CHAPTER 3

CONTROL OF *MELOIDOGYNE INCOGNITA* RACE 2 ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA)* BY MEANS OF SOIL AMENDMENTS WITH POULTRY AND CATTLE MANURE.

ABSTRACT

The effects of soil amendment with cattle and poultry manure on bambara groundnut (*Vigna subterranea* (L.) Verdc) variety DIPC were evaluated in the greenhouse. Treatments consisted of soil amended with 0.2, 0.4 and 0.8 kg/m^2 of each manure. Control treatments consisted of non-amended soil and a treatment with commercial fertilizer, $(NH_4)_2SO_4$. A nematicide treatment with fenamiphos was added for comparison. All treatments were evaluated with and without nematodes. The nematode population decreased as the manure application rate was increased. Poultry manure at 0.8 kg/m^2 was as effective as fenamiphos in reducing the nematode population. However, phytotoxicity was observed on plants receiving these treatments. Shoot mass was increased by 132 %-270 % in plants treated with cattle manure at 0.4 kg/m^2 and 0.8 kg/m^2 compared to the untreated control. A decrease in weight was recorded with the 0.8 kg/m^2 poultry manure, fenamiphos and fertilizer treatments. Cattle manure at 0.4 kg/m^2 was less phytotoxic than poultry manure at the same rate. The fertilizer treatment and the untreated control did not differ significantly with regard to nematode reduction, plant growth and yield.

3.1 Introduction

Organic soil amendment is widely used by farmers throughout the world with the aim of improving soil fertility and plant growth. The practice has been found to improve the physical characteristics of the soil and to reduce soil-borne pests and diseases (Stirling, 1991). According to Bridge (1996), amendments improve the nutrient and water-holding capacity of the soil thereby improving plant growth and hence increasing tolerance to nematodes. Riegel, Fernandez, & Noe (1996) suggested that organic amendment stimulate microbial activity in the soil. Some of these microorganisms are antagonistic to nematodes. A wide range of materials including amongst others poultry and cattle manure help control nematodes (Bridge, 1987; Rodriguez-Kabana, 1986; Chindo & Khan, 1990; Poswal & Akpa, 1991).

Incorporation of chicken manure into nematode-infested soil is reported to have reduced *M. incognita* numbers by 80 % (Kaplan & Noe, 1993). Previously, chicken litter was shown to reduce *M. incognita* populations on tomato with a concomitant increase in growth (Chindo & Khan, 1990). Riegel et al. (1996) reported low population density of *M. incognita* on cotton grown in plots amended with chicken litter.

Very little work has been done involving the use of cattle manure as an amendment to control *M. incognita*. Poswal & Akpa (1991) reported successful control of *M. incognita* with cow dung in Nigeria. Apart from this study most of the work involving cattle manure is unpublished. In Botswana small-scale farmers incorporate cattle manure into the soil mainly to improve soil fertility. It has been observed that addition of cattle manure to the soil reduces soil-borne pests and diseases including root-knot nematodes (unpublished observation).

Control of root-knot nematodes is best achieved through the use of nematicides. Nematicides are generally very expensive and their supply is erratic in rural areas. They are also hazardous to the environment as well as humans and livestock. It is therefore necessary to investigate non-chemical strategies like organic soil amendment using materials such as poultry and cattle manure as alternatives to nematicides.

The objectives of the study were therefore to investigate the efficacy of poultry and cattle manure on *M. incognita* race 2 and to determine the effective application dosage.

3.2 Materials and Methods

The experiment was conducted from January to April 1998 in the glasshouse at the University of Pretoria. The soil used in the experiment was a steam-pasteurized sandy loam soil (80 % sand, 4 % silt, 14 % clay and pH 6.0) prepared by mixing top soil with river sand at the ratio of 2:1. Twenty-five centimeter diameter plastic pots were each filled with 8 kg of soil and treated with a basal dressing of NPK (2:3:2) applied at the rate of 500 kg/ha.

The experiment consisted of 6 replicates of the following treatments:

- Soil amended with 0.2 kg/m^2 cattle manure
- Soil amended with 0.2 kg/m^2 poultry manure
- Soil amended with 0.4 kg/m^2 cattle manure

- Soil amended with 0.4 kg/m^2 poultry manure
- Soil amended with 0.8 kg/m^2 cattle manure
- Soil amended with 0.8 kg/m^2 poultry manure
- Untreated control
- Control supplemented with $(NH_4)_2SO_4$ applied at the rate of 80 kg N/ha to supply the equivalent amount of N as in 0.4 kg/m^2 poultry manure
- Fenamiphos applied at 2 liter/ ha included as a separate treatment for comparison.

Manure samples were ground into a fine powder, sifted to remove large particles, and subsequently added to the soil. All treatments except fenamiphos were applied separately to *M. incognita* race 2-inoculated and non-inoculated soil. Fenamiphos was applied to *M. incognita*-inoculated soil only. The amendments were thoroughly mixed with soil and watered to field capacity using a watering can. Chemical analysis of the two types of manure was done by the soil analysis laboratory, Department of Plant Production and Soil Science, University of Pretoria.

Inoculation was done immediately after application of amendments to the soil. Each treatment received 1 g of heavily galled tomato roots infected with a South African isolate of *M. incognita* race 2 (egg mass index = 5). Each egg mass contained an estimated 250 eggs. Roots were cut into 1-cm-long pieces, mixed with 5 ml of tap water, and incubated in petri dishes for 24 hours to allow eggs to hatch. The resulting inoculumn consisted of a mixture of eggs and juveniles. The inoculumn was placed in depressions in the center of each pot and covered with soil. Amended treatments were set aside for a week to allow the manure to decompose before planting.

Planting was done one week after inoculation and the fenamiphos treatment was applied at this stage. Three seeds of bambara groundnut variety DIPC were planted per pot. Treatments were arranged on benches in the greenhouse in a completely randomized design and watered daily. Seedlings were thinned to one seedling per pot four weeks after planting. To assess the effects of treatments at midseason (four weeks after planting), one plant per pot was removed, shoots were cut and dried in an oven at 60 °C for three days and weighed. Roots were washed free of soil and stained in 0.15 g/liter aqueous solution of Phloxine B for 15 minutes and evaluated for galls and egg masses using a gall/egg mass rating scale of 0-5 as described by Taylor & Sasser (1978). Root mass was not determined at midseason.

Plants were harvested after ten weeks and evaluated following the same procedures used at midseason. In addition, roots were gently washed free of soil and their fresh mass determined. Roots were then evaluated for galls and egg masses as previously explained. Pods were counted and oven-dried at 60 °C for three days to determine their dry mass. Data were analyzed by ANOVA and the means separated by Duncan's multiple range test (SAS, BMDP Statistical Software, Los Angeles, CA). Gall and egg mass index data were ranked and means separated using Kruskal-Wallis tests for non-parametric data (Steel & Tourie, 1981). Because the gall and egg mass index values were the same, only the gall index data are presented.

3.3 Results

Results of the chemical analysis of manure samples are presented in Table 3.3a. According to this chemical analysis, poultry manure contained more nutrients than cattle manure. Phytotoxicity was observed in all plants that received the 0.8 kg/m^2 dosage of poultry manure and in plants treated with fenamiphos. These treatments had to be terminated because they died prematurely. No galls or egg masses formed on all plants grown in soil amended with 0.8 kg/m^2 poultry manure and in soil treated with fenamiphos. Phosphorus deficiency symptoms (dark leaves) were noticed in the control, fertilizer, cattle manure (0.2 kg/m^2 and 0.4 kg/m^2) and poultry manure (0.4 kg/m^2) treatments. Leaves were darker green in these plants than in other treatments. There was great variation in leaf growth among plants. Treatments that received a lower dosage of both poultry and cattle manure tended to have tiny, sharp pointed leaves while those receiving higher dosages had broader leaves.

The effects of the different amendments on *M. incognita* at midseason are presented in Table 3.3b. Treatments showed significant differences with regard to gall index, egg mass index and dry mass of shoots. The non-amended control had the highest gall and egg mass index compared to other treatments. No significant differences in gall and egg mass index occurred in plants receiving cattle manure applied at all three dosages, poultry manure applied at 0.2 kg/m^2 and 0.4 kg/m^2 and the control amended with $(NH_4)_2SO_2$.

Plants that received cattle manure at the rate of 0.4 kg/m^2 and 0.8 kg/m^2 had the highest increase in shoot weight compared to the non-amended control (147 % and 162 % respectively). The lowest increase in shoot weight (22 %) was recorded in plants grown in soil treated with cattle manure at the rate of 0.2 kg/m^2. A decrease in weight was recorded in plants that received poultry manure at the rate of 0.8 kg/m^2, plants treated with fenamiphos, and in plants grown in soil amended with $(NH_4)_2SO_4$ (Table 3.3b).

At harvest, the gall indices of plants grown in the non-amended control soil were significantly different from those treated with fenamiphos and poultry manure applied at 0.4 kg/m^2. The gall index was higher in plants grown in non-amended soil than in any of the treatments. Other treatments were not significantly different from the non-amended control in gall indices (Table 3.3c).

Cattle manure increased the dry mass of shoots by 270 %, 132 % and 49 % when it was applied at rates of 0.8 kg/m^2, 0.4 kg/m^2 and 0.2 kg/m^2 respectively compared to the non-amended control. No significant differences occurred in the dry mass of shoots of plants from soil treated with poultry manure at the rate of 0.2 kg/m^2 and 0.4 kg/m^2 (Table 3.3c). A decrease in shoot mass was recorded in plants grown in soil treated with fenamiphos and in those from the control supplemented with $(NH_4)_2SO_4$. The dry mass of shoots of plants from $(NH_4)_2SO_4$-supplemented control was not significantly different from those from the non-amended non-supplemented control (Table 3.3c).

Treatment of soil with fenamiphos, and $(NH_4)_2SO_4$ reduced the fresh weight of bambara groundnut roots compared to the non-amended control. Root weight was increased by the addition of other treatments (Table 3.3 c). There was no significant increase in root weight between poultry manure applied at the rate of 0.2 kg/m^2, and 0.4 kg/m^2, and cattle manure applied at all three rates.

Yield (number and dry mass of pods) was increased in plants treated with cattle manure applied at all three rates and poultry manure applied at the rate of 0.2 kg/m^2 and 0.4 kg/m^2 compared to the non-amended control. Treatment of soil with fenamiphos, and $(NH_4)_2SO_4$ resulted in a decrease in yield (Table 3.3c)

3.4 Discussion

The general decrease in galling and reproduction of *M. incognita* race 2 on plants grown in soil amended with poultry and cattle manure at midseason confirms earlier report that addition of manure to soil reduce population densities of plant-parasitic nematodes (Main & Rodriguez-Kabana, 1982). Effective control was achieved when poultry manure was applied at the rate of 4-10 ton/ha. In the present study, poultry manure applied at the rate of 0.8 kg/m^2 (8 ton/ha) was as effective as fenamiphos applied at 2 liter/ha. Phytotoxicity due to poultry manure only occurred at 0.8 kg/m^2 and not at the lower rates.

The phytotoxicity observed with fenamiphos-treated plants suggests that this nematicide is not suitable for use on bambara groundnut. The nematicide has not been tested on this crop before and is therefore not registered on bambara.

Plants from soil amended with cattle manure at 0.4 kg/m^2 and 0.8 kg/m^2 had the highest increase in shoot weight probably because of an increase in nutrients. Unlike poultry manure, cattle manure was not phytotoxic when applied at high dosages. The general decrease in shoot weight observed in treatments receiving poultry manure at 0.8 kg/m^2, fenamiphos, and $(NH_4)_2SO_4$ can be attributed to phytotoxicity.

The gall index on plants grown in soil amended with poultry manure at 0.4 kg/m^2 was significantly lower than in other treatments except fenamiphos where no galls formed. Gall indices declined with increasing rates of amendments thus confirming findings by Chindo & Khan (1990). In soil amended with 0.4 kg/m^2 poultry manure, there was a sustained reduction in gall formation (reflected in the gall index) up to harvest. Poultry manure applied at the rate of 0.2 kg/m^2 was not different from the control. For soil amendment to be effective against root-knot nematodes, the application rate, which is lower than the threshold for phytotoxicity but still effective against the nematode, should be applied. In this study it is 0.4 kg/m^2 (4 ton/ha) and corresponds to previous findings by Chindo & Khan (1990).

Cattle manure applied at 0.4 kg/m^2 and 0.8 kg/m^2 was as effective as poultry manure at 0.4 kg/m^2 in reducing galling. This efficacy along with the fact that it was less phytotoxic gives cattle manure an advantage over poultry manure. When cattle manure was applied at 0.4 kg/m^2 and 0.8 kg/m^2, it had an added advantage in that it increased shoot weight of bambara groundnut plants by 132 % and 270 % respectively compared to 62 % for poultry manure at 0.4 kg/m^2. All the amendments caused an increase in root weight over the untreated control. Cattle manure applied at 0.4 kg/m^2 resulted in 134 % increase in yield (number of pods) compared to the non-amended control. The consistent increase in yield and plant growth makes this treatment viable for effective nematode management. A dosage of

0.8 kg/m^2 (8 ton/ha) is equally effective but this quantity will be uneconomic to use when 4 ton/ha could give the same results.

The $(NH_4)_2SO_4$ treatment was not different from the control with regard to nematode reduction, and growth and yield parameters. According to Sinclair (1975), for inorganic fertilizers to be effective as nematicides, the material must be applied in excess of those required for crop fertilization. The fertilizer in this treatment was applied at the rate equivalent to the amount of N in 0.4 kg/m^2 poultry manure. This produced similar results to poultry manure at the rate of 0.4 kg/m^2 in terms of gall indices thus supporting previous reports (Riegel et al., 1996). However, growth and yield decreased indicating phytotoxicity, probably due to the high rate of N provided.

The results obtained in this study suggest that the control achieved when using poultry and cattle manure could be a result of increased nutrient availability to the plant or production of toxicants fom decomposition of the manure (Chindo & Khan, 1990). Production of toxic products can further be supported by the phytotoxicity observed on plants receiving these amendments at high dosages. It is also possible that control could be due to changes in microbial activity in the soil as a result of addition of organic matter (Riegel et. al., 1996). Soil microorganisms were reported to have been stimulated by the addition of manure to the soil (Rodriguez-Kabana, 1986). According to Rodriguez-Kabana (1986), some of these microorganisms may be antagonistic or predators of the nematode.

It has been established in this study that control of *M. incognita* race 2 on bambara groundnut by means of soil organic amendment using cattle manure, rather than poultry manure, can be more advantageous because of the lower phytotoxicity associated with cattle manure. The increase in growth and yield were also more pronounced on plants receiving cattle manure than in those receiving poultry manure.

3.5 REFERENCES

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Table 3.3a: Results of chemical analysis for poultry and cattle manure samples

Manure	N %	P %	K %	CA %	Mg %	Na %	SO4 %	Cu $mgkg^{-1}$	Fe $mgkg^{-1}$	Mn mg/kg^{-1}	Zn mg/kg^{-1}	C %
Poultry	3.50	1.90	2.25	7.44	1.42	0.30	11.25	44	2796	425	306	1.19
Cattle	2.15	1.60	1.85	2.56	1.29	0.70	1.93	56	1496	42	318	1.18

Table 3.3b: Effect of different organic amendments on galling and reproduction of *M. incognita* race 2 on *Vigna subterranea* at midseason.

Treatment	Ranked gall index	Dry wt. of shoots (g)	% Increase in shoot weight
Untreated control	275.50a	2.98c	-
$(NH_4)_2SO_4$	214.00ab	2.56cd	-14 %
Poultry manure (0.4 kg/m^2)	178.50ab	5.01b	68 %
Poultry manure (0.2 kg/m^2)	163.00ab	5.47b	84 %
Cattle manure (0.2 kg/m^2)	159.50ab	3.64c	22 %
Cattle manure (0.4 kg/m^2)	159.50ab	7.36a	147 %
Cattle manure (0.8 kg/m^2)	143.00ab	7.81a	162 %
Fenamiphos (2 liters/ha)	96.00c	1.63 de	-45 %
Poultry manure (0.8 kg/m^2)	96.00c	1.02e	-66 %

Each value is the mean of 6 replicates. Means in each column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. Ranked gall indices followed by the same letter do not differ significantly at $P \leq 0.05$ according to Kruskal-Wallis test for non-parametic data.

Table 3.3c: Effect of different organic amendments on galling caused by *M. incognita* race 2 and on growth and yield of *Vigna subterranea* at harvest

Treatment	Ranked gall index	Dry wt. of shoots (g)	Fresh wt. of roots (g)	Number of pods	Dry wt. of pods (g)
Untreated control	273.00a	3.62d -	33.14bc -	11.33bc -	0.16bc -
Poultry manure (0.2 kg/m^2)	210.00ab	5.90c (63 %)	56.12a (69 %)	15.33ab (35 %)	1.53a (856 %)
Cattle manure (0.2 kg/m^2)	183.00ab	5.40c (49 %)	56.61a (71 %)	16.50ab (46 %)	0.76abc (375 %)
$(NH_4)_2SO_4)$	158.50abc	2.53de (-30 %)	19.20c (-42%)	10.00bc (-12 %)	0.48bc (200 %)
Cattle manure (0.4 kg/m^2)	129.50abc	8.40a (132 %)	57.02a (72 %)	26.50a (134 %)	1.27ab (694 %)
C attle manure (0.8 kg/m^2)	103.00abc	13.40a (270 %)	59.55a (80 %)	16.83ab (49 %)	0.99abc (519 %)
Poultry manure (0.4 kg/m^2)	95.00bc	5.88c (63 %)	47.41ab (43 %)	18.50ab (63 %)	1.05ab (556 %)
Fenamiphos (2 liter/ha)	24.00c	1.93e (-47 %)	18.13c (-45 %)	2.50c (-78 %	0.16c (-)

Each value is the mean of 6 replicates. Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. Ranked gall indices followed by the same letter do not differ significantly at $P \leq 0.05$ according to Kruskal-Wallis test for non-parametic data.

Figures in brackets represent % increase in growth and yield compared to control.