Research Article

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Trichoderma asperellum affects *Meloidogyne incognita* infestation and development in *Celosia argentea*

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Abstract: Due to the rise in cost and detrimental health and environmental consequences that accompany the use of nematicides, there is a need for a more eco-friendly and less expensive alternative to control root-knot nematode (*Meloidogyne incognita*). Nematode infestation reduces the quality and quantity of *Celosia argentea* Linn. A pot experiment was conducted in a greenhouse to determine the biocontrol efficacy of *Trichoderma asperellum* against *M. incognita* in *C. argentea*. The treatments consisted *M. incognita* infected *C. argentea* inoculated with 0, 2.2×10^7, 4.4×10^7, or 6.6×10^7 cfu/pot of *T. asperellum*. All doses of *T. asperellum* reduced the root-knot nematode population and root gall index. Growth and development of *C. argentea* were improved, indicating that *T. asperellum* has the potential to be used as a biocontrol agent in *C. argentea* production. The biocontrol activity of *T. asperellum* in *C. argantea* increased as the week went by until the plants attained full maturity. Hence, the control of *M. incognita* by *T. asperellum* depends on the developmental stage of the plant root system.

Keywords: agriculture, microbial inoculant, biocontrol, *Meloidogyne incognita*, *Celosia argentea*

1 Introduction

Celosia argentea Linn. (family Amaranthaceae) is an annual, herbaceous, vegetable whose leaves, tender stems, and young flower spikes are eaten cooked (Daramola et al. 2015). The dried, ripe seed is reported to have medicinal properties because of its β-carotene, vitamin E, folic acid, ascorbic acid, phosphorus, calcium, iron, and protein contents (Tang et al. 2016). The leaves contain amaranthine (betacyanin), oxalic acid, and phytic acid (Tang et al. 2016). The production of *C. argentea* is challenged by the sedentary endoparasite root-knot nematode (*Meloidogyne incognita*; Daramola et al. 2015; Fabiyi et al. 2016), which causes 60–80% yield loss of *C. argentea* (Tang et al. 2016) due in part to stunted growth, rotted, and galled roots (Anwar et al. 2009). Current management of *M. incognita* in *C. argentea* production is with synthetic nematicides. Human health risk and environmental consequences accompany the use of synthetic nematicides (Fabiyi et al. 2016; Alori and Babalola 2018). Synthetic nematicides are also very expensive, thereby increasing the cost of *C. argentea* production. Hence, the need for a more eco-friendly and less expensive alternative that will pose no health risk.

Microbial inoculants are an effective, environmentally safe, alternative method of controlling plant diseases affecting vegetables (Radwan et al. 2012).

Microbial inoculants can improve plant growth and yield and can act as biocontrol agents against many crop pathogens (Zakaria et al. 2013; Alori and Babalola 2018). Whether they are efficacious in the production of *C. argentea* needs ascertaining. The study therefore evaluated the

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efficacy of *Trichoderma asperellum* as a biocontrol agent against *M. incognita* on *C. argentea*.

2 Materials and method

2.1 Collection of soil sample and sterilization of soil for pot experiment

Soil samples were randomly collected from a 0 to 15 cm depth at the Landmark Teaching and Research Farm Omuaran, Kwara State, Nigeria, at 80°9′N latitude and 50°61′E longitude. Soil samples were bulked to form a representative sample that was transported to the laboratory for testing. Samples were air-dried for 3 days and passed through a 2 mm sieve in preparation for analysis. Samples were wet sterilized in an autoclave at 121°C for 20 min.

2.2 Sources of *C. argentea* seeds and *T. asperellum*

Seeds of *C. argentea*, var. TLV8, were purchased from the National Horticultural Research Institute, Ibadan, Nigeria, and *T. asperellum* was obtained from the Microbiology (Nematology) Department, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania.

2.3 Extraction of nematode juveniles

Galled roots of *C. argentea* plants were sourced from the Teaching and Research Farm of Kwara State University, Malete, Kwara State, Nigeria. Galled roots were washed gently with water to remove soil particles. Nematode eggs were extracted from 5 g of chopped galled roots (Hussey and Baker 1973). Density of extracted eggs was assessed by placing 2 mL of nematode suspension into dishes and counting was done at ×40 with a stereomicroscope (Doncaster 1962).

2.4 Pot experiment

Twelve plastic pots, 30 cm diameter, filled with 10 kg of sterilized soil were arranged in a completely randomized design in a screenhouse of the Landmark University Teaching and Research Farm. Four pots containing sterilized soil were inoculated with 0, 2.2×10^7, 4.4×10^7, or 6.6×10^7 cfu/pot of *T. asperellum*, replicated 3 times to make 12 pots. Two days after inoculation of the fungus, seeds of *C. argentea* were planted in pots, and the plants were thinned to two per pot after emergence. The plants were inoculated with approximately 1,000 second-stage juveniles (J2) of *M. incognita*/pot 1 week after emergence, which were added in 3 cm-deep holes around the plant base using the method of Iheukwumere et al. (1995). The experiment was comprised of the following treatments: 1,000 J2 of *M. incognita* only, 2.2×10^7 cfu/pot of *T. asperellum* + 1,000 J2 of *M. incognita*, 4.4×10^7 cfu/pot of *T. asperellum* + 1,000 J2 of *M. incognita*, or 6.6×10^7 cfu/pot of *T. asperellum* + 1,000 J2 of *M. incognita*, each replicated three times. Average temperature of the screenhouse during the experimental period was 25°C. Plants were supplied with tap water to field capacity at 6 am and 6 pm daily with a watering can.

Evaluations of responses began 3 weeks after planting (WAP). Data were taken weekly on plant height, number of leaves, and stem diameter. Root gall indices and egg counts were recorded after harvest. The scale used for rating roots for galling, or the root gall index, was based on Taylor and Sasser (1978), where 0 = no infestation, 1 = 1–5% of the root galled, 2 = 6–25% of the root galled, 3 = 26–50% of the root galled, 4 = 51–75% of the root galled, and 5 = 76–100% of the root galled.

2.5 Analysis of soil chemical characteristics

Soil chemical properties were determined before and after the experiment, such as soil pH with an electronic soil pH meter (Model 215; Denver Instrument, Colorado, USA), soil particle size analysis by the hydrometer method (Gee and Or 2002), organic matter content was determined using the wet oxidation method (Shamshuddin et al. 1994), and exchangeable bases (K, Mg, Na, and Ca) were determined by ammonium acetate method (Chapman 1965). To determine exchangeable acidity, 5 g of air-dried soil (sieved through 2 mm sieve) was weighed into a 250 mL conical flask. Fifty milliliters of 1 N potassium chloride (KCl) solution was added to the soil sample in the conical flask. The flask was shaken on a reciprocating shaker for 1 h, and the content was filtered through Whatman No. 42 filter paper. Twenty-five milliliters of the filtrate was pipetted into a 100 mL conical flask and 50 mL distilled water was added along with 5 drops of

phenolphthalein indicator. The resulting solution was titrated with 0.01 N sodium hydroxide (NaOH) to a permanent pink end point. The volume of the base used was recorded to calculate the total exchangeable acidity (H + Al); the effective cation exchange capacity was determined by the summation of the exchangeable bases (Ca, Mg, Na, and K) and exchangeable acidity expressed in cmol/kg; the total soil nitrogen was determined by Macrokjedahl method (Bremner 1965); and the Bray1 method was used to determine the available phosphorus (Murphy and Riley 1962).

2.6 Data analysis

All data from the experiment were subjected to analysis of variance (ANOVA) using IBM SPSS statistical package (2012). Means were separated using Duncan's multiple range test ($P \leq 0.05$).

3 Results

3.1 Effects of *T. asperellum* on the population of nematode on gall index in *C. argentea* infected with *M. incognita*

Table 1 shows the population of *M. incognita* in infected *C. argentea* inoculated with *T. asperellum*. *M. incognita* was found to be the highest in plants treated with 1,000 second-stage juveniles (J2) of *M. incognita* only and lowest in plants that received 1,000 second-stage juveniles (J2) of *M. incognita* + 6.6×10^7 cfu/pot of *T. asperellum*.

The *C. argentea* that received 1,000 second-stage juveniles (J2) of *M. incognita* only had the highest mean for gall index at 3.3 and this mean was significantly different ($P \leq 0.05$) from those plants treated with *T. asperellum* suspension except for those that received 1,000 second-stage juveniles (J2) of *M. incognita* + 2.2×10^7 of *T. asperellum* suspension.

3.2 Effect of *T. asperellum* on plant height of *C. argentea* infected with *M. incognita*

Table 2 reports the impact of *T. asperellum* on the height of *C. argentea* infected with *M. incognita*. It was observed that at 5 and 6 WAP, *M. incognita*-infected *C. argentea* inoculated with *T. asperellum* at the rate of 6.6×10^7 cfu/mL had significantly higher height than the untreated control. However, at 7 and 8 WAP, the application of *T. asperellum* at the rate of 2.2×10^7, 4.4×10^7, and 6.6×10^7 cfu/mL resulted in significantly higher height of *C. argentea* when compared to the control that was not treated with *T. asperellum*.

Table 2: Effect of *Trichoderma asperellum* on plant height of *Celosia argentea* infected with *Meloidogyne incognita*

Trichoderma asperellum application rate (cfu/mL)	Plant height (cm)					
	3WAP	4WAP	5WAP	6WAP	7WAP	8WAP
0	8.5[a]	10.8[a]	14.5[b]	16.6[b]	8.8[c]	20.7[c]
2.2×10^7	8.8[a]	12.0[a]	16.3[ab]	19.6[ab]	21.4[b]	22.5[b]
4.4×10^7	8.5[a]	11.8[a]	16.6[ab]	20.1[ab]	23.2[ab]	25.9[ab]
6.6×10^7	9.0[a]	13.7[a]	19.5[a]	23.0[a]	26.3[a]	28.5[a]

WAP = weeks after planting.
Each value is a mean of three replicates.
Means followed by the same letter along the same column are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

Table 1: Effect of treatment on final nematode population and gall index

Treatment	Nematode population		Root gall index
	Initial	Final	
1,000 second-stage juveniles (J2) of *M. incognita* only	1,000	2,098a[a]	3.3b
2.2×10^7 cfu *Trichoderma asperellum*/pot + 1,000 second-stage juveniles (J2) of *M. incognita*	1,000	920b	3.0ab
4.4×10^7 cfu of *T. asperellum*/pot + 1,000 second-stage juveniles (J2) of *M. incognita*	1,000	440c	2.3a
6.6×10^7 cfu of *T. asperellum*/pot + 1,000 second-stage juveniles (J2) of *M. incognita*	1,000	230d	1.7a

[a]Values in columns followed by the same letter are not significantly different, $P \leq 0.05$, Duncan multiple range test.

Table 3: Effect of *Trichoderma asperellum* on the number of leaves of *Celosia argentea* infected with *Meloidogyne incognita*

Trichoderma asperellum application rate (cfu/mL)	Number of leaves					
	3WAP	4WAP	5WAP	6WAP	7WAP	8WAP
0	7.0[a]	9.0[a]	12.0[b]	13.7[b]	15.3[c]	16.7[c]
2.2×10^7	6.7[a]	9.7[a]	13.3[a,b]	16.3[a,b]	18.3[b]	20.3[b]
4.4×10^7	6.7[a]	10.0[a]	14.0[a,b]	17.3[a,b]	19.3[a,b]	22.7[a,b]
6.6×10^7	7.0[a]	12.0[a]	16.0[a]	19.3[a]	21.7[a]	24.0[a]

WAP = weeks after planting.
Each value is a mean of three replicates.
Means followed by the same letter along the same column are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

3.3 Effect of *T. asperellum* on number of leaves of *C. argentea* infected with *M. incognita*

The effect of *T. asperellum* on the number of leaves of *C. argentea* infected with *M. incognita* is presented in Table 3. At weeks 5 and 6, the application of *T. asperellum* at 6.6×10^7 cfu/mL significantly increases the number of leaves compared with the uninoculated *C. argentea* in the presence of *M. incognita*. However, at weeks 7 and 8, the application of *T. asperellum* at 2.2×10^7, 4.4×10^7, and 6.6×10^7 increased the number of leaves of *C. argentea* infected with *M. incognita*.

3.4 Interactive effects of *T. asperellum* and WAP on growth parameters of *C. argentea* infected with *M. incognita*

Table 4 reports the impact of the interactive effects of *T. asperellum* and WAP on the growth parameters of *C. argentea* infected with *M. incognita*. The treatment (inoculants, WAP) effects on measured plant growth parameters were significant ($P \leq 0.05$). It was observed that inoculation of *M. incognita*-infected *C. argentea* with *T. asperellum* significantly increased the plant height and the number of leaves.

Likewise, the interactive effect (inoculant × weeks) on measured growth parameters was significant. This implies that the interaction between *T. asperellum* with the *C. argantea* seedling root increases as the week goes by.

3.5 Effects of *T. asperellum* on chemical composition of the soil used

The result in Table 5 shows that the application of microbial inoculants caused a significant change in the mineral composition of the soil after harvesting. The pH of the soil where plants were treated with *M. incognita* only increased by 13%, while the soil where plants were treated with *M. incognita* + 2.2×10^7 cfu/pot of *T. asperellum*, *M. incognita* + 4.4×10^7 cfu/pot of *T. asperellum*, and *M. incognita* + 6.6×10^7 cfu/pot of *T. asperellum* caused up

Table 4: Interactive effect of *Trichoderma asperellum* and WAP on growth parameters of *Celosia argentea* infected with *Meloidogyne incognita* measured over time

	Plant height	Number of leaves
Weeks after planting		
3	8.70e	6.85f
4	12.08d	10.18e
5	16.73c	13.83d
6	19.83b	16.65c
7	19.93b	18.65b
8	24.41a	20.93a
Microbial inoculants		
1,000 second-stage juveniles (J2) of *M. incognita* only	13.33d	12.28d
2.2×10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita*	16.77c	14.10c
4.4×10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita*	17.68b	15.00b
6.6×10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita*	20.00a	16.67a
ANOVA response		
Week	0.00	0.00
Inoculants	0.00	0.00
Week × inoculants	0.00	0.00

Values in columns followed by the same letter are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

Table 5: Effects of *Trichoderma asperellum* on chemical composition of the soil used

Soil chemical property	1,000 second-stage juveniles (J2) of *M. incognita* only	2.2×10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita*	4.4×10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita*	6.6×10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita*	Initial chemical property of soil	Value
pH (H_2O) 1:2	6.56a	5.43d	5.48c	5.71b	pH (H_2O) 1:2	5.84 ± 0.1
Total N (%)	2.20b	2.69ab	2.65ab	2.85a	Total N (%)	0.30 ± 0.1
Avail. P (g/kg)	16.99c	14.88d	27.56b	29.67a	Avail. P (g/kg)	12.50 ± 0.5
Organic matter (%)	1.52c	2.00a	1.74b	1.95a	Organic matter (%)	1.8 ± 0.01
Na^+ (cmol/kg)	0.74d	0.78c	0.88a	0.80b	Na^+ (cmol/kg)	0.05 ± 0.01
K^+ (cmol/kg)	0.47c	0.45c	0.55b	0.61a	K^+ (cmol/kg)	0.07 ± 0
Ca^{2+} (cmol/kg)	3.28c	3.16d	3.97b	4.11a	Ca^{2+} (cmol/kg)	0.4 ± 0.1
Mg^+ (cmol/kg)	0.95d	1.01c	1.23b	1.42a		
Al^+ H (cmol/kg)	0.05a	0.07a	0.07a	0.06a		
ECEC (cmol/kg)	5.48c	5.47c	6.70b	7.00a		

ECEC = effective cation exchange capacity.
Values in rows followed by the same letter are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

to 7% reduction in pH. There was an increase in all the mineral contents of the soil of plants treated with *M. incognita* + 2.2×10^7 cfu/pot of *T. asperellum*, *M. incognita* + 4.4×10^7 cfu/pot of *T. asperellum*, and *M. incognita* + 6.6×10^7 cfu/pot of *T. asperellum* when compared with the composition at the start of the experiment (Table 5).

4 Discussion

4.1 Effects of *T. asperellum* on the population of nematode on gall index in *C. argentea* infected with *M. incognita*

The higher the rate of application of *T. asperellum*, the lower the population of *M. incognita* in infected *C. argentea*. This may be due to the ability of the fungi conidial to get attached to the eggs and cause immobilization of the second-stage juvenile of the nematode (Mascarin et al. 2012). Second, *Trichoderma* strains are reported to colonize root systems in many plants, thereby coordinating the defense mechanisms of host plant (Hermosa et al. 2012).

The result on galling index reflects a similar trend with regard to the number of nematodes because nematodes determine the presence of root galls in the root system of *C. argentea* plants, meaning the number of nematodes is directly proportional to galling index. Since *T. asperellum* caused immobilization of the second-stage juvenile of the nematode, galling cannot occur. Mechanisms of *Trichoderma* spp. to control plant pathogen include parasitism and antagonism. *Trichoderma* spp. induce systemic resistance in plants (Harman 2006).

4.2 Effect of *T. asperellum* on growth parameters of *C. argentea* infected with *M. incognita*

Significant increase in plant height and the number of leaves observed by reason of inoculation of *M. incognita*-infected *C. argentea* could be attributed to the reduction in nematode population and galling index as shown in Table 1. *T. harzianum* (T22) was reported to increase the growth of maize (Akladious and Abbas 2012). Also, according to Bíró-Stingli and Tóth (2011), northern root-knot nematode-infected green pepper treated with *T. asperellum* experiences up to 18% increase in height compared to the untreated control. *T. harzianum* caused an increase in the growth of *M. javanica*-infected tomato (Javeed and Al-Hazm 2015).

4.3 Interactive effects of *T. asperellum* and WAP on growth parameters of *C. argentea* infected with *M. incognita*

Increased plant height and number of leaves could be attributed to the reduction in nematode population and galling index (Table 1). The improved growth recorded in this research could also be attributed to increased nutrient status of the soil because of the application of *T. asperellum* as seen in Table 5. Alori and Babalola (2018) reported that microbial inoculants effectively control plant pathogens by either direct or indirect mechanism. They improved plant growth by the production of plant growth hormones. However, *T. asperellum* spp. increased plant growth due to increased nutrient uptake (Harman 2006).

The interaction between *T. asperellum* and WAP depends on the developmental stage of the plant root system, which improves with the age of the plant. Idowu et al. (2016) reported a similar effect.

4.4 Effects of *T. asperellum* on the chemical composition of the soil used

A reduction in pH recorded in pots treated with *T. asperellum* probably accounts for the reduction in nematode population and root gall index.

Also, increase in all the mineral contents of the soil when compared with the composition at the start of the experiment as recorded in Table 5 agrees with the findings of many researchers; Marathe et al. (2011) reported that microbial inoculant application resulted in improved soil fertility status and plant nutrient uptake. A significant increase in organic carbon content of the soil was noted by reason of inoculation of groundnut with microbial inoculants such as *T. viride*, *T. harzianum*, and *Bacillus megaterium* (Prasad et al. 2017). This could be due to the ability of these organisms to accelerate decomposition of organic matter and the mineralization of both micro- and macronutrients in the soil (Xu and Li 2017; Table 5).

5 Conclusion

Trichoderma asperellum improved the growth and development of *C. argentea* infected with *M. incognita*, indicating that *T. asperellum* has the potential to be used as a biocontrol agent in *C. argentea* production. The biocontrol activity of *T. asperellum* in *C. argantea* increased as the week went by until the plants attained full maturity. Hence, the control of *M. incognita* by *T. asperellum* depends on the developmental stage of the plant root system.

Conflict of interest: The authors declare no conflicts of interest.

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