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Pathogenicity of *Meloidogyne incognita* Race 1 on Turmeric (*Curcuma longa* L.) as Influenced by Inoculum Density and Poultry Manure Amendment

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Abstract: A glasshouse experiment was conducted to assess the pathogenicity of *Meloidogyne incognita* race 1 on turmeric (*Curcuma longa* L.) at three inoculum densities (0, 2500, 5000 eggs plant^{-1}) and soil amendment with different levels of Poultry Manure (PM) (0, 10, 20, 30 and 40 t ha^{-1}). The results obtained showed that root-galling and egg production decreased in a linear response to increasing PM level at both low and high inoculum densities ($r = -0.88$ and $r = -0.85$, respectively; $p \leq 0.01$). The lowest gall index and egg mass index of 2.0 were recorded in plants amended with 40 and 30 t ha^{-1} PM, respectively. The high nematode density was more pathogenic ($p \leq 0.05$) than the low density. Shoot length, fresh shoot and root weights responded in a curvilinear pattern with increasing levels of PM. In general, these growth variables increased with increase in PM level up to 20 t ha^{-1} and then declined with further increase. At high inoculum density, shoot growth was enhanced by 242 and 58% with 20 and 40 t ha^{-1} PM relative to unamended soil, respectively. Conversely, fresh root weight was increased by 120% and reduced by 12.8% with 20 and 40 t ha^{-1} PM, respectively. From this study, it could be inferred that higher levels of PM were highly nematicidal but phytotoxic to turmeric plants; the optimal rate of PM amendment is predicted to be 18-22 t ha^{-1}. However, for this method to be incorporated into Integrated Nematode Management Programme of turmeric, field trials are needed for the optimization of the quantity, time and method of application.

Key words: Turmeric, *Meloidogyne incognita*, poultry manure, soil amendment, root-knot nematode, tolerance, *Curcuma longa*

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a rhizomatous, herbaceous perennial flowering plant of the ginger family, Zingiberaceae. The plant is best known as a condiment, although it has uses in the social and religious lives of people in South-East Asia, its probable centre of origin. It is grown mostly in India and small quantities are produced in China, Indonesia, Peru and Jamaica. According to Selvan (2002), the total area under cultivation in India as at 1999-2000 was 161,300 ha with a production figure of 653,600 metric tonnes. The crop is less produced in Nigeria compared to its close relative, ginger. The rhizome is indispensable in the preparation of curry powder and is also an important source of natural yellow dye. Turmeric is also used as a colouring additive in the drug, confectionery and food industries (Khanna, 1999). Of late, it has been reputed for its medicinal properties. Turmeric contains bout 5% essential oil, 3% curcumin, polyphenols and some curcuminoids (Khanna, 1999). Curcumin and various curcuminoids have been reported to exhibit a wide range of biological activities against some pathogenic bacteria, fungi and insects (Phan, 2001; Tripathi *et al*., 2002). Turmeric is also associated with the lowering of cholesterol level and inhibition in the oxidation of LDL in rabbits with experimental atherosclerosis (Rarminez-Tortosa *et al*., 1999).

The cultivation of *C. longa* L. is constrained by both biotic and abiotic stress factors. A number of species of plant parasitic nematodes have been reported to attack turmeric (Bai *et al.,* 1995) of which *Meloidogyne* sp., *Radopholus similis* and *Pratylenchus coffeae* are of economic importance. Turmeric plants infected with *M. incognita* are reported to have large root galls, stunted growth, yellowing, marginal and tip drying of leaves and reduced tillering with galling and rotting of roots (Ray *et al*., 1995; Koshy *et al*., 2005). Mani *et al.* (1987) observed that infested rhizomes tended to loss their bright yellow colour. Levels of protein, carbohydrate, chlorophyll a and b and curcumin were lower in plants infested with *M. incognita* (Poornima and Sivagami, 1998). The inoculum density accounts in part for the severity of

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damage induced by root-knot nematodes (Udo and Nwagwu, 2007) on crops. One hundred juveniles of *M. incognita* caused significant reduction in growth characters of turmeric (Haidar *et al*., 1998a). Similarly, Poornima and Sivagami (1998) reported that an initial inoculum level of more than 5,000 *M. incognita* larvae/plant was highly pathogenic to turmeric.

The management of root-knot disease on turmeric with chemical nematicides has been very effective with a yield increase over 70% (Gunasekharan *et al*., 1987; Haidar *et al*., 1998b) and nematode population reduction of over 80% (Mani *et al*., 1987). However, the prohibitive cost, adverse effects on non-target organisms and the environment as well as associated health hazards are serious drawbacks to the use of synthetic chemical nematicides. Plant resistance has also been utilized in the management of this pest on turmeric (Gunasekharan *et al*., 1987; Mani *et al*., 1987; Eapen *et al*., 1999) but the problem of resistance-breaking pathotypes developing and availability of planting materials are also serious constraints (Castagnone-Sereno, 2002). The quest for alternative management strategies of root-knot disease due to the removal of some effective nematicides (Methyl bromide, EDB, DBCP, etc.) from the pesticide market has spurred research on the role of organic amendments in suppressing nematode population in the soil (Ogbuji, 1981; Chindo and Khan, 1990; Nwanguma and Awoderu, 2002; Nwanguma and Fawole, 2004; Agu, 2008). Although turmeric is less cultivated compared to ginger in Nigeria, it is fast gaining wide popularity among progressive farmers and root-knot problem may reach economic proportion if not checked. This study therefore aims at evaluating the effects of different inoculum densities and levels of poultry manure amendments on the pathogenicity of *Meloidogyne incognita* race 1 on turmeric (*C. longa* L.) in a derived savanna zone of Nsukka, South Eastern Nigeria.

MATERIALS AND METHODS

The study was conducted in a sun-lit glasshouse of the Department of Crop Science, University of Nigeria, Nsukka between April and August, 2008. Nsukka lies in a derived savanna belt of Nigeria (Lat. 6°52^{1}N, Long. 7°24^{1}E and 419 m a.s.l.). The glasshouse mean day temperature and relative humidity were 30±2°C and 77±3%, respectively for the period of study.

Turmeric (*Curcuma longa* L. CV. NECLV1) rhizomes were procured from the National Root Crop Research Institute, Umudike, Abia State, Nigeria and used for the study. Fingers were obtained from the rhizomes and weighed 15-20 g as planting materials. Top soil was collected from a fallow land dominated by guinea grass (*Panicum maximum* Jacq.). The organic amendment used in this trial was poultry manure obtained from a broiler hen pen in the livestock unit of Teaching and Research Farm, University of Nigeria, Nsukka. The pen was cleaned approximately once a month. The primary constituents of the poultry manure were chicken excrement and wood-shaving bedding. Physical and chemical properties of the soil was determined using standard procedures (IITA, 1989). The poultry manure was analyzed for its chemical properties by methods outlined by Tel (1984). The soil was sandy loam (76.0% sand, 18.0% clay and 6.0% silt) in texture, pH 4.50 (H_2O), OM 1.35%, total N5.6 g kg^{-1} available P 57.71 mg kg^{-1}, the values for exchangeable K, Ca, Mg and Na were; 0.07, 1.40, 0.20 and 0.10 Cmol kg^{-1}, respectively. The poultry manure had a pH of 7.74 (H_2O), total organic carbon 38.70 g kg^{-1}, total N 8.02 g kg^{-1}, available P 3.30 g kg^{-1}, exchangeable K, Ca, Mg and Na of 1.73, 1.98, 1.86 and 0.85 Cmol kg^{-1}, respectively. Fresh poultry manure was air-dried in the glasshouse for two weeks and then ground to powder. The soil was steam-sterilized and air-dried. Three kilogrammes of the air-dried soil was thoroughly mixed with each level of poultry manure (10, 20, 30 and 40t ha^{-1}) corresponding to 15, 30, 45 and 60 g per 3 kg of soil, while no amendment (0 t ha^{-1}) served as control. The soil mixture was used to fill plastic pots with diameter 18 cm and depth 20 cm. The turmeric rhizomes (fingers) were planted two per pot and later thinned to one before nematode inoculation. Nsukka population of *M. incognita* race 1 maintained on begonia plants (*Begonia rex-cultorum)* served as an inoculum source. This population was multiplied on Ceylon spinach (*Bassela alba)* in the glasshouse. Root-knot nematode eggs were extracted from the heavily galled roots of Ceylon spinach using sodium hypochlorite (NaOCl) technique (Hussey and Barker, 1973). Five milliliters of the inoculum suspension contained approximately 2,500 eggs by count. Four weeks after emergence of the turmeric rhizome, each plant was inoculated with 2,500 eggs or 5,000 eggs by pouring five or ten milliliters of the inoculum suspension to four holes made at the base of each plant. Uninoculated plants served as control. The pots were arranged in a randomized complete block fashion on the glasshouse benches. Each treatment was replicated four times. It was a 3×5 factorial experiment laid out in RCBD. The inoculum density (0, 2500 and 5000 eggs plan t^{-1}) was the first factor combined with the second factor, poultry manure levels (0, 10, 20, 30 and 40 t ha^{-1}). The pots were watered once daily (300 mL) for the first 6 weeks after inoculation and 400 mL for the remaining period of growth. The following data were collected twelve weeks after nematode inoculation: number of galls per root system, shoot length, fresh weights of root and shoot per plant. For egg mass count, fresh root was stained with phloxine B (0.15 g L^{-1}) for 15 min (Daykin and Hussey, 1985). Root gall or egg mass index was determined on a 0-5 scale rating according to Taylor and Sasser (1978); 0=0, 1=1-2, 2=3-10, 3=11-30, 4=31-100 and 5= more than 100 galls or egg masses per root system.

A two-way analysis of variance (ANOVA) was used to test the significance of the treatments. Significant treatment means were separated using Fisher's Least Significant Difference (F-LSD) at 5% level of probability. Regression analysis was used to determine plant growth and nematode responses to increasing levels of amendments. All statistical analyses were performed with MINITAB 15 statistical software.

RESULTS

The number of galls induced by *M. incognita* at both inoculum densities (2,500 and 5,000 eggs $plant^{-1}$) decreased in a linear response to increasing poultry manure (PM) levels (Fig. 1a), r = -0.88 ($p<0.01$). The roots of the turmeric plants had more galls at high inoculum density than low density. Gall indices also decreased significantly ($p\leq0.05$) with successive increment in PM level (Table 1). Interaction between inoculum density and organic amendment level was significant. The lowest gall index of 2.0 was observed in plants amended with 40 t ha^{-1} of manure and inoculated with 2,500 eggs. The number of egg masses produced by the root-knot nematode species followed the trend of root galling (Fig. 1b). Egg mass production decreased in a linear response to increasing poultry manure levels (r = -0.84 and -0.85 for low and high inoculum densities, respectively). Egg mass indices (EMI) decreased significantly ($p\leq0.05$) with increase in PM level (Table 1). The lowest EMI of 2.0 was observed at 30 t ha^{-1} PM combined with low inoculum density and at 40 t ha^{-1} PM combined with high nematode density.

Shoot length was increased with each unit increase in PM up to 20 t ha^{-1} but decreased at 30 t ha^{-1} and above. The greatest growth enhancement was obtained with 20 t ha^{-1} PM amendment at all inoculum densities while the greatest growth inhibition occurred in unamended soils inoculated with nematodes. Shoot length was increased by 242 and 58% with 20 t ha^{-1} and 40 t ha^{-1} PM amendment, respectively compared with unamended soil at high inoculum density. The relationship between shoot length and PM amendment levels was expressed with quadratic regression equations for uninoculated control, low and high inoculum densities

Table 1: Gall Index (GI) and Egg Mass Index (EMI) of *Curcuma longa* infected with *M. incognita* at varying levels of poultry manure

	Poultry manure level (t ha^{-1})					
Inoculum density	0	10	20	30	40	Mean
Gall index*						
2,500 eggs/plant	5.00	4.00	3.00	2.50	2.00	3.30
5,000 eggs/plant	5.00	4.00	3.00	3.00	2.50	3.50
Mean	5.00	4.00	3.00	2.75	2.25	
Egg mass index*						
2,500 eggs/plant	4.50	3.50	2.25	2.00	2.00	2.85
5,000 eggs/plant	5.00	4.00	3.50	3.00	2.00	3.50
Mean	4.75	3.75	2.88	2.50	2.00	

LSD (0.05) for comparing inoculum density (I) means (GI) = 0.16; LSD (0.05) for comparing inoculum density (I) means (EMI) = 0.21; LSD (0.05) for comparing poultry manure level (PM) means (GI) = 0.25; LSD (0.05) for comparing poultry manure level (PM) means (EMI) = 0.33; LSD (0.05) for comparing I×PM Interaction means (GI) = 0.35; LSD (0.05) for comparing I×PM Interaction means (EMI) = 0.47; *0 = Immune; 1 = Highly resistant; 2 = Resistant; 3 = Moderately Susceptible; 4 = Susceptible; 5 = Highly Susceptible

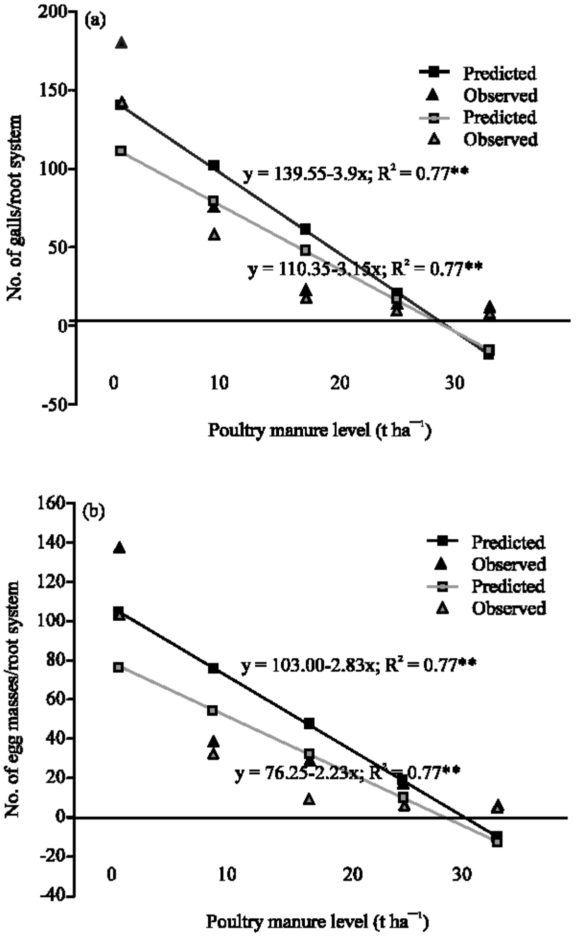

Fig. 1: Effects of increasing levels of poultry manure on the number of (a) galls/root system and (b) egg masses/root induced by *M. incognita* at 2,500 eggs/plant and 5000 eggs/plant

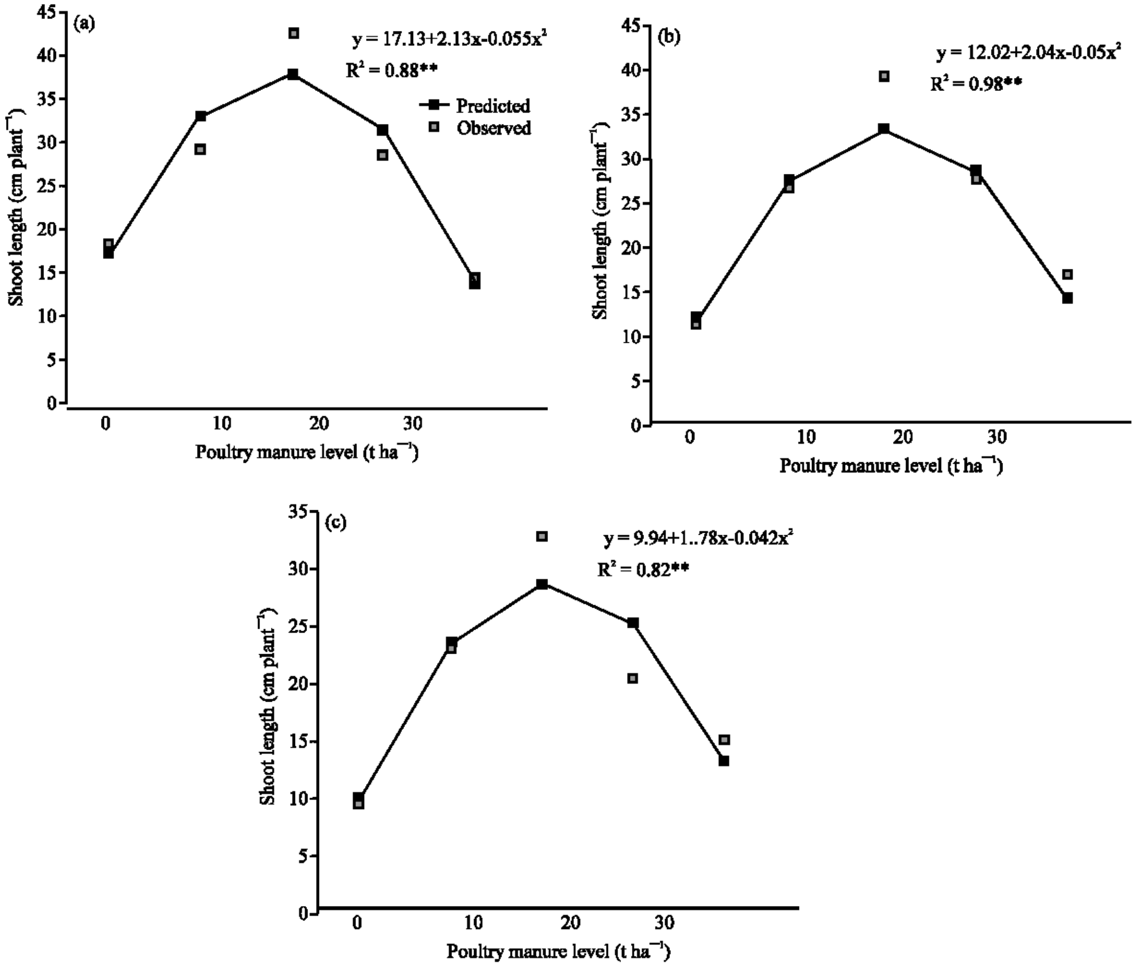

Fig. 2: Effects of increasing poultry manure levels on shoot length (cm)/plant of turmeric at (a) 12 weeks without rook-knot nematode inoculation (control) and (b) 12 weeks after inoculation with 2,500 eggs of *M. incognita* and (c) 12 weeks after inoculation with 5, 000 eggs of *M. incognita*

in Fig. 2a-c, respectively. In general, shoot length increased with increase in PM levels, it peaked at 20t ha^{-1} and decreased sharply with further increase in PM. The R^2 values were quite high (R^2 = 0.88, 0.98 and 0.82, respectively; $p \leq 0.01$). The greatest growth enhancement by PM amendment was predicted to be 18-20, 20-21 and 20-22 t ha^{-1} with uninoculated, low and high nematode densities, respectively.

The results of the effects of root-knot infection and PM amendments on fresh shoot weight followed the trend of shoot length (Table 2). Fresh shoot weight increased significantly ($p \leq 0.05$) with increase in PM level-up to 20 t ha^{-1}. No significant reduction effect was observed between low and high nematode density on fresh shoot weight. Plants amended with 20 t ha^{-1} PM had the highest fresh shoot weight at all inoculum densities; while plants unamended with PM but inoculated with nematodes had the lowest fresh shoot weight. Fresh shoot weight was increased by 287% and 48% with 20 and 40 t ha^{-1} PM amendment, respectively compared with unamended soil at high nematode density.

Table 2: Influence of soil amendment with poultry manure on fresh shoot weight (g)/plant of *Curcuma longa* L. 12 weeks after inoculation with *M. incognita*

	Poultry manure level (t ha^{-1})					
Inoculum density	0	10	20	30	40	Mean
0 eggs/plant(control)	23.00	36.25	50.50	25.00	14.75	29.90
2,500 eggs/plant	15.25	32.75	47.00	21.00	15.75	26.35
5,000 eggs/plant	11.50	29.50	44.50	23.25	17.00	25.15
Mean	16.58	32.83	47.33	23.08	15.83	

LSD (0.05) for comparing Inoculum density (I) means = 1.55; LSD (0.05) for comparing Poultry Manure (PM) means = 2.00; LSD (0.05) for comparing I x PM (Interaction) means = 3.46

Root-knot nematode inoculation reduced significantly ($p \leq 0.05$) the fresh root weight of turmeric plants (Table 3). Soil amendment with PM upto 30 t ha^{-1} increased significantly fresh root weight relative to

Table 3: Influence of soil amendment with poultry manure on fresh root weight (g)/plant of *Curcuma longa* L. 12 weeks after inoculation with *M. incognita*

	Poultry manure level (t ha^{-1})					
Inoculum density	0	10	20	30	40	Mean
0 eggs/plant(control)	24.67	32.08	43.85	33.14	22.79	31.31
2,500 eggs/plant	21.05	24.31	41.65	31.37	20.44	27.76
5,000 eggs/plant	17.40	21.08	38.25	27.03	15.17	23.79
Mean	21.04	25.82	41.25	30.51	19.47	

LSD (0.05) for comparing Inoculum density (I) means = 0.85; LSD (0.05) for comparing Poultry Manure (PM) means = 1.10; LSD (0.05) for comparing I x PM (Interaction) means = 1.91

unamended soils. However, amendment rate of 40 t ha^{-1} PM reduced significantly fresh root weight compared with other levels of PM. The highest fresh root weight (41.25 g plant) was obtained with 20 t ha^{-1} PM. Interaction between PM level and inoculum density was significant. The highest fresh root weight was obtained with 20 t ha^{-1} PM while the lowest was obtained with 40t ha^{-1} PM at all inoculum densities. Fresh root weight was increased by 120 and decreased by 12.8% with 20 and 40 t ha^{-1} PM amendment, respectively, compared with unamended soil.

DISCUSSION

The results obtained in this study showed that root galling and egg production by *M. incognita* on turmeric (*C. longa* L.) was inversely related to the applied levels of PM amendments. The greatest inhibition of nematode damage and egg production was observed at the highest level of PM amendment (40 t ha^{-1}). At this level, plants were rated resistant with a gall index and egg mass index of about 2.0. This finding corroborates the results of earlier investigators on other crops (Ogbuji, 1981; Chindo and Khan, 1990; Kaplan and Noe, 1993; Nwanguma and Awoderu, 2002; Nwanguma and Fawole, 2004; Agu, 2008). Ogbuji (1981) observed an increase in root galling by *M. incognita* on carrot with a soil: poultry manure mixture of 10:1 but traces of galls at a ratio of 10:2. Chindo and Khan (1990) reported a significant reduction in the population density of *M. incognita* and gall index on tomato with poultry manure amendments of 4 t ha^{-1} and above, while Nwanguma and Awoderu (2002) observed that 16 t ha^{-1} of poultry manure was comparatively as effective as a systemic nematicide (Mocap) in suppressing *M. incognita* population. Similarly, Nwanguma and Fawole (2004) and Agu (2008) evaluated the effects of different organic amendments on okra and African yam bean respectively, both reported the superiority of poultry manure to other organic amendments. In this trial and even other studies cited, it is apparent that the nematode-suppressing ability of any organic amendment depends on the quantity applied and its quality. Many researchers have attributed the nematicidal properties of poultry manure to its toxic decomposition products such as ammoniacal nitrogen, increased hydrogen ion concentration (acidity) and induced hypersonic solution (Chindo and Khan, 1990; Kaplan and Noe, 1993; Nwanguma and Awoderu, 2004). The C:N ratio of organic amendments is implicated to play a significant role in nematode suppression and phytotoxicity (Mian and Rodriguez-Kabana, 1982). The poultry manure used in this study had a very low C:N ratio of 4.83. Organic amendments with low C:N ratios when applied to the soil at a rate of 1.0%w/w or above are highly nematicidal and phytotoxic (Mian and Rodriguez-Kabana, 1982). From the results of this trial, it is evident that higher levels of PM amendments (>20t ha^{-1}) were highly nematicidal and phytotoxic to the test plant. Phytotoxicity could be attributed to the accumulation of salts or ionic species like nitrites, nitrates and ammonia. Similar results were obtained by Ogbuji (1981), Kaplan and Noe (1993), Mian and Rodriguez-Kabana (1982) and Nwanguma and Fawole (2004). However, Kaplan and Noe (1993) observed that phytotoxicity was expressed at the early of growth of tomato plants but was not apparent at the later growth stage. Conversely, McSorley and Gallaher (1995) did not obtain any significant reduction in nematode density nor yield increases in some vegetable crops amended with 269 t ha^{-1} of yard waste compost. Also, they observed that this rate was not phytotoxic to the crops tested. Phytotoxicity was expressed on turmeric plants as reduced shoot length, fresh shoot weight and fresh root weight. Although 40t ha^{-1} PM amendment induced resistance on turmeric to *M. incognita* infection, fresh root weight (newly formed rhizome inclusive) was significantly reduced compared with the unamended soil. This underscores the relevance of choosing an optimal rate of an organic amendment for nematode control in a particular crop. The high inoculum density of *M. incognita* was more pathogenic than the low inoculum density validating the report of Udo and Nwagwu (2007) and calls for the establishment of an economic threshold level for this pathogen on this crop in this agroecological zone.

Apart from its nematicidal properties, organic amendment of soil tends to maintain soil structure, is less disruptive to the soil environment, encourages population of beneficial soil microbes, facilitates crop rooting, improves water retention capacity and results in a more even distribution of nutrients in the soil profile (Arden-Clarke and Hodges, 1988). In this trial, the predicted optimal rate of PM amendment for moderate root-knot disease infection and maximum growth of turmeric plant is 18-22 t ha^{-1}. It is likely that this rate may have contributed indirectly to a healthy soil environment and thus the induced nematode tolerance observed.

In conclusion, poultry manure amendment of soil presents a veritable tool which could be incorporated into Integrated Nematode Management Programme in turmeric. The problem of phytotoxicity could be remedied by early preplant or post-harvest application and/or widening of C: N ratio of PM by addition of other sources of carbon. However, before the adoption of this method of control, field trials are needed for the optimization of the quantity, time and method of application.

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