Research Article

Alori Elizabeth Temitope*, Aluko Ajibola Patrick, Joseph Abiodun, Adekiya Aruna Olasekan, Aremu Charity Onye, Adebiyi Ojo Timothy Vincent, Adegbite Kehinde Abodunde, Ejue Wutem, Rutazaha JoanPaula Elliseus

Trichoderma asperellum affects Meloidogyne incognita infestation and development in Celosia argentea

https://doi.org/10.1515/opag-2020-0075
received March 27, 2020; accepted June 04, 2020

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. argentea 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...
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°6′1′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 \( \times 40 \) 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 \( \times 10^7 \), 4.4 \( \times 10^7 \), or 6.6 \( \times 10^7 \) 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 \( \times 10^7 \) cfu/pot of *T. asperellum* + 1,000 J2 of *M. incognita*, 4.4 \( \times 10^7 \) cfu/pot of *T. asperellum* + 1,000 J2 of *M. incognita*, or 6.6 \( \times 10^7 \) 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 $\times$ 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 $\times$ 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 $\times$ 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 $\times$ 10$^7$, 4.4 $\times$ 10$^7$, and 6.6 $\times$ 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) | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|-------------------------------------------------|------|------|------|------|------|------|
| 0                                               | 8.5a | 10.8a | 14.5b | 16.6b | 8.8c | 20.7c |
| 2.2 $\times$ 10$^7$                              | 8.8a | 12.0a | 16.3ab | 19.6ab | 21.4b | 22.5b |
| 4.4 $\times$ 10$^7$                              | 8.5a | 11.8a | 16.6ab | 20.1ab | 23.2ab | 25.9ab |
| 6.6 $\times$ 10$^7$                              | 9.0a | 13.7a | 19.5a | 23.0a | 26.3a | 28.5a |

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 |
|-----------|---------------------|-----------|
| Initial | Final | index |
| 1,000 second-stage juveniles (J2) of *M. incognita* only | 1,000 | 2,098a$^*$ | 3.3b |
| 2.2 $\times$ 10$^7$ cfu *Trichoderma asperellum*/pot + 1,000 second-stage juveniles (J2) of *M. incognita* | 1,000 | 920b | 3.0ab |
| 4.4 $\times$ 10$^7$ cfu of *T. asperellum*/pot + 1,000 second-stage juveniles (J2) of *M. incognita* | 1,000 | 440c | 2.3a |
| 6.6 $\times$ 10$^7$ cfu of *T. asperellum*/pot + 1,000 second-stage juveniles (J2) of *M. incognita* | 1,000 | 230d | 1.7a |

$^*$Values in columns followed by the same letter are not significantly different, $P \leq 0.05$, Duncan multiple range test.
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 $6.6 \times 10^7$ cfu/mL significantly increased 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 $2.2 \times 10^7$, $4.4 \times 10^7$, and $6.6 \times 10^7$ increased the number of leaves 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. argentea* seedling root increases as the week goes by.

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* + $2.2 \times 10^7$ cfu/pot of *T. asperellum*, *M. incognita* + $4.4 \times 10^7$ cfu/pot of *T. asperellum*, and *M. incognita* + $6.6 \times 10^7$ cfu/pot of *T. asperellum* caused up

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

| *Trichoderma asperellum* application rate (cfu/mL) | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|--------------------------------------------------|------|------|------|------|------|------|
| 0                                                | 7.0a | 9.0a | 12.0b| 13.7b| 15.3c| 16.7c|
| $2.2 \times 10^7$                                 | 6.7a | 9.7a | 13.3a|b    | 16.3a|b    | 18.3b| 20.3b|
| $4.4 \times 10^7$                                 | 6.7a | 10.0a| 14.0a|b    | 17.3a|b    | 19.3a|b    | 22.7a|b    |
| $6.6 \times 10^7$                                 | 7.0a | 12.0a| 16.0a| 19.3a| 21.7a| 24.0a|

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

| Weeks after planting | Plant height | Number of leaves |
|----------------------|--------------|------------------|
| 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 |
|----------------------------------------------------------|--------|--------|
| $2.2 \times 10^7$ cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita* | 16.77c | 14.10c |
| $4.4 \times 10^7$ cfu/pot of *T. asperellum* + 1,000 second-stage juveniles (J2) of *M. incognita* | 17.68b | 15.00b |
| $6.6 \times 10^7$ 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 \((J_2)\) of *M. incognita* only | 2.2 \(\times\) 10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles \((J_2)\) of *M. incognita* | 4.4 \(\times\) 10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles \((J_2)\) of *M. incognita* | 6.6 \(\times\) 10^7 cfu/pot of *T. asperellum* + 1,000 second-stage juveniles \((J_2)\) 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                                          | 1.95a                                           | Avail. P (g/kg)   | 12.50 ± 0.5 |
| Organic matter (%)    | 1.52c                                           | 2.00a                                           | 1.74b                                           | 1.8a                                            | 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 ± 0.0 |
| Ca\(^2+\) (cmol/kg)   | 3.28c                                           | 3.16d                                           | 3.97b                                           | 4.1a                                            | Ca\(^2+\) (cmol/kg)| 0.4 ± 0.1 |
| Mg\(^+\) (cmol/kg)    | 0.95d                                           | 1.01c                                           | 1.23b                                           | 1.42a                                           | Mg\(^+\) (cmol/kg)| 0.05 ± 0.01 |
| Al\(^3+\) H (cmol/kg) | 0.05a                                           | 0.07a                                           | 0.07a                                           | 0.06a                                           | Al\(^3+\) H (cmol/kg)| 0.05 ± 0.01 |
| ECEC (cmol/kg)        | 5.48c                                           | 5.47c                                           | 6.70b                                           | 7.00a                                           | ECEC               | 5.88 ± 0.1 |

ECEC = effective cation exchange capacity.

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

### 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*. Inoculation of *C. argentea* infected with *M. incognita* led to a 7% reduction in pH. There was an increase in all the mineral contents of the soil of plants treated with *M. incognita*. Since the root system of the nematode is directly proportional to galling index, the higher the rate of application of *T. asperellum*, the lower the population of *M. incognita* in infected *C. argentea*.

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

The result on galling index reflects a similar trend. The reactivity of *M. incognita* in infected *C. argentea* is due to the ability of the fungi conidial to get attached to the eggs and cause immobilization of the nematode. Mechanisms of defense include parasitism and antagonism. *Trichoderma* spp. induce systemic resistance in plants (Harman 2006).

---

**4 Discussion**

The higher the rate of application of *T. asperellum*, the lower the population of *M. incognita* in infected *C. argentea*. It 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 because nematodes are reported to colonize the defense mechanisms of host plants (Hernadez et al. 2012). Second, *Trichoderma* strains are reported to colonize the root systems in many plants, thereby coordinating the second-stage juvenile of the nematode, galling cannot occur. Mechanisms of *Trichoderma* spp. to control plant pathogens include parasitism and antagonism. *Trichoderma* spp. induce systemic resistance in plants (Harman 2006).
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. argentea* 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.

**References**

[1] Akladious SA, Abbas SM. Application of *Trichoderma harzianum* T22 as a biofertilizer supporting maize growth. Afr J Biotechnol. 2012;11(35):8672–83.

[2] Alori ET, Babalola OO. Microbial inoculant for improving crop quality and human health. Front Microbiol. 2018;9:1–12.

[3] Anwar SA, Zia A, Shaked Q. A root-knot nematode pathogenic to cock’s comb, *Celosia argentea*. Pak J Nematol. 2009;27:309–15.

[4] Biró-Stüngli T, Tóth F. The effect of trifender (*Trichoderma asperellum*) and the nematode-trapping fungus (*Arthrobotrys oligospora*) fresenius on the number of the northern root-knot nematode (*Meloidogyne hapla*) Chitwood) in green pepper. J Plant Prot Res. 2011;51(4):371–6.

[5] Bremer JM. Cation exchange capacity. In: Black CA, editor. Methods of soil analysis part 2. Madison, Wisconsin, USA: American Society of Agronomy Inc; 1965. p. 1149–78.

[6] Chapman HD. Cation exchange capacity. In: Black CA, editor. Methods of soil analysis part 2. Madison, Wisconsin, USA: American Society of Agronomy Inc; 1965. p. 891–901.

[7] Daramola F, Popoola J, Eni AO, Sulaiman O. Characterization of root-knot Nematodes (*Meloidogyne spp*) associated with *Abelmoschus esculentus*, *Celosia argentea* and *Corchorus olitorius*. Asian J Biol Sci. 2015;8:42–50.

[8] Doncaster CC. *A* counting dish for nematodes. Nematol. 1962:7:334–6.

[9] Fabiyi OA, Olununj GA, Olagbenro MO. Response of the root-knot nematode-infected celosia argentea to bark extracts of *Khaya ivorensis*. Ile J Agric. 2016;28(2):24–36.

[10] Gee WG, Or D. Methods of soil analysis. In: Dane J, Topp GC, editors. Particle size analysis. Soil Science Society of America; 2002. p. 253–93.

[11] Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol. 2006;96:190–4.

[12] Hermosa R, Viterbo A, Chet I, Monte E. Plant-beneficial effects of *Trichoderma* and of its genes. Microbiol. 2012;158:17–25.

[13] Hussey RS, Baker KR. A comparison of methods of collecting inocula for *Meloidogyne* spp including a new technique. Plant Dis Report. 1973;57:1025–8.

[14] IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

[15] Idowu OO, Olawole OI, Idumuu OO, Salami AO. Bio-control effect of *Trichoderma asperellum* (Samuels) Lieckf. and *Glomus intraradices* (Schenk) on Okra seedlings infected with
Pythium aphanidermatum (Edson) Fitzp and Erwinia caroto-vora (Jones). Amer J Exp Agric. 2016;10(4):1–12.

[16] Ihekwumere CC, Atiri GI, Fawole B, Dashiell KE. Evaluation of some commonly grown soybean cultivars for resistance to root-knot nematode and soybean mosaic virus in Nigeria. Fitopatol Bras. 1995;20:190–3.

[17] Javeed MT, Al-Hazm AS. Effect of Trichoderma harzianum on Meloidogyne javanica in tomatoes as influenced by time of the fungus introduction into soil. J Pure Appl Microbiol. 2015;9:535–9.

[18] Marathe A, Chandra R, Maity A, Sharm J, Jadhav VT. Effect of different microbial inoculants on soil properties, nutrient acquisition and growth of pomegranate (Punica granatum). Indian J Agric Sci. 2011;81(7):622–7.

[19] Mascarin GM, Bonfim Junior MF, Filho JV. Trichoderma harzianum reduces population of Meloidogyne incognita in cucumber plants under greenhouse conditions. J Entomol Nematol. 2012;4(6):54–7.

[20] Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31–6.

[21] Prasad S, Syed I, Anuradha P. Effect of different microbial inoculants on yield, microbial population and chemical properties in soil of groundnut grown on vertisol. Int J Microbiol Res. 2017;9(1):831–3.

[22] Radwan MA, Farrag SAA, Abu-Elamayem MM, Ahmed NS. Biological control of the root knot nematode, Meloidogyne incognita on tomato using bioproducts of microbial origin. Appl Soil Ecol. 2012;56:58–62.

[23] Shamshuddin J, Jamilah I, Ogunwale JA. Organic carbon determination in acid sulphate soils. Pertanika J Trop Agric Sci. 1994;17:197–200.

[24] Tang Y, Xin H-L, Guo M-L. Review on research of the phytochemistry and pharmacological activities of Celosia argentea. Rev Bras Farmacogn. 2016;26(6):787–96.

[25] Taylor AL, Sasser JN. Biology, identification and control of root-knot nematodes (Meloidogyne species). USA: Department of Plant Pathology, North Carolina State University, United States Agency for International Development. Raleigh, North Carolina; 1978.

[26] Xu P, Li J. Effects of microbial inoculant on physical and chemical properties in pig manure composting. Compost Sci Utilization. 2017;25(supp. 1):S37–42.

[27] Zakaria HM, Kassab AS, Shamseldean MM, Oraby MM, El-Moursheyed MMF. Controlling the root knot nematode Meloidogyne incognita in cucumber plants using some soil bioagents and some amendments under simulated field conditions. Ann Agric Sci. 2013;58(1):77–82.