Management of *Meloidogyne javanica* on Tomato (*Solanum lycopersicum*) with Bio-control Agents and Soil Amendments

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Abstract

Plant-parasitic nematodes, especially the root-knot nematodes are one of the major constraints to tomato production in Nigeria. In this study, biological control agents were evaluated singly, and in combination with biochar and rice husk as soil amendments to control Meloidogyne javanica. The experiment was laid out in a completely randomized design having four replications in a screenhouse. The treatments were Paecelomyces lilacinnus (1 g/pot), Trichoderma asperellum (1.5 x10^6 spores/pot) and Arbuscular mychorrizal fungi (AMF) (15 g/pot) as biocontrol agents applied singly and in combination with palm bunch biochar (15 g/pot) and rice husk (40 g/pot); uninoculated plants and plants with no treatment constituted the controls. Treatments were applied 5 days after tomato plants were inoculated with 2,000 eggs of M. javanica. Plant height, number of leaves, number of days to flowering, fruit weight, galling index, nematode populations (roots and soil) and nematode reproductive factor were the data taken from the plants. Analysis of variance was carried out and significant means were separated using Student Newman Keuls at $P \leq 0.05$. All the treatments improved plant growth significantly more than the inoculated control. The highest fruit yield was obtained from treatments with biochar followed by Paecilomyces lilacinus and its combinations with biochar and rice husk. Plants that received Trichoderma asperellum, rice husk+ T. asperellum, biochar + T. asperellum, biochar + P. lilacinus, Biochar + AMF and AMF alone provided between 64% and 79.5% reduction in nematode population. Bioagents provided some protection against Meloidogyne javanica by reducing its population, and rate of reproduction, their effect was further enhanced with the application of soil amendments more so with biochar than with rice husk.

Keywords: Glomus spp., rice husk, root-knot nematode, Paecilomyces, palm-bunch biochar.

Introduction

Tomato is one of the world's largest vegetable crops in terms of production (Olaniyi *et al*., 2010). About 161.8 million tonnes of tomatoes were produced worldwide, with China being the largest producer and accounting for about one quarter of the global output, followed by India and the United States (FAOSTAT, 2014). It is cultivated on 85% of farmlands in Nigeria (Olaniyi *et al*., 2010), and regarded as the most important vegetable with production of 2.1 million tonnes compared to 739 thousand tonnes of pepper and 235 thousand tones of onions (FAOSTAT, 2014).

Plant-parasitic nematodes are one of the most important biological factors limiting tomato production all over the world (Fawole *et al.*, 1992; Olowe, 2009). Due to a wide range of hosts, root-knot nematodes (*Meloidogyne* spp.) are regarded as the most economically important plant parasitic nematodes (Sasser *et al*., 1984; Imafidor and Nzeako, 2007). The root-knot nematode is a major pathogen on tomatoes where they cause considerable losses in yields.

Nematicides have been used in controlling nematode pests with remarkable results. However, public concerns on residues in food

and environment and the development of resistance to nematicides by nematodes have led to the search for alternative means of managing them (Sahebani and Hadavi, 2008). Biological control of soil-borne plant pathogens including nematodes by antagonistic microorganisms is a potential non-chemical means of plant disease control. Examples of effective agents include arbuscular mycorrhizal fungi (AMF) which form symbiotic relationships with plant roots. Disease reduction within host plants colonized by AMF is the result and output of the complex interactions between pathogens, AMF and the plant (Harrier and Watson, 2004). AMF symbiosis has been shown to reduce the damage caused by soil borne pathogens (Azcon-Aguilar *et al*., 2002). Several strains of *Trichoderma* have been developed as biocontrol agents to manage diseases of plants (Harman, 2006). The various mechanisms include antibiosis, parasitism, inducing host-plant resistance and competition. *Trichoderma* also have been shown to have antagonistic activity towards root-knot nematodes (Windham *et al*., 1989; Sharon *et al*., 2001). *Paecilomyces lilacinus* has been considered to have great potential for application as bio-control agent in sub-tropical and tropical agricultural soils (Morgan-Jones *et al.,* 1984). *Paecilomyces lilacinus* is reported to inhibit egg hatch, root galls and egg masses (Cabanillas *et al.*, 1988).

The main objective of this study was to use biological agents for the management of *Meloidogyne javanica*. Specifically, the study evaluated the effects of bio-control agents alone and in combination with biochar and rice husk as soil amendments on *Meloidogyne javanica* infecting tomato plants.

Materials and Methods

The study was conducted at the Roof-top Garden, Nematology and Pathology Research Laboratories in the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. Sandy - Loam Top soil was collected from the Crop garden of the Department and mixed with river sand at a 1:1 ratio . The soil-sand mixture was sterilized at 170°C for two hours, using an electrical soil sterilizer. Soil was allowed to cool and filled into 5-litre (23 cm diameter) pots.

The seeds of the tomato cultivar Rio-Grand were purchased from a commercial seed marketer. Arbuscular mycorrhizal fungi (*Glomus musseae* + *G. etinicatum*) were obtained from pot cultures, while *Paecilomyces lilacinus* was a commercial product and *Trichoderma asperellum* isolates were collected from pure cultures in the Pathology laboratory of the Department.

Nematode Extraction and Inoculum Estimation

Meloidogyne javanica inoculum was obtained from galled roots of *M. javanica*-infected tomato plants cultured from a single egg mass population. The nematode eggs were extracted from the roots following the procedure of Hussey and Barker (1973). Galled roots were rinsed with water to remove soil and debris. Roots were later chopped into 1-2 cm pieces using a pair of scissors. A solution of 0.5% sodium hypochlorite (NaOCl) was prepared from Chlorox bleach in a 500 ml measuring cylinder. The NaOCl solution was poured into a wide-mouthed 500 ml glass jar containing the chopped roots to cover the chopped roots. The container was covered tightly and shaken vigorously for four minutes then poured into a stack of sieves of 75, 45 and 28 µm aperture sizes. The eggs collected in the 28 µm sieve

size were rinsed several times with tap water to remove all traces of NaOCl. The eggs were then rinsed out of the sieve with distilled water into a beaker using a wash bottle.

The extracted nematode egg suspension was made up to 200 ml with water in a beaker. While stirring with a magnetic stirrer, 1 ml aliquot of the egg suspension was taken using hypodermic syringe and released into a counting dish. Nematode eggs in counting dish were counted using a tally counter while viewing under microscope at 10X magnification. The nematode egg population was determined by extrapolation to the volume of 200 ml. The process was repeated thrice and the average taken as the population of nematode eggs/ml. The nematode egg suspension was then adjusted by adding water to dilute the population to a concentration of 1000 eggs/ ml of suspension.

Estimation of spore population of biocontrol agents

The conidia suspension was prepared by adding 10 ml of distilled water unto the surface of each of the plates containing the pure culture of *Trichoderma asperellum*. A sterile spatula was used to dislodge the culture and subsequently washed into a sterile beaker. The solution was later filtered through four layers of sterile cheese cloth. The filtrate containing the spores was collected and made up to 50 ml by adding sterile distilled water. Using a sterile syringe, 0.1 ml of homogenous conidial suspension was loaded into Neubauer heamocytometer through the V-shape groove. The suspension was allowed to settle for two minutes after which the spores in two chambers were counted. An average count of 20.6 spores was obtained by counting five small squares in each chamber of the heamocytometer. The number of conidia per ml was calculated using the formula below:

$$X = A + B \times 50{,}000;$$

Where A = average number of spores in the first chamber, and B = average number of spores in the second chamber. The total number of spores per ml was 1.4×10^6

Paecilomyces lilacinus was applied in a powder carrier commercially sold as Paecilo® at the rate of 1 g estimated to contain 1×10^8 cfu/g, while the AMF *Glomus* spp. was applied in a sand mixture at the rate of 15 g estimated to contain 100 infective propagules/g.

Collection and Preparation of soil amendments

Rice husk was obtained from Rice Farmers Cooperative Processing Unit in Ibadan and stored in bags until required. Palm bunches from which the oil nuts had been previously harvested were collected from the Palm Oil Processing Unit, Teaching and Research Farm of the University of Ibadan. The bunches were sun dried for two weeks and then taken to the Department of Mechanical Engineering for processing into biochar. Biochar was produced under no-oxygen, pyrolysis condition using a patent facility in the Department. The reactor was heated by a step-wise procedure from 200°C, then elevated to 250°C, 300°C, and finally to 400°C. The process was maintained for 1.5 h at each temperature. The whole process was brought to an end when no further smoke came out from the gas exit pipe. The cooled biochar was collected in bags and stored until required.

Treatments and experimental design

Tomato cultivar Rio Grand™ seeds were sown in sterilized soil in nursery trays and maintained for three weeks after which they were transplanted into 5 kg pots containing the

sterilized soil. The pots were arranged in completely randomized design with four replicates. There were three bio-agent treatments and two soil amendment treatments applied singly and in combination to nematode inoculated plants. The two control treatments comprised uninoculated plants, and inoculated-untreated plants. The treatment application rates used per 5 kg soil were; *AMF at 15 g , Trichoderma asperellum at* 1.5 x10^6 spores, *Paecilomyces lilacinus* at 1 g, Bio-char at 15 g, Rice husk at 40 g, Rice husk at 40 g + *Trichoderma at* 1.5 x10^6, Rice husk at 40 g + *Paecilomyces at* 1 g, Bio-char at 15 g + *Trichoderma at* 1.5 x10^6, Bio-char at 15 g + *Paecilomyces at* 1g, Bio-char 15g + *AMF at* 15 g, Rice husk at 40 g + *AMF at* 15 g, only nematodes at 2000 eggs and plants with no nematode inoculation or soil treatment.

Plants were inoculated one week after transplanting with 2000 eggs of *Meloidogyne javanica*. A 5 ml capacity syringe was used to take 5 ml of the nematode inoculum and to release the inoculum into four 2 cm deep holes made close to each plant. Application of bio-control agents and soil amendments was done five days after inoculation. A ring was drawn 2 cm away and 2 cm deep around each plant to receive the treatments and the appropriate treatments were incorporated into the soil and covered with soil. The experiment was repeated a second time to validate the results earlier obtained.

Data collection and analysis

Data collection commenced immediately after the application of treatments, and was taken weekly for plant height (cm) and number of leaves. Number of days to appearance of the first flower of each plant was recorded as they appeared. Number of flowers on each stand was counted and the number of fruits picked from each stand was recorded. Fruit weight (g) was measured with a Ohaus™ balance. Each experiment was terminated 10 weeks after inoculation by separating each plant shoot from the roots. Individual pots were then upturned over polythene sheets and roots were separated from soil. The roots where then rinsed in water to remove soil and debris. Weight (g) of the fresh shoots and roots were taken with an electronic balance (Ohaus™). Data on galling index, number of nematodes in soil and roots, and reproductive factor were also taken.

Galling index which is an estimation of damage to root caused by root-knot nematodes was rated per plant. The whole root system was visually rated for galling on a 1-5 scale. where; 1= no galls, 2=1-15% of galled root, 3=16-30%, 4=31-60%, 5=61-100%. (Claudius-Cole, 2005).

The nematode eggs were extracted from the roots following the procedure of Hussey and Barker (1973). Nematode extraction from soil was carried out using the extraction tray method (Coyne *et al*., 2007). Soil measuring 250 cm^3 from each treatment was spread thinly on facial tissue placed on a plastic sieve, which was sitting on a 25 cm diameter plastic tray. Water was added to the tray until sample was at field capacity. The soil samples were left for 48 hours, after which the water containing the extracted nematodes was poured into plastic cups. Each extract was concentrated to 50 ml from which two separate 2-ml aliquots was taken for counting. Average counts per sample was used to estimate the nematode population per sample. The nematode population per pot was estimated using:

$$\frac{\text{Number of nematodes in 250 cm}^3}{250\ \text{cm}^3} \times 5000\ \text{cm}^3$$

Reproductive Factor (RF) was calculated by dividing the total number of nematodes found in each treated pot at the end of the experiment by number of nematodes inoculated in the pot:

$$RF = \frac{Pf}{Pi}$$

where P_f is final population (soil + root populations) and P_i is initial population (2000 eggs).

Data were processed in Microsoft Excel and submitted to the analysis of variance (ANOVA) using the SAS statistical package (SAS Institute, 2003). Significant treatment means were separated by using Student Newman Keuls Test (SNK) at 5% level of probability.

Results

There were no significant differences in the growth and yield parameters measured in the first and second trials except for the number of flowers produced which was more (P=0.05) in the second trial than in the first trial (Figure 1). The total number of nematodes extracted from soil and roots of tomato plants was also not significantly different although values obtained in the second trail were higher than in the first trial (Figure 2). Similarly this trend was observed for the galling index and reproductive factor of the nematode (Figure 3). Data from both experiments were therefore pooled as similar trends were observed among the treatments in both trials.

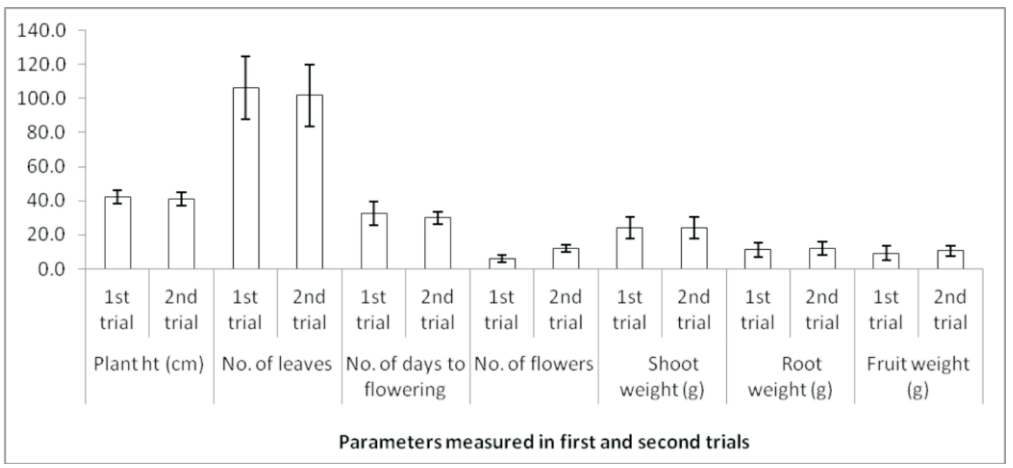

Figure 1: Mean values for growth and yield parameters of treated tomato plants in the first and second trials.
Bars represent standard error of means

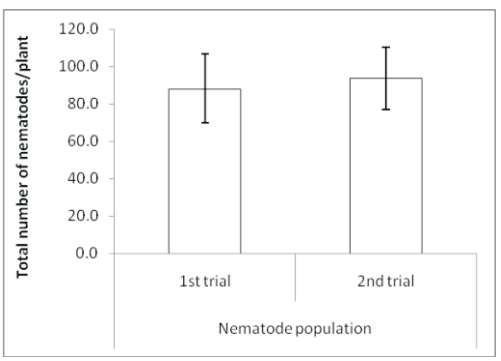

Figure 2: Total number of nematodes extracted from roots and soil of *Meloidogyne javanica* infected plants treated with bioagents and soil ammendments.

Bars represent standard error of means; values presented are transformed (square root of x+0.5) means of nematode populations.

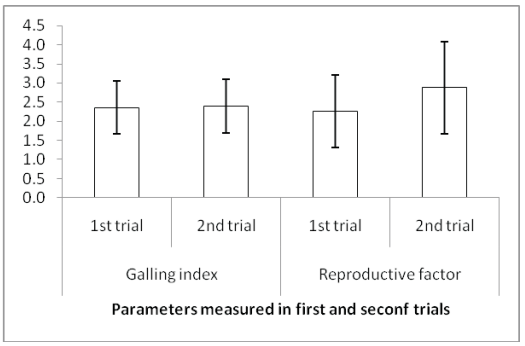

Figure 3. Galling index and reproductive factor of *Meloidogyne javanica* infected plants treated with bioagents and soil ammendments.

Bars represent standard error of means; Galling index on 1-5 scale where, 1= no galls, 2=1-15% of galled root, 3=16-30%, 4=31-60%, 5=61-100%. Rf = P_f/P_i, where P_f is final population (soil + root populations) and P_i is initial population (2000 eggs).

Effect of treatments on growth of tomato plants between two to ten weeks after inoculation

There were significant ($P \leq 0.05$) differences in plant height among treatments from 2 weeks after inoculation (WAI). Plants treated with AMF alone were shorter than other treatments from 2-6 weeks after inoculation (WAI). However, by 10 WAI, AMF-treated plants were not significantly different in height from the uninoculated control (Figure 4a). *Meloidogyne javanica* inoculated-untreated control plants were the shortest. At 10 WAI, All the treated plants were significantly ($P \leq 0.05$) taller than the inoculated and untreated control plants . Nematode-infected plants treated with Biochar + AMF were the tallest ($P \leq 0.05$) compared to other treatments including the uninoculated plants. This was followed by plants treated with *T. asperellum,* AMF, and rice husk + AMF, all of which were significantly taller than the inoculated control.

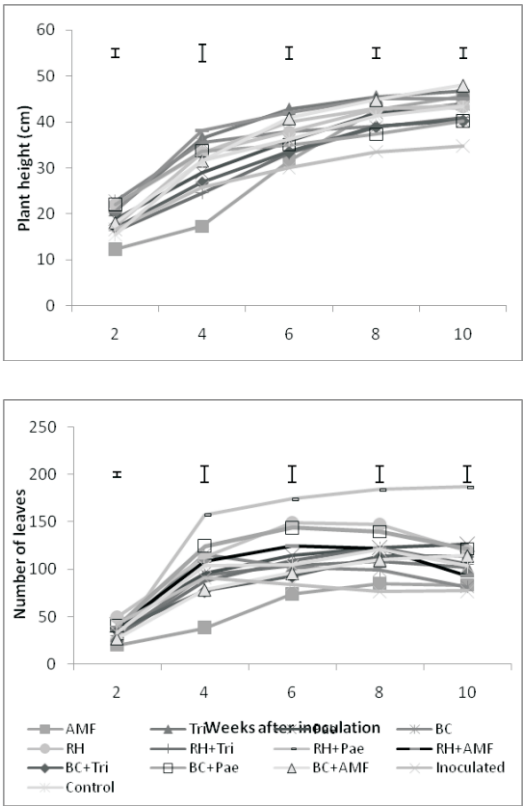

Figure 4a: Growth of *Meloidogyne javanica* - inoculated plants treated with soil amendments and biocontrol agents singly and in combinations.

Error bars represent Standard error of means; AMF: Arbuscular mycorrhiza fungi (*Glomus* spp.); Tri: *Trichoderma asperellum*; Pae: *Paecilomyces lilacinus;* BC: Biochar; RH: Rice husk; RH+T: Rice husk + *T. asperellum*; RH+P: Rice husk+ *P. lilacinus* : RH+A: Rice husk+ AMF :BC+T: Biochar + *T. asperellum:* BC+P: Biochar + *P. lilacinus*: BC+A: Biochar + AMF: Nem: Nematode inoculated plants : Control - untreated and uninoculated plants.

The number of leaves also showed significant differences from two weeks after inoculation, however wider differences were observed from 3-8 WAI (Figure 4b). Plants

with AMF alone had fewer number of leaves throughout the experiment and this was significantly ($P \leq 0.05$) lower than other treatments including the inoculated control at 3-5 WAI. At 3 WAI and onwards, rice husk combined with *P. lilacinus*, Biochar with *P. lilacinus* and rice husk alone improved leaf production significantly more than the uninoculated control plants and other treatments. Significantly ($P \leq 0.05$) higher leaf production was observed in nematode-infected plants treated with rice husk + *P. lilacinus, P. lilacinus* alone and Biochar + *P. lilacinus.* These were higher than the uninoculated control and plants treated with T. asperellum and its combinations with soil amendments. Plants treated with AMF alone, biochar alone and the nematode inoculated plants had the lowest ($P \leq 0.05$) number of leaves.

Effect of treatments on yield parameters of tomato plants at ten weeks after inoculation

Delayed flowering occurred in plants treated with AMF alone, these plants flowered significantly ($P \leq 0.05$) later than all other treatments and compared to the uninoculated control, they flowered 16 days later. (Table 1). The earliest plants to flower were those treated with biochar + *P. lilacinus* followed by plants treated with biochar alone, rice husk + *P. lilacinus,* rice husk +AMF and the uninoculated plants. Furthermore, infected plants treated with *T. asperellum*, rice husk, biochar + AMF, and rice husk + *T. asperellum* flowered earlier (not statistically different) than the inoculated control plants.

Plants treated with rice husk + AMF had the highest ($P \leq 0.05$) fresh shoot weight compared to other treatments, although the shoot weight was not significantly different from that of uninoculated plants (Table 1). The inoculated control plants had the lowest shoot weight and was similar to inoculated plants treated with AMF alone, rice husk + *T. asperellum and biochar + AMF.* Plants treated with *P. lilacinus* had the highest fresh root weight (19.3 g), while the *M. javanica*-inoculated plants recorded the least root weight (4.5 g). Infected plants treated with *T. asperellum,* rice husk + *P. lilacinus,* and rice husk + AMF had similar root weight with the uninoculated control plants.

The highest number of fruits was produced by plants treated with rice husk + *P. lilacinus, P. lilacinus* alone and biochar + *P. lilacinus* and the fruits were more ($P \leq 0.05$) than the number produced by the control plants. The fewest ($P \leq 0.05$) fruits were produced by plants treated with rice husk alone and the inoculated plants. Other treatments produced either more or comparable number of fruits with the control (uninoculated) plants.

Plants treated with the biochar as soil amendment recorded the highest fruit weight (24.8 g) followed by Biochar + *P. lilacinus*-treated plants. While the lowest ($P = 0.05$) fruit weight was recorded from inoculated plants and was not significantly different from fruit weight in plants treated with, Arbuscular mycorrhizal fungi alone. Plants treated with rice husk alone, rice husk + *T. asperellum,* rice husk + *P. lilacinus,* rice husk + AMF, biochar + AMF and *P. lilacinus* were comparable in fruit weight with the uninoculated control plants.

Table 1: Effect of soil amendments and biocontrol agents on yield parameters of tomato plants inoculated with *Meloidogyne javanica*.

Treatment	No. of days to flowering	Shoot weight (g)	Root weight (g)	No. of fruits	Fruit weight (g)
Inoculated control	33.0bc	17.5c	4.5d	2.3e	1.2d
AMF	45.0a	16.3c	7.3cd	6.9bc	5.2.0d
Rice husk + *T. asperellum*	34.0bc	18.2c	7.5cd	7.3bc	17.0b
Biochar + AMF	32.0c	19.8c	8.5c	5.0cd	12.5bc
Biochar + *P. lilacinus*	26.0d	20.7bc	7.8cd	9.2ab	21.0ab
Rice husk	30.0cd	22.0bc	10.8cd	2.3e	17.8b
Rice husk+ *P. lilacinus*	28.0cd	24.5bc	13.8b	10.7a	16.3b
Trichoderma asperellum	30.0cd	25.0b	13.8b	4.6d	9.5cd
Paecilomyces lilacinus	33.0bc	25.5b	19.3a	9.3ab	9.0cd
Biochar + *T. asperellum*	40.0ab	26.0b	11.0cd	4.6d	12.0bc
Biochar	27.0cd	26.5b	12.0bc	2.3e	24.8a
Rice husk+ AMF	28.0cd	39.75a	15.3ab	4.4de	14.5bc
Uninoculated control	29.0cd	30.8ab	13.0b	4.8d	12.7bc

Means with the same letter in a column are not significantly different using SNK at $P \leq 0.05$
AMF = Arbuscular Mycorrhizal Fungi (*Glomus* spp.).

The highest GI (30% root galling) was recorded in the plants inoculated with nematodes but received no treatment, and they were not significantly different from GI in plants treated with *T. asperellum*, Biochar + *T. asperellum,* Biochar alone, and Rice husk + AMF. Plants that received treatment of rice husk alone and biochar alone recorded the highest galling index. The lowest nematode populations was observed in tomato plants treated with AMF (Table 2). High nematode populations were observed in roots and soil of plants treated with rice husk alone and all its combinations as well as biochar treatment alone and these were comparable to the inoculated control. Reproductive factor (RF) for nematode-infected plants that received AMF was less <1 and was not significantly different from the zero value recorded in plants that were not inoculated (Table 2). However, RF from the AMF treatment was not significantly different from RF in plants treated with all the combinations of biocontrol agents and soil amendments except for Rice husk + *P. lilacinus* and rice husk + AMF. Reproductive factor from single treatment with biochar and rice husk was not different from RF in inoculated control plants.

Table 2: Nematode population, galling index (GI) and reproductive factor (RF) of *Meloidogyne javaniva*-infected plants treated with soil amendments and biocontrol agents singly and in combinations

Treatment	Galling index	Total nematode population[a]	Reproductive factor
Uninoculated control	1.0d	0.7d	0.0d
AMF	2.0bc	63.2c	0.8cd
Biochar + AMF	1.5cd	70.7bc	1.0c
Trichoderma asperellum	3.3a	74.2bc	1.1bc
Rice husk + *T. asperellum*	1.8c	80.6bc	1.3bc
Biochar + *P. lilacinus*	2.0bc	83.7b	1.4bc
Biochar + *T. asperellum*	3.0a	89.4b	1.6bc
Paecilomyces lilacinus	2.3b	92.2b	1.7bc
Rice husk+ *P. lilacinus*	2.3b	94.4b	1.8b
Rice husk+ AMF	2.8ab	100.0b	2.0b
Biochar	2.8ab	137.8a	3.8a
Rice husk	2.5b	139.6a	3.9a
Inoculated control	3.3a	126.7a	3.2a

[a]Transformed ($\sqrt{n+0.5}$) means of nematode population
Means with the same letter in a column are not significantly different using SNK at $P \leq 0.05$
AMF = Arbuscular Mycorrhizal Fungi (*Glomus* spp.). Galling index on 1-5 scale 1= no galls, 2=1-15% of galled root, 3=16-30%, 4=31-60%, 5=61-100%. Rf = P_f/P_i, where P_f is final population (soil + root) and P_i is initial population (2000 eggs)

Discussion

Growth of tomato in terms of plant height and number of leaves on AMF treated pots was slower in comparison to other treatments. In a study by Smith *et al.* (2004), tomato did not respond positively to three AM fungi and showed significant growth depressions even though colonization of AMF occurred. In their study, positive response of the tomato plants was observed 12 weeks after planting which was similar to the results in this study where growth of the plants began to improve after 8 weeks of observation and by 10 WAI, plants were similar in height to the uninoculated control. There is a possibility that AMF lengthened the vegetative phase of the plants or that the process of root colonization could lengthen the time to flowering. Rice husk addition to soil is reported to reduce nematode population in field trials with an accompanied increase in growth and yield of plants (Hassan *et al.*, 2010). Fruit yield of tomato plants treated with Rice husk + *P. lilacinus, P. lilacinus* and Biochar + *P. lilacinus* ranked among highest of all the treatments. The positive results obtained from combination of

P. lilacinus with rice husk or biochar may be due to the provision of substrate which probably improved growth of *P. lilacinus* in the soil environment thus enhancing colonization of eggs of the root-knot nematode leading to reduced nematode populations. *Paecilomyces lilacinus* is reported to reduce egg population in egg masses, number of root galls, and also inhibits giant cell formation in host plants (Cabanillas *et al.*, 1988). On its own, biochar provided the high tomato fruit yield, and contributed to the increased benefit of the bioagents it was combined with. Biochar application to soil improves soil productivity and increases water soil holding capacity (Lehmann and Joseph, 2009; Koide *et al.,* 2011), these conditions may have contributed to improved efficiency of *P. lilacinus* in causing reduction in *M. javanica* egg population. Hu *et al*. (2014) found that biochar amended soil were enriched with *Actinobacteria, Trichoderma* and *Paecilomyces* compared to unamended soil.

While yield improved with the soil amendments (biochar and rice husk) alone, they did not reduce nematode populations or galling index in plants, however single and combined application of bio-agents significantly reduced the ability of nematodes to reproduce. Application of AMF alone and Biochar +AMF had the least reproductive factor and also showed lower galling. Plants that received *Trichoderma asperellum*, rice husk+ *T. asperellum*, biochar + *T. asperellum,* and biochar+ *P. lilacinus* provided between 64% and 79.5% reduction in nematode population. Each of the three bioagents evaluated provided some level of protection for the tomato plants; however, this protection was enhanced with the addition of biochar or rice husk. This may be due to the slow decomposition rate of rice husk compared to biochar. The combinations of biochar with biocontrol agents provided greater reduction in nematode populations compared to rice husk combinations. According to Orlando (2016), biochar is a viable option for use in the control of plant-parasitic nematodes because of its potential to reduce plant-parasitic nematodes and increase yields. Biochar peharps interacted with mycorrhizae to lower parasitic nematode populations. While further field studies need to be conducted, this study found that biological agents remain a viable treatment option for the control of plant-parasitic nematodes. Application of biochar was found to improve the beneficial effect of biological control agents.

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