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Management of Nematode Pests on Celosia Argentea with Selected Organic Composts and Biological Nematicides

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ABSTRACT

Ban on the importation of synthetic pesticides, particularly nematicides, has gingered nematologists to seek for the environmental friendly alternatives. However, use of organic compost and bio-nematicide have been suggested. The current field experiment was carried out at LadokeAkintola University Teaching and Research Farm, Ogbomoso, Nigeria. Celosiaargentea seedlings were inoculated with root knot nematode while some were not inoculated. Different organic compost-type and bio-nematicides were used as treatments for the management of the nematodes. The experiment was fitted into randomized complete block design with 15treatments and four replicates. Data were collected on plant height, number of leaf per plant, gall index, nematode population in the soil and root at planting and harvest. It was observed that all the treated C. argentea have significantly higher plant height, higher number of leaves, reduced gall index, lower population of nematodes in the root and soil when compared with the control. C. argentea, treated with Cassava peel and neem composts without root knot inoculated plants, significantly performed better than other treatments with respect to all measured parameters. There was no significant difference between the measured parameterson C. argentea treated with organic compost and also bio-nematicide. The study concluded that organic compost and bio-nematicide can be adopted as treatments in the management of nematodes on C. argentea.

Keywords

Celosia argentea, Bio-nematicides, Organic composts, Nematodes.

Introduction

Celosia argentea L. is one of the common leafy vegetables in Africa which is mostly cultivated by peasant farmers, especially in Nigeria. It is the cheapestgreen leafy vegetables and readily available natural source of nutrients for human nutrition [1]. This vegetable contains considerable amounts of vitamins and mineralsas well as moisture, fibre and ash essential for good health [2], *C. argentea* has also been reportedly used traditionally as food and medicine.

In view of the aforementioned numerous benefits of *C. argentea*, its cultivation becomes difficult due to nematode infestation [3]. Nematodes reduce plant vigor, cause root lesions, rotting and deformation. Damage due to the feeding activity of the nematodes on the vegetables could result in the reduction in quality and quantity of the harvested vegetable crops [4]. Affected plants may show slow or stunted growth, yellowing of the leaves, wilting of the plant and seedlings produce few roots [5]. Devastating effecting of nematode on leafy vegetable calls for its management.

Use of synthetic chemicals has been reported to be effective and quick in action in the control of nematode. Literatures have shown that synthetic chemical results into environmental problems. Several techniques such as solarization, flooding, use of resistant cultivars and use of cover crops have been practiced [6] but they are not feasible in all locations. However, use of organic compost and bio-control in the management of nematode has been considered as the preferred alternatives due to their cheapness, availability, and the little technique it involved in the preparation.

Incorporation of organic matter into the soil may suppress nematodes through several mechanisms. Organic matter may support higher populations of natural pests of nematodes such as bacteria and/or fungi, it may also release toxic compounds to nematodes during decomposition and may increase soil nutrients and water levels thus enabling plants to escape damage by nematodes. While, bio-control involves reduction of nematode populations by use of natural enemies which include parasites, predators and antagonists of adult nematodes, juveniles and eggs [7]. Plant health promoting rhizo-bacteria such as *Bacillus*spp, *Pseudomonas* spp., and *Telluriachitinolytica* are a promising group of micro-organisms that may have some effect in reducing damage by nematodes. Therefore, this experiment was conducted to determine the efficacy of organic composts and bio-nematicides on nematode population and their effects on the growth and yield of *C. argentea*.

Materials and Methods

Experimental Site

This experiment was conducted at the LadokeAkintola University of Technology (LAUTECH), Ogbomoso, Nigeria.Teaching and Research Farm (latitude 8.17000N, longitude 4.26360E).

Land Preparation and Experimental Design

The experimental land were ploughed and arrowed after which the field lay out was mapped out with pegs. The land was prepared and partition into vegetable bed of 1 m x 2 m sizes. Seeds of *C.argentea*, TLV 8 cultivar, obtained from National Horticultural Research Institute (NIHORT), Ibadan was sown at three seed per planting hole. Two weeks after planting, seedlings were thinned to one healthy plant per stand. The experiment was arranged and demarcated in a Randomised Complete Block Design (RCBD) with four replications.

Nematodes Egg Extraction

Eggs of root knot nematode, *Meloidogyne incognita*, were obtained from International Institute of Tropical Agriculture (IITA), Ibadan and cultured on *C.argentea* at the Teaching and Research Farm, LAUTECH, Ogbomoso. Root-knot nematodes galledwere obtained from an infected *C.argentea*roots. The galled roots were washed with tap water in the laboratory, then chopped into small pieces (1-2 cm seized) and soaked in 0.5% Sodium hypochlorite (NaOC1) inside a conical flask, then the content was shaken for 5 minutes in order to remove the gelatinous matrix encasing the eggs. The content was allowed to pass through a 200 mm mesh sieve in order to collect the plant debris. The filtrate that contains the eggs was then passed through a 0.001mm micro mesh sieve in order to collect the eggs. The eggs were then rinsed with distilled water in order to wash off any sodium hypochlorite (NaOC1) that may remain on the eggs.

Preparation of organic Composts and Bio-nematicides

Organic composts preparation: Organic composts were prepared from neem leaf, cassava peel and Tithonia leaf atLAUTECH Organic House, Ogbomoso. The preparation of the organic composts took four (4) months using windrow method.

Bio-nematicides preparation: *Azadirachtaindica*leaf and *Khayaivorensis* bark were collected separately. Aqueous extract of the neem leaf was obtained using Soxhlet extractor and *Khayaivorensis* bark. 500 kg of blended neem leaves and *Khayaivorensis*bark were added to 500 ml distilled water in a litre sized round bottom flask which was then fixed to a Soxhlet extractor. The extraction was separately carried out for 5 hours. The content extracted was taken as 100% concentration of neem leaf.

Treatment Application and Management: The experimental field was ploughed and arrowed. There were fifteen (15) treatments with four (4) replicates. The treatment were: Neem compost without nematode inoculation, Cassava peel without nematode inoculation, Neem compost with nematode inoculation, nematode inoculated Celosia, Tithonia compost with nematode inoculation, Cassava peal compost with nematode inoculation, Trichoderma + Khayaivorensis with nematode inoculation, Trichoderma + neem leaf with nematode inoculation, No plant extract with nematode inoculation, No plant extract without nematode inoculation, Trichoderma + Neem seed with nematode inoculation, Trichoderma + Khayaivorensis without nematode inoculation, Trichoderma + Neem seed without nematode inoculation, and Trichoderma + Neem leaf without nematode inoculation were incorporated into the soil two weeks before planting. The experiment was laid out in randomized complete block design (RCBD).

At four weeks after planting, treatments were applied and six weeks after planting the test plants were inoculated with 2000 *Meloidogyne incognita* juveniles. Weeding was done at when due. Data collection and Statistical Analysis Data were collected on the plant height(cm), number of leaf per plant, nematode population changes and root gall index was determined at the final stage using the method described by Sasser et al., all the data were subjected to statistical analysis of variance and means were partitioned using Duncan's Multiple Range Test at 5% probability level.

Analysis of Phytochemical compounds in the Composts

Test for Flavonoids (Shinoda test): 1 m of NaOH was added to 4 ml of extracts of each compost. A yellow colour observed indicates the presence of flavanoids, while any other colour aside yellow indicates absence of flavanoids.

Test for tannins: Two drops at 5% ferric chloride was added to 4 ml of extract indicate the absence of tannins, other colour aside orange shows the presence of tannins.

Test for glycosides: 1 ml of 50% concentrated sulphuric acid was added to 10 ml of each crude extract of each compost in test tube. The mixture in boiling water for 15 minutes fehling's solution (5 ml each of fehling's solution A and B) was added and the mixture was boiled. A colourless solution observed indicates the absence of glycosides, other colour aside shows the presence of glycosides.

Test for alkaloids: 1ml of 1% hydrochloric acidwas added to 3 ml of crude extract of each compost in the test tube. The mixture will be treated with two drops each of Mayer's and Wayner's reagent separately. A colourless (Mayer's reagent) an orange colour (Wayer's reagent) if observed indicates the absence of alkaloids,

other colour shows the presence of alkaloids.

Results

The result presented in table 1 indicates the presence of phytochemical compounds in the different composts used for the experiment. Five different compounds were tested. All the tested composts contained alkaloids. Tannins were not also present in *Trichoderma* +*Khayaivorensis*. However, glycosides only present in *Trichoderma* + *Khayaivorensis*. Saponin was found in all tested composts. Flavonoid was detected in neem compost, *Trichoderma* + *Khayaivorensis*, *Tithonia* compost and *Trichoderma* + neem seed composts.

Table 1: Phyto-Chemical Analysis of Compost and Bio-Nematicides Treatments.

Treatments	Alkaloids	Tannins	Glycocides	Saponnins	Flavonoids
Neem Compost	+	+	_	+	+
CassavaPeel Compost	+	+	_	+	_
Tithonia Compost	+	+	_	+	+
Trichoderma + *Khayaivorensis*	+	_	+	+	+
Trichoderma + neem leaf	+	+	_	+	_
Trichoderma + neem seed	+	+	_	+	+

As presented in Table 2, significant difference was detected among the treatments. It was observed that the application of neem compost without inoculation resulted into highest number of leaves (23.2) followed by the plots treated with no plant extract without inoculation which had 22.7. No significant difference was observed among the plants treated with neem compost with inoculation, *Tithonia* compost with inoculation and *Tithonia* compost without inoculation. Celosia treated with *Trichoderma* + *Khayaivorensis* with inoculation and *Trichoderma* + neem leaf with inoculation significantly had higher number of leaves than *Trichoderma* + *Khayaivorensis* without inoculation at 6 weeks after planting. Cassava peel compost without inoculation produced higher number of leaves (21.3) than cassava peel with inoculation (13.5) where as*Tithionia* compost with inoculation and *Tithonia* compost with inoculation had the same number of leaves. However, *Celosia* treated with neem compost without inoculation had higher number of leaves (23.3) than plants treated with neem compost with inoculation (19.2) while plants treated with *Trichoderma* +*Khayaivorensis* inoculation led to higher number of *Celosia* leaves (12.5) than *Trichoderma* + neemleaf with inoculation at 6 WAP. The leaves obtained from Celosia treated with *Trichoderma* + Neem seed with inoculation was significantly lower (18.1) than that of Celosia treated with *Trichoderma* + Neem seed without inoculation. Similar result was obtained in the plots treated with *Trichoderma* +Neem seed with inoculation and *Trichoderma* + Neem seed without inoculation at 6 WAP. At 8 weeks after planting, the result shows that the effectiveness of the *Trichoderma* + neem seed with inoculation was significantly the same with *Trichoderma* + neem leaf without inoculation in respect to number of leaves. The quantity of leaves (26.7) obtained from *Celosia* treated with *Trichoderma* + *Khayaivorensis* without inoculation was comparable with that of Celosia treated with *Trichoderma* + Neem leaf without inoculation. However, plants treated with no plant extract with inoculation had the highest number of leaves at 8 weeks after planting closely followed by the application of cassava peel compost without inoculation and *Trichoderma* + neem leaf with inoculation. Lownumber of leaves were recorded from *Celosia* plants treated with *Tithonia* Compost with inoculation compared with plants treated with Tithonia compost without inoculation. Similar trend was observed in the plots treated with Cassava peel compost with inoculation and Cassava peel compost without inoculation. The application of cassava peel compost with inoculation significantly performed better than *Trichoderma* +*Khayaivorensis*with inoculation in respect to number of leaves. All the applied treatments resulted into higher number of leaves than the *Trichoderma* + neem leaf without inoculation and no plant extract with inoculation. *Celosia* sprayed with Neem compost without inoculation had higher number of *Celosia* leaves (27.7) when compared with the plots treated with Neem Compost with inoculation at 8 WAP.

Table 2: Effect of treatment on number of leaves of *Celosia argentea* planted on nematode infested soil (field trial) for 6 and 8 weeks after planting respectively.

Number of Leaves Treatments	6WAP	8WAP
Neem Compost without inoculation	23.2a	27.7abcd
Cassava peel without inoculation	21.3c	32.2ab
Neem Compost with inoculation	19.2c	24.7abcd
Inoculated celosia	18.2d	29.0abc
Tithonia Compost with inoculation	15.4c	24.5abcd
Tithonia Compost without inoculation	14.0c	22.7bcd
Cassava peel compost with inoculation	13.5i	22.5bcd
Trichoderma + Khayaivorensis with inoculation	12.5j	21.7cd
Trichoderma + neem leaf with inoculation	10.2k	18.5d
No plant extract with inoculation	20.4d	34.0a
No plant extract without inoculation	22.7b	28.7abc
Trichoderma + Neem seed with inoculation	18.1g	31.7ab
Trichoderma + Khayaivorensis without inoculation	18.8i	26.7abcd
Trichoderma + Neem seed without inoculation	11.2i	18.2d
Trichoderma + Neem seed without inoculation	19.4h	26.7abcd

Keyword: WAP- Week After Planting

Means followed by different letters along the same column are not statistically different at 5% probability level.

The result presented in table 3 shows that the applied treatments had effects on *Celosia* plant height. Among the treatments, *Celosia* treated with *Trichoderma* + Neem seed with inoculation had highest plant (12.3 cm) at 6 WAP. No significant difference was detected on *Celosia* plant height treated with Cassava peel without inoculation and Cassava peel with inoculation. Similar observation was recorded on the inoculated plants treated with neemcompost and neemcompost without inoculation. The same significant effect was observed on the plants treated with *Trichoderma* + Neem seed without inoculation and *Trichoderma* +Neem leaf without inoculation at 6 WAP. At 8 WAP, highest plant height (28 cm)

was recorded on the *Celosia* plants with no plant extracts with inoculation. Tithonia Compost without inoculation and Cassava peal compost with inoculation had the same significant impact on the *Celosia* plant height. Cassava peel without inoculation had higher plant height (26.0 cm) than Cassava peel compost with inoculation. *Celosia* with Tithonia compost with inoculation had higher Celosia plant height (20.2) than plants sprayed with Cassava peel compost with inoculation. However, Neem Compost without inoculation had the same significant effects on *Celosia* plant height with Neem Compost with inoculation. Similar observation was recorded between *Trichoderma* + Neem seed with inoculation and *Trichoderma* + Neem seed without inoculation. Inoculation and Trichoderma + neem seed with inoculation singnificantly higher plant height than the plant treated with *Tithonia* compost without inoculation. At 8 weeks after planting, the effectiveness of *Tithonia* compost without inoculation was not different from cassava peel with inoculation. Among all the treatments, plot treated with no plant extract with inoculation had the highest plant height at 8 WAP.

Table 3: Effect of treatments on plant height of *Celosia argentea* planted on nematode infested soil (field trial) at 6 and 8weeks after planting respectively.

Number of Leaves		
Treatments	**6WAP**	**8WAP**
Neem Compost without inoculation	11.2b	23.7ab
Cassava peel without inoculation	11.2b	26.0ab
Neem Compost with inoculation	8.7b	18.7ab
Inoculated celosia	10.7d	22.5ab
Tithonia Compost with inoculation	10.1d	20.2ab
Tithonia Compost without inoculation	7.7d	17.0b
Cassava peel compost with inoculation	8.7b	17.0b
Trichoderma + Khayaivorensis with inoculation	9.2d	19.7ab
Trichoderma + neem leaf with inoculation	8.8b	19.7ab
No plant extract with inoculation	10.5d	28.0a
No plant extract without inoculation	11.5b	20.0ab
Trichoderma + Neem seed with inoculation	12.3a	24.5ab
Trichoderma + Khayaivorensis without inoculation	9.7i	22.2ab
Trichoderma + Neem seed without inoculation	10.1c	22.7ab
Trichoderma + Neem seed without inoculation	11.1c	20.5ab

Keyword: WAP-Week After Planting

Means followed by the different letters along the same column are not statistically different at 5% probability level.

As presented in Table 4, significant difference was detected among the treatments. Highest root gall index (4.7) was observed from the inoculated *Celosia* plants whereas significant different was not detected in the root gall index between the soil treated with *Tithonia* Compost with inoculation and Cassava peel compost with inoculation. The soil treated with Neem Compost with inoculation had significantly higher root index (2.5) than the soil treated with neem compost without inoculation which had 1.2 root gall index meanwhile, soil treated with *Trichoderma* + *Khayaivorensis* without inoculation had significant lower root gall index (1.0) when compared with *Trichoderma* + *Khayaivorensis*with inoculation. Similar trend was recorded in the soil treated with *Trichoderma* + neem leaf without inoculation and *Trichoderma* + neem leaf with inoculation. Soil with no plant extract with inoculation had higher root gall index (1.5) than soil no plant extract without inoculation, the applied treatments had different significant root gall index. Meanwhile, inoculated *Celosia* plants had highest root gall index. The same root gall index was detected in the harvested *Celosia* plants from plants treated with *Trichoderma* + *Khayaivorensis* without inoculation and Trichoderma + Neem seed without inoculation. Whereas, Celosia from *Trichoderma* + *Khayaivorensis* with inoculation had higher root gall index (1.7) than *Trichoderma* + *Khayaivorensis* without inoculation which had 1.0 root gall index. Significantly lower root gall index (2.2) was recorded in the *Celosia* treated with *Tithonia* Compost without inoculation when compared to*Celosia* plants treated with *Tithonia* Compost with inoculation which had 3.5 root gall index. However, significant different was recorded in the root gall index from *Celosia* plants treated with no plant extract with inoculation and No plant extract without inoculation. Similar trend was recorded in the *Celosia* plants from Neem Compost with inoculation and *Tithonia* Compost without inoculation.

Table 4: Effect of treatments on root gall (Field Trial).

Treatments	Root Gall Index
Neem Compost without Inoculation	1.2cd
Cassava peel without inoculation	1.0e
Neem Compost with inoculation	2.5bcde
Inoculated celosia	4.7a
Tithonia Compost with inoculation	3.5ab
Tithonia Compost without inoculation	2.2bcde
Cassava peel compost with inoculation	3.5ab
Trichoderma + Khayaivorensis with inoculation	1.7cde
Trichoderma + neem leaf with inoculation	2.7bcd
No plant extract with inoculation	1.5cde
No plant extract without inoculation	1.0c
Trichoderma + Neem seed with inoculation	3.0bc
Trichoderma + Khayaivorensis without inoculation	1.0e
Trichoderma + Neem seed without inoculation	1.0e
Trichoderma + Neem seed without inoculation	1.2de

Means followed by the different letters along the same line are not statistically different at 5% probability level

Discussion

The use of compost in the cultivation of crops has been reported to have had positive effects on the target plants therefore this experiment was conducted to determine the effect of compost and bio-nematicide on the growth and yield of nematicide infected *Celosia argentea*. The result of the phytochemical analysis of the tested treatments revealed that alkaloid, tannins, glycosides, saponins, and flavonoids were detected but in varied quantity. All the tested nematicides had alkaloids. Among the treatments, only Trichoderma + Khayaivorensis had Glycosides. Saponin was found in all tested nematicides. Olayinka et al., reported presence of saponins, glycosides, alkaloid and tannins in Kyayaivorensis compost. The report has it that plants that have high quantity of Alkaloid and Flavonoids exhibited higher ovivicidal effects on *Meloidogynespp*. The result clearly revealed that there was significant low number of leaves from the inoculated plants with

no plant extracts compared with other applied treatments. Root knot nematode infection has been reported to have caused low yield [8]. This is an indication that the application of *Trichoderma*, neem compost, *Tithonia* compost had positive effect in the control of nematode infection thereby leading to higher number of leaves, this also suggests that it is very necessary for the farmers to control the nematode infestation on the field in other to have higher number of yield leaves which is the ultimate aim of every potential farmer. However, single application of neem, *tithonia* and cassava peel compost on the plants that were not inoculated had significant higher leaves and plant height than combination of *Trichoderma* + neem seeds with inoculation. This is a pointer to the fact that neem, *Tithonia* and cassava peel compost prevented nematode infection on the Celosia plants. Reduction of nematode eggs and gall index can be attributed to the nematicidal activities of the treatments used Also, these results obtained on the field suggest that the application of neem compost also effectively suppressed root knot nematode infection. For instance, plant that were treated with neem compost with inoculation had higher number of nematode population than the plants that were treated with neem compost without inoculation. These indicate that the introduction of nematode population increased level of infestation by the nematode and also the neem compost effectively suppressed the nematode infestation, that is why we had lower number of nematode population on the plant that were treated with compost without inoculation than the plant that were treated with neemcompost with inoculation. The findings of this study collaborates with the observations by Joshua [9] who reported that neem, *tithonia* and *Tephrosiavogelii*extracts suppressed parasitic nematodes by changing soil physical and chemical properties and enriched the soil with beneficial microflora. It was also observed that combination of *Trichoderma* plus neem leaf had nematicidal effect than the single application of compost because the plant that were treated with neem compost had higher number of nematode infestation than the plant that were treated with combination of *Trichoderma* plus neem leaf as well as *Trichoderma* plus neem seed also *Trichoderma* plus neem seed performed better than the combination *Trichoderma* plus neem leaf in the control of nematode infection, cassava peel compost with inoculation also had lower number of nematode population which is 140 than the plant treated with *Tithonia* compost without inoculation which had 154 nematode population. Literature has shown that root colonization of *Trichoderma* impeded nematode performance at multiple stages of parasitism invasion, galling and reproduction in tomato. Among all the treatments, plant treated with *Trichoderma* plus neem seed without inoculation had the least nematode population, this shows that the combination of *Trichoderma* plus neem seed had suppressing effect on the nematode infection. This observation concurs with Murslain et al., who reported that combination of *Moringaoleifera* with *Trichoderma* effectively suppressed nematode populations, nematode egg hatching and juvenile numbers of Meloidogynejavanica. It has been reported in this result that nematode infection had negative effect on the plant height. For instance, the plants that were not treated but that was inoculated had lower plant height than the plants that were treated without inoculation. It has been reported that nematodes damage plants by feeding on the roots, weakening the plants ability to take up water and nutrients [10]. Also, reports by William and Roberts revealed that nematode infection result to wilting and stunted growth. These report further underscores the necessities for root knot nematode control in the cultivation of *Celosia argentea*. Root gall index population density is an indication of intensity of nematode infestation. The data collected on the field revealed that nematode attacks *Celosia argentea* and its infestation can be reduced by application of composts and *trichoderma*, However, inoculated plants without the application of compost or *Tricoderma* had higher significant root gall index and there was significant difference in the rate of root gall index with respect to the applied treatments. For instance, the treatment with neem composts had significant lower root gall index than those treated with cassava peel and *Tithonia* composts. The implication is that neem compost exhibited higher nematicidal effects than *Tithonia* and cassava peel composts. The presence of alkaloids such as nimbine, nimbinine, nimbidine, hionemone in neem leaves might have an adverse effect on nematode biology [11]. These compounds are toxic to nematodes and either kill them or reduce their mobility. It has been reported that certain micro-organisms that contribute to the decomposition of oil-cake produce certain products like ammonia, fatty acids, formaldehyde and phenolics [12-14]. The effect of these combined factors leads to reduced nematode development. However, *Trichoderma* + neem seeds and *Trichoderma* + Khayaivoreinsis treated Celosia plants had significant lower root gall index than the single application of neem, *Tithonia* and cassava peel compost. This suggests that there is synergistic action between the treated combination against nematode infection. *Trichoderma* strains are able to colonise root systems in many plants thereby resulting in the coordinating of defense mechanism of host plants [15]. *Trichoderma* also induces systemic resistance in plants [16].

Conclusion

Based on the data collected both on the field and at screen house, it is clearly shown that neem, *Tithonia* and cassava peel compost had sufficient quantity of phytochemical compounds which are capable of controlling the nematode. However, nematicidal efficacy of neem and *Tithonia* was higher than that of cassava peel compost at the screen house. Under field condition, the three tested compost materials exhibited the same nematicidal effects. Combination of *Trichoderma* + *Khayaivoreinsis* effectively suppressed nematode populations meanwhile efficacy of neem, *Tithonia* and cassava composts were not comparable to the combination of *Trichoderma* +*Khayaivoreinsis*. Therefore, use of neem, *Tithonia* and cassava peel composts coupled with the combination of *Trichoderma* + *Khayaivoreinsis* in the cultivation of *Celosia argentea* is recommended.

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