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Effect of time of application of moringa (*Moringa oleifera* L.) leaf powder on root-knot nematode (*Meloidogyne* spp.) infecting cowpea (*Vigna unguiculata* L. walp)

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ABSTRACT

The root-knot nematode, *Meloidogyne* spp. is a major economic pest of cowpea in Northern Ghana. The use of moringa leaf powder is an economical and environmentally friendly alternative for nematode management. The effectiveness of time of application of moringa (*Moringa oleifera*) leaf powder on the management of root-knot nematode (*Melodogyne* spp.) for cowpea (*Vigna unguiculata*) was evaluated at University for Development Studies, Nyankpala, Ghana using a Randomized Complete Block Design. Treatments were; application at planting (AP), application one week after planting (1 WAP), application two weeks after planting (2 WAP) and application three weeks after planting (3 WAP). Application at 1 WAP showed better root-knot nematode suppression resulting in fewer root galls and greater plant growth. There was also an increase in grain yield. Application of moringa leaf powder at 1 WAP has a longer potency and efficacy in reducing or controlling the population of root-knot nematodes of cowpea.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is the most widely cultivated important grain legume crop in West and Central Africa (Fatokun *et al*., 2012; Directorate Plant Production, 2014). Worldwide, cowpea production was estimated at 3.3 million metric tons of dry grain (FAO, 2009) with 70% of this obtained from the Northern savanna zones of Africa. In Ghana, cowpea is cultivated in both humid and semi-arid regions, but yields are low. Production was estimated at 64,680 metric tons in 2004 from Northern Ghana, which encompasses the Guinea and Sudan Savanna ecologies (SRID, 2005). On average, cowpea grains contain 25% protein, 53.2 mg/kg iron, 38.1 mg/kg zinc, 826 mg/kg calcium, 1915 mg/kg magnesium, 14,890 mg/kg potassium, and 5055 mg/kg phosphorus (Boukar *et al*., 2011). High protein content of cowpea makes it an exceptional crop for alleviating malnutrition in developing countries where animal protein is beyond the reach of most people. Fresh pods of some varieties are used as vegetables. Boiled cowpea leaves are kneaded to a pulp and then squeezed into golf ball-sized pellets that are dried in the sun and stored (Madamba *et al*., 2006). The rapid growth rate of cowpea provides good ground cover beneficial to soil erosion control measures. Crop residues are nitrogen rich helping to improve soil fertility and structure which together have made cowpea an important component of subsistence agriculture, particularly in the dry savanna of sub-Saharan Africa (Singh *et al*., 1997).

Root-knot nematodes (RKN), *Meloidogyne* spp., are serious pests in most cowpea-growing regions of the world and have been reported to cause significant cowpea yield losses in Burkina Faso (Castagnone-Sereno, 2006; Sawadogo *et al*., 2009). Siddiqi (2000) reported that RKN can cause 50–80% loss in vegetable crops. Nematodes that cause serious damage to cowpea in Ghana are *Meloidogyne incognita, Pratylenchus brachyurus* and *Rotylenchulus reniformis* (Adomako *et al*., 2016). In Ghana, Kankam *et al*. (2015) identified *Meloidogyne* spp. as one of the plant-parasitic nematodes of economic importance to cowpea production.

Methods of controlling nematodes include; soil sterilization, use of pathogens free subsoil, use of soil amendments such as compost and manure, alternate wetting and ploughing, use of trap crops such as Crotalaria and Mucuna in the rotation, and the use of nematicides such as Furadan (Carbofuran) (GIDA-JICA, 2004; Andres *et al*., 2012; Kankam and Adomako, 2014; Kankam *et al*., 2014). Application of leaf residue as soil organic amendment agents has proven to be effective in managing nematodes (Youssef and Lashien, 2013). Research work on the control of nematodes with botanicals has been done but findings are largely based on laboratory experiments. Extracts from parts of certain plants such as moringa (*Moringa oleifera*, Lam) and neem (*Azardirachta indica* L.) have been reported to have nematicidal properties that inhibit the growth and development of *Meloidogyne* spp. (Sowley *et al*., 2014; Kankam *et al*., 2015). However little is known about their time of application for nematode management. The objective of this study was to determine the effect of the time of application of moringa leaf powder on incidence of RKN infecting cowpea.

Materials and Methods

Experimental site

The study was conducted at the experimental farm of the University for Development Studies, Nyankpala, Northern Region, Ghana during the 2014 major cropping seasons. The Nyankpala campus is situated at a latitude 9° 25° 41’N longitude 0° 58° 45’W with an altitude of 200 mm. Annual rainfall is unimodal with 1000-1200 mm occurring from April to November. Temperatures range from 21°C to 34.1°C. The area has a minimum relative humidity of 53% and a maximum of 80% (SARI Annual report, 2012). The area is characterized by natural vegetation that is dominated by few shrubs (SARI, 2012).

Field layout and Experimental design

A local white-seeded and brown-eyed cowpea variety known as “Apagbaala” was used in this study. The seeds were obtained from Savanna Agricultural Research Institute (SARI) at Nyankpala. The variety takes 45 days to mature. The cowpea seeds were sown on raised beds after the land was ploughed and harrowed to a fine tilt. Experimental plots were 3.0 m x 3.0 m (9 m^2) in size. The blocks were 1.5 m apart with 1.0 m furrows between plots in a block. Sowing was done on 15th May, 2014 at a rate of two seeds per hill planted at a depth of about 3.0 cm. Plants were thinned to one per hill one week after emergence. Number of plants per plot was 36 at a spacing of 50 cm x 75 cm and six rows of each plot. Moringa leaves were collected in Nyankpala, shade-dried, pounded into a powder in a mortar.

The experimental design used was a Randomized Complete Block Design, with four treatments each replicated four times. The treatments were the different times of application of moringa leaf powder at 60 g per plot. Experimental treatments were four application times as follows: 1) application at planting (AP), 2) application one week after planting (1 WAP), 3) application two weeks after planting (2 WAP), and 4) application three weeks after planting (3 WAP). For each treatment, 60g of moringa leaf powder was applied to each plot. The powder was spread closer to the base of the cowpea plants during the application. The plants were sprayed with Karate 2.5 EC (Lambda-cyhalothrin) at a rate of 800 ml/ha weekly starting one week after emergence until harvesting to control leaf-eating and pod-sucking insects.

Data collection

Data were recorded for plant height, number of leaves, chlorophyll content, root weight, grain yield, root galling index, and root-knot nematode populations. Eighteen (18) plants were sampled from each plot for measurement of each parameter.

Plant height (cm) was measured from the soil surface to the last petiole of the leaf and the readings were recorded.

Leaf number was determined by counting the number of fully expanded leaves and the number recorded.

Chlorophyll content (mg) was determined using a chlorophyll meter (Minolta). Three leaves were randomly selected from each plant for measurement. The mean of the three measurements was recorded.

For plant height, number of leaves, and chlorophyll content, data collection started the fourth week after planting and continued at two week intervals until the tenth week after planting.

Fresh root weight (g) was determined by removing the plants from the plots, rinsing the roots with tap water, blotting the roots dry, and weighed.

Dry grain yield was estimated from the six rows of each plot, after the pods were harvested, sun dried, to reduce the moisture content to 12%, threshed and winnowed to obtain the pure seeds. The results were extrapolated to kilograms per hectare (kg/ha) for each treatment using the following formula (Asante *et al.*, 2001):

$$\text{Grain yield/ha} = \frac{10{,}000}{Area\ harvested} \times \text{Grain yield/plot}$$

The roots of the harvested cowpea plants were each washed separately and dabbed dry with tissue paper. The galling index was assessed on a 0-10 scale according to Bridge and Page (1980) were 0 = No knots on roots, 1 = Few small knots. Difficult to find, 2 = Small knots only but clearly visible. Main root clean**,** 3 = Some larger knots visible. Main roots clean**,** 4 = Larger knots predominate but main roots clean, 5 = 50% of roots infested. Knotting on some main roots. Reduced root system, 6 = Knotting on main roots, 7 = Majority of main roots knotted, 8 = All main roots, including tap root, knotted. Few clean roots, 9 = All roots severely knotted. Plant usually dying, 10 = All roots severely knotted. No root system. Plant usually dies.

Twenty core soil samples were taken from each plot and thoroughly mixed to form a composite sample. Root-knot nematode juveniles (J_2) were extracted from 200 cm^3 soil samples using a series of sieves (850, 250, 75 and 38 µm) and a 48 h decanting period through a Baermann funnel (Christie and Perry, 1951). Counting of J_2 was carried out with stereoscopic microscope. Juveniles (J_2) of *Meloidogyne* spp. were identified based on Siddiqui (2000) identification key. Measurements before planting to show the baseline for the population was also done. Root weight, grain yield, root galling index and nematodes population were taken at harvest.

Data analysis

Data were subjected to analysis of variance (ANOVA) using computer statistical package

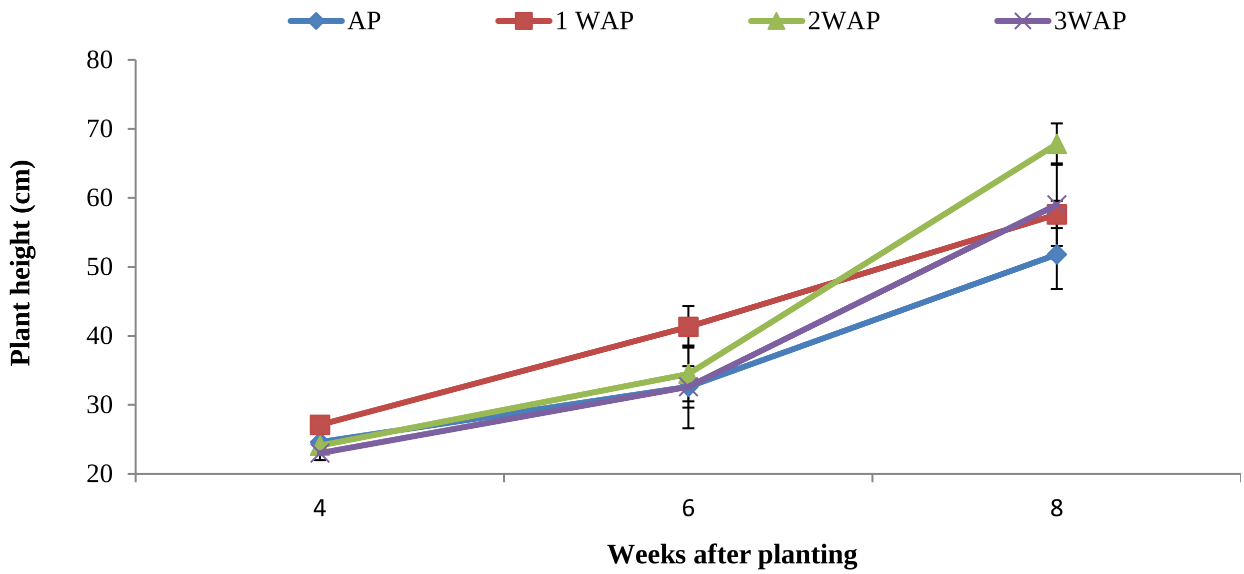

Figure 1. Effect of time of application of moringa (*Moringa oleifera*) leaf powder on cowpea (*Vigna unguiculata*) plant height (cm). Bars represent SEM.

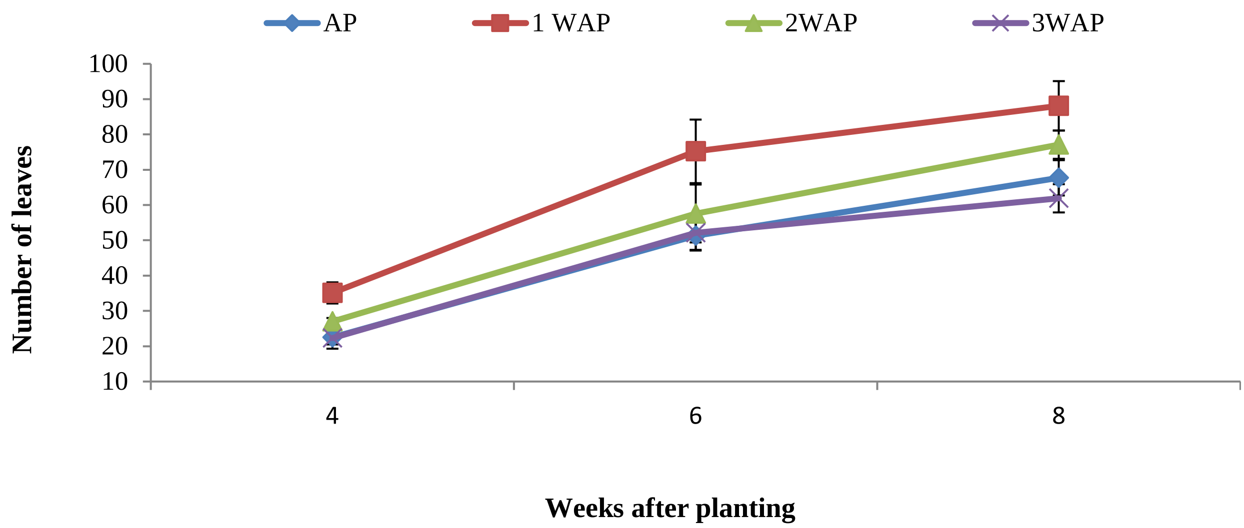

Figure 2. Effect of time of application of moringa leaf powder on number of leaves of cowpea. Bars represent SEM.

Genstat (version 11) and treatment means were separated using least significant difference (LSD) at 5% probability. All count data were transformed using square root transformation of $\sqrt{(x+0.5)}$, where x is the mean count.

Results

Plant height

At 6 and 8 WAP, there were significant differences ($P < 0.05$) among treatments (Figure 1). The 1 WAP treatment produced the tallest plants at 4 and 6 WAP

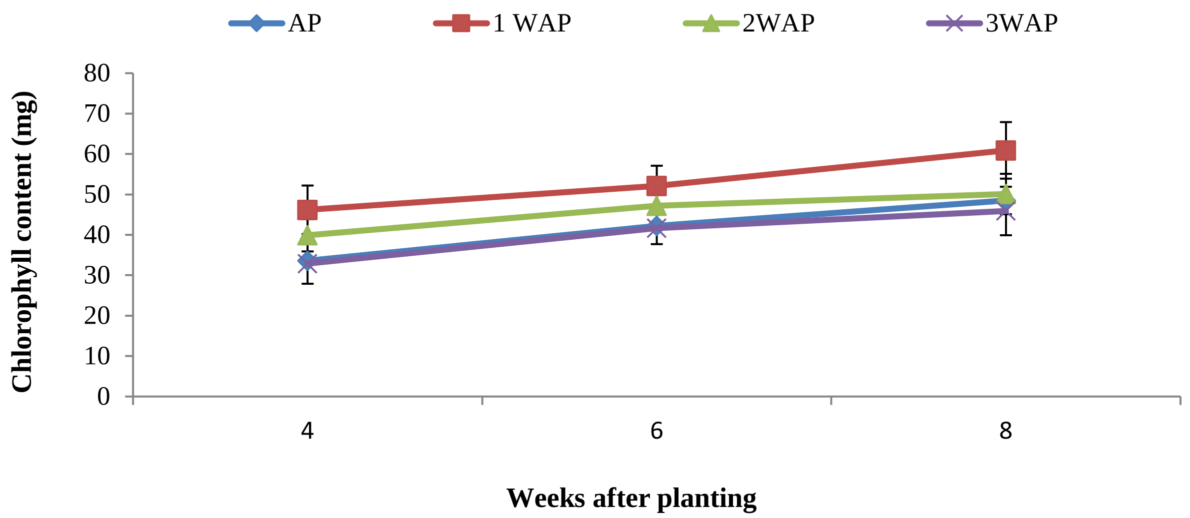

Figure 3. Effect of time of application of moringa leaf powder on chlorophyll content (mg) of cowpea. Bars represent the standard error of the mean chlorophyll content for each treatment.

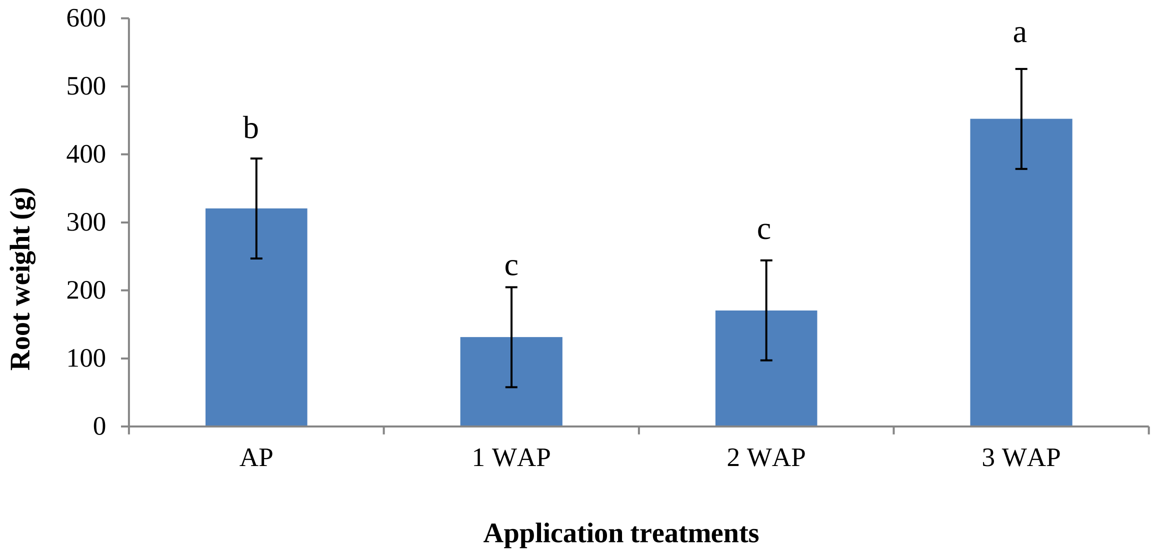

Figure 4. Effect of time of application of moringa leaf powder on root weight of cowpea at maturity. Bars represent the standard error of the mean root weight for each treatment. Lengths with different letters are significantly different at $P < 0.05$.

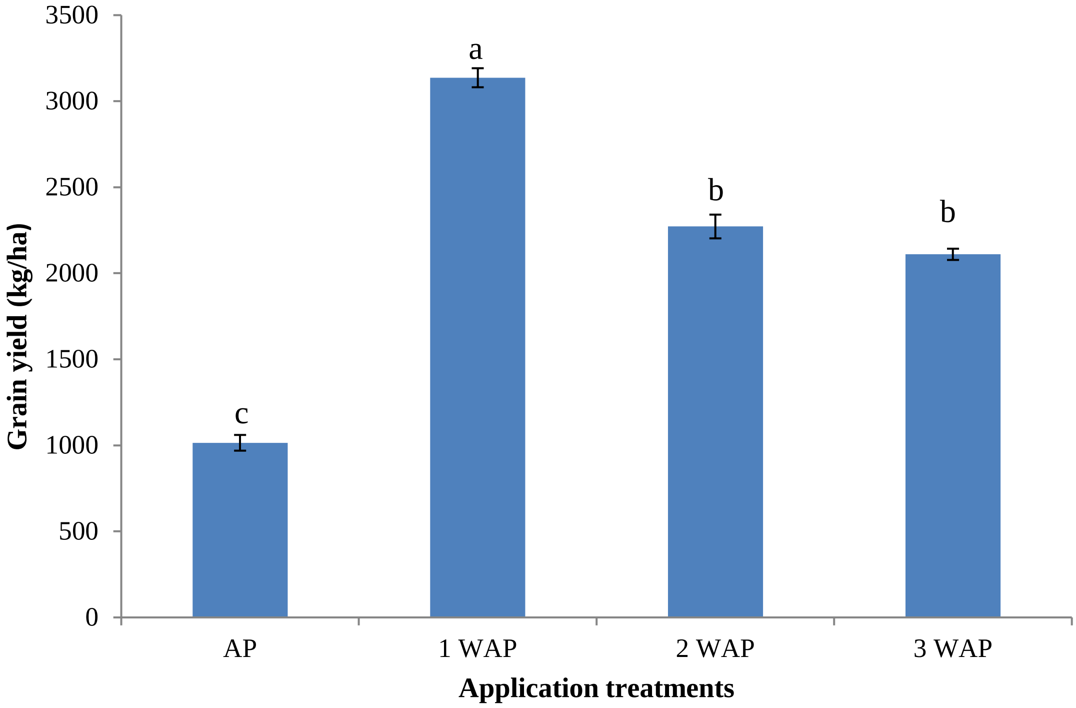

Figure 5. Effect of time of application of moringa leaf powder on grain yield of cowpea after harvest. Bars represent the standard error of the mean grain yield for each treatment. Lengths with different letters are significantly different at $P < 0.05$.

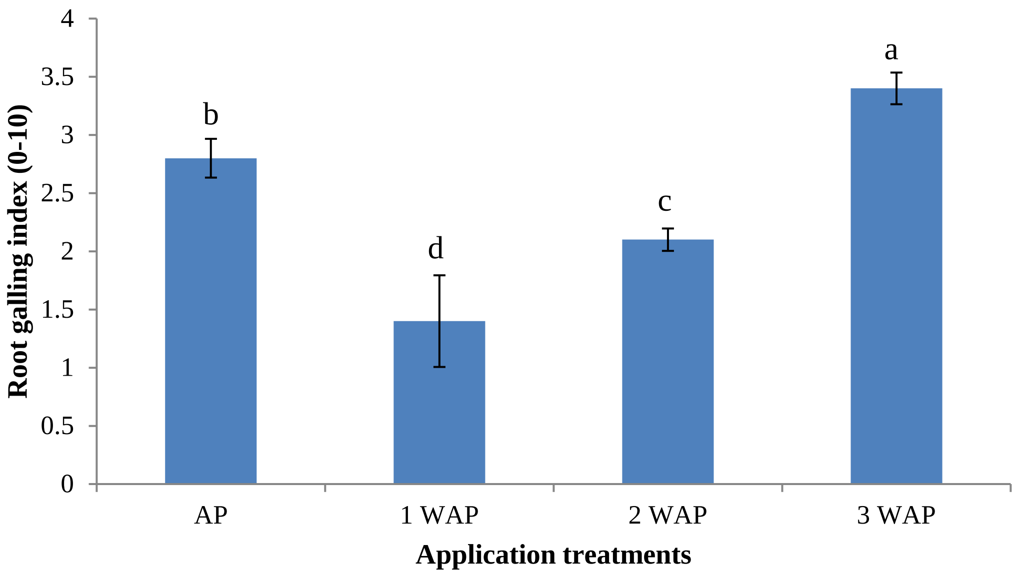

Figure 6. Effect of time of application of moringa leaf powder on root galling index at harvest. Bars represent the standard error of the mean root gall for each treatment. Lengths with different letters are significantly different at $P < 0.05$.

but by 8 WAP plants from the 2 WAP treatment were the tallest.

Number of leaves

There were significant differences ($P < 0.05$) among the treatments for leaf number (Figure 2). Plants of the 1 WAP treatment consistently produced a significantly higher ($P < 0.05$) number of leaves than those of the other treatments throughout the growing season.

Chlorophyll content

Significant differences ($P < 0.05$) were observed among treatments for chlorophyll content (Figure 3). Plants treated with moringa at 1 WAP consistently produced a significantly higher ($P < 0.05$) chlorophyll content than those of the other treatments throughout the cropping season.

Root weight

There were significant differences ($P < 0.05$) in root weight among the treatments (Figure 4). Plants treated with moringa leaf powder at 3 WAP had the heaviest roots while those treated at 1 WAP had the lightest.

Grain yield

There were significant differences in grain yield among treatments (Figure 5). Plants treated with moringa leaf powder at 1 WAP produced significantly more ($P < 0.05$) grain than those of the other treatments. Plants treated with the moringa leaf powder produced least amount of grain (100 kg/ha).

Root galling index

There were significant differences ($P < 0.05$) in root galling index at harvest among the treatments. Plants treated with the moringa leaf powder at 3 WAP had the highest galling index while those of the 1 WAP treatment had the lowest gall formation (Figure 6).

Incidence of Root-knot nematode juveniles in the soil

Plants treated with moringa leaf powder at planting had significantly higher ($P < 0.05$) number second stage juveniles of *Meloidogyne* spp. than those of the other treatments. Plants treated with moringa leaf powder at 1 WAP had the lowest incidence of second stage juveniles but it was not significantly different from the other treatments. Generally, application of moringa leaf powder after planting resulted in a significant reduction in number of second stage juveniles of *Meloidogyne* spp. population density compared to the application at planting (Table 1).

Discussion

The results of this study have shown that application of moringa leaf powder at different times is effective against *Meloidogyne* spp. under field conditions. Plants treated with moringa leaf powder at 1 WAP had the tallest plants which produced more leaves than those of the other treatments. It is possible that application of the moringa leaf powder promoted plant growth which confirms Fuglie (2001) report that moringa leaf extract contains some micronutrients and macronutrients such as calcium, potassium, iron, and magnesium which enhance plant development. Unlike the 2 WAP and 3 WAP treatments which were applied at a later stage, the 1 WAP treatment applied earlier, supplied the essential nutrients required for maximum growth and proper respiration (Reyhan and Amiraslani, 2006). Eisenback *et al*. (1991) also observed that heavily diseased plants do not respond to water because of the severity of damaged caused by nematodes to conducting tissues of the plant at the

Table 1. Effect of time of application of moringa leaf powder on incidence of *Meloidogyne* spp. J_2 in 200 cm^3 soil at harvest of cowpea.

Weeks after planting	**Untransformed mean of *M.* spp.**	**Transformed mean of *M.* spp.**
AP	1019.8a	31.94a
1	16.0b	4.12b
2	70.5b	8.43b
3	118.2b	10.89b
LSD (5%)	112.03	5.93

* $\sqrt{x+0.5}$ transformed, where x is mean count. Figures in a column followed by different letters are significantly different ($P < 0.05$).

root level resulting in reduction of top growth of the plant. His view holds true with the present findings where plant growth was proportionately affected with increase in number of galls and RKN population.

Chlorophyll content varied among treatments. This variation could be attributed to the nutritional composition of the extract used. This agrees with Mahmood *et al*. (2007) report an increase in chlorophyll content in Soybean due to application of moringa leaf powder. Macronutrients contained in the extracts as reported by Fuglie (2001) might have also contributed to the increase of the chlorophyll content of the cowpea plants. This finding agrees with Anjorine *et al*. (2001) who reported that *M. oleifera* leaf is rich in zeatin and other growth enhancing compounds like ascortase, phenolic and minerals that make it an excellent crop growth enhancer.

Variation in root weight demonstrated that application of moringa leaf powder responded differently at different times. This response could be attributed to nematicidal properties of the powder. This finding agrees with Knoblock *et al*. (1989) reported that nematicidal effects of moringa leaf powder on nematodes in the soil is probably due to the interference of the powder with the roots thereby dissolving the proteins.

The application of moringa leaf powder increased the yield of cowpea. The increase in yield could have resulted from a decrease in nematode population or from enhancement of the nutrients. The highest grain yield at 1 WAP may be attributed to the time of application and nutritional composition of the moringa leaf powder. This confirms Mahmood *et al*. (2007) report that application of moringa leaf extract resulted in an increase in yield of soybean. Yasmeen *et al.* (2012) also reported that moringa leaf extract contains several minerals that could increase the yield of wheat. Ploeg (2001) also reported that root-knot nematodes have greater effect on crop yield when they attack the roots of seedlings immediately after seed germination.

Application of moringa leaf powder at different times had a significant effect on both root galls and root-knot nematodes population after harvest. The 1 WAP treatment recorded the lowest population density of *Meloidogyne* spp. while the AP treatment recorded the highest. A significant increase in number of galls was also observed at all application times. Plants treated with moringa leaf extract at 3 WAP produced the highest number of galls followed by those treated at planting with those treated at 1 WAP as the least. The density of root-knot nematode multiplication was increased with the corresponding increase in application times (weeks) of moringa leaf powder. This might be due to the destruction of root system by the root-knot nematode which led to competition for food and nutrition among the developing nematodes within the root rhizosphere. The inability of juveniles to identify new infection courts for subsequent generation might also account for the reduction (Ogunfowora, 1977). The 1 WAP treatment recorded the lowest count of root galls which might be due to the low presence of root-knot nematodes. It might also be attributed to the nematicidal properties in the moringa that could have possibly been released at the right period to reduce the nematode population. Claudius-Cole *et al*. (2010) reported that water extracts of moringa were toxic to *Meloidogyne* spp. Sowley *et al*. (2014) also found aqueous extract of moringa leaf to be toxic to *Meloidogyne* spp. populations.

The findings of this study disagree with Youssef and Lashien (2013) report that application of 10 g of crushed cabbage leaf residue before planting was more effective in suppressing number of galls and number of juveniles of *Meloidogyne* spp. since 1 WAP treatment of moringa leaf powder in this study had a better effect. It was also observed that, as nematode population increases in the rhizosphere of the cowpea plant, the presence of root galling of cowpea plant roots also increases.

Conclusion

The study has proved that, application of moringa leaf powder at 1 WAP is the most effective for the control of root-knot nematodes of cowpea. Farmers should use moringa leaf powder at one week after planting to control root-knot nematodes on their cowpea farms since it is economic and environmental friendly.

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