 ^a | 2.79 ^a | 4.43 ^a | 0.34 ^a | 0.81 ^a | 1.91 ^a | 0.00 ^a | 0.00 ^a |
| Control | 1.60 ^a | 3.10 ^{ab} | 3.76 ^a | 0.37 ^a | 1.46 ^{ab} | 1.92 ^a | 2.00 ^a | 25.00 ^b |
| LSD $\alpha=0.05$ | 1.39 | 1.71 | 7.95 | 0.20 | 0.65 | 2.70 | 4.07 | 17.78 |
| <i>P</i> values | 0.44 | 0.02 | 0.20 | 0.55 | 0.02 | <0.01 | <0.01 | <0.01 |

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

139 **3.3 Root knot Nematode Population and Reproduction Factor**

140 Final nematodes population and reproduction factor is an indication of nematode multiplication.
 141 Biochar treatment resulted in significant reduction in final nematode population over the control at
 142 termination of both experiments (Table 3). 2B1S recorded the highest reduction in final nematode
 143 population which is significantly different from 1B1S and 1B2S. The control however showed a
 144 significant increase in final nematode population at the end of both experiment ($P < .05$).

145 Nematode reproduction factor, as indicated in Table 2, also showed significant differences among the
 146 treatments with 2B1S recording the lowest reproduction factor less than 1 for both experiment,
 147 followed by 1B1S and 1B2S respectively. The control recorded the highest reproduction factor which
 148 was greater than 1 for both experiment. This suggested that, root knot nematode may not multiply in
 149 biochar amended soils. It is generally observed that, nematode population and reproduction factor
 150 decreased as the concentration of biochar in the medium increased showing the nematicidal potential
 151 of biochar against RKN. Biochar soil amendments was targeted to highly weathered and acidic soil
 152 because biochar has been reported to increase soil pH and moisture content [22, 11]. Kung et al. [12]
 153 reported a sharp decrease in nematode population when the pH of the soil became alkaline. 2B1S
 154 recorded the highest alkaline pH of 8.62, followed by 1B1S with a pH of 7.46 and 1B2S with a pH of
 155 7.12 at the end of the experiment. The control soil was however acidic with a pH of 6.20. Since
 156 biochar amended soil becomes alkaline at the end of both experiments, the reduction in final
 157 nematode population and decreased reproduction factor in biochar amended soil may be attributed to
 158 increased pH of the medium.

159 **Table 3. Effect of biochar concentration on final nematode population and reproduction factor**

| Treatment | Final nematode population per ml | | Reproduction factor | |
|-------------------|----------------------------------|--------------------|---------------------|--------------------|
| | Infested soil | Inoculated soil | Infested soil | Inoculated soil |
| 1B:1S | 21.00 ^b | 23.00 ^b | 0.66 ^b | 0.72 ^{ab} |
| 1B:2S | 27.00 ^c | 32.00 ^c | 0.84 ^b | 1.00 ^b |
| 2B:1S | 0.00 ^a | 13.00 ^a | 0.00 ^a | 0.41 ^a |
| Control | 47.00 ^d | 61.00 ^d | 1.47 ^c | 1.91 ^c |
| LSD $\alpha=0.05$ | 2.14 | 2.33 | 0.18 | 0.56 |

P values <0.001 <0.001 <0.01 <0.01

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

161 3.4 Root Galling

162 Root knot nematode infection is manifested by the development of galls or giant cells on the root
 163 accompanied by stunted growth, chlorosis and loss of energy by the plant [23]. Biochar lessened the
 164 adverse effects of nematodes, resulting in decline in galling and an improvement in the growth and
 165 yield of the tomato, but the effect differs based on the treatment applied and parameters measured.
 166 Application of biochar treatment significantly reduced the formation of galls on the root of tomato as
 167 shown in Table 4. The number of galls or knots varied with the concentration of the biochar treatment.
 168 Results revealed that, extent of gall formation on the roots were significantly lower in higher biochar
 169 treated medium with 2B1S recording the lowest root galling followed by 1B1S and 1B2S respectively.
 170 It may be observed that, as the biochar concentration increased, the extent of gall formation on the
 171 roots of tomato decreased. Root galling was not observed during the first 2WAP, at 4WAP, significant
 172 galling occurred on the roots which increased at 6WAP. The absence of galls during the first 2WAP
 173 may be due to the fact that, most RKN has a life cycle of at least three (3) weeks [24]. The root knot
 174 nematodes may have penetrated the roots but may have not reproduced to establish permanent
 175 feeding sites in the roots which lead to the formation of galls.

176 Moreover, the control recorded higher number of root galls, where most of the plants showed
 177 symptoms of wilting during the day and most died before maturity. This agrees with the findings of
 178 Mitkowski and Abawi [25] who reported wilting and stunted growth in lettuce as a result of RKN
 179 infection. It is observed that, the extent of gall formation on the roots correlated with egg mass indices
 180 analyzed. Treatments that recorded higher root gall index had higher egg masses (Table 4). Biochar
 181 amended soils had lower egg masses in which no significant differences occur among the three
 182 biochar concentrations but all were significantly different from the control. Hence biochar may have
 183 the potential to manage gall formation on the roots of tomato.

184

185 **Table 4. Effect of biochar concentration on root gall formation and egg masses**

| Treatment | Root gall index | | | | Egg mass index | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | Infested soil | | Inoculated soil | | Infested soil | Inoculated soil |
| | 4WAP | 6WAP | 4WAP | 6WAP | | |
| 1B1S | 1.80 ^b | 2.00 ^a | 2.10 ^a | 2.60 ^b | 1.10 ^a | 1.60 ^a |
| 1B2S | 2.80 ^c | 2.80 ^a | 3.00 ^a | 3.10 ^b | 1.62 ^a | 1.81 ^a |
| 2B1S | 1.00 ^a | 1.00 ^a | 1.20 ^a | 1.30 ^a | 0.00 ^a | 0.60 ^a |
| Control | 6.80 ^d | 8.00 ^b | 6.40 ^b | 8.10 ^c | 3.67 ^b | 3.50 ^b |
| LSD $\alpha=0.05$ | 0.77 | 2.68 | 2.17 | 1.57 | 1.65 | 1.29 |

P Value <0.01 <0.01 <0.01 <0.01 <0.01 <0.01

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

187

188 **4. CONCLUSION**

189 The effectiveness of biochar against root knot nematodes may be confirmed by an increment in shoot
 190 growth, plant biomass, fruit number and weight which are due to decline in nematode attack as
 191 indicated by decreased final nematode population in biochar treated soils. It was assessed from the
 192 study that, root knot nematodes density decreased whilst plant growth parameters were enhanced
 193 significantly due to biochar application. Biochar increased the pH of the soil to become alkaline at the
 194 end of the experiment. Soil pH control should be carried out after biochar application to a range that is
 195 suitable for the growth of tomato.

196

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