Effects of Crop Residues on Bacteria and Nematode Populations in Soil Planted with Plantain.

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ABSTRACT

Plant parasitic nematodes constitute major threat to the growth and yield of plantain crop. The application of synthetic nematicides is widely accepted among farmers for the control of nematode infections on crops, but the attendant problems posed on non-target toxicity including consumers, soil and environmental pollutions are quite worrisome for the users and consumers. It is therefore imperative to devise alternative approaches that are eco-friendly, affordable yet potent in the management of nematode infection on crops. This study which was carried out in two locations: Federal University Technology, Akure (FUTA) and College of Agriculture, Osun State University, Ejigbo campus, Nigeria. The study evaluates the potential of three crop residues (biochar, cocoa pod husk and rice bran) in the management of soil inhabiting nematodes as well as the soil microbial loads. The crop residues were applied on soil grown with plantain. The initial and final soil inhabiting nematodes and bacteria populations were determined on the soil grown with plantain. Phytochemical composition of the crop residues was done in the laboratory following standard methods. The application of the crop residues resulted in a significant decrease in the the populations of the four nematode genera (*Meloidogyne, Radopholus, Helicotylenchus* and *Tylenchulus*), while the microbial load increased significantly compared to the control. The phytochemical composition of the crop residues revealed the presence of saponin, tannin, alkaloids, polyphenols, terpenoids, and glycosides. The results of this study showed that the crop residues have the potential to suppress the population of soil nematodes and application of crop residues are suggested as suitable and sustainable measure for the management of soil inhabiting nematodes.

Keywords: Crop residues, nematodes, bacteria, phytochemicals, soil

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INTRODUCTION

The impacts of pathogen infections on the quality and yield of plantain crop are well documented in the literature. Pathogen infections have resulted in various pathological disorders such as wilting and yellowing of leaves, root necrosis, root rot and breakage and toppling of psoudostem. (Danny *et al*. 2013). Nakato *et al*. (2018) reported that in East Africa, approximately $2-8 billion have been lost to infection by the bacteria, *Xanthomonas* within the last decade. In Indonesia, field observational study revealed that infection by the parasitic nematode, *Radopholus* caused more than 75% yield loss of plantain. Also, in the Southern Nigeria, the black sigatoka disease of plantain has been attributed to the fungus, *Mycosphaerella fijiensis*, causing leaf necrosis (Uzakah, 2020). In Cameroon, Nkendah and Akyeampong (2003) reported that infection by the nematode, *Meloidogyne* species, caused about 60% yield loss of plantain.

The conventional approaches toward curtailing nematodes threats are based on the use of expensive chemicals such as methyl bromide, usually applied as a pre-planting fumigant, which have been shown to have adverse effects on the soil health (Adegbite and Adesanya, 2005; Olabiyi, 2016). In the recent times, organic farming system had laid a serious restriction on the use of most of these synthetic nematicides and fertilizers. There is, therefore, an urgent need to develop sustainable methods of nematode management that are potent and ecologically safe (Noling and Becker, 1994; Martin, 2003).

The use of organic amendments especially crop residues for the management of pathogen infection on crop has been reported in the literature (Ntalli and Caboni, 2012; Ntalli *et al*., 2020) Crop residues had been reported to possess nematicidal property that can be equated to that of biological control agents in many crops (Tomer *et al*., 2015). Oka (2010) reported the efficacy of some organic amendments toward controlling soil nematodes of certain crops. .

The precise manners in which organic amendments affect plant-nematode relationship are yet to be fully elucidated since available results till date are inconsistent and somewhat undefined. McIntyre *et al*. (2000) in field experiments showed that organic inputs improved crop growth and yield (due to greater soil fertility), while plant-parasitic nematodes were not suppressed. However, Pattison *et al*. (2006) reported significant reduction in the nematodes densities especially those of *Radopholus similis* when organic amendments were applied. From the foregoing, it can be inferred that some controversies still remain about the reliability of organic amendments for nematode management. Also, mechanisms of action of organic soil amendments in relation to nematode management are yet to be fully explored. The present study is therefore aimed at investigating the potentials of crop residues to reduce the population of soil pathogenic nematodes and bacteria in soils in which plantains are cultivated.

MATERIALS AND METHODS

Experimental sites description

The study was carried out in two locations: Teaching and Research Farms of Osun State University, Ejigbo (Lat. 7.9045° N, Long. 4.3052° E) and the Federal University of Technology, Akure (FUTA) (Lat. 7.3070° N, Long. 5.1398° E). The two locations fall within the rain forest zone of southwest Nigeria, with altitude of 370 m above sea level. The rainfall pattern in the two locations is bimodal with an annual average of 1200 and 1400 mm for FUTA and Ejigbo respectively. The textural soil class in FUTA is sandy loamy while for Ejigbo is sandy clay.

Field preparation and crop establishment

The experimental sites in the two locations were established in the month of May, 2017 and terminated in the month of October, 2019. The fields were ploughed and laid out into plots of 1 m inter plot spacing and 2 m spacing between replicates. Two healthy suckers were planted per experimental unit and thinned to one vigor sucker after three weeks of establishment on field, prior to application of crop residues.

Collection of crop residues and preparation of Biochar

The crop residues under investigation were Biochar, Rice bran and Cocoa pod husk. Cocoa pod husk was collected from a cocoa plantation in Ifewara, Osun State, Nigeria (Lat. 7.4581° N, Long. 4.6796° E), Rice bran was collected from a local rice mill in Ede, Osun State, Nigeria (Lat. 7.7349° N, Long. 4.4439° E). The mineral fertilizer (NPK 15-15-15) was purchased from a local retail agrochemical store in Ejigbo, Osun State, Nigeria (Lat. 7.9045° N, Long. 4.3052° E) The collected cocoa pod husk and rice bran were sun-dried for three days after which the dry rice bran was packaged and stored in sack bags at room temperature until use, while the cocoa pod husk was ground into granule using electric mill machine. The ground cocoa pod husk was packaged into sack bags and stored until use.

The Biochar used in the study was locally produced from mango tree due to non-availability of Pyrolizer. Logs of wood were obtained from mango trees; anaerobic condition was created for the incomplete combustion of the woods by covering the logs with soils excavated from the surrounding while leaving some holes for the passage of air. Later, fire was introduced into the buried logs through the holes and the temperature was checked using a thermometer on the 2nd, 4^{th,} and 6th days after the fire has been introduced into the system; the average temperature was found to be 150 ± 22 °C. The whole process was completed in seven days.

Experimental design and treatments application

The experimental design followed a completely randomized block design. The crop residues were applied at four rates: 4, 6, 8, and 10 dry matter tonnes per hectare while mineral fertilizer was applied at three rates; 100, 200, and 300 kg NPK per hectare. A block contains 16 experimental units, this gives 64 experimental units in each location.

Phytochemical composition of crop residues

The phytochemical chemical screening of the crop residues for the presence of secondary metabolites such as saponin, tannin, alkaloids, polyphenols, terpenoids, and glycosides was performed following the procedures of Farnsworth (1966).

Soil sample collection for the determination of nematodes and microbial populations

Nematodes count

Soil auger was used to collect approximately 250 g of soil samples at depth 10- 20 cm at two different time points; initial soil samples were collected after the harvest of the parent crops (15 months after crop residues application) while the final soil samples were collected after the harvest of ratoon crops (30 months after crop residues application). The samples were taken from a distance close to the plantain roots such that some portions of roots were deliberately cut alongside with soil. The collected soil samples were packed inside a sealed nylon bag and transported to the laboratory. In the laboratory, the samples were later removed from the bags and wrapped in a tissue paper. The tissue papers with soil samples were placed inside bowls of water, and were left for two days to allow the nematodes to crawl into the bowls of water. The whole set up were covered with foil paper to prevent dryness. The nematodes were identified under a dissenting microscope using morphological keys (Siddiqi, 2000).

Microbial count

An aliquot soil sample was prepared aseptically by transferring 1 g of soil into sterilized bottles containing 9 mL of distilled water. The mixture was mixed thoroughly after which 1 mL of the aliquot was taken and plated on petri dish containing nutrient agar. The plates were incubated in the oven at 37 °C for 24 hour. Total bacterial count was done using a colony counter.

Statistical analyses

Data on nematode and bacterial counts for each location were subjected to a two-way analysis of variance, the fa ctors being the different crop residues (Biochar, rice bran and cocoa pod husk) and locations (FUTA and Ejigbo). The interactive effect of the two factors was also tested. The means were separated using Tukey's tests. The initial and final count data for each location were compared using T-test analyses. Statistical analyses were performed using the GraphPad Prism software (version 5). Data were reported as mean ± standard error, and statistical significance was assumed at $p < 0.05$.

RESULTS

Phytochemical analysis of the crop residues

The phytochemical compositions of the crop residues were shown in Table 1. The analysis showed that all the three crop residues contained secondary metabolites such as saponin, tannin, alkaloids, polyphenols, terpenoids, and glycosides (Table 1).

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Table 1: Phytochemical composition of the crop residues

Phytochemicals	Biochar	Rice Bran	Cocoa Pod Husk
Saponin	+	+	+
Tannin	+	+	+
Alkaloids	+	+	+
Polyphenols	+	+	+
Terpenoids	+	+	+
Glycosides	+	+	+

+ indicates Present

Table 2: *Meloidogyne* spp. population in soils amended with crop residues in FUTA and Ejigbo

	FUTA		EJIGBO	
Treatment	Initial	Final	Initial	Final
BCH	64.83±3.18^{bB}	46.25 ± 2.05^{dA}	71.83±3.08^{dB}	65.00 ± 3.61^{dA}
CPH	48.42±3.96^{aB}	37.42 ± 3.25^{cA}	31.17±2.70^{cB}	27.92 ± 1.49^{cA}
MF	72.11±1.25^{cB}	14.22 ± 2.37^{aA}	68.10±1.66^{bB}	12.67 ± 1.23^{bA}
RB	50.67±2.84^{aB}	29.00 ± 1.75^{bA}	22.83±1.84^{aB}	5.60 ± 0.72^{aA}
CL	81.33±3.33^{dA}	80.56 ± 4.42^{dA}	88.67±6.04^{eA}	99.33 ± 11.32^{eA}

BCH: Biochar, CPH: Cocoa pod husk, RB: Rice bran, MF: Mineral fertilizer, CL : Control

Means with the same lower case alphabets are not significantly different across the column while means with the same upper case alphabets are statistically the same for the same parameters between FUTA and Ejigbo along the row.

Effects of amendment of soil with crop residues on the population of *Meloidogyne* spp

The effect of amendment of soil with the different crop residues on the population of *Meloidogyne* spp was shown on Table 2. There was a significant decrease in the population of *Meloidogyne* spp after the 15 months of application of the crop residues ($F_{4, 86} = 98.53$, $p < 0.0001$). The population of *Meloidogyne* spp differed significantly across the locations ($F_{4, 86} = 21.14$, $p < 0.0001$). There was a strong interactive effect of crop residues and location on the population of *Meloidogyne* spp ($F_{4, 86} = 6.65$, $p = 0.0001$). After the harvesting of the ratoon crops, the population of *Meloidogyne* spp differed significantly for the different crop residues ($F_{4, 86}$ = 136.86, p < 0.0001). Also, the population differed significantly across the locations ($F_{4, 86}$ = 7.54, p < 0.0001). Similarly, there was a strong interactive effect of the crop residues and location on the population of *Meloidogyne* spp ($F_{4, 86}$ = 6.89, p < 0.0001). Generally, the final population of *Meloidogyne* spp decreased significantly compared to the initial population for all the treatments at both locations.

Effects of amendment of soil with crop residues on the population of *Helicotylenchus* spp

The effect of amendment of soil with the different crop residues on the population of *Helicotylenchus* spp nematode was shown on Table 3. There was a significant effect of crop residues on the population of *Helicotylenchus* spp after the first year of application of the crop residues ($F_{4, 86}$ = 136.05, p < 0.0001). After, the first year of application, the population of *Helicotylenchus* spp did not differ significantly across the locations ($F_{4, 86}$ = 2.15, p = 0.1458). There was a strong interactive effect of crop residues and location on the population of *Helicotylenchus* spp ($F_{4, 86}$ = 38.78, p < 0.0001). After the harvesting of the ratoon crops, the population of *Helicotylenchus* spp differed significantly for the different crop residues ($F_{4, 86}$ = 136.86, p < 0.0001). Also, the population differed significantly across the locations ($F_{4, 86}$ = 7.54, p < 0.0001). Similarly, there was a strong interaction between the crop residues and location on the population of *Helicotylenchus* spp ($F_{4,86}$ = 6.89, p < 0.0001). Generally, the final population of *Helicotylenchus* spp decreased significantly compared to the initial population for all the treatments at both locations.

Table 3: *Helicotylenchus* spp. population in the soils amended with crop residues in FUTA and Ejigbo

	FUTA		Ejigbo	
Treatment	Initial	Final	Initial	Final
BCH	75.08 ± 3.09^{cA}	62.67 ± 4.41^{dA}	55.08 ± 2.09^{cB}	38.58 ± 3.69^{bA}
CPH	45.00 ± 1.43^{bB}	27.50 ± 2.09^{bA}	38.75 ± 1.89^{aB}	17.17 ± 1.77^{aA}
MF	60.44 ± 2.74^{bB}	17.33 ± 1.24^{aA}	49.67 ± 1.87^{aB}	12.56 ± 1.64^{aA}
RB	46.00 ± 1.81^{bB}	29.38 ± 1.58^{aA}	24.75 ± 2.18^{aB}	10.42 ± 0.58^{aA}
CL	34.88 ± 1.7^{aA}	37.67 ± 3.72^{cA}	62.54 ± 8.39^{bA}	60.37 ± 10.12^{cA}

BCH: Biochar, CPH: Cocoa pod husk, RB: Rice bran, MF: Mineral fertilizer, CL : Control

Means with the same lower case alphabets are not significantly different across the column while means with the same upper case alphabets are statistically the same for the same parameters between FUTA and Ejigbo along the row.

Table 4: *Radopholus similis* population in soils amended with crop residues in FUTA and Ejigbo

	FUTA		Ejigbo	
Treatment	Initial	Final	Initial	Final
BCH	104.80 ± 2.89^{aA}	92.40 ± 3.75^{bA}	87.80 ± 1.89^{cB}	55.58 ± 2.95^{bA}
CPH	109.10 ± 8.17^{cB}	94.80 ± 3.67^{bA}	45.67 ± 4.17^{aB}	23.50 ± 2.54^{bA}
MF	102.3 ± 7.10^{aB}	51.56 ± 7.34^{aA}	59.67 ± 4.02^{bB}	14.78 ± 4.08^{bA}
RB	118.30 ± 7.50^{bB}	92.08 ± 4.02^{aA}	67.33 ± 3.14^{bB}	45.08 ± 2.37^{bA}
CL	124.50 ± 2.63^{bA}	123.00 ± 1.56^{cA}	114.70 ± 4.25^{dB}	110.30 ± 6.36^{aB}

BCH: Biochar, CPH: Cocoa pod husk, RB: Rice bran, MF: Mineral fertilizer, CL: Control

Means with the same lower case alphabets are not significantly different across the column while means with the same upper case alphabets are statistically the same for the same parameters between FUTA and Ejigbo along the row.

Effects of amendment of soil with crop residues on the population of *Radopholus similis*

The effect of amendment of soil with the different crop residues on the population of *Radopholus similis* was shown on Table 4. There was a significant effect of crop residues on the population of *Radopholus similis* after the first year of application of the crop residues ($F_{4,86} = 11.75$, $p < 0.0001$). After, the first year of application, the population of *Radopholus similis* differed significantly across the locations ($F_{1,86} = 98.00$, $p < 0.0001$). There was a strong interactive effect of crop residues and location on the population of *Radopholus similis* ($F_{4,86} = 42.40$, $p < 0.0001$). After the harvesting of the ratoon crops, the population of *Radopholus similis* differed significantly for the different crop residues ($F_{4,86} = 66.38$, $p < 0.0001$). Also, the population differed significantly across the locations ($F_{1,86} = 169.61$, $p < 0.0001$). Similarly, there was a strong interaction between the crop residues and location on the population of *Radopholus similis* ($F_{4,86} = 31.99$, $p < 0.0001$). Generally, the final population of *Radopholus similis* decreased significantly compared to the initial population for all the treatments at both locations.

Effects of amendment of soil with crop residues on the population of *Tylenchulus* spp

The effect of amendment of soil with the different crop residues on the population of *Tylenchulus* spp was shown on Table 5. There was a significant effect of crop residues on the population of Tylenchulus after the first year of application of the crop residues ($F_{4, 86} = 59.83$, $p < 0.0001$). After, the first year of application, the population of *Tylenchulus* spp differed significantly across the locations ($F_{1, 86} = 96.63$, $p < 0.0001$). There was a strong interactive effect of crop residues and location on the population of *Tylenchulus* spp ($F_{4,86} = 23.49$, $p < 0.0001$). After the harvesting of the ratoon crops, the population of *Tylenchulus* spp differed significantly for the different crop residues ($F_{4,86} = 88.03$, $p < 0.0001$). Also, the population differed significantly across the locations ($F_{1, 86} = 120.19$, $p < 0.0001$). Similarly, there was a strong interaction between the crop residues and location on the population of *Tylenchulus* spp ($F_{4, 86} = 29.69$, $p < 0.0001$). Generally, the final population of *Tylenchulus* spp decreased significantly compared to the initial population for all the treatments at both locations.

Table 5: *Tylenchulus* spp population in soils amended with crop residues in FUTA and Ejigbo

	FUTA		Ejigbo	
Treatment	Initial	Final	Initial	Final
BCH	58.50 ± 3.24^{bA}	57.50 ± 2.92^{dA}	45.50 ± 3.14^{cB}	28.17 ± 1.57^{cA}
CPH	46.67 ± 1.52^{aB}	15.92 ± 1.70^{aA}	28.167 ± 0.76^{aA}	15.92 ± 4.85^{aB}
MF	44.22 ± 3.66^{aB}	14.09 ± 3.64^{bA}	29.67 ± 1.08^{bB}	12.22 ± 1.69^{bA}
RB	48.00 ± 1.94^{aB}	35.19 ± 2.07^{aA}	27.75 ± 1.83^{bB}	21.75 ± 1.36^{bA}
CL	49.50 ± 0.29^{aA}	46.67 ± 1.67^{cA}	51.50 ± 2.00^{cB}	49.68 ± 2.40^{dA}

BCH: Biochar, CPH: Cocoa pod husk, RB: Rice bran, MF: Mineral fertilizer, CL : Control

Means with the same lower case alphabets are not significantly different across the column while means with the same upper case alphabets are statistically the same for the same parameters between FUTA and Ejigbo along the row.

Effects of amendment of soil with crop residues on the soil microbial load

The microbial load in soil amended with the different crop residues was shown on Table 6. There was a significant effect of crop residues on the microbial load after the first year of application of the crop residues ($F_{4, 86}$ = 89.94, p < 0.0001). After, the first year of application, the microbial load differed significantly across the locations ($F_{1,86}$ = 9.40, p = 0.0029). There was a strong interactive effect of crop residues and location on the soil microbial load ($F_{4,86}$ = 78.64, p < 0.0001).

After the harvesting of the ratoon crops, the microbial load differed significantly for the different crop residues ($F_{4, 86}$ = 225.94, p < 0.0001). Also, the microbial load differed significantly across the locations ($F_{1,86}$ = 13.81, p = 0.0004). Similarly, there was a strong interaction between the crop residues and location on the microbial load ($F_{4, 86}$ = 45.39, p < 0.0001). Generally, the final microbial load increases significantly compared to the initial microbial load for all the treatments at both locations.

Table 6: Bacterial loads in soils amended with crop residues in FUTA and Ejigbo

	FUTA		Ejigbo	
Treatment	Initial	Final	Initial	Final
BCH	123.50 ± 1.41^{aA}	127.25 ± 1.75^{cB}	126.50 ± 4.57^{cA}	173.25 ± 3.90^{cB}
CPH	127.20 ± 0.77^{aA}	$164.50\pm3..52^{eB}$	110.80 ± 6.66^{bA}	220.80 ± 8.51^{dA}
MF	122.60 ± 1.94^{aB}	87.56 ± 2.53^{aA}	101.60 ± 5.88^{aB}	29.89 ± 4.13^{aA}
RB	127.10 ± 0.59^{aA}	138.61 ± 1.75^{dB}	124.20 ± 4.65^{cA}	167.61 ± 4.68^{cA}
CL	127.25 ± 0.88^{aA}	120.70 ± 8.84^{bA}	103.25 ± 4.4^{aA}	92.33 ± 7.17^{bA}

Means with the same lower case alphabets are not significantly different across the column while means with the same upper case alphabets are statistically the same for the same parameters between FUTA and Ejigbo along the row.

DISCUSSION

The present study investigated the effects of application of three crop residues: Biochar, Rice bran, and Cocoa pod husk on the population dynamics of pathogenic soil nematodes and bacteria. The study was carried out in two different locations: FUTA and Ejigbo. All the crop residues significantly reduced the populations of the four nematodes investigated in this study especially the Biochar and rice bran. These observations corroborate the findings of other studies that has shown that organic amendment has the potential to reduce the population of soil nematodes. Rosskopf *et al*. (2020) reported the suppressive effects of organic amendments in managing soil borne plant pathogens and plant-parasitic nematodes, using annual fruits and vegetable as test crops. Senthilnathan *et al*. (2017) also reported that the application of different concentrations of humic acid (0.04%, 0.08%, 0.2% and 0.4%) significantly reduced the nematode soil density by 53.5%–56.7%, root infection by 61.9%– 63.8%, egg population by 61.9%–63.8% and reproduction rate by 55.7%–56.6%. Also, Ikwunagu (2019) reported that biochar obtained from sawdust and cassava peel reduced the population of root knot nematodes on the soil planted with mungbeans. In another study, Olabiyi and Ojo (2017) showed that application of leaf residues of certain plants significantly reduced the nematode root damage as well as the nematode population in soil and root of cowpea.

The ability of the plant residues to significantly reduce the population of soil nematodes could be due to the presence of certain phytochemicals with nematicidal property in the crop residues. Indeed, the phytochemical screening of the crop residues indicated the presence of notable phytochemicals such as saponin, alkaloids, tannins, and polyphenols. There are reports of plant wastes containing phytochemicals; Siddiqui *et al*. (2018) reported the presence of various phytochemicals such as terpenoids, tannins, alkaloids and saponins in the peels of banana and pawpaw. Also, Thakur *et al*. (2020) reported the presence of terpenoids, flavonoids, saponins, tannins, and phenols in orange peels. These phytochemicals have been demonstrated to possess nematicidal activities in various studies (Aissani *et al*., 2018; Desmedt *et al*., 2020).

The final population of nematodes decreased significantly after the application of the crop residues. The noticed differences between the initial and final population of nematodes were probably due to more availability of nematicidal constituents of the crop residues as a result of microbial degradation. This assertion was supported by the microbial counts data in this study which was significantly higher after the harvesting of the ratoon crops. Essentially, the higher bacterial population would result in greater biodegradation of the crop residues by bacteria (Hellequin *et al*., 2018; Arcand *et al*., 2016).

The results of this study showed a marked difference in the initial and final populations of the nematodes across the two study locations. To a large extent, the nematode populations in Ejigbo were significantly lower than those in FUTA. The differences may be attributed to the differences in the textural soil classes of the locations. Indeed, there are reports of sandy soil favouring the proliferation of *Meloidogyne* spp compared to clay soil (Fajardo *et al*., 2011; El-Hadad *et al*., 2011; Oka *et al*., 2010), and this could explain the reason for a higher population of *Meloidogyne* spp in FUTA.

In conclusion, the findings of this study showed that crop residues possess nematicidal property that make them suitable alternatives to synthetic chemicals for the management of soil nematodes. The application of these crop residues promotes proliferation soil microbes which aid in the decomposition of the crop residues thereby making the active nematicidal components of the crop residues more available in the soil.

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