



INTEGRATED USE OF POULTRY MANURE AND *Trichoderma reesei* FOR THE CONTROL OF (*Meloidogyne arenaria*) ON INDIAN SPINACH (*Basella alba*) IN CALABAR

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ABSTRACT

Root-knot nematode (*Meloidogyne arenaria*) is a serious pathogen affecting Indian spinach production. The high rate of infestation of this pathogen on Indian spinach in Calabar has led to decrease in Indian spinach production. Research into the control of *M. arenaria* was carried out in screenhouse Botanic Garden University of Calabar, Calabar to evaluate the efficacy of poultry manure in combination with inoculum density of *Trichoderma reesei* in the management of *M. arenaria* infesting Indian spinach (*Basella alba*). One gram of spore load of *T. reesei* at 3.0×10^7 spore/ml, 2.8×10^7 spores/ml, 1.30×10^7 spores/ml and 2.0×10^6 spore/ml were applied singly or in combination with poultry manure at 0, 5 t/ha, 10 t/ha and 15 t/ha per pot. Soil unamended with poultry manure and un-inoculated with *T. reesei* serves as the control. There were 20 treatments laid out in a completely randomized design (CRD) with three replications. Two weeks old Indian spinach plants were inoculated with 10 ml (3,000) second stage larvae of *M. arenaria* in soil. The result revealed that Indian spinach plant was highly susceptible to *M. arenaria* infection as gall index was rated (G.I = 5.00) without poultry manure and *T. reesei*. Successive increase in poultry manure rates and inoculum density of *T. reesei* inoculated individually significantly ($p < 0.05$) reduced root gall in plant. Application of various rates of poultry manure in combination with *T. reesei* significantly ($p < 0.05$) inhibited root galling than single application. Inoculation of *T. reesei* at 3.0×10^7 spore/ml with 15 t/ha of poultry manure significantly decreased the susceptibility to (GI = 2.00). The combination of *T. reesei* with poultry manure significantly ($p < 0.05$) enhanced the growth parameters, number of pods and fresh pod weight. The highest fresh pod (101.00 g/ plant) was obtained when 3.0×10^7 spores/ml of *T. reesei* was combined with 15 t/ha of poultry manure.

KEYWORDS: *Meloidogyne arenaria*, Indian spinach (*Basella alba*), Poultry manure, *Trichoderma reesei*, Biocontrol.

INTRODUCTION

Background of the study

Indian spinach (*Basella alba* L.), a fast-growing leafy vegetable widely consumed across tropical and subtropical regions, plays an essential role in the diet of humans due to its high nutritional content, including vitamins A and C, iron, calcium, and antioxidants (Sharma, Sharma, & Singh, 2018).

However, the productivity and market value of Indian spinach are significantly constrained by plant-parasitic nematodes, particularly the root-knot nematode species, a notorious pest in tropical and subtropical agriculture. *M. arenaria* induces characteristic root galls that interfere with the uptake of water and nutrients, resulting in stunted growth, gall formation, chlorosis, reduced leaf size, and ultimately, poor yield (Karssen & Moens, 2006; Khan, Menon, & Khan, 2019).

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Traditionally, the management of *M. arenaria* has relied on synthetic *nematicides*, but their usage is increasingly discouraged due to environmental concerns, human health risks, and the development of nematode resistance (Haydock, Woods, Grove, & Hare, 2013). Consequently, sustainable and eco-friendly alternatives such as organic amendments and biological control agents are gaining prominence. Among the organic amendments, poultry manure has shown great potential due to its high nitrogen content, rapid mineralization rate, and ability to improve soil structure and microbial activity (Olabi, Oyedunmade, & Ojo, 2010; Adegbite and Agbaje, 2007). Poultry manure does not only enhance soil fertility but also exhibits nematicidal properties through the release of ammonia and other toxic metabolites during decomposition, thus suppressing nematode populations (Nchore, Waceke & Kimenju, 2011).

Biological control using beneficial fungi such as *Trichoderma spp.* has emerged as a complementary approach in integrated nematode management. *T. reesei*, a well-known cellulolytic fungus, has gained attention for its antagonistic effects against a wide range of soil-borne pathogens, including nematodes (Harman *et al.*, 2004).

This study aimed at evaluating the effectiveness of poultry manure and *T. reesei* in managing *M. arenaria* infestation on Indian spinach. By understanding the interactive effects of these two treatments, this research will contribute to the development of an integrated and environmentally friendly nematode management strategy.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at screenhouse (Botanic Garden) University of Calabar, Calabar Nigeria. Other laboratory works were carried out in Botany Postgraduate Research Laboratory University of Calabar.

Sources of experimental materials

Seeds of Indian Spinach were obtained from Cross River State Ministry of Agriculture, other materials such as pure stock culture of *M. arenaria* and *T. reesei* were provided by the Department of Plant and Ecological studies.

Soil sample collection, sterilization and poultry manure analysis

Soil sample was collected from the faculty of Agriculture research Farm University of Calabar, the soil samples were collected at the depth of 0 – 15 cm using a soil auger, placed in nylon bags and labelled carefully. The soil sample was transported from the field to the laboratory where it was mixed thoroughly and sterilized by heating in enamel pot to a temperature of 100°C for 15 minutes to kill other pathogens. The heat sterilized soil was spread out in a shade for 7 days to cool before used for planting.

The soil sample was subsequently sieved in the laboratory using 2mm mesh size sieve. After that, the physico-chemical properties of the soil such as sand, silt, clay, textural class, pH in H₂O, EC, organic carbon (%), available P (mg/kg) and total nitrogen (g/kg) and poultry manure such as, pH in H₂O, organic carbon (%), EC (ds/m), total nitrogen (%) P (mg/kg), K (mg/kg), Ca (mg/kg) and Mg (mg/g) present were analyzed in Soil Science Laboratory University of Calabar, Calabar.

Nematode inoculum preparation

Nematode inoculum preparation was carried out according to Etim *et al.* (2025). Heavily galled roots of Indian spinach which were gently uprooted from the soil and soil particles adhering to the roots of the plant were removed by gently rinsing in water. Galled roots were chopped into small pieces and put into electric blender, little quantity of water was added to the galled tissues and blended into slurry. In order to avoid inactivating the infective nematode larvae, the blending was done for five seconds at each interval. The blended substrate was emptied into beakers as more water was added and stirred. 10 ml of the suspension (3,000) larvae was used to count under stereomicroscope and also used to inoculate into the plants.

Preparation of media for subculturing stock culture of *T. reesei*

Media preparation for subculturing of fungi was carried out according to methods of Umana *et al.*, 2014 using Potato dextrose agar (PDA). 9.5 grams was measured using sensitive weighing balance and was poured into 250 ml conical flask. 250 ml of distilled water was added and stirred using magnetic stirrer until the solution gave a paste. Non-absorbent cotton wool was covered with aluminum foil and used to cork the mouth of the conical flask which was wrapped again with aluminum foil up the neck of the flask. The medium was placed in autoclave at 121°C for 15 minutes. 250 milligrams of chloramphenicol were added to the solution, and allowed to cool before removing from the autoclave. Medium was poured into sterile petri dishes and labelled.

Spore count of *T. reesei*

Spore count was carried out according to Pitt and Poole (1981) where hemacytometer (single chamber) was used to count the spores placed under the microscope. The spore loads derived were used for inoculation. The following spore loads of 3.0×10^7 spores/ml, 2.80×10^7 , 1.30×10^7 , 2.0×10^6 spores/ml were generated for the experiment.

Preparation of carrier for *T. reesei*

Preparation of carrier for *T. specie* was done according to Sivan *et al.*, (1984). where 100g of millet grain was weighed using Ohaus sensitive weighing balance. The millet was soaked in distilled water for 24 hours to ferment before used. Normal sterilization of millet inside Bema (bottle) was done by autoclaving at 121°C for 15 minutes.

The sterilized millet inside the bottles were allowed to cool and a sterilized 5mm cork borer was used to pick the pure culture of *T. reesei* and inoculate into the sterilized PDA. Filter paper was used to cover the mouth of the Bema bottles and the bored holes of the cover bottles were used to seal the mouth after inoculation. Substrates were kept in the laboratory for 2 to 4 days for sporulation and inoculation into soil in screenhouse.

Poultry manure treatment, Planting of Indian spinach and inoculation of *M. arenaria* in soil

Poultry manure was cured by using the in-house manure drying system on slats method according to Elson and King (2010). The poultry manure was kept for two weeks under a shade before applying on pots. Three seeds of Indian spinach were sown in 5kg of sterilized loamy soil and later thinned to one plant stand per pot at two weeks after planting. *M. arenaria* was inoculated two weeks by excavating holes around the base of the plant after which the inoculum load (10ml) of the suspension was inoculated into the soil. The soil around the roots was later covered.

Application of poultry manure and inoculation of *T. reesei* on Indian spinach plant infested with *M. arenaria*

Poultry manure were applied at the rate of 5 t/ha, 10 t/ha and 15 t/ha equivalent to 20, 30 and 50 grams introduced into Indian spinach along with *T. reesei* at different spore load at 3 weeks after planting on pots receiving treatments. Pots without treatment application serves as control. The soil was later covered.

Experimental design, data collection and statistical analysis

The screenhouse trial was arranged in a completely randomized design (CRD) having 20 treatments and three replications. Data on number of galls, gall index, plant height (cm), number of leaves, number of nematodes per plant, number of pods per plant and fresh pod weight per plant of Indian spinach plant were collected at two weeks interval starting from three weeks after planting. Data collected were subjected to analysis variance (ANOVA) and significant means were separated using Duncan New Multiple Range Test (DNMRT) at 5% probability level.

RESULTS

The effects of poultry manure application and *T. reesei* inoculation on number of galls, gall index per plant and number of nematodes per plant of Indian spinach plants inoculated with *M. arenaria*

Soil amendment with poultry manure significantly ($p < 0.05$) reduced root galling by the nematode species with the highest rate of poultry manure (P3) recording the least number of galls as presented in Table 1. Infestation of soil with *T. reesei* with the least inoculum density 1.30×10^7 spores/ml to 2.0×10^7 spores/ml (TR3 to TR4) recorded plant stands with significantly ($p < 0.05$) fewer galls than poultry manure with the highest rate. Successive increase in inoculum density of *Trichoderma* led to significant increase in root galling. The combination of poultry manure with *Trichoderma* resulted to decrease in the number of galls except in the case of 1.30×10^7 spores/ml with 5t/ha (TR3+P1). However, plants inoculated with the highest inoculum density of *Trichoderma* (3.0×10^7 spores/ml) in combination with poultry manure (15t/ha) produced plants with least number of galls.

The Indian spinach plant was highly susceptible to *M. arenaria* as gall index was rated 5.00 as presented in Table 1. Poultry manure reduced the susceptibility rating from highly susceptible (5.00) to susceptible (G.I = 2.42). Soil inoculated with *Trichoderma* alone at 3.0×10^7 spores/ml reduced the rating to moderately, the trend was also observed when poultry manure was inoculated in combination with *Trichoderma*. However, the combination of *T. reesei* (3.0×10^7 spores/ml) with poultry manure (15t/ha) change the susceptibility to (G.I= 2.00).

The number of nematodes per plant was significantly ($p < 0.05$) reduced with increase in poultry manure rates and inoculum density of *Trichoderma* compared with control Table 1. However, at each inoculum densities, *T. reesei* at 3.0×10^7 to 2.0×10^6 spores/ml (TR1 to TR4) significantly ($p < 0.05$) gave a lower nematodes per plant. The combination of different inoculum densities of *Trichoderma* with poultry manures produced plants with significantly ($p < 0.05$) lesser number of nematodes compared to control. However, plants treated with *Trichoderma* at inoculum density of 3.0×10^7 spores/ml in combination with the highest rate of poultry manure (15 t/ha) significantly gave the least number of nematodes per plant followed by 2.80×10^7 spores/ml in combination with P3 (15 t/ha).

TABLE 1: Effects of poultry manure and *T. reesei* on number of galls, gall index and number of nematodes per plant of Indian spinach plant inoculated with *M. arenaria*

Treatments	Number of galls	Gall index	Number of nematodes per plant
P0 – (control)	186.00 ± 6.00 ^a	5.00±0.00 ^a	9342.40±1.921 ^t
P1 – 5 t/ha	94.16 ± 2.603 ^b	4.00±0.00 ^b	6240.50±1.067 ^p
P2 – 10 t/ha	74.33 ± 1.202 ^c	4.00±0.00 ^b	4243.20±1.824 ^o
P3 – 15 t/ha	65.00 ± 1.732 ^d	4.00±0.00 ^b	724.40±1.925 ^{mn}
TR1 - 3.0 x 10 ⁷ spores/ml	58.00 ± 1.453 ^{k-m}	2.42±0.222 ^c	1241.20±1.521 ^q
TR2 - 2.80 x 10 ⁷ spores/ml	51.00 ± 0.882 ^{hi}	4.00±0.00 ^b	1803.20±1.423 ^{rst}
TR3 - 1.30 x 10 ⁷ spores/ml	46.00 ± 0.577 ^f	4.00±0.00 ^b	1945.50±0.614 ^{rs}
TR4 – 2.0 x 10 ⁶ spores/ml	43.67 ± 1.453 ^e	4.00±0.00 ^b	1174.60±0.457 ^{rst}
TR1 + P1	41.67 ± 1.155 ^{o-q}	3.00±0.00 ^d	1295.40±1.443 ^k
TR2 + P1	41.00 ± 1.453 ^{mm}	3.00±0.00 ^d	1442.30±1.137 ⁱ
TR3 + P1	38.00 ± 0.577 ^{ij}	3.00±0.00 ^b	1614.00±1.224 ^{mn}
TR4 + P1	37.67 ± 1.202 ^{gh}	4.00±0.00 ^b	1352.20±1.456 ⁿ
TR1 + P2	35.33 ± 0.667 ^{s-u}	3.00±0.00 ^d	725.20±0.177 ^{efg}
TR2 + P2	33.00 ± 1.000 ^{p-r}	3.00±0.00 ^d	924.30±0.467 ^{ij}
TR3 + P2	32.33 ± 1.155 ^{n-p}	3.00±0.00 ^d	824.30±0.222 ^j
TR4 + P2	31.00 ± 0.882 ^{kl}	3.00±0.00 ^d	1428.60±0.222 ^k
TR1 + P3	10.00 ± 0.577 ^a	2.00±0.00 ^a	634.20±0.330 ^a
TR2 + P3	17.00 ± 0.882 ^{uv}	3.38±0.00 ^f	843.50±1.553 ^{bc}
TR3 + P3	16.00 ± 0.577 ^{r-t}	3.00±0.00 ^d	986.20±0.624 ^{def}
TR4 + P3	20.00 ± 0.667 ^{op}	3.00±0.00 ^d	1324.60±0.771 ^{fg}

* Means within a column followed by the same letter of alphabet are not significantly different from one another based on Duncan's New Multiple range test (DNMT) at 5% probability level.

P = Poultry manure

T = *T. reesei*

The effect of poultry manure and *T. reesei* inoculation on Plant height (cm) of Indian spinach plant inoculated with *M. arenaria*.

Three weeks after planting (3WAP) application of poultry manure and inoculation of *Trichoderma* significantly ($p<0.05$) enhanced the growth of Indian spinach compared with the control Table 2. Increase in application rate of poultry manure led to significant ($p<0.05$) increase in height. However, there was no significant ($p>0.05$) difference in height between plants inoculated with various inoculum densities of

Trichoderma. Significant enhancement was observed on height of plants when poultry manure was combined with *Trichoderma* than single rates. At 5 and 7 WAP followed the trend of 3WAP. However, at 5WAP, plants inoculated with 2.0×10⁶ spores/ml of *Trichoderma* (TR4) was similar in height with control (PO) and the same was shown for those inoculated with 1.30×10⁷ spores/ml (TR3). However, *Trichoderma* spores at 3.0×10⁷ spores/ml in combination with 15 t/ha of poultry manure (P3) significantly ($p<0.05$) produced the tallest plants.

TABLE 2: Effect of poultry manure and *T. reesei* inoculation on Plant height (cm), number leaves of Indian spinach inoculated with *M. arenaria*

Treatment/concentration	Plant height			Number leaves		
	3WAP	5WAP	7WAP	3 WAP	5 WAP	7 WAP
P0 (control)	8.78± 0.33 ^p	19.67± 0.431 ^t	27.17± 0.441 ^t	3.67 ± 0.333 ^k	5.67 ± 0.541 ^m	9.33 ± 0.333 ^{no}
P1 (5 t/ha)	8.72± 0.33 ^{mno}	21.33± 0.333 ^r	32.33± 0.601 ^{pq}	5.00 ± 0.142 ^{ghi}	6.67 ± 0.233 ^{ki}	11.00 ± 0.577 ^{im}
P2 (10 t/ha)	11.00± 0.167 ^{jk}	24.17± 0.167 ^{no}	39.50± 0.289 ^{kl}	5.67 ± 0.243 ^{efg}	8.34 ± 0.67 ^{fgh}	13.33 ± 0.333 ^{fg}
P3 (15 t/ha)	12.00± 0.167 ^{hi}	26.00± 0.289 ^j	42.00± 0.289 ^j	6.34 ± 0.333 ^{cde}	8.67 ± 0.333 ^{efg}	15.00 ± 0.577 ^{cd}
TR1 (3.0x10 ⁷ spore/ml)	10.00± 0.167 ⁱ	20.33± 0.333 ^{lm}	30.17± 0.726 ^t	4.67±0.333 ^{hij}	6.00 ± 0.577 ^{im}	8.67 ± 0.333 ^{op}
TR2 (2.80x10 ⁷ spore/ml)	10.30± 0.00 ^{lm}	21.33± 0.333 ^r	28.50± 0.289 ^s	4.34 ± 0.333 ^{ijk}	6.67 ± 0.333 ^{kl}	8.67 ± 0.333 ^{op}
TR3 (1.30x10 ⁷ spore/ml)	8.28± 0.00 ^{no}	20.83± 0.167 ^{rs}	27.83± 0.441 st	4.34 ± 0.00 ^{lm}	6.00 ± 0.00 ^{lm}	8.33 ± 0.333 ^{op}
TR4 (2.0x10 ⁶ spore/ml)	8.54± 0.33 ^p	21.50± 0.289 ^{kl}	31.83± 0.928 ^q	4.00 ± 0.00 ^{jk}	5.67 ± 0.333 ^m	8.00 ± 0.00 ^p
TR1+ P ₁	12.00± 0.167 ^{hi}	26.17± 0.167 ^{ij}	35.67± 0.333 ⁿ	6.00 ± 0.000 ^{def}	8.00 ± 0.000 ^{ghij}	13.33 ± 0.333 ^{fg}
TR2+ P ₁	11.17± 0.154 ^{jk}	24.67± 0.333 ^{mn}	34.17 ± 0.167 ^o	5.67 ± 0.333 ^{efg}	7.67 ± 0.333 ^{hijk}	12.33 ± 0.333 ^{ijk}
TR3+ P ₁	11.17± 0.177 ^{jk}	23.50± 0.289 ^p	33.50 ± 0.289 ^o	5.34± 0.333 ^{fgh}	8.34 ± 0.333 ^{fgh}	11.33 ± 0.241 ^{ki}
TR4+ P ₁	9.83± 0.167 ^{lm}	21.33± 0.333 ^r	33.17 ± 0.167 ^{op}	5.34 ± 0.333 ^{fgh}	7.34 ± 0.333 ^{ijk}	10.33 ± 0.333 ^m
TR1+ P ₂	13.83± 0.167 ^{ef}	28.17± 0.441 ^{fg}	43.50 ± 0.289 ⁿ	7.00 ± 0.00 ^{bc}	9.34 ± 0.333 ^{def}	14.00 ± 0.541 ^{ef}
TR2+ P ₂	12.50± 0.289 ^{hi}	27.50± 0.289 ^{gh}	41.17 ± 0.136 ^{ij}	6.34 ± 0.333 ^{cde}	8.00 ± 0.00 ^{ghij}	13.00 ± 0.532 ^{gh}
TR3+ P ₂	11.83± 0.167 ^{jl}	25.83 ± 0.167 ^{jk}	40.33 ± 0.447 ^{ki}	6.00± 0.00 ^{def}	8.34± 0.333 ^{fgh}	12.33 ± 0.333 ^{ijk}
TR4+ P ₂	11.17± 0.167 ^{jk}	25.83 ± 0.167 ^{jk}	39.33 ± 0.447 ⁱ	6.00 ± 0.00 ^{def}	7.67 ± 0.333 ^{hijk}	11.33 ± 0.333 ^{kl}
TR1+ P ₃	16.17± 0.156 ^a	30.67± 0.333 ^a	47.50 ± 0.322 ^a	9.28± 0.333 ^a	14.00 ± 0.333 ^a	17.00 ± 0.333 ^a
TR2+ P ₃	15.17± 0.156 ^c	29.17± 0.177 ^{de}	45.33 ± 0.333 ^f	6.67± 0.333 ^{bcd}	8.21 ± 0.577 ^{def}	15.00 ± 0.351 ^{ef}
TR3+ P ₃	14.83 ± 0.333 ^{cd}	26.83 ± 0.457 ^{hi}	44.83 ± 0.153 ^{fg}	6.34± 0.333 ^{cde}	9.00 ± 0.000 ^{defg}	12.33 ± 0.333 ^{ijk}
TR4+ P ₃	14.33 ± 0.333 ^{de}	25.83 ± 0.247 ^{jk}	43.67 ± 0.322 ^h	4.00± 0.00 ^{def}	8.34± 0.333 ^{fgh}	11.67 ± 0.333 ^{kl}

*Mean within a column followed by the same letter of alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at 5% probability level.

P – Poultry manure

T – *T. reesei*]

The effect of poultry manure and *T. reesei* inoculation on number of leaves of Indian spinach inoculated with *M. arenaria*.

At three weeks after planting (3WAP), application of poultry manures significantly ($p < 0.05$) enhanced leaf production in Indian spinach plants compared with the control as presented in Table 2. Infestations of Soil with *Trichoderma* at 1.30×10^7 and 2.0×10^6 spores/ml did not significantly ($p > 0.05$) enhance leaf production compared with the control. The combination of *Trichoderma* with poultry manure led to increase in the number of leaves produced compared with individual application. At 5 and 7 WAP poultry manure at 10t /ha and 15t /ha significantly enhanced leaf production compared with the control. However, soil inoculated with *Trichoderma* alone irrespective of the inoculum densities produced plants with similar number of leaves compared with the control. The combination of *T. reesei* with 10 t/ha and 15t /ha of poultry manure led to a significant enhancement in leaf production compared with the control. The highest leaf production was obtained when *T. reesei* (3.0×10^7 spores/ml) was combined with 15 t/ha of poultry manure (TR1 + P3).

The effects of poultry manure and *Trichoderma reesei* inoculation on number of pods per plant

and fresh pod weight per plant of Indian spinach inoculated with *M. arenaria*.

Soil amended with poultry manure significantly ($p < 0.05$) enhanced Indian spinach plant compared with the control as presented in Table 3. Soil inoculated with *Trichoderma* alone did not significantly ($p > 0.05$) increase the number of pods produced by the plants compared with the control. The combined amendment of poultry manure and inoculation of *Trichoderma* significantly ($p < 0.05$) produced plant with greater number of pods than individual application. However, the highest inoculum density (3.0×10^7 spores/ml) of *Trichoderma* in combination with poultry manure at 15 t/ha (TR1 + P3) produced plants that significantly ($p < 0.05$) had the highest number of pods.

Increase in rate of poultry manure significantly ($p < 0.05$) increased fresh pod weight of Indian spinach compared with the control Table 3. Soil inoculated with the highest inoculum density of *Trichoderma* alone (TR1 and TR2) significantly ($p < 0.05$) enhanced fresh pod weight compared with the control. However, the combination of the highest rate of poultry manure at 15 t/ha (P3) with inoculum density of *Trichoderma* (TR1 + P3) significantly ($p < 0.05$) produced plants with the highest fresh pod weight.

TABLE 3: Effects of poultry manure and *T. reesei* inoculation on number of pods and fresh pod weight per plant of Indian spinach inoculated with *M. arenaria*

Treatment/combination	Number of pods per plant	Fresh pod weight (g)
P0 (control)	1.80 ± 0.321 ^t	12.00 ± 1.752 ^t
P1 (5 t/ha)	4.46 ± 0.214 ^p	34.78 ± 0.234 ^p
P2 (10 t/ha)	6.27 ± 0.215 ^o	39.87 ± 1.251 ^o
P3 (15 t/ha)	9.83 ± 0.354 ^m	48.23 ± 1.737 ^{mn}
TR1 (3.0 x 10 ⁷ spores/ml)	2.87 ± 0.334 ^{rs}	26.67 ± 1.426 ^{mn}
TR2 (2.80 x 10 ⁷ spores/ml)	2.23 ± 0.921 ^s	19.20 ± 1.352 ^q
TR3 (1.30 x 10 ⁷ spores/ml)	1.79 ± 0.193 ^t	19.99 ± 1.774 ^{rs}
TR4 (2.0 x 10 ⁶ spores/ml)	1.37 ± 0.176 ^t	18.41 ± 0.426 ^{rst}
TR1 + P ₁	8.23 ± 0.214 ^l	62.40 ± 1.774 ^k
TR2 + P ₁	7.97 ± 0.521 ^m	55.40 ± 1.342 ^l
TR3 + P ₁	6.84 ± 0.601 ⁿ	48.31 ± 1.521 ^{mn}
TR4 + P ₁	9.42 ± 0.224 ^j	44.99 ± 1.042 ⁿ
TR1 + P ₂	13.68 ± 0.231 ^{ef}	82.41 ± 1.320 ^{efg}
TR2 + P ₂	12.45 ± 0.562 ^h	74.27 ± 1.624 ^{ij}
TR3 + P ₂	11.86 ± 0.333 ^{ij}	71.24 ± 0.416 ^j
TR4 + P ₂	9.23 ± 0.533 ^k	64.07 ± 4.213 ^k
TR1 + P ₃	18.45 ± 0.143 ^a	101.00 ± 1.751 ^a
TR2 + P ₃	17.24 ± 0.225 ^b	85.04 ± 0.423 ^{def}
TR3 + P ₃	15.56 ± 0.237 ^d	82.92 ± 0.562 ^{fg}
TR4 + P ₃	13.98 ± 0.247 ^{efg}	72.21 ± 1.246 ^{hi}

*Mean within a column followed by the same letter of alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at 5% probability level.

P – Poultry manure

T – *T. reesei*

DISCUSSION

The Indian spinach used in this study was found to be highly susceptible to *M. arenaria* infection as gall index was rated 5.00 in soils not amended with poultry manure and uninfected with *T. reesei*. Increase in rate of poultry manure led to a significant reduction in root galls, nematode in plant compared with the control, this is in line with Olewe, (2018) severe root galling in plant can be reduced as the organic amendment increases. *M. arenaria* infestation in Indian spinach can also lead to poor plant growth, reduced leaf quality, and significant yield loss (Chitwood, 2003). Infestation of soil with *T. reesei* alone significantly parasitized nematode, root galling in plant. The highest spore load alone was effective in decreasing root galling in nematode compared to the control. These findings validate the work of Sharon *et al.*, (2021).

Several studies have documented the susceptibility of Indian spinach to *M. arenaria* and the nematode's damaging effects. According to Sasser and Freckman (2021), *M. arenaria* is among the most economically important root-knot nematodes globally, and its wide host range includes *Basella alba*. The nematode penetrates the root system and causes hypertrophy and hyperplasia of root tissues, resulting in gall formation and restricted nutrient and water uptake (Moens *et al.*, 2009). In Indian spinach, these

physiological disruptions manifest as stunted growth, chlorosis, wilting, and reduced leaf yield.

Research by Sharma *et al.* (2015) showed that *M. arenaria* infestation in Indian spinach led to significant reductions in plant height, fresh biomass, and leaf area. Plants heavily infested with the nematode displayed poor root development and extensive galling, which compromised their overall vigor. In their study, nematode populations as low as 500 juveniles per kg of soil were sufficient to cause noticeable reductions in growth and leaf production, highlighting the crop's sensitivity to nematode pressure.

Yield losses due to *M. arenaria* in Indian spinach can be severe under field conditions. Kumar *et al.* (2020) observed that untreated nematode-infested plots yielded up to 60% less leaf mass compared to non-infested controls. They also reported that nematode infestation significantly delayed leaf regrowth after harvesting, thus affecting the crop's marketable yield and economic value.

M. arenaria poses a significant threat to Indian spinach cultivation by impairing root function, reducing vegetative growth, and decreasing yield. The nematode's ability to persist in soil and infect successive crops necessitates integrated management strategies, especially in smallholder farming systems where Indian spinach is grown intensively.

Soil amendments with organic matter have been reported to stimulate soil food webs, thus increasing the population of free-living nematodes, plant growth promoting rhizobacteria, fungi and nematode antagonist (Oka, 2010). The role of organic amendment could be complementary as it may help the antagonist like *Trichoderma* species to established in the soil. The highest root gall reduction and suppression of *M. arenaria* population was obtained with the highest spore load of 3.0×10^7 spores/ml of *T. reesei* in combination with the highest rate of poultry manure 15 t/ha indicating that the combination of various mechanisms of nematode control by the two agents as discussed previously may likely account for this observation. Amendment of soil with poultry manure and its combination with various spore load enhanced growth, earliness to anthesis and fruit yield of Indian spinach.

CONCLUSION

A comparative study between different rates of poultry manure and different inoculum density of *T. reesei* showed that increase poultry manure concentration and inoculum density of *Trichoderma* applied singly significantly reduce root galling of Indian spinach plant. However, the combination of different inoculum density of *T. reesei* with poultry manure rates significantly inhibited root galling than single application of each control agent. The combination of *T. reesei* at 3.0×10^7 spores/ml with 15 t/ha of poultry manure significantly reduced the susceptibility of Indian spinach from 5.00 to (G.I = 2.00). Inoculation of *T. reesei* with poultry manure significantly enhanced plant height, number of leaves, number of pod/plants, fresh pod weight. The highest fresh pod weight was obtained when 15 t/ha of poultry manure was combined with *Trichoderma* highest spore load of 3.0×10^7 spores/ml.

RECOMMENDATIONS

1. Poultry manure at the rate of 15 t/ha may be used in combination with 3.0×10^7 spore/ml of *T. reesei* in the management of *M. arenaria* infecting Indian spinach in Calabar after confirmation in field trials.
2. Other indigenous species of *Trichoderma* should be evaluated for their efficacy in controlling other species of *Meloidogyne* prevalence in Calabar and its environs.
3. Research should be geared towards isolating indigenous *Trichoderma* species at commercial level for nematode management in Nigeria.

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