Research Article

Antihelminthic Effects of Sawdust Mixed with Eucalyptus Biochar against Plant-Parasitic Nematodes Associated with Beniseed (*Sesamum indicum* L.) Crop in Makurdi, North-Central Nigeria

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Abstract | A field trial was conducted at the Teaching and Research Station of University of Agriculture, Makurdi during the 2019 cropping season to investigate the effect of sawdust mixed with eucalyptus biochar on nematodes associated with beniseed as well as their impact on yield. A total of nine treatments combinations were evaluated using a 3 x 3 factorial experiment laid out in a Randomized Complete Block Design (RCBD) in three replications. The treatments combinations consisted of single applications of sawdust and eucalyptus biochar, and mixed formulations of sawdust + eucalyptus biochar applied in powder forms at the rates of 0 t/ha, 2.5 t/ha and 5 t/ha. Nematode and yield data were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability. Results showed that use of 2.5 t/ha and 5 t/ha of sawdust mixed with eucalyptus biochar reduced the population of plant-parasitic nematodes by 21.6%, and 37.8%, respectively. Although yields obtained from plots treated with the two rates of mixed formulation were 0.51 t/ha and 0.49 t/ha, the observed difference was not statistically significant but was higher than yields obtained when either eucalyptus biochar or sawdust was applied singly. The study showed evidence of nematode population control and yield improvement when mixed formulations of sawdust-eucalyptus biochar was applied at 2.5 and 5.0 t/ha and hence recommended for inclusion in the integrated pest management program for beniseed production in Makurdi.

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Introduction

Beniseed (*Sesamum indicum* L.) is an oil crop belonging to the family Pedaliaceae. The crop is grown in both tropical and sub-tropical regions of Africa, Asia and Latin America. FAO (2020) report showed that Asia, Africa and America were accounted for 54.4%, 41.2% and 4.4%, respectively of the 6,549,725 tonnes of global beniseed production in 2019. The report (FAO, 2020) further ranked Nigeria the third largest producer of beniseed in Africa with a production statistic of 480,000 tonnes behind Sudan (1,210000 t) and United Republic of Tanzania (680,000 t). Ten beniseed-producing states were reported in publication dubbed "Overview of the Nigerian Sesame Industry and presented in Figure

1 (Chemonics International Inc., 2002). However, the major producing areas in order of priority are Nasarawa, Jigawa and Benue States. Other important areas of production are found in Yobe, Kano, Katsina, Kogi, Gombe and Plateau States (Katanga *et al*., 2019). Because of the drought-resistant nature of the plant, it thrives well in the northern part of Nigeria and some parts of the West. It can also be grown in some parts of South-East and South-South Nigeria. It has been successfully grown in Ebonyi, Cross River and Delta. Although the yield was sub-optimal. the leaves were unusually broader (Toungos, 2020).

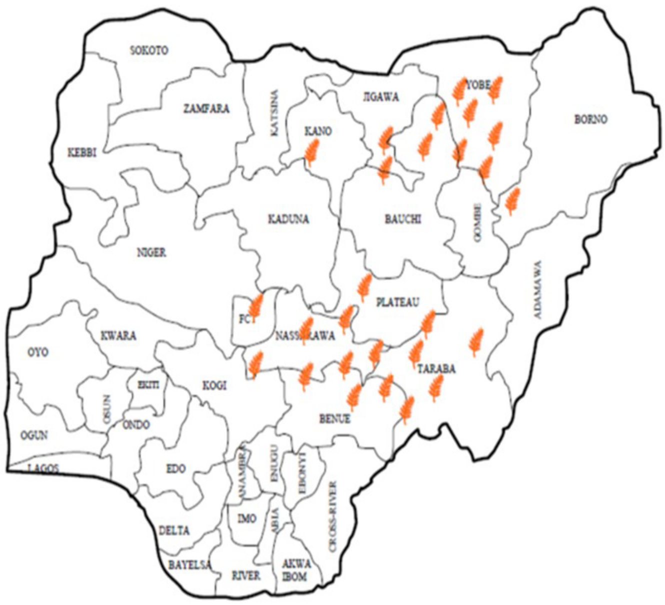

Figure 1: *Map of Nigeria showing beniseed producing states.*
Source: *Chemonics International Inc. 2002.*

The seeds, which yield half of their weight in oil, are most commonly used in soups, while the young leaves are used as a soup vegetable. Various parts of the plants are also used in native medicines. The stems are usually burned as fuel where firewood is scarce and the ash is commonly used for local soap production. The pressed cake remaining after the oil is removed which is a rich source of protein for farm animals (Falusi and Salako, 2001).

Plant-parasitic nematodes (PPNs) are a major problem affecting the growth and yield of sesame. According to a nematode survey conducted in an experimental area of Embrapa Cerrados, Planaltina, Federal District of Brazil in1993, nematodes found to be associated with sesame included: *Meloidogyne* spp., *Pratylenchus brachyurus*, *Aphelenchoides* sp., *Ditylenchus* sp., *Helicotylenchus dihystera*, *Paratrichodorus minor*, *Xiphinema* sp., *Tylenchorhynchus* spp, *Heterodera* spp., and *Criconemella ornate* (Sharma and Amabile, 1998; Kepenekcü, 2002). The effects of PPNs on beniseed ranged from stunted growth, yellowing and wilting of foliage to death of the plant (Sharma and Amabile, 1998). They are responsible for poor yield in quantity and quality (Atungwu and Afolami, 2003). However, PPNs vary over time and space and need to be routinely identified at definite location and time for them to be effectively managed (Mondal *et al*., 2020). While use of chemical nematicides have its advantages in the control of PPNs, the environmental and health consequences of some of these chemicals have been widely campaigned against. Some cases have been advanced for the use of non-chemical options for the management of PPNs, most of which are either direct or indirect soil amendments.

Sawdust and biochar are two classical examples of soil amendments that have been used to control certain nematodes on crops. They were reported to promote soil microflora, microfauna and soil chemical properties (Ikwunagu *et al*., 2019; Liu *et al*., 2020; Eche and Okafor, 2020). Use of sawdust and biochar derived from various plant and animal sources is becoming popular as a strategy for plant-parasitic nematode mitigation (Hassan *et al*., 2010; Eche and Okafor, 2020). The term ‘biochar’ refers to a biomass-derived carbon-rich material subjected to pyrolysis and thermal decomposition of organic material in an oxygen-deficient environment at high temperature (Lehmann and Joseph, 2009). This process takes place in a reactor and transforms the organic material into different amounts of solid, liquid and volatile products. Although it is gaining popularity as a soil amendment in North-Central Nigeria, evidence of its antihelminthic potential against plant-parasitic nematodes in a myriad of crops cultivated in the region remain scanty. Furthermore, recommended quantities of sawdust and biochar were mostly in the ranges of 5 /t ha and 10 t/ha which usually questions the feasibility as well as the sustainability of deploying such practice, especially on fields bigger than 1 hectare. As it is with most of the recommendations linked to organic materials with nematicidal properties, this may be discouraging, labourious and unattractive. Hence, the need to test lower rates of the duo for possible PPNs control. It is against this backdrop that a preliminary study was carried-out to investigate the effects that mixed formulations of sawdust and eucalyptus biochar had on plant-parasitic nematodes associated with beniseed and its yield of the crop.

Materials and Methods

Experimental site and land preparation

Field experiment was conducted at the Teaching and Research Station of University of Agriculture, Makurdi (UAM) located at latitude 07°45'N to 07°50'N, longitude 08°45'E to 08°50'E with an average altitude of 98m. The experimental site falls within the South Guinea Savannah Agro-ecological zone of Nigeria. A land area of 600 m^2 was measured out on the experimental area using a 50 m synthetic measuring tape. The marked area was manually cleared of weeds and ridged using cutlass and hoe. A total of fifteen ridges (circa. 75cm wide and 36m long) were made. The experimental area was, then subdivided into 27 experimental units (plots). Each plot size was 4.0 m x 5.0 m (20 m^2) in dimension and had five ridges within.

Source of seed and planting

Seeds of beniseed variety Yandev-55 were obtained from the National Cereals Research Institute (NCRI), Yandev Outstation, Benue State, Nigeria. Selection of the variety used in the experiment was based on its adaptability and wide acceptance in the study area. Planting was done manually by drilling on the ridges constructed at a seeding rate of 10 kg/ha.

Composition of sawdust and biochar formulation

A total of 300 kg freshly blended sawdust made up of obeche (*Triplochiton scleroxylon*) and mahogany (*Swietenia macrophylla*) was obtained from a wood mill located at Timber Shade, Industrial Layout in Makurdi, Benue State. The sawdust was air-dried at room temperature for four weeks under shade. A total of 300 kg branches cut from mature eucalyptus (*Eucalyptus globulus*) plants within the environs of UAM was air-dried at room temperature for four weeks. Dried branches were further chipped and carbonized for five hours at 500°C (The highest treatment temperature) in a locally fabricated bioreactor. Biochar was produced through a process of pyrolysis according to George *et al.* (2016). One hundred kilogrammes (100 kg) of the chipped eucalyptus branches were mixed with 10 kg of sand and poured into a 210-L bioreactor to establish an oxygen-reduced atmosphere during carbonization. The resulting carbonized materials were separated from sand, using a sieve. The biochars were crushed into powder using a mortar and passed through a 1 mm sieve and fractions less than 1 mm were used for the experiment. The prepared biochar was wrapped in aluminum foil and stored at room temperature.

Treatments and experimental design

A total of nine treatments combinations were evaluated using a 3 × 3 factorial experiment laid out in Randomized Complete Block Design (RCBD) in three replications. The treatments combinations consisted of single application of sawdust and eucalyptus biochar, and mixed formulations of sawdust +eucalyptus biochar applied in powder form at the rates of 0 t/ha (0 kg/plot), 2.5 t/ha (5 kg/plot) and 5 t/ha (10 kg/plot).

Soil sampling and nematode extraction

Soil samples were randomly taken from each of the 27 experimental units established on the experimental site. Each soil sample consisted of six cores (2.5 cm in diameter and 20 cm deep) at harvest. The six core samples taken from each experimental unit were bulked into a composite sample from which a subsample of 250 g was finally collected in a polythene bag and properly labeled. Soil samples collected from each experimental unit at harvest were taken to the Specialized Equipment Centre, UAM for nematode extraction. Nematode extraction from the soil was done using modified sieving and centrifugation procedure according to Whitehead and Hemming (1965) while sampled roots were macerated done according to Zuckerman *et al.* (1990). Mai and Lyon (1975) pictorial keys was used to identify genera of plant-parasitic nematodes recovered.

Data collection and statistical analysis

Data collected included pre-planting nematode populations (Pi) and post-planting nematode populations (Pf) obtained from soil and root, and yield per plot. Yield data was standardized to t/ha. Data was subjected to a two-way analysis of variance (ANOVA) using GENSTAT (14^{th} Edition). Count data were square-root transformed ($\sqrt{x+1}$) to improve the homogeneity of variance. Means were separated using Duncan's New Multiple Range Test at 5% level of probability. The reproduction factor (RF determined) and rate of population change (RPC%) were calculated using the formulae presented below: $RF = P_f/P_i$ (Oostenbrink, 1966); RIP= $P_f - P_i/P_i \times 100$ [where *Pf* = final nematode population, *Pi* = initial nematode population].

Links Researchers

Results and Discussion

Effects of different rates of single and combined applications of sawdust (SD) and eucalyptus biochar (EB) on plant-parasitic nematode populations, reproduction factor (RF) and yield of beniseed are presented in Table 1. The least nematode population (6 nematodes/10 g root) and RF (0.69) were recorded in plots treated with 5 t/ha of the mixed formulation of EB + SD. On the contrast, the highest nematode populations and RF were recorded in the untreated plots. Although single applications of 2.5 t/ha or 5 t/ha of either EB or SD, respectively also resulted in lower nematode populations and RF compared to the untreated plots, a total of 14 nematodes/10 g of root sample was recovered from plots treated with EB applied at 2.5 and 5 t/ha with RF value of 0.93 or 0.92, respectively as presented in Table 1. The highest beniseed yields recorded were 0.53 t/ha and 0.49 t/ha from plots treated with 5 t/ha and 2.5 t/ha of the mixed formulation, respectively. The difference between the two highest yields was not statistically significant ($p \geq 0.05$), but significantly differed significantly from yields obtained from the other plots when either biochar or sawdust was applied ($p \leq 0.05$).

A total of eight plant-parasitic nematode genera were recovered from the rhizosphere of beniseed in field, before, and after soil amendment using single or combined application rates of sawdust and eucalyptus biochar (Table 2). The recovered nematode genera were *Meloidogyne*, *Criconema*, *Tylenchus*, *Helicotylenchus*, *Pratylenchus*, *Rotylenchus*, *Hirschmaniella* and *Dorylaimus*. Although the least nematode populations (circa. 6 nematodes/250 g of soil) was recorded in plots treated with 5 t/ha of mixed formulation, the difference observed when the same combination at 2.5 t/ha of the mixed formulation was applied (circa. 7 nematodes/250 g of soil) was not statistically significant ($p \geq 0.05$). However, the highest populations of plant-parasitic nematodes in the soil were recorded in the untreated plots and had average values of approximately 44, 18 and 33 nematodes/ 250 g soil in the untreated plots in the respective treatments.

Table 1: *Effects of single and combined applications of sawdust and eucalyptus biochar applied on plant-parasitic nematode population (root) and yield of beniseed in Makurdi.*

Amendment	Rates (t/ha)	No. of PPNs per 10g of root (Pi)	Reproduction factor (RF)	Yield (t/ha)
	0	31[a]	3.52[a]	0.19[c]
Sawdust	2.5	26[b]	1.11[c]	0.24[c]
	5	23[bc]	1.01[c]	0.38[b]
	0	33[a]	2.52[b]	0.20[c]
Eucalyptus biochar	2.5	15[c]	0.93[d]	0.33[b]
	5	14[c]	0.92[d]	0.30[b]
	0	27[b]	3.07[a]	0.24[c]
Eucalyptus biochar + Sawdust	2.5	9[d]	0.81[d]	0.49[a]
	5	6[e]	0.69[e]	0.53[a]
F pr. ($p \leq 0.05$)		0.04	0.01	0.02
SED (±)		0.75	0.01	0.17
CV (%)		8.11	1.03	3.07

PPNs; plant-parasitic nematode; Means followed by the same letter in columns indicate no significant differences based on Duncan's New Multiple Range Test; Each value is an average of three replications; CV: Coefficient of Variation; SED: Standard Error Difference.

Table 2: *Major plant-parasitic nematode genera recovered from soil samples at harvest/250 g soil.*

Amendment	Rate (t/ha)	Meloid-ogyne	Cricone-ma	Tylen-chus	Helicoty-lenchus	Pratylen-chus	Roty-lenchus	Hirschman-iella	Doryl-aimus	[x]Aver-age
Sawdust (SD)	0	108a	77a	10b	13b	52a	41a	83a	14a	44.2a
	2.5	32d	18d	2c	0d	11d	10c	23e	6b	11.6d
	5	43c	25c	6b	9c	33b	33b	54c	10a	29.8c
Eucalyptus Biochar (EB)	0	115a	43b	19a	17b	20c	9c	37d	5b	17.9d
	2.5	20e	16d	8b	10c	8d	2d	18f	0c	8.5de
	5	20e	10e	8b	10c	18c	10c	10g	4b	10.3d
EB + SD	0	70b	45b	11b	37a	31b	33b	61b	13a	33.4b
	2.5	17f	14d	10b	5d	5d	5cd	10g	0c	7.2e
	5	10f	7e	3c	4d	9d	7c	6g	5b	6.2e
F.pr ($P \leq 0.05$)		<0.001	0.02	0.01	<0.001	0.05	0.01	<0.001	0.03	0.02
CV (%)		82.84	80.17	58.47	92.11	73.85	87.93	80.59	80.12	11.50

x = average of plant-parasitic nematode across column. Means followed by the same letter in columns indicate no significant differences based on Duncan's New Multiple Range Test; Each value is an average of three replications; CV: Coefficient of Variation.

In accordance, populations of plant-parasitic nematodes in the untreated plot increased by 224.8% during the growth period of beniseed which represented the highest rate of population increase recorded in the experiment, followed by 125% and 191.8% in the respective treatments (Figure 2). However, decreases in the rates of nematode populations were observed in the plots treated with both either single or mixed formulations of EB and SD at 2.5 t/ha and 5 t/ha. Plant-parasitic nematode populations decreased by 37.8% in plots treated with mixed formulation of at 5 t/ha of EB + SD, closely followed by the same formulation at 2.5 t/ha of EB and SD (21.6%) (Figure 2).

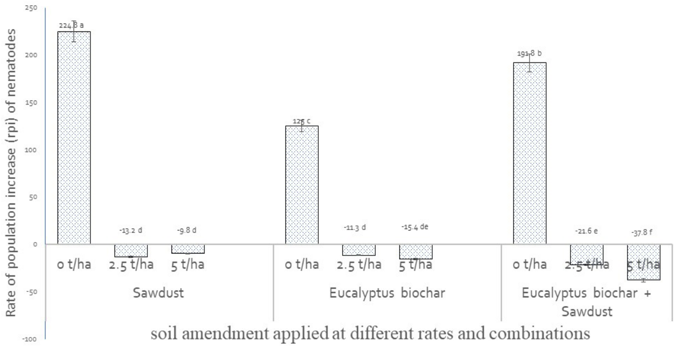

Figure 2: *Rate of PPN Population increase.*

The current study has shown that mixed formulations of sawdust + eucalyptus biochar resulted in better suppression of plant-parasitic nematodes infecting beniseed when compared to applying the soil amendments singly, even at higher rates. Even though the use of single applications of sawdust and or eucalyptus resulted in reduced populations of plant-parasitic nematodes compared to the untreated plots, the yield was still sub-optimal when compared to yields obtained from plots treated with the combination. Formulations of sawdust and eucalyptus biochar. The combined effects of sawdust +eucalyptus biochar on the crop could be due to the fact that, while sawdust improves the soil physical characteristics, the biochar must have resulted in modification of soil quality in terms of nutrient availability and abiotic conditions or activated sorption of allelopathic, and phytotoxic compounds that were likely injurious to the plant-parasitic nematodes (Bonanomi *et al*., 2015).This is in conformity with the work of Hale *et al*. (2013) who reported that biochar used as a soil amendment can boost soil fertility and improve soil quality by reducing soil acidity, increasing moisture holding capacity, attracting more beneficial fungi and microbes, improving cation exchange capacity and retaining nutrients in the soil.

In their global meta-analysis, Jeffery *et al*. (2017) concluded that most biochar experiments carried out globally in agroecosystems demonstrated positive effects of biochar on crop yield in the short- to medium-term, with the greatest positive results using biochar additions over 100 t/ha. This effect was attributed to its liming capacity and to improved soil water retention, but was later more specifically ascribed to tropical soils, which are typically nutrient-poor, acidic, and with low nutrient retention capacity compared to temperate soils, in which biochar improvements are not found (Jeffery *et al*., 2017). Besides, the plausible physiochemical impacts of biochar addition on soils that may affect crop production, a plethora of biological mechanisms might be also acting, ranging from changes in microorganism communities and to the associated changes in mineralization processes and primary production (Lehmann *et al*., 2011; George *et al*., 2016), and shifts in microbivore fauna that regulate decomposers to impacts on plant-parasitic nematodes (Domene, 2016).

Also, findings of Liu *et al*. (2020) who published data from 59 pot experiments and 57 field experiments from 21 countries found that crop productivity was increased by 11% on average when biochar was used. They also discovered that field application of biochar at a rate of 10 t/ha caused increase in crop productivity which varied with crop type with greater increases for legume crops (30%), vegetables (29%) and grasses (14%) compared to cereal crops like corn (8%), wheat (11%) and rice (7%). The positive effects of the sawdust + eucalyptus biochar on sesame plant can be attributed to a synergy of actions between the sawdust and eucalyptus biochar. While this study opened new areas for research, especially on the possibility of mixing non-chemical substrates against PPNs, further multilocational trials will provide deeper insight to the role that our environment plays and site-specific recommendations for adoption by end-users.

Conclusions and Recommendations

This study has provided evidence that eucalyptus biochar mixed with sawdust when applied at the rate of 2.5 t/ha suppressed plant-parasitic nematodes associated with beniseed in Makurdi. Unlike previous studies that have focused on use of eucalyptus biochar or sawdust usually at rates ranging between 5 t/ha and 10 t/ha, the current study has shown that with lower rates of 2.5 or 5 t/ha of eucalyptus biochar mixed with sawdust, the populations of plant-parasitic nematodes were suppressed by 21.6 % and by 37.8%, respectively. The yield of beniseed was significantly improved following application of eucalyptus biochar mixed with sawdust when applied at the rate of 2.5 t/ha.

Novelty Statement

The present study has provided evidence that application of mixed formulations of Eucalyptus biochar and Sawdust at the rates of 2.5 t/ha or 5 t/ha can be used to suppress populations of plant-parasitic nematodes associated with beniseed crop and substantially increased yield of the crop.

Author's Contribution

Christopher Oche Eche conceptualized and designed the study and prepared the first draft and final manuscript for publication. Juliana Iye Oluwatayo participated in the study design and reviewed the first draft of manuscript. Demben Moses Esang participated in field layout of the experiment and interpretation of analyzed data. Paul Madina reviewed first draft of manuscript. Alexander Uloko was involved in data collection and statistical analysis of data collected for purposes of the experiment. All authors have read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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