CHAPTER 5

COMPARISON OF SOIL AMENDMENTS, BIOFUMIGATION AND BIOLOGICAL CONTROL AGENTS FOR CONTROL OF *MELOIDOGYNE INCOGNITA* RACE 2 ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA*).

ABSTRACT

A study was conducted in the greenhouse to compare a range of non-chemical control measures for the control of *M. incognita* race 2 on bambara groundnut. Treatments consisted of soil amendments with cabbage applied at the rate of 4 kg/m^2, cattle manure at 4 kg/m^2, and a commercial biocontrol agent Biostart® 2000, aldicarb, fertilzer supplement of KNO_3 plus $Ca(NO_3)_2$, and a fertilizer supplement of $(NH_4)_2NO_3$. The effects of the treatments on gall and egg mass indices, and on growth of infected bambara groundnut were evaluated eight weeks after planting. All treatments reduced galling and egg mass index to some degree. There was no significant difference in gall and egg mass indices between plants treated with cattle manure, cabbage, Biostart® 2000, and fertilizer supplements. Aldicarb resulted in the greatest reduction of the nematode population.

5.1 Introduction

Root knot nematode, *Meloidogyne incognita* race 2 on bambara groundnut was successfully controlled in previous experiments by various control methods such as soil organic amendments, biofumigation, solarization and the use of nematicides. These control methods

gave various degrees of success in controlling *Meloidogyne* species on other crops such as tomato (Chindo & Khan, 1990; Kaplan & Noe, 1993; Stapleton & Duncan, 1998), citrus (McLeod & Da Silva, 1994), potato (Motjahedhi, Santo, Hang & Wilson, 1991; Motjahedhi, Santo, Wilson & Hang, 1993), and water melon (Keinath, 1996) to mention a few. However it was observed that some of these control methods were not very effective when used individually and needed to be complemented by other methods in order to improve control (Keinath, 1996). It is therefore necessary to evaluate these control methods together and compare their effects in order to determine how they can be used in an IPM program for *M. incognita* race 2 on bambara groundnut.

The objective of this study was therefore to determine the effectiveness of a range of non-chemical control measures in comparison with a commercial nematicide for control of *M. incognit*a race 2 on bambara groundnut.

5.2 Materials and methods

The study was conducted in the greenhouse with temperatures maintained between 20 and 30 °C. The soil used was a steam pasteurized sandy loam soil (80 % sand, 4 % silt, 14 % clay and pH 6.0) prepared by mixing topsoil with river sand at the ratio of 2:1. The volume of soil contained in a 25 cm diameter plastic pot was weighed and the amount of plant material required as amendment per pot calculated accordingly. The leaves and stems of three and a half months old cabbage (*Brassica oleracea capitata* L.), variety Drumhead were cut into 1cm long pieces and weighed to determine the amount of amendment needed per pot. Cattle manure was ground into a fine powder and sifted to remove large particles. The

manure was subsequently added to dry soil at the rate of 0.8 kg/m^2. A commercial biological control product Biostart® 2000 was obtained from Microbial Solutions (Pty) Ltd (P.O. Box 103, Kya, Sand, 2163, South Africa). The product contains *Bacillus laterosporous*, *Bacillus chitinosporous*, and *Bacillus licheniformis*.

The experiment comprised of the following six treatments replicated six times and arranged in a completely randomized design:

- Soil amended with cabbage variety Drumhead at the rate of 4 kg/m^2
- Soil amended with cattle manure at the rate of 0.4 kg/m^2
- Biostart® 2000 (1:1 mixture of biostart and microboost dissolved in 100 ml water) applied at the rate of 5 ml/pot
- Aldicarb applied at the rate of 5 g/m^2
- Supplement of KNO_3 and $Ca(NO_3)_2$ applied at the rate of 60 kg N/ha
- Supplement of $(NH_4)_2NO_3$ applied at the rate of 60 kg N/ha.

The two fertilizer supplements were included as controls to compare the effects of the different nitrogen sources on the nematode population as was previously reported by (Rodriguez-Kabana, 1986). For the biofumigation treatment, Quantities of 9 kg of soil were thoroughly mixed with cabbage residues, watered to field capacity, put in plastic bags and placed on the floor in the greenhouse. The cattle manure was incorporated into the soil and put in 25 cm diameter plastic pots and after thorough mixing watered to field capacity. The pots were placed on benches in the greenhouse. Each treatment was applied separately to *M. incognita* race 2 inoculated and non-inoculated soil.

The *Meloidogyne* inoculumn consisted of a suspension of eggs and juveniles prepared as follows: tomato roots heavily infected with *M. incognita* race 2 (egg mass index = 5) were washed free of soil, cut into 1 cm long pieces and incubated in water for 12 hours to allow for hatching of the eggs. A 5ml suspension of eggs and juveniles was poured into a recess in the soil and recesses were subsequently covered with soil. The nematode inoculum was applied at the time of planting except for the soil amendments where inoculum was incorporated into the soil together with the amendment to allow for biofumigation and biocontrol activities to take effect.

Three seeds of bambara groundnut variety DIPC were planted in each pot and thinned to one seedling per pot four weeks after emergence. The Biostart®2000 treatment was applied every two weeks and terminated three weeks before harvest. Plants were harvested ten weeks after planting and assessed for nematode damage. The fresh root mass was determined prior to evaluating roots for gall and egg masses. Roots were stained in a 0.15g/liter aqueous solution of Phloxine B (Hussey & Barker, 1973), and evaluated for gall and egg mass indices using a rating scale of 0-5 (Taylor & Sasser, 1978). Shoots were dried in an oven at 60 °C for three days and the dry mass determined. All data were analyzed statistically by ANOVA and means separated using Duncan's multiple range test.

5.3 Results

There were significant differences between treatments with regard to gall and egg mass indices. Plants from the control supplemented with $(NH_4)_2NO_3$ had the highest gall and egg mass indices while the lowest indices were recorded in the aldicarb treatment. Gall and egg

mass indices were reduced by 8-58 % in plants treated with Biostart® 2000, cattle manure, fertilizer, cabbage and aldicarb respectively, compared to the control supplemented with $(NH_4)_2NO_3$ (Table 5.3 a). There was no significant difference between the Biostart®2000 treatment and the $(NH_4)_2NO_3$ control in terms of gall and egg mass indices. No significant differences in gall and egg mass indices occurred between treatments with a combination of KNO_3 plus $Ca(NO_3)_2$, cattle manure and Biostart® 2000 (Table 5.3a).

The nematode had a dramatic effect on the root mass of inoculated plants compared to the non-inoculated control plants (Fig 5.1). Plants grown in non-inoculated soil had greater root mass than the *M. incognita* race 2-infected plants. (Table 5.3b). In plants treated with KNO_3 plus $Ca(NO_3)_2$, cattle manure, and $(NH_4)_2NO_3$, root mass was greater than in infected plants treated with cabbage, Biostart ® 2000 and aldicarb There were significant differences between the fresh weight of roots of inoculated and non-inoculated plants from KNO_3 plus $Ca(NO_3)_2$ treatment and the $(NH_4)_2NO_3$ control. No significant differences in weight occurred between roots of inoculated and non-inoculated plants receiving cattle manure. There were significant differences between root weight of inoculated and non-inoculated plants treated with Biostart® 2000, and aldicarb. No significant differences in weight occurred between roots of inoculated and non-inoculated plants from cabbage treatments (Table 5.3b). Phytotoxicity symptoms ocurred on plants treated wih cabbage, Biostart® 2000 and aldicarb. Roots of plants receiving these treatments were rotten and stunted compared to the other treatments.

Significant differences occurred in the dry mass of shoots between inoculated and non-inoculated plants from soil treated with KNO_3 combined with $Ca(NO_3)_2$ and the $(NH_4)_2NO_3$ control (Table 5.3b). No significant differences in dry mass were observed in other treatments. The dry mass of shoots of plants inoculated with *M. incognita* race 2 in soil treated with KNO_3 combined with $Ca(NO_3)_2$, and cattle manure, was significantly different from that of the other treatments. Dry mass of shoots of inoculated plants treated with cattle manure, aldicarb, and control supplemented with $(NH_4)_2NO_3$ did not differ significantly. However, plants treated with cattle manure were significantly different from those treated with cabbage and Biostart® 2000 (Table 5.3b).

5.4 Discussion

All the treatments evaluated in this study were able to give some degree of control of *M. incognita* race 2 on bambara groundnut although the effect of Biostart® 2000 was insignificant. Although the control obtained was not dramatic, the results confirmed the potential of using cabbage residues, cattle manure, fertilizer (KNO_3 plus $Ca(NO_3)_2$, Biostart® 2000, and aldicarb as IPM components for *M. incognita* race 2 on bambara groundnut. Incorporation of cabbage residues into the soil reduced galling of bambara groundnut roots and incidence of *M. incognita* race 2 egg masses by 33 % compared to the $(NH_4)_2NO_3$ control, whereas aldicarb resulted in a 58 % reduction. Previously, *M. incognita* population was successfully controlled by the incorporation of cabbage residues into the soil on other crops (McLeod & Da Silva, 1994; McLeod, Somers & Gendy, 1995). It has been reported previously that this practice on its own is not very effective unless it is combined with other control methods to enhance its efficacy (Gamliel & Stapleton, 1997).

Aldicarb has been used successfully to control nematodes on cowpea, a relative of bambara groundnut, but it has not been tested on bambara groundnut before. The 58 % control achieved in this study warrants further evaluation of aldicarb on this crop.

Addition of KNO_3 plus Ca $(NO_3)_2$ to the soil reduced galling of bambara groundnut roots and egg mass index of *M. incognita* race 2 by 22 % compared to the $(NH_4)_2NO_3$ control confirming previous reports that nitrogenous fertilizer have detrimental effects on nematodes (Rodriguez-Kabana, 1986). According to Rodriguez-Kabana (1986), inorganic and organic fertilizers containing ammoniacal nitrogen have the greatest effect on nematode populations. The nitrogenous fertilizer used in this study was applied at a very low rate hence it can not be accurately concluded that the reduction in nematode population was due to the nitrogen released into the soil. Riegel, Fernandez & Noe (1996) evaluated KNO_3 against *M. incognita* on cotton and observed an increase in juvenile population in plants receiving this treatment. In this study, the combination of KNO_3 with Ca $(NO_3)_2$ resulted in a 22 % reduction in *M. incognita* race 2 population. According to Rodriguez-Kabana (1986), inorganic nitrogenous fertilizers are effective against nematodes when they are applied at levels far in excess of those required for normal fertilization. Since such levels are toxic to plants, in this study, fertilizers were applied at rates required for normal fertilization and resulting in only 22 % reduction in nematode population

In the present study, soil amendment with 0.4 kg/m^2 cattle manure reduced gall formation by *M. incognita* with 21 % compared to the control. This reduction was similar to that obtained when the soil was treated with a combination of the two mineral fertilizers (KNO_3 and

$Ca(NO_3)_2$. Cattle manure was previously reported to be effective against *Meloidogyne* species (Poswal & Akpa, 1991). As in the case of mineral fertilizers, the control achieved could be as a result of nutrients available to the plant and release of chemicals such as ammonia that are detrimental to the nematodes (Stirling, 1991).

Treatment of soil with Biostart ®2000 only reduced galling of bambara groundnut roots and incidence of *M. incognita* race 2 egg masses by 8 % compared to the $(NH_4)_2NO_3$ control According to the suppliers of Biostart® 2000, the product acts by dissolving the chitin of the eggshell and this inhibits egg development. The inoculum used in this study consisted of egg masses, which were incubated for 12 hours to allow eggs to hatch so that the final inoculumn could include some juveniles. It is possible that most eggs hatched and resulted in more juveniles than eggs, hence the Biostart® 2000 treatment being ineffective. The Biostart® 2000 treatment was applied every second week. Since the life span of the bacteria is approximately eight days, the population probably declined to small numbers before the next application. Ideally, the product should have been applied weekly.

Addition of cattle manure and fertilizer to the soil improved the plant growth, corresponding with previous reports (Poswal & Akpa, 1991; Riegel et al., 1996). Although the mode of action of organic matter is complex and a number of mechanisms appear to be involved, this improvement in plant growth could be attributed to the changes in the nutrient status and physical characteristics of the soil (Stirling, 1991). It common knowledge that addition of inorganic fertilizer to the soil results in improvement of plant growth (Riegel et al., (1996)

Treatment with cabbage, Biostart® 2000, and aldicarb did not improve plant growth. This could be attributed to phytotoxicity that was observed in plants receiving these treatments confirming earlier findings in this study. Although Biostart® 2000 is reported to promote plant growth (G. Limmerick, P. O. Box 103, Kya Sand, 2163, South Africa, personal communication), it was not confirmed in this study.

5.5 REFERENCES

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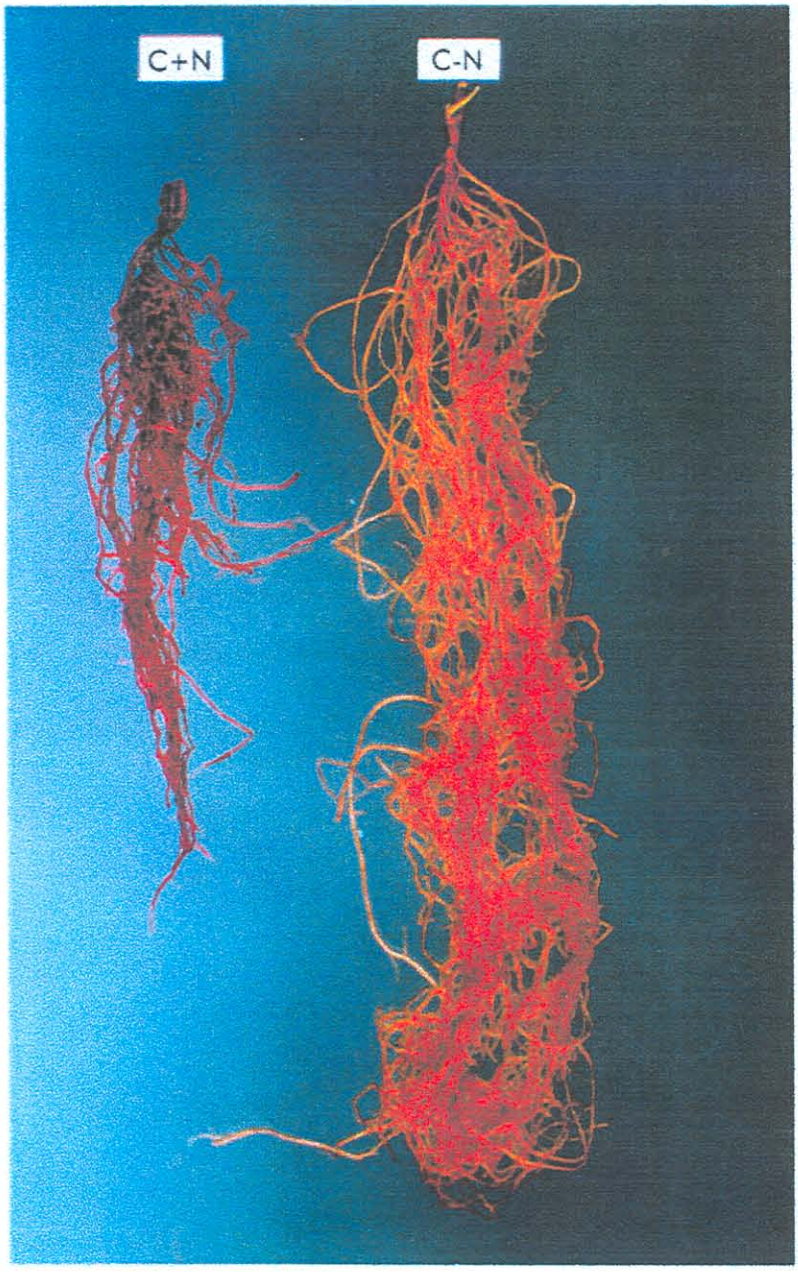

Fig. 5.1: Effects of *M. incognita* infection on roots of bambara groundnut after ten weeks in the greenhouse. C + N = Control with nematodes, C – N = Control without nematodes. Roots were stained with Phloxine B to enhance egg masses.

Table 5.3a: Effect of different soil treatments on galling of *Vigna subterranea* ten weeks after planting in the greenhouse in soil inoculated with *M. incognita* race 2.

Treatments	Ranked gall index
$(NH_4)_2NO_3$ (Control 1)	67.0a
Biostart ®2000	61.9ab (8)
Cattle manure	52.6bc (21)
KNO_3 and $Ca(NO_3)_2$ (Control 2)	52.0bc (22)
Cabbage	44.6c (33)
Aldicarb	28.3d (58)

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. Figures in brackets represent the % increase or decrease compared to the $(NH_4)_2SO_4$ control.

Table 5.3b: Effect of different soil treatments on growth of *Vigna subterranea* inoculated with *M. incognita* race 2, ten weeks in the greenhouse.

Treatment	Fresh weight of roots (g)		Dry weight of shoots (g)	
	Inoculated	Non-inoculated	Inoculated	Non-inoculated
KNO_3 and $Ca(NO_3)_2$ (Control 2)	7.35aA	10.83aB	1.82aA	2.37aB
Cattle manure	6.93aA	7.61abcA	1.62abA	1.57bcA
$(NH_4)_2NO_3$ (Control 1)	4.38abA	7.92abB	1.19bcA	1.79abB
Cabbage	3.50bA	4.24cA	1.13cA	0.93cA
Biostart®2000	3.27bA	6.11bcB	0.91cA	1.43bcA
Aldicarb	3.25bA	7.56abcB	1.24bcA	1.49bcA

Each value is the mean of 6 replicates. Means in each vertical column followed by the same lower case letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test. Means within each parameter followed by the same upper case letter do not differ significantly according to the t test.