

NEMATICIDAL EFFECTS OF DIFFERENT BIOCHAR SOURCES ON ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPP.) EGG HATCHABILITY AND CONTROL ON MUNGBEAN (*VIGNA RADIATA* (L.) WILCZEK)

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ABSTRACT: *The nematicidal effects of different biochar sources and a synthetic nematicide on Root-Knot Nematode (*Meloidogyne* spp.) egg hatchability and control on mungbean were compared in Petri dishes and pot experiments. Each Petri dish contained 100 eggs ml⁻¹ of nematode eggs suspension and were arranged in CRD with six treatments and a control replicated thrice. Treatments included: biochars of bitter leaves, cassava peels, sawdust and poultry waste, a synthetic nematicide (Carbofuran 3G) and tap water (control). Suspension of the biochars and nematicide were applied at 0.1 g/ml and 0.015 g/ml respectively. Eggs hatched were observed for 24, 48 and 72 hours. In the pot experiment, each pot (except the control) was inoculated with 2,000 nematode eggs and was arranged in CRD. Similar treatments as in egg hatchability were used. The biochars and nematicide were applied as powder at 20 g/pot and 3 g/pot respectively. Data collected included: growth parameters, pod and grain yield, shoot and root parameters, nematode galls and population. They were subjected to ANOVA and means were compared using LSD at 5% probability level ($P < 0.05$), using computer software "Genstat Discovery Edition 4". Results showed that bitter leaves biochar compared favourably with Carbofuran on nematode egg hatchability. Generally, biochars of cassava peels and sawdust compared favourably with nematicide on the control of RKN on mungbean but did not significantly affect yield and nodulation.*

KEY WORDS: Biochar, Carbofuran, *Meloidogyne* spp., Petri dish, Pot experiment, *Vigna radiata*

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is an erect or leguminous climbing bean relating to cowpea (Agugo, 2003; Fuller, 2007). It is a dicotyledonous plant commonly referred to as green or golden gram, moong, ludou (Chinese), green soy and chop bean. (Agugo, 2003; Leung, 2007). It is native to the Indian subcontinent (Walshaw, 2010). It is a warm-season crop, requiring about 75-90 days to mature (Directorate Plant Production, 2010). It is mostly grown in Asian countries like Thailand, Burma, Indonesia and Philippines. It is now widely cultivated in Africa, South America, Australia and the United States (Kim *et al.*, 2007). It has been successfully grown in some Mid-Western parts and Southern parts of Nigeria (Agugo, 2003; Mensah and Tope, 2007).

The beans are small, cylindrical or ovoid, globular or oblong in shape with the seed colour usually dark olive green, bright green skin or yellow. Some cultivars produce brown or speckled black seed (Rubatzky and Yamaguchi, 1997).

Mungbean is widely used as human food, green manure, forage for livestock and for medicinal purposes (Huijie *et al.*, 2003; Ugese and Avan, 2005). As human food, it is consumed generally as boiled, or cooked with vegetables or meat, as well as a dessert or incorporated in bread or cake. It can be used to make sprouts for egg rolls and other vegetable dishes (Mendoza *et al.*, 2001).

It is very rich in protein and essential amino acids with the exception of the sulphur amino acids, methionine and cysteine which may be nutritional limited. It is a good source of soluble carbohydrate, and contains very high amount of crude fiber (Duke, 1983).

Among various pests and diseases, nematodes particularly *Meloidogyne* spp. poses a great problem to the cultivation of pulse crops causing severe yield losses (De *et al.*, 2000; Mahapatra and Swain, 2001). Researchers have recorded high yield losses of mungbean due to *M. javanica* and *M. incognita* under field conditions (Gupta and Verma, 1990; Sharma and Sharma, 2000). According to Taniwiryo *et al.* (2007), synthetic nematicides have been mostly used by farmers over the years, leading to their excessive and unsafe usage. This led to the need for alternative control options that will be of economic importance, effective as the synthetic nematicides, non-toxic and readily available (Fernandez *et al.*, 2001). Such alternatives are the use of botanical nematicides (nematicides of plant origin) and agro wastes (Javed *et al.*, 2006).

Onuju *et al.* (2014) stressed the benefits and possible use of constituents of higher plants in controlling plant diseases. Organic amendments have advantageous effects on the soil's nutrient, physical conditions and biological activity thereby improving the health of plants as well as decrease the population of the nematode (Oka and Yermiyahu, 2000). Many researchers have focused on the use of plant extracts as organic amendments but little or no research has been done on the use of biochar as regards the control of plant parasitic nematodes. However, some research works done on the use of biochar as organic amendment have been found to increase soil pH (Onwuka *et al.*, 2016).

Incorporation of biochars, the solid, carbon (C)-rich products of biomass pyrolysis, into soil or potting mixes provide nutrients and improve nutrient solubilization and uptake, thereby promoting plant growth and resistance against organism (Atkinson *et al.*, 2010). They induce systemic plant defense against pathogens by increasing populations of certain bacteria, actinomycetes, yeasts and fungi (Elad *et al.*, 2010). It has also been found to decrease the populations of plant-parasitic nematode (Zhang *et al.*, 2013). Lehmann *et al.* (2011) reported that mixing of biochar into soils frequently stimulates microbial growth as well as activates soil microorganisms that are dormant in the soil, thereby resulting in direct protection against soil pathogens through microbial production of antibiotics (antibiosis), competition for resources, or parasitism of the nematodes.

This study therefore aims at:

1. determining the nematicidal potentials of biochars of bitter leaves, cassava peels, sawdust and poultry waste on egg hatchability of root-knot nematode (*Meloidogyne* spp.);
2. comparing the nematicidal effects of synthetic nematicide (Carbofuran 3G) with the biochars in the control of root-knot nematode (*Meloidogyne* spp.) on mungbean.
3. assessing the effect of the individual biochars on the growth, nodulation and yield of mungbean.

MATERIALS AND METHODS

EXPERIMENTAL LOCATION

The experiment was carried in the green house of the Department of Plant Health Management, Michael Okpara University of Agriculture Umudike (MOUUAU), Abia State, Nigeria. It's located at latitude 5⁰2'N and longitude 7⁰33'E. It lies in the humid tropical rain forest zone with annual rainfall of 1916mm per annum, altitude of 112m above sea level and relative humidity of 76% with temperature range of 19-35⁰C (N.R.C.R.I, 2010).

EXPERIMENTAL MATERIALS

Planting material: Bold seeded variety, Vc6372 (45-8-1) of mungbean susceptible to root-knot nematode (*Meloidogyne* spp.) was obtained from the Department of Agronomy, College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike.

Treatments: The treatments included the biochars of bitter leaves, cassava peels, sawdust and poultry waste and a synthetic nematicide (Carbofuran 3G).

Treatments' sources and biochar preparation: Sawdust was gotten from timber market, cassava peels, poultry waste and bitter leaves were gotten from nearby farms in Michael Okpara University of Agriculture, Umudike, while the synthetic nematicide (Carbofuran 3G) was gotten from a chemical store in Umuahia. Following the method of Onwuka *et al.*, (2016), the biochar of the sawdust, cassava peels, poultry manure and bitter leaves were each prepared using a simple pyrolysis drum. They were sun dried prior to heating. The pyrolysis temperature was maintained at 450^oc for 1 hour.

Preparation of nematode inoculum: Root-knot nematode eggs were obtained from a culture of nematode infected roots of *Basella alba* (Ceylon spinach). Galled root pieces of *Basella alba* (Ceylon spinach) containing egg masses were cut into small pieces and placed in a beaker of 500 ml capacity with 200 ml of 0.5% chlorox (Sodium hypochlorite, NaOCI solution shaken vigorously by hand for 4 min (Hussey and Baker, 1973). This was done in order to dissolve the gelatinous matrix encasing the eggs. The solution was poured through two nested sieves, 200 mesh (75 µm) and 500 mesh (25 µm). Eggs in the 500 mesh sieve were washed free of NaOCI solution with slow stream of cold tap water into a beaker previously marked to contain 1 L. The cut roots in the original beaker were washed twice with water to obtain additional eggs. The number of eggs per ml of water were estimated by counting 3 samples of 1 ml each using Domncaster's counting dish under an electronic stereomicroscope and a working mean of eggs/ml estimated.

PETRI DISH EXPERIMENT

Treatments and biochar application: The effect of the biochar compared to Carbofuran 3G on the hatchability of eggs of *Meloidogyne* spp. was determined in 18 Petri dishes. The stock solution of the biochar was prepared by dissolving 20 g each of the biochar in 200 ml of distilled water. The suspension was equivalent to 1 g of the biochar per 10 ml of water. Also, the stock solution of Carbofuran 3G was prepared by dissolving 3 g of the Carbofuran in 200 ml of distilled water. This was equivalent to 0.15 g of the Carbofuran 3G per 10 ml of water. Nematode eggs suspension at a concentration of 100 eggs/ml of the suspension was introduced into each of the 18 Petri dishes, followed by 10 ml each of the biochar suspensions, 10 ml of Carbofuran 3G suspension and 10 ml of tap water which served as control respectively.

Experimental Design: The Petri dishes were placed on a laboratory bench and were arranged in a completely randomized design with the six treatments replicated three times including the control.

Data Collection: The eggs hatched were observed at 24, 48 and 72 hours by viewing 1 ml of the suspension from each Petri dish under an electronic stereomicroscope to count the hatched juveniles.

POT EXPERIMENT

Experimental Design: The experiment was laid in a completely randomized design using plastic buckets with seven treatments replicated three times including the control.

Soil sterilization and preparation: Top soil (0-15 cm) was collected from Michael Okpara University of Agriculture, Umudike farm. The soil mixture was moistened and then put into a cut drum, covered and heated until it reached a temperature of about 80°C and maintained at this temperature for 20 minutes (Ononuju *et al.*, 2014). After cooling down, 7 kg of the soil was separately filled in each of the 21 plastic buckets.

Planting of seeds: Mungbean seeds were pre-soaked for six hours and sown three seeds per hole in the plastic buckets containing 7 kg of the sterilized soil. Two weeks after, the plants were thinned down to a healthy seedling per pot, and fertilizer (NPK 15-15-15) applied at 156 kg/ha (Polthanee *et al.*, 2015).

Inoculation of the nematode eggs to the plants: Three weeks after emergence, each pot (except the second control pots) was inoculated by pouring a volume of 20 ml of root-knot nematode eggs suspension containing 2,000 eggs extracted by Hussey and Baker (1973) method near the plant by making a groove around it.

Treatments and biochar application: Seven days after the inoculation, 20 g each of the biochar was separately applied by spreading them evenly on the soil surface around the mungbean roots. Also, the synthetic nematicide (Carbofuran 3G) was applied at 3 g/plant. The control consist of pots with nematode but no treatment application and pots with no nematode and treatment application. The plants were watered daily and weeded as at when due.

Data Collection: The following data were taken at the end of the experiment after twelve weeks:

- **Growth/yield parameters:** Visual observations and measurements were taken from plant in each plastic bucket and recorded. The parameters recorded were: plant height (using a meter rule) and number of leaves (by counts) every three weeks interval, number of pods, number of seeds/pod (by counts), weight of pods, weight of seeds, fresh root weights, fresh and dry shoot weights (using a digital laboratory weighing balance) and number of nodules.
- **Nematode population in the soil:** 200 ml of soil samples were taken from each plant stand. Nematodes were extracted from soil samples using the modified Baermann technique (Hooper, 1969). Nematode counts were made after 24 hours using a stereoscopic microscope (Ononuju and Nzenwa, 2011), and were log transformed [$\log x$] prior to analysis.
- **Nematode eggs in root:** Nematode eggs in the roots were estimated by cutting each of the root samples into small pieces, placed in a beaker of 500 ml, and thereafter processed as previously described in extraction of nematode population from soil (Ononuju and Nzenwa, 2011). Data were log transformed [$\log x$] prior to analysis.
- **Root knot index:** Gall rating was obtained by observing the number of galls on the roots of the mungbean plants after harvest. This was done on a 0-4 scale (Agu and Ogbuji, 1996).

Score	Rating	Reaction
0	No gall present	No infection
1	1-3 galls present	Rare infection
2	4-10 galls present	Slight infection
3	11-30 galls present	Moderate infection
4	more than 30 galls present	Severe infection

STATISTICAL ANALYSIS

The data collected in the two experiments were subjected to Analysis of Variance (ANOVA) and means were compared using Least Significant Difference (LSD) at 5% probability level ($P < 0.05$) by using computer software “Genstat Discovery Edition 4”.

RESULTS AND DISCUSSION

RESULTS

The effects of the different biochars and Carbofuran 3G on cumulative egg hatch of *Meloidogyne* spp. at 24, 48 and 72 hours are shown in figure 1. Highest number of eggs (9, 18 and 28 eggs at 24, 48 and 72 hours respectively) were hatched when bitter leaves biochar was applied. This was followed by those obtained when poultry waste biochar was applied (4, 12 and 22 eggs). The least egg hatchability (0, 3 and 6 eggs) occurred when Carbofuran 3G was applied.

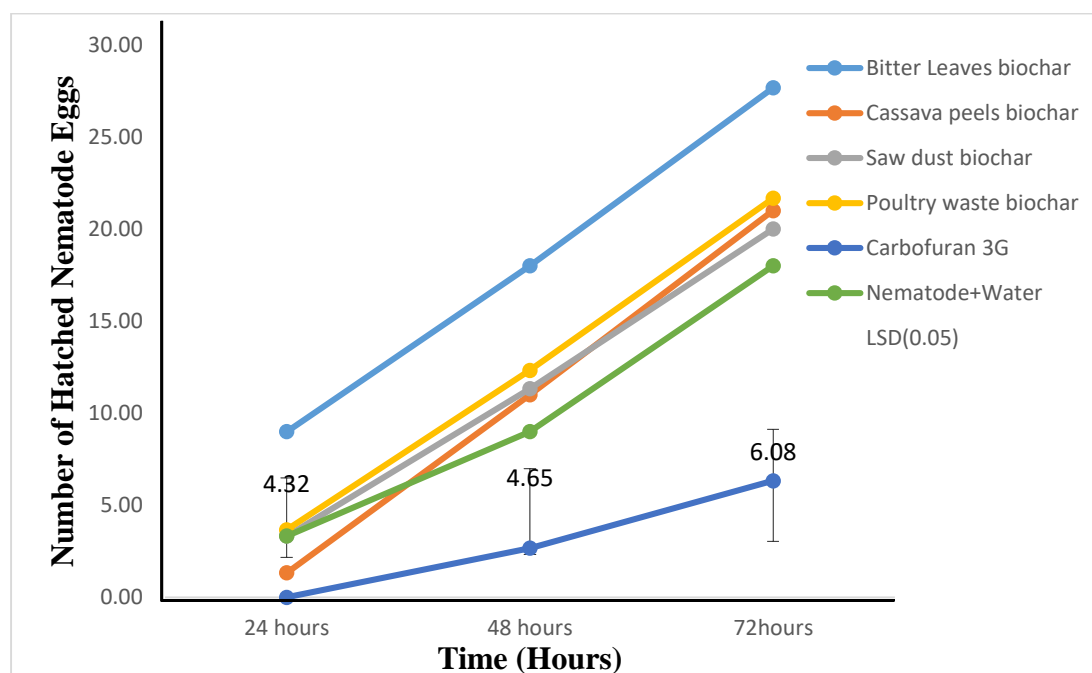


Figure 1: Effects of biochar and Carbofuran 3G on cumulative egg hatch of *Meloidogyne* spp. over time. (*the bars represent the $LSD_{(0.05)}$ with their corresponding values)

Figure 2 shows the effects of the biochars and Carbofuran 3G on vegetative growth of mungbean infected with root-knot nematode in the pot experiment. No significant difference was observed on the vegetative growth across the weeks. Plants with no nematode recorded the highest plant height at three (13.87 cm), six (27.50 cm) and twelve weeks after planting (22.83 cm). Plants treated with carbofuran 3G only had the highest plant height (23.30 cm) on week nine. The least plant heights were recorded in plants treated with biochars of sawdust 3 WAP (11.67 cm), poultry waste 6 WAP (17.00 cm), and bitter leaves 9 WAP (15.00) and 12 WAP (15.00 cm). More leaves were produced in plants with nematode alone 3 WAP (7), sawdust biochar 6 WAP (16) and 9 WAP (19) and poultry waste biochar 12 WAP (24). Plant treated with bitter leaves biochar had the least number of leaves 3 WAP (5), 6 WAP (13) and 12 WAP (12), whereas plants treated with cassava peels biochar had the least number of leaves 9 WAP (8).

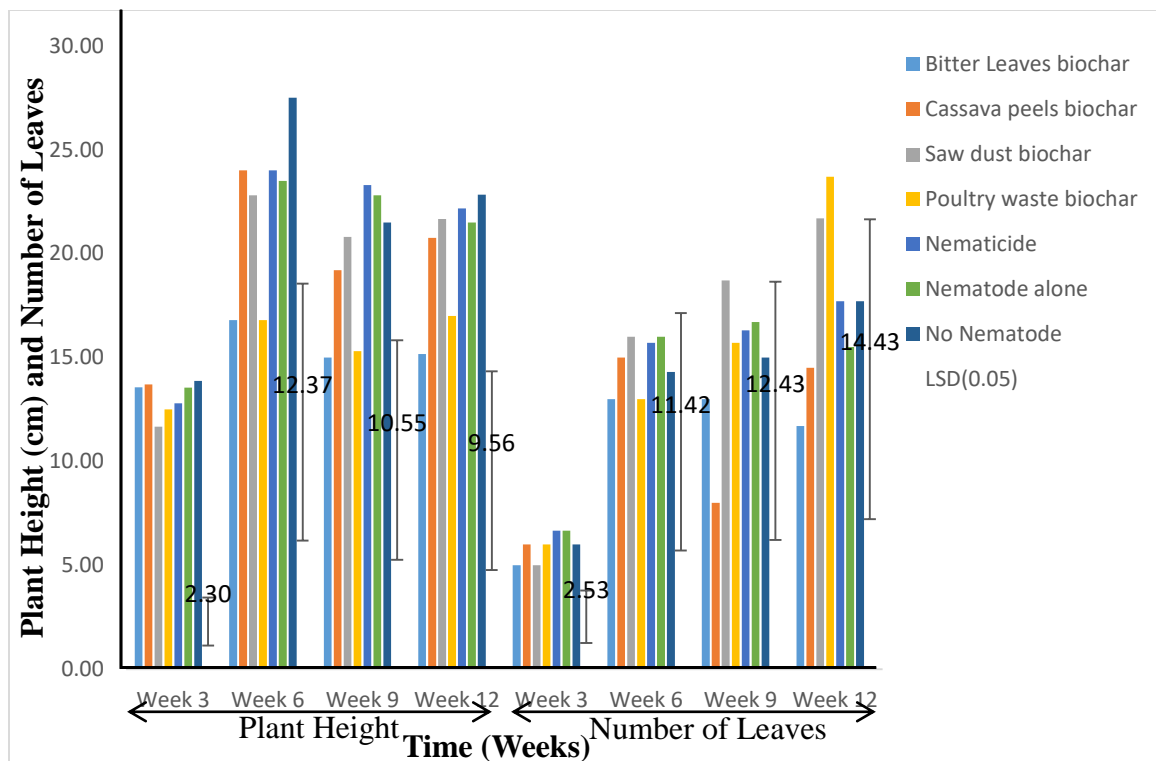


Figure 2: Effects of biochar and Carbofuran 3G on vegetative growth of mungbean infected with root-knot nematode in pot. (*the bars represent the $LSD_{(0.05)}$ with their corresponding values)

The effects of the biochars and Carbofuran 3G on pod and grain yield of mungbean infected with root-knot nematode in the pot experiment are shown in Figure 3. Number and weight of pods did not differ significantly among the treatments. However, the highest number and weight of pods were recorded for plants with no nematode (4 and 3.55 g respectively), whereas the least number and weight of pods were recorded for plants with nematode alone (1 and 1.02 g respectively). Plants with no nematode compared favourably with those treated with biochars of cassava peels, bitter leaves and poultry waste in number and weight of pods. There were no significant difference ($P \leq 0.05$) in the number and weight of seeds between plants treated with biochars of poultry waste, bitter leaves, sawdust and

nematicide. However, they did not compare favourably with plants with nematode alone. Similarly, on the seed weight, significant differences were not observed among the treatments but the plant with no nematode recorded the highest weight (1.8 g) while the plants treated with cassava peels biochar recorded the least weight (0.7 g).

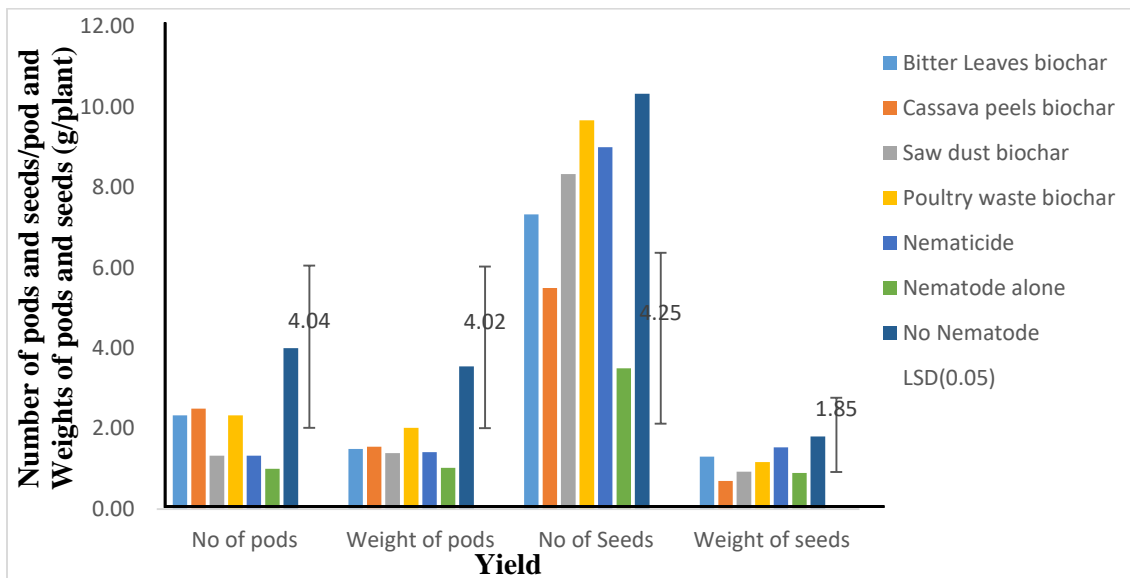


Figure 3: Effects of biochar and Carbofuran 3G on pod and grain yield of mungbean infected with root-knot nematode in pot. (*the bars represent the $LSD_{(0.05)}$ with their corresponding values)

Figure 4 shows the effects of the biochars and Carbofuran 3G on shoot and root weight and nodulation of mungbean infected with root-knot nematode in the pot experiment. On fresh and dry shoot weights, significant differences ($P \leq 0.05$) were not observed among the treatments. However, the highest fresh and dry shoot weights (11.53 g and 4.48 g respectively) came from plants treated with Carbofuran 3G whereas the least values of 5.53 g and 2.27 g respectively were recorded with bitter leaves biochar. Also, on fresh root weight of the mungbean, there were no significant differences ($P \leq 0.05$) among the treatments although plants with no nematode had the least weight (4.37 g) while the highest weight (6.95 g) was recorded with cassava peels biochar. On the number on nodules, significant differences were equally not observed among the treatments, however, the highest number of nodules (9) were recorded on plants with no nematodes.

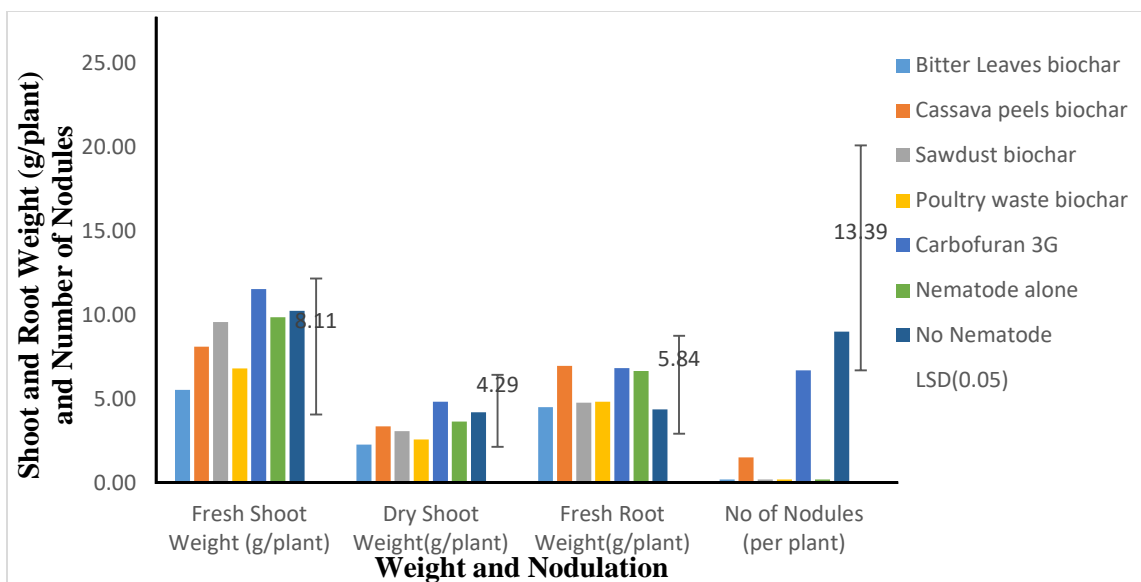


Figure 4: Effects of biochar and Carbofuran 3G on shoot and root weight and nodulation of mungbean infected with root-knot nematode in pot. (*the bars represent the LSD_(0.05) with their corresponding values)

Table 1 shows the effects of the biochars and Carbofuran 3G on number of galls, population and reproductive factor of root-knot nematode in mungbean in pot experiment. Application of bitter leaves and cassava peels biochars significantly ($P \leq 0.05$) suppressed galling incidence on mungbean. Uninoculated mungbean plants had no gall but nodules while plants treated with either cassava peels biochar or bitter leaves biochar were slightly galled. Severe galling however occurred when no treatment was applied. Number of eggs produced was significantly ($P \leq 0.05$) reduced when Carbofuran 3G was applied. Number of Juveniles/200ml soil was significantly ($P \leq 0.05$) suppressed by sawdust biochar and Carbofuran 3G applications. Reproductive factor of the nematode was significantly ($P \leq 0.05$) decreased by all the biochars and the Carbofuran 3G. This was more pronounced when carbofuran 3G and sawdust biochar were applied.

Table 1: Effects of biochar and Carbofuran 3G on root-knot nematode gall, population and reproductive factor on mungbean in the experiment

Treatments	Gall index	*No of Nematode Eggs	*No of Juveniles/ 200 ml Soil	Reproductive Factor
Bitter leaves	2.67	3.08 (1667)	2.64 (500)	1.08
Cassava peels	2.50	2.87 (1500)	2.65 (450)	0.97
Sawdust	3.00	2.90 (833)	2.42 (333)	0.58
Poultry waste	3.33	3.17 (1867)	2.85 (733)	1.30
Carbofuran	3.00	2.79 (833)	2.49 (467)	0.65
Nematode alone	4.00	3.66 (4800)	3.20 (1750)	3.28
No Nematode	0	- (0)	- (0)	0.00
LSD(0.05)	1.23	0.85	0.66	1.54

*Figures in parentheses are transformed means [log x] of number of nematode eggs and juveniles respectively

DISCUSSION

On the cumulative egg hatch of *Meloidogyne spp.* for 24, 48 and 72 hours, more eggs of the root-knot nematodes hatched with increase in the time of exposure (3 days). This means that, the biochar could not suppress or inhibit egg hatching instead stimulated them. The reason might be that the egg shell was weakened or even dissolved by the biochar, thereby releasing more juveniles. Jada *et al.* (2013) validated this findings that some plant extracts have the ability of increasing nematode egg hatchability. Riga *et al.* (2000) also observed the effect of some plant extracts in increasing egg hatching in nematodes on soya bean by 46.6%. On the other hand, the poor hatchability of the eggs in the nematicide could be attributed to the inhibitory effects of the nematicide on the nematode's eggs. This is consistent with the works of Oudejans, (1991) and Ononuju and Fawole (2000) who independently reported the inhibitory effects of nematicide on nematode's eggs hatchability.

The no significant difference ($P \leq 0.05$) recorded on the initial plant height and number of leaves of mungbean in the pot might be due to the normal growth of the mungbean in the absence of nematode infection and treatment. It was also observed that plants with no nematode increased in height across the weeks due to the absence of the nematode. This

was confirmed by Ononuju *et al.* (2014) who observed stunted growth and wilting of leaves due to the presence of nematodes on okra.

Observation on the lack of significant effect of the treatments on the number of leaves in the pot is similar to that of Claudius-Cole *et al.* (2010) and Ononuju *et al.* (2014) who observed that there was no significant difference in total number of leaves of cowpea and okra treated with some plant extracts respectively.

The poor yield in pod and grain can be attributed to the high susceptibility of mungbean to root-knot nematodes. This tallies with the findings of De *et al.* (2000), Stirling *et al.*, (2005) and Mahapatra and Swain (2001) who on their works respectively observed the high susceptibility of mungbean to root-knot nematode. The lack of significant effect on the yield of the crop might be attributed to the biochar as reported by Nelissen *et al.*, 2015 and Bass *et al.*, 2016. However, the potted plants that had no nematode gave the highest number of pods and yield.

The non-significant effect of the treatments on fresh and dry shoot weights of mungbean could be due to the slow rate to action of the biochar (Barman and Das, 1996) or due to the poor nutrient and water flow as a result of the formed galls on the plant roots (Ploeg, 2001). The increase in the weights of the fresh root can be attributed to the formed galls which increased the root weight. Plants with no nematode in pot had the least weight due to the absence of galls. Similar observation was made in a work done by Nwankwo *et al.* (2016), the fresh root weight of cowpea was found to increase with increasing nematode galls.

Plants with no nematode in the pots had the highest number of nodules due to the absence of nematodes which decreases their number. Khan and Kounsar (2000) acknowledged that nematode infection decreases the number of root nodules and that root system of infected plants appear smaller and has fewer bacterial nodules than roots of healthy plants.

Gall index ranging from slight to severe as observed indicated the proliferation of nematodes and their active penetration due to the absence or lower rate of treatments. Ononuju, (1999), Ononuju *et al.* (2014) and Nwankwo *et al.* (2016) made similar observations as they observed severe galling of the roots of plantain, okra and soya bean in their respective works. Stirling *et al.*, (2005) equally reported the high susceptibility of mungbean to *Meloidogyne* spp. which is seen in the severe galling of the roots.

The biochar and nematicide differed significantly from the pots with nematode on the log transformed number of eggs due to the ability of the biochar to rapidly stimulate egg hatchability, thereby increasing the number of juvenile in the soil. This is in line with the reports of Jada *et al.* (2013) that some plant extracts (such as root exudates of plants) has the ability of increasing egg hatchability. Riga *et al.* (2000) equally observed the effect of extracts from the roots of soya bean in increasing egg hatching in nematodes by 46.6%.

The significant difference observed between the sawdust biochar and the pot with nematode alone on the log transformed number of juveniles is consistent with the reports of Ozores-Hampton (2002) that the use of organic amendments suppressed soil phyto-parasitic nematode populations. Oka and Yermiyahu, (2000) equally reported the

suppressive effect of organic amendments on the population of soil nematode. This they did by releasing some toxic chemicals to the nematodes (Tobih *et al.*, 2011). Zhang *et al.*, 2013 equally reported the effectiveness of poultry waste biochar in decreasing the population of plant-parasitic nematodes.

The reproductive capacity of the nematode was significantly affected by the biochar and the nematicide in the pot experiment and contributed in the decrease of the juveniles' population and hatching of eggs. Zhang *et al.*, (2013) equally reported the effectiveness of biochar (poultry waste) in decreasing the population of plant-parasitic nematodes.

CONCLUSION AND RECOMMENDATIONS

The incorporation of biochar to agricultural soil or potting mix has been found to be beneficial in the control of root-knot nematode infestation on mungbean. It has also been found as a good substitute to synthetic nematicide (Carbofuran 3G). Biochars are non-toxic to farmers and environment, and easily available since the feedstock biomasses are of plant origin.

Farmers are hereby advised to adopt the use of biochars particularly, of cassava peels and sawdust origin in an integrated approach for the management of root-knot nematode problems in their farms. We need to investigate further on the rates of application of the biochar both in green house and field trials in order to determine the best application rate of the biochar. We equally recommend their application in different environmental condition as well as on different crops.

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