Fertilizer application enhances establishment of cacao seedlings in plantparasitic nematodes infected soil

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Abstract: Low soil fertility, pests and diseases are major problems of growth and establishment of cacao seedlings on the field. Cocoa production increases by new plantings and rehabilitation of moribund farms, but a build-up of plantparasitic nematodes (PPN) causing dieback and declining soil fertility has discouraged many farmers, leading to a reduction in crop productivity. In this study, the potentials of some organic wastes as fertilizers and their effects on establishment of cacao seedlings in PPN infected soils was investigated at Ibadan and Owena of Southwestern Nigeria. Goat dung (GD), organic fertilizer (OF), organo-mineral fertilizers (OMF) and NPK 15:15:15 were applied at 200, 400 and 600 kg ha-1, respectively, to cacao seedlings one month after transplanting, while unfertilized served as control. Results from the experiments showed a significant increase in percentage survival of cacao seedlings under organic fertilizers at Ibadan and Owena compared to NPK and control even at the lowest rate of 200 kg ha⁻¹ 3 years after transplanting. The incorporation of GD, OF and OMF significantly reduced the population densities of PPN compared to control. Therefore, GD, OF and OMF at 200 kg ha⁻¹ are recommended for soil application to enhance the field establishment of cacao seedlings in the soil infected with PPN.

Key words: fertilizers; plant-parasitic nematodes; cacao seedlings; establishment; organic wastes

Uporaba gnojil pospešuje rast sadik kakavovca v tleh okuženih s parazitskimi ogorčicami

Izvleček: Slaba rodovitnost tal, škodljivci in bolezni so glavni problem pri vzgoji sadik kakavovca na prostem. Pridelava kakava se povečuje z novimi nasadi in obnovo zanemarjenih kmetijskih zemljišč, a pojav parazitskih ogorčič (PPN), ki povzročajo propad sadik in zmanjšana rodovitnost tal jemljeta pri tem mnogim kmetom pogum, kar vodi v zmanjšanje v pridelavi te kulture. V tej raziskavi je bil preučevan potencial nekaterih organskih ostankov kot gnojil in njihov vpliv na rast sadik kakavovca v z ogorčicami (PPN) okuženih tleh v Ibadanu in Oweni, v jugovzhodni Nigeriji. Uporabljeni so bili kozji gnoj (GD), organska gnojila (OF), organsko-mineralna gnojila (OMF) in NPK 15 : 15 : 15 v odmerkih 200, 400 in 600 kg ha-1 , v nasadu kakavovca en mesec po presaditvi in kot kontrola nepognojen nasad. Rezultati poskusa so pokazali značilno povečanje preživetja sadik kakavovca pri gnojenju z organskimi gnojili v Ibadanu in Oweni v primerjavi z gnojenjem s NPK in kontrolo, celo pri najmanjšem gnojenju z organskimi gnojili, 200 kg ha-1, 3 leta po presaditvi. Vnašanje GD, OF in OMF v tla je značilno zmanjšalo gostoto populacij ogorčic v primerjavi s kontrolo. Zaradi tega priporočamo gnojenje z GD, OF in OMF v odmerku 200 kg ha-1 za uspešno rast sadik kakavovca v tleh okuženih s parazitskimi ogorčicami.

Ključne besede: gnojila; rastlinske parazitske ogorčice; sadike kakavovca; uspešna vzgoja; organski odpadki

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1 INTRODUCTION

Cocoa (*Theobroma cacao* L.) is cultivated in the humid tropics of the world (Yanelis et al., 2012) with more than 70 % production coming from Africa as a source of income for producing countries (Simo et al., 2018). The crop production is dominated by small-scale farmers who live and work in the cocoa belt providing them employment and income (Minimol et al., 2015; Ngoh Dooh et al., 2015). However, cocoa production has witnessed a downward trend due to declining soil fertility, pests and diseases, aging trees and low yields from smallholder farms. Low farm gate prices paid to farmers make it difficult for them to afford expensive inputs to increase soil fertility and yield, such as mineral fertilizers, and pesticides to control pests and diseases adverse effects. There are also concerns that the projected global temperature rise and subsequent increase in potential evapotranspiration and demand for plant water may lead to further drought stress during the dry season and deterioration of cocoa climate conditions (Läderach et al., 2013; Schroth et al., 2016). Cocoa production increases through new plantings and rehabilitation of moribund farms, but the build-up of plant-parasitic nematodes causing die-back of cacao seedlings in nurseries and young plantations and declining soil fertility caused many farmers to be discouraged leading to a reduction in crop productivity (Orisajo et al., 2012; Orisajo, 2018). The need to pay attention to soil fertilization is now almost as important as the control of pests and diseases in cocoa. Tropical soils are inherently low in soil organic matter and fertility status; hence external fertilizer supply is a key factor in raising crop production.

Fertilization is an indispensable agricultural practice in which organic and inorganic fertilizers are used primarily to improve plant nutrition and hence crop productivity (Tian et al., 2015; Francioli et al., 2016). Inorganic fertilizers which perform a decisive role in improving crop productivity are wildly applied. The production and application of these fertilizers cause serious environmental damage like greenhouse gas emissions, eutrophication (Copetti et al., 2016), pollution (De Notaris et al., 2018), leaching and contamination of groundwater thereby posing risk to human health (Huang et al., 2018; Jalali & Latifi, 2018). The continuous application of NPK leads to increase in the soil compactness, decrease in the soil pH (Adamtey et al., 2016), soil porosity, and organic carbon level (Chaudhary et al*.,* 2017) as well as decrease in soil beneficial microorganism populations (Wei et al*.,* 2017). Continuous excessive applications of inorganic fertilizer can also lead to nutrient accumulation in soil, and eventual P and N loss from soil to aquatic ecosystems (Qiao et al., 2012; Yan et al., 2013). Excessive N and P applications will also deteriorate the soil quality and reduce the soil's

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production levels (Zhang et al., 2015). With rising costs of chemical fertilizer and the aforementioned growing concerns over the environmental impact of excessive fertilizer application, there has been an increasing scrutiny on how nutrients are managed on farms (Chen et al., 2014).

Organic fertilizers (manures) are gaining attention as the alternative to inorganic fertilizers. Organic manure produced from biomass and animal conventionally plays an important role in recycling of nutrients (Hasler et al., 2015). When added to soils, organic manure enhances soil fertility by increasing nutrient availability (Cavagnaro, 2014), soil organic carbons (Xie et al., 2014), available N and P, micronutrients, soil aggregation, and water holding capacity, as well as leading to a high soil buffering capacity against external disturbances (Yu et al., 2012; Liang et al., 2012; Chaudhary et al., 2012; Sogn et al., 2018). Though, the benefits associated with organic amendments majorly depend upon the type and application rate of organic fertilizers (Jones & Healey, 2010).

The application of organic material, though a traditional practice to improve soil fertility and structure, is also known as a control method for soil- borne diseases, including plant-parasitic nematodes (Hassan et al., 2010; Houx et al., 2014). In recent years, a variety of organic materials, such as animal and green manures, compost, and proteinaceous wastes, are used for this purpose (Summers, 2011; Stirling et al., 2011; Renco & Kovacik, 2012; Olabiyi & Oladeji, 2014; Abolusoro et al., 2015; Rudolph & DeVetter, 2015; Tiyagi et al., 2015; Briar et al., 2016; Forge et al., 2016; Atandi et al., 2017; Shiferaw et al., 2017). Incorporation of organic amendments has been shown to be detrimental to plant parasitic nematodes (Wang et al*.,* 2004) due to release of NH_{4} , formaldehyde, phenol, volatile fatty acids and toxic compounds (Oka, 2010; McSorley, 2011; Briar et al., 2016). It was generally postulated that the adverse influence of organic amendment on plant-parasitic nematode is referred to increasing host resistance to nematode infection and enhancement of growth performance (Country & Millon, 2008).

This work aims to examine the effects of organic and organo-mineral fertilizers on plant-parasitic nematodes, cacao seedlings growth and establishment on the field. This will possibly ameliorate the current frustration faced by small-scale farmers on poor establishment of cacao seedlings and thereby increasing the crop production and income.

2 MATERIALS AND METHODS

2.1 STUDY AREA

Field experiments were carried out at the Cocoa Re-

search Institute of Nigeria (CRIN) experimental farms in Ibadan, Oyo State and Owena, a CRIN Substation in Ondo State, Nigeria. Ibadan lies between the latitude 7⁰ 30' N and longitude 3° 54' E at an altitude of 1222 m above sea level. It is located in the tropical rain forest ecosystem with mean solar radiation of 18MJ m^{-2} day⁻¹ and an annual average rainfall of 2000 mm with a bimodal pattern. Owena lies between the latitude 7° 15' N and longitude 5° 12' E at an altitude of 367 m above sea level. It is located in the tropical rain forest ecosystem with mean solar radiation of 30MJ m^{-2} day⁻¹ and an annual average rainfall of 1500 mm with a bimodal pattern.

The experiment was conducted over three years on the False horn plantain (*Musa* spp. L., AAB – group 'Agbagba') as shade crop planted with cacao (*Theobroma cacao* 'F3 Amazon') in Ibadan and Owena. The experiment was set as a randomized complete block design involving four fertilizer types: goat dung (GD), organic (OF), organo-mineral fertilizer (OMF) and NPK 15:15:15, which were separately applied at 200, 400, 600kg ha⁻¹ and unfertilized served as control. Each treatment had 3 replications. Healthy sword suckers of plantain of approximately uniform size (50-60 cm tall, 30-40 cm pseudostem girth) pared to remove lesions were planted at a spacing of 3 x 3 m. Cocoa seedlings of 5 months old were planted four weeks later at the same spacing.

2.2 PROCUREMENT OF FERTILIZERS AND PROXIMATE ANALYSIS

Organic (OF) and organo-minerals fertilizers (OMF) used for the experiments were obtained from the Sunshine Fertilizers, Ministry of Agriculture, Ondo State. They were manufactured in 2016 with batch numbers 30172, 30110, respectively. Goat dung (GD) was collected from Goat farms in Ilesha Garage, Akure, Ondo State. The GD was collected from pens with good farm sanitation, air-dried, carefully sorted to remove foreign materials and packed in 50 kg bags. The analysis was conducted to determine the nutrient content of the fertilizers using the wet digestion method (Odu et al., 1986). After drying, the fertilizer sample was finely ground in a mortar at approximately 80 °C for 12 hours. The 0.5 g sample was then weighed into a 100-ml Berzelius beaker. Five millilitres (5 ml) of nitric acid (HNO₃) and 2 ml of perchloric acid (HClO $_4$) were added, covered with a watch glass and digested by heating to a final volume of 5 ml. Ten millilitres (10 ml) of water was then added and the digested solution was filtered through an acid-washed filter paper into a 50 ml volumetric flask. The filter paper was washed with water and the filtrate diluted to volume with deionized water. The filtrate was read under atomic adsorption spectrometer, flame photometer and colorimeter for macro and micronutrients in the sample.

2.3 SOIL SAMPLES COLLECTION AND ANALYSIS

Soil samples were collected randomly from each of the experimental sites at both locations (Ibadan and Owena) with the aid of soil auger at 0 - 30 cm depth. For the pre-cropping analysis, the samples were bulked together and mixed thoroughly, air dried at room temperature and analysed for various elements. Particle analysis was determined using the hydrometer method (Kettler et al., 2001). Organic carbon determination was by the potassium dichromate oxidation method (Zhang et al., 2001). The total nitrogen (N) was determined by Kjeldahl method; available P by ammonium-vanadomolybdate colorimetric method; exchangeable K and Na by flame photometer; and exchangeable Mg, Ca and Mn were determined using atomic absorption spectrophotometer (Ryan et al., 2001). Soil pH was read on pH meter (1:1 water). Soil was assayed to confirm the presence and the initial population density of the plant-parasitic nematodes (Coyne et al., 2007). Aliquots of 100 ml soil was put into a set up that has two plastic sieves with extractor tissue sandwiched in between. The plastic sieves with the soil were thereafter placed in a plastic bowl, and water was added to the extraction bowl just enough to wet the soil. The set-up was left undisturbed for 48 hours. Thereafter, the plastic sieve containing the soil was removed briskly, and the nematode suspension in the bowl was poured into a nalgene wash bottle and allowed to settle. The supernatant was siphoned out, and the suspension containing nematodes was then poured into a labelled beaker, and adjusted to 10 ml by adding water. This was homogenized and 1ml of the suspension was taken with the use of pipette, dispensed into the nematode counting dish and examined under a high power stereomicroscope. Nematodes were transferred with a picker to a slide with a drop of water, covered (with a cover slip) and examined under an Olympus compound microscope for identification using taxonomic keys (UNL, 2019) and counted. The identification and counting was repeated three times and mean population of nematodes per sample calculated. Two grams (2 g) each of the organic fertilizers used were also analysed for nutrient composition.

2.4 FERTILIZER APPLICATION AND DATA COL-LECTION

The fertilizers were applied to treatment plots one month after transplanting using ring method of appli-

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cation at 5 cm away from the base of cacao. Monthly Data collection on growth parameters (plant height, stem girth, number of leaf, and leaf area and number of branches) commenced 3 months after transplanting. Leaf samples $(4th$ leaf) were collected from 4 tagged cocoa seedlings at 12 months after transplanting and were analysed in the laboratory for chemical composition. The experiments were monitored for 36 months (144 weeks after planting). Survival count was carried out 12 months after transplanting. At 15 months after transplanting, soil samples were collected from treatment plots and were processed and analysed for physical properties (sand silt, loam, clay, soil moisture content and soil bulk density), chemical properties (soil organic matter, soil pH, N, P, K, Mg, Ca, and Na), and plant-parasitic nematodes population densities using aforementioned standard procedures.

2.5 DATA ANALYSIS

Nematode population densities were $log_{10}(x + 1)$ transformed and percentage data were square-root-transformed prior to analysis to stabilize variances (Gomez & Gomez, 1984), while the other data collected were not transformed. Only the predominant plant-parasitic nematode species were included in the data analysis. Analyses of variance (ANOVA) were carried out to test for main effects and interactions. Pre-planned comparisons between treatment combinations were tested with linear contrasts. All analyses were performed using GENSTAT.

3 RESULTS AND DISCUSSIONS

3.1 NUTRIENT COMPOSITION OF THE ORGANIC MATERIALS

The nutrient composition of the organic materials ap-

Table 1: The nutrient composition of the organic materials

plied to the soil is presented in Table 1. The C : N ratio of the organic fertilizers used are 8.2, 9.4, 9.8 for goat dung, organo-mineral fertilizer and organic fertilizer, respectively. Changes in the C : N ratio of aggregates may reflect the degree of organic materials decomposition within aggregate fractions (Baldock et al., 1992). Higher C : N ratios of aggregates suggest that soil organic C is relatively fresh or little altered, whereas, soil organic C is more decomposed and relative aged when the C : N ratio of aggregates is low (Chen et al., 2010). Difference in soil organic matter quality within aggregate fractions will result in difference in the types of nutritional substrates available, which may directly affect the natural microbial communities (Bending et al., 2002). In general, amending the soil with organic materials having low C : N ratio (less than 20) resulted in rapid mineralization of N in the form of NH_{4+} or NO_3 – for absorption and uptake by plant roots (Powers & McSorley, 2000). The fertilizers used in these experiments have low C : N and this appeared to have positive effects on the survival of the cacao seedlings.

3.2 SURVIVAL AND GROWTH OF CACAO SEED-LINGS AS AFFECTED BY FERTILIZER APPLICA-**TION**

Results indicated that fertilizers applied significantly ($p < 0.05$) increased the survival of cocoa seedlings 12 months after planting in the field. The percentage survival of cacao seedlings under organic fertilizers at Ibadan and Owena increased significantly compared to NPK and control even at the lowest rate of 200 kg ha⁻¹ used in the experiment (Table 2). However, application of 600 and 400 kg ha-1 of NPK enhanced the survival of the cacao seedlings compared to the control. In the same vein, growth of cacao seedlings was consistently improved by the fertilizer application compared with the control at both locations (Table 3). Application of goat dung, organo-mineral fertilizer and organic

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fertilizer at 200, 400 and 600 kg ha $^{-1}$ led to a significant increase in the height of cacao compared with NPK and control (Table 3). Similar pattern was observed for other growth parameters measured. In contrast, there was a significant reduction in plant height, stem girth, number of leaves, leaf area and number of branches of cacao in unfertilized plots. The increase in growth parameters could be attributed to the enhanced nitrogen and phosphorus uptake by the plant using organic amendments (Pandit et al., 2018). Organic manures have been shown to supply required plant nutrients, improve soil structure and promote plant growth (Agbede et al., 2014, 2017). The addition of organic manure in soil may encourage the immobilization of bioavailable nitrogen and phosphorus, which may otherwise be lost through leaching or emissions in the environment (Sun et al., 2018). The inclusion of organic manure may also generate higher transpiration rates leading to higher water retention in the soil. Hence, more availability of water soluble nutrients may cause the crop yield improvement (Doan et al., 2015).

Application of inorganic fertilizer, NPK, even at the lowest rate 200 kg ha-1 also improved cacao growth significantly compared with the control (Table 3). This is in agreement with the earlier study that the use of appropriate levels of NPK fertilizers have good effects on plant growth factors (Irshad et al., 2006). NPK application enriched the availability of macro nutrients, nitrogen, phosphate, and potassium in the soil. These nutrients therefore, were readily absorbed by the crops. In crop metabolism, these nutrients are utilized in carbohydrate synthesis, cellulose, proteins, hormones, and enzymes. All these processes triggered the growth of plant organs such as plant height, stem diameter, number of leaves, leaf area and number of branches as reported in this present study. This result was in line with the previous studies conducted by Mandal et al.(2009) and Bandyopadhyay et al. (2010). In their studies, applications of NPK also triggered the growth of vegetative crops.

3.3 RELATIONSHIPS BETWEEN PLANT-PARASITIC NEMATODES AND CACAO GROWTH

Relationships between the predominant plant-parasitic nematode population densities recovered and vegetative growth of young cacao revealed various statistically significant interactions (Table 4). *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Pratylenchus coffeae* Goodey, 1951 and *Radopholus similis* (Cobb, 1893) Thorne, 1949 population densities were negatively correlated with the survival percentage of the cacao seedlings $(r = -0.69, p < 0.01; r = -0.58, p < 0.05$ and $r = -0.46, p <$ 0.05, respectively). Furthermore, *M. incognita* was negatively correlated with the plant height ($r = 0.91$, $p < 0.01$), leaf area ($r = -0.61$, $p < 0.01$) and number of branches ($r =$ -0.51 , $p < 0.05$). This confirmed the previous reports that root-knot nematodes, *M. incognita*, damage on cacao seedlings led to stunted growth of the plants (Afolami & Caveness, 1983; Afolami & Ojo, 1984) Similarly, *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956, *P*. *coffeae* and *R*. *similis* population densities were negatively correlated with plant height ($r = -0.46$, $p < 0.05$; $r = -0.51$, $p < 0.05$; $r = -0.43$, $p < 0.05$, respectively), while they have no significant correlation with leaf area and number of

Table 2: Survival rate (%) of cacao seedlings as affected by fertilizer application at Ibadan and Owena (12 months after transplanting)

Treatment means within each column followed by the same letters are not significantly different from each other using Tukey's HSD at 5 % level.

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	Hm	$P_{\mathcal{C}}$	Rs	Survival $(\%)$	Plant height (cm)	Leaf area $\rm (cm^2)$	Branches (no)
M. incognita (J2)	0.96 **	0.41^*	$0.67**$	-0.69 **	-0.91 **	-0.61 **	-0.51 [*]
H. multicinctus	$\overline{}$	0.46^*	$0.72**$	0.24	-0.46 [*]	-0.12	-0.24
P. coffeae		$\overline{}$	$0.84**$	-0.58 [*]	-0.51 [*]	-0.15	-0.18
R. similis			$\overline{}$	-0.46 [*]	-0.43 [*]	-0.15	-0.17
Survival (%)				$\overline{}$	$0.89**$	$0.63**$	0.51^*
Plant height (cm)					$\overline{}$	0.71 **	0.53^*
Leaf area $\text{(cm}^2\text{)}$						٠	0.28

Table 4: Linear correlation matrix (half) of mean values for plant-parasitic nematode population densities / 100 g soil, percentage survival, plant height, leaf area and branches of young cacao

Mi: Meloidogyne incognita; Hm: Helicotylenchus multicinctus; Pc: Pratylenchus coffeae; Rs: Radopholus similis. Correlation coefficient significant at $p < 0.05$, $\rightarrow p < 0.01$.

branches (Table 4). However, plant height was positively correlated with survival percentage ($r = 0.89$, $p < 0.01$), leaf area ($r = 0.71$, $p < 0.01$) and number of branches ($r =$ 0.53, $p < 0.05$).

3.4 EFFECTS OF ORGANIC FERTILIZERS ON POPULATION DENSITIES OF PLANT-PARA-SITIC NEMATODES

The incorporation of goat dung, organo-mineral fertilizer and organic fertilizer at 200, 400 and 600 kg ha-1 led to a significant reduction in the population densities of these plant-parasitic nematodes compared with NPK fertilizer and control (Table 5). This is in agreement with earlier studies that soil amendments with different types of organic manures are effective in reducing the population densities of many soil-borne plant pathogens including plant-parasitic nematodes (Hassan et al., 2010; Shiferaw et al., 2017). Organic manure has been reported to be rich in several compounds especially nitrogen and phenolics (Hassan et al., 2010; Renco & Kovacik, 2012). Nitrogen in the organic manure after conversion into ammonia (Thoden et al., 2011) has been reported to kill several plant parasitic nematodes (Lazarovits et al., 2001). Phenols and other nematostatic chemicals released from organic matters into amended soil significantly decreased the nematodes population (Oka 2010; Briar et al., 2016). Several researchers using organic soil amendments have reported satisfactory results on the plant growth and yield in a variety of crops with marked reduction in the population of plant-parasitic nematodes (Orisajo et al., 2008; Pakeerathan et al., 2009; Iqbal et al., 2012; Chaudhary & Kaul, 2013; Abolusoro et al., 2015; Adepoju et al., 2017). All the treated plants showed significant and satisfactory results when compared to untreated control. Our findings in this study are similar with the aforementioned earlier reports. In the same vein, application of NPK at 200, 400 and 600 kg ha $^{-1}$ 600 also had a significant lower population densities of *M*. *incognita*, *H*. *multicinctus*, *P*. *coffeae* and *R*. *similis*. Our findings were consistent with earlier studies that the use of appropriate levels of NPK fertilizers have good effects on plant growth factors with resultant reductions in plant-parasitic nematode populations (Irshad et al., 2006; Ameen et al., 2013; Osman et al., 2015; Kolawole et al., 2018). Contrarily, nematode populations were reported to have increased due to NPK and manure combined with chemical fertilizer (Hu et al., 2018). Other studies also reported an increase in the total number of nematodes due to the use of chemical fertilizers (Li et al*.*, 2016; Hu et al*.*, 2017).

4 CONCLUSION

Improving the agronomic conditions for plant growth is an important factor for increasing the plant tolerance to plant-parasitic nematodes (Charegani et al., 2010). Results from this study have shown that the addition of fertilizers to the soil will improve the survival and growth of cacao seedlings. With rising costs of chemical fertilizer and the growing concerns over the environmental impact of excessive fertilizer application, goat dung, organo-mineral fertilizer and organic fertilizer at 200 kg ha⁻¹ are recommended for soil application. These have been shown to enhance the field establishment of cacao seedlings in the soil infected with plant-parasitic nematodes.

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