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Comparative Efficacy of Powders and Water Extracts of Chromolaena Odorata (L) and Annona Senegalensis (Pers) in Management of Root-Knot Disease of Pepper

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Abstract
Root-knot nematode, *Meloidogyne incognita* is an important pest of pepper. Various control measures have been employed in managing root-knot disease of pepper. The problems arising from chemical control have resulted in a search for plant toxicants for nematode control. This study investigated the performance of powders and water extracts of *Chromolaena odorata* and *Annona senegalensis* in the management of *Meloidogyne incognita* infection on pepper.Pepper cultivar Ex-Sam-St was raised in the nursery and transplanted into polypots and fields, inoculated with 5000 *M. incognita* eggs extracted with the sodium hypochlorite method. After inoculation, dry powders at 80 kg/ha, water extracts at 100,000 mg/kg, carbofuran 3G at 3 kg ai/ha and water were applied in pot and field experiments. Gallings on roots rated, final nematode population and reproductive factor were calculated. The data were log-transformed, analyzed with ANOVA, means separated with LSD at 5% probability level.*Annona senegalensis* leaf powder and its water extract were the most outstanding in performance next to carbofuran in reducing final nematode population, reproductive factor and galling index. Dry powders were more effective than water extracts in reducing the nematode infection. These botanicals can be used as alternatives to synthetic nematicides in the management of the nematode.
Keywords: Botanicals, Final nematode population, Galling Index, Inoculation, Reproductive Factor, Root damage.

1. Introduction

Pepper, is one of fruit vegetables grown worldwide (Fayemi, 1999) for human consumption supply of raw materials, medicinal purposes, storage and pest control measures (Celocia *et al*., 2006). The root knot nematode, *Meloidogyne incognita* is a major nematode pest of pepper (Sikora and Fernandez, 2005) that have reduced the output of pepper, 74-100% reduction in yield (Sogut and Elekcioglu, 2007; Olabiyi and Oyedunmade, 2008) have been reported. The application of pesticides has been banned due to human health and environmental pollution effects (Noling and Becker, 1994; W.H.O., 2008). To avoid the hazards associated with the synthetic nematicides arose the need to search for naturally occurring toxicants in plants, which are apparently effective, cheaper available and environment friendly (Park *et al*., 2005; Khan *et al*., 2008). The botanicals used in this study were Siam weed (*Chromolaena odorata*), family-Asteraceae and African custard apple (*Annona senegalensis*), family-Annonaceae (Gill, 1988; Odugbemi and Akinsulire, 2006). *Chromolaena odorata* extracts have been reported as being nematicidal (Adekunle and Fawole, 2003; Adegbite and Adesiyan, 2005; Thoden *et al*., 2009). *Annona senegalenis* was reported as an effective antihelmintic on livestock (Abdu *et al*., 2000), *A. muricata* and *A. squamosa* were reported to be nematicidal (Salawu, 1992; Abid *et al*., 1997).

This study was aimed at investigating the use of these botanicals for the management of the Root-knot nematode infection on pepper.

1.1 Materials and methods

Collection and preparation of botanicals

The *Chromolaena odorata* leaves and roots were collected from the Crop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, and *Annona senegalensis* bark and leaves were collected from Otukpo village in Benue State. The collected plant parts were air-dried for three months at room temperature. The dried plant parts were reduced to powders with a grinding machine and stored in plastic bags. Water extracts of the plant parts were prepared by addition of 10 g of the powders to 100 ml of cold water in a beaker left for one week and filtered with muslin cloth. Dilution was done by the addition of 10 ml of the suspension to 90 ml of water that resulted in 100,000 mg/kg concentration that was used in both the pot and field experiments. The dry powders were quantified to give an application rate of 80kg/ha (0.2g/pot and per plant stand on the field.

Extraction and collection of inocula

Meloidogyne incognita was maintained on celosia plants, and the eggs were extracted with the sodium hypochlorite method (Hussey and Barker, 1973) and calibrations were done to determine the number of eggs/ml and initial population level of 5000 *M. incognita* eggs was used to inoculate the pepper plants in pot and field experiments. Pot and field experiments

The pepper cultivar, Ex-Sam-St was sourced from the Department of Crop Protection, Ahmadu Bello University, Zaria. It was rated susceptible to *M. incognita* in a screening test. This cultivar was raised in the nursery in heat-sterilized soil and transplanted at one mouth old into five-litre polypot that contained heat-sterilized soil and to the field at spacing of 60 cm x 60 cm. The pot experiment was set up on the roof-top of the Department of Crop Protection and Environmental Biology, University of Ibadan, mIbadan. Nigeria, while the field experiment was carried out in the Crop Garden of the Department from 2009 to 2011. The soil type was identified as loamy sandy.

The following treatments were used

a. *Chromolaena odorata* leaf powder at 80 kg/ha
b. *Chromolaena odorata* root powder at 80 kg/ha
c. *Annona senegalensis* leaf powder at 80 kg/ha
d. *Annona senegalensis* bark powder at 80 kg/ha
e. *Chromolaena odorata* leaf water extract at 100,000 mg/kg
f. *Chromolaena odorata* root water extracts at 100,000 mg/kg
g. *Annona senegalensis* leaf water extracts at 100,000 mg/kg
h. Annona senegalensis bark water extracts at 100,000 mg/kg
i. Carbofuran 3G at 3 kg ai/ha
j. water (control)

The dry powders were applied to the polypots and plots two weeks before the transplanting of the pepper seedling with daily watering to ensure complete decomposition of the organic materials. The water extracts were applied as soil drenches 24 hours after inoculation of the seedlings with 5000 *M. incognita* eggs when poured into 2-4 holes, 2-4 cm deep around the base of the plants. The treatments were applied in Completely Randomized Design with four replicates in pots and Randomized Complete Block Design with four replicates in the field experiments. The pots and plots were weeded, watered as when necessary. Plant damage was done visual rating of the galling on the root (Taylor and Sasser, 1978) on a 0 to 5 Scale. Final nematode population = root population (eggs) + soil population (number of second-stage juveniles).

Reproductive Factor (RF) = Pf/Pi where Pf = final nematode population
and Pi = initial population density

All data collected were log transformed and Analysis of variance carried out with SAS (2002) and the means separated with LSD at 5% probability level.

1.1.1 Results:

Table 1: Biomass, yield and *Meloidogyne incognita* multiplication rate on Ex-Sam-St pepper amended with botanicals and carbofuran at harvest eight weeks after inoculation with *M. incognita (Pot experiment)**

Treatment	Application rate	Fresh shoot weight(g)	Dry shoot weight(g)	Fresh root weight(g)	Number of fruits/plant	Fresh fruit weight/plant (g)	Number of eggs	Number of J2 5kg soil	Final population (E+J2)	RF	G1
C. odorata leaf	80kg/ha	12.2±4.6	5.4±2.0	7.1±2.6	1.2±0.9	18.2±3.9	8322.0±3902.6(3.9)	6250.0±4732.4(3.7)	14572.0±5813.2(4.1)	2.9±0.2(0.5)	2.2±0.6(0.5)
C. odorata root	80kg/ha	8.1±2.8	3.6±1.2	7.1±1.3	2.0±1.4	13.7±2.0	9353.0±3335.4(3.9)	2500.0±1443.3(3.3)	14353.0±6503.5(4.1)	2.8±2.1(0.5)	2.5±0.6(0.5)
A. senegalensis leaf	80kg/ha	11.6±5.7	4.8±1.8	7.2±1.6	2.2±0.4	13.2±3.7	4763.0±1728.9(3.6)	200.0 ±0.0(2.3)	4963.0±1728.9(3.6)	0.9±0.1(0.2)	2.2±0.4(0.5)
A. senegalensis bark	80kg/ha	8.2±2.5	3.9±1.0	9.7±2.5	0.7±0.1	5.6±2.1	12446.0±5060.9(4.0)	2500.0±1443.3(3.3)	14940.0±5815.9(4.1)	2.9±1.0(0.5)	2.7±0.2(0.5)
C. odorata lwext	100000mg/kg	9.8±3.4	4.4±1.5	12.8±3.5	1.7±0.4	19.1±2.8	13935.0±5268.1(4.1)	5000.0±3535.5(3.6)	18529.0±6931.1(4.2)	3.5±1.3(0.6)	2.7±0.2(0.5)
C. odorata rwext	100000mg/kg	7.3±1.8	2.9±1.0	6.4±1.7	1.0±0.1	7.8±2.0	11146.0±3021.0(4.0)	5000.0±2041.0(3.6)	16146.0±3371.1(4.2)	3.2±1.1(0.6)	2.2±0.6(0.5)
A. senegalensis lwext	100000mg/kg	10.1±2.7	5.0±2.3	6.2±1.2	0.2±0.2	2.8±2.8	2771.0±780.5(3.4)	5000.0±5000.0(3.6)	7771.0±4730.5(3.8)	1.5±0.2(0.3)	1.5±0.2(0.3)
A. senegalensis bwext	100000mg/kg	9.2±1.7	4.7±0.7	9.7±2.8	0.7±0.4	8.6±2.0	13974.0±2055.7(4.1)	100.0±0.0(2.0)	14074.0±2055.7(4.1)	2.8±0.6(0.5)	2.7±0.4(0.5)
Carbofuran 3G	3kgai/ha	10.5±0.7	4.2±0.2	7.2±0.9	1.0±0.1	9.2±2.3	3425.0±426.5(3.5)	120.0±0.0(2.0)	3545.0±426.5(3.5)	0.7±0.1(0.2)	2.2±0.2(0.5)
Control (water)		7.6±2.9	3.6±1.1	8.2±3.0	2.5±0.9	4.6±0.1	16279.0±7669.8(4.0)	6250.0±2393.5(3.7)	22529.0±7045.6(4.2)	4.5±2.3(0.7)	3.5±0.2(0.6)
LSD 0.05		9.6	4.3	6.5	2.5	23.9	11336(4.0)	8306.5(3.9)	14617(4.1)	1.3(0.3)	1.2(0.3)

*Data are means of four replicates, *C. odorata* = *Chromolaena odorata, A. senegalensis* = *Annona senegalensis, C. odorata* lwext = water extract of *Chromolaena odorata* leaf, *C. odorata* rwext = water extract of *Chromolaena odorata* root, *A. senegalensis* lwext = water extract of *Annona senegalensis* leaf, *A. senegalensis* bwext = water extract of *Annona senegalensis* bark, Pi = 5000 *M. incognita* eggs, RF = Reproductive Factor, GI = Galling index, Final population = Number of eggs + Number of J2.

Table 2: Effects of amendments with botanicals and carbofuran on yield, biomass and root population, soil population, final population, reproductive factor, galling index on Ex-Sam-St pepper cultivar inoculated with *Meloidogyne incognita*. (Field experiment)

Treatment	Application rate	Number of fruits	Yield tons/ha	Fresh shoot weight(g)	Dry Shoot Weight(G)	Fresh root weight(g)	Root population (No of eggs)	Soil population (J2/200 ml soil)	Final Nematode population	Reproductive factor (RF)	Galling index (Gl)
C . o leaf	80 kg/ha	2.2±2.2	0.4±0.1	22.2±7.0	10.6±3.3	8.4±2.0	8265.0±1407.2(3.9)	850.0±478.7(2.9)	9115.0±1824.8(3.9)	1.7±0.3(0.2)	2.5±0.2(0.3)
C . o root	80 kg/ha	3.2±1.8	0.8±0.1	20.3±7.5	9.7±3.6	6.1±1.7	12625.0±5912.0(4.0)	1200±879(3.0)	13825.0±5912.0(4.0)	2.7±1.1(0.3)	2.7±0.4(0.4)
A.s leaf	80 kg/ha	0.0±0.0	0.0±0.0	16.0±4.6	7.6±2.2	6.0±1.1	6655.0±835.4(3.8)	225.0±193.1(2.3)	6880.0±784.4(3.8)	1.3±0.1(0.1)	2.7±0.4(0.4)
A.s. bark	80 kg/ha	2.5±1.6	0.4±0.1	23.5±6.4	11.2±3.1	8.8±1.6	7425.0±911.3(3.8)	925.0±512.1(2.9)	8350.0±526.7(3.9)	1.6±0.0(0.2)	2.7±0.4(0.4)
C.o. Lwext	100,000mg/kg	3.7±2.0	0.8±0.1	22.0±4.1	10.5±3.9	8.0±0.9	12350.0±2043.4(4.0)	1450.0±607.5(3.1)	13800.0±2475.2(4.1)	2.7±0.5(0.4)	3.0±0.4(0.4)
C.o. rwext	100,000mg/kg	1.0±1.0	0.1±0.1	19.0±6.3	9.1±3.0	6.5±0.2	14275.0±4418.9(4.1)	25.0±25.0(1.3)	14300.0±4404.7(4.1)	2.8±0.8(0.4)	3.5±0.6(0.5)
A.s lwext	100,000mg/kg	1.2±0.9	0.1±0.1	12.5±4.4	5.9±2.1	6.0±1.1	8775.0±1557.9(3.9)	100.0±0.0(2.0)	8875.0±1557.9(3.9)	1.7±0.3(0.2)	3.2±0.6(0.5)
A.s bwext	100,000mg/kg	0.5±0.5	0.1±0.1	14.3±7.3	6.8±3.5	5.5±2.0	15938.0±4766.7(4.2)	100.0±100.0(2.0)	16038.0±4731.6(4.2)	3.1±0.9(0.5)	3.2±0.6(0.5)
Carbofuran	3kgai/ha	1.7±0.6	0.2±0.1	35.9±13.0	17.2±6.2	9.7±1.7	5721.0±1319.8(3.7)	400.0±400.0(2.6)	6121.0±1633.9(3.7)	1.2±0.3(0.0)	2.2±0.2(0.3)
Control (water)	-	0.7±0.6	0.1±0.1	11.1±1.4	5.3±0.7	5.6±0.5	72920.0±25689.5(4.8)	2250.0±2250.0(3.3)	75170.0±26484.0(4.8)	14.9±5.3(1.1)	4.5±0.2(0.6)
Uninoculated control	-	0.0±0.0	0.0±0.0	16.4±2.1	7.8±1.0	6.3±1.0	5888.0±298.8(3.7)	25.0±25.0(1.3)	5913.0±322.9(3.7)	1.1±0.0(0.0)	2.0±0.0(0.3)
LSD (0.05)		3.9	0.1	20.8	9.9	4.1	23885(4.3)	1247.9(3)	24689(4.3)	4.9(0.7)	1.2(0.3)

* Data are means of four replicates, *C. o = Chromolaena odorata, A.s = Annona senegalensis, C. o*lwext = water extract of *C. odorata* leaf, *C.o* rwext = water extract of *C. odorata* root, *A.s* lwext = water extract of *A. senegalensis* leaf, *A.s* bwext = water extract of *A. senegalensis* bark, Pi = 5000 *M. incognita* eggs, RF = Reproductive Factor, GI = Galling index, Final nematode population = Root population + Soil population.

In the pot experiments, there were significant (P≤0.05) differences in the final population of M. incognita on the pepper cultivar among water (control) and Annona senegalensis leaf powder 77.9%, water extract of A. senegalensis leaf 65.5%, carbofuran 84.3%, A. senegalensis bark (Table 1). Equally there were significant differences in the final populations of the nematode among C. odorata leaf and root, water extracts and the dry powders excepts C. odorata root powder (Table 2) in the field experiments. Final nematode population was lowest in carbofuran-treated plants with reduction of 91.8% in final nematode population, followed by A. senegalensis leaf powder with 90.8% reduction, water extracts of A. senegalensis leaf 88.1% among the water extracts (Table 2).

In the pot experiments, there were significant (P≤0.05) differences in Reproductive Factor (RF) of the nematode among water (control) and carbofuran 84.4%, A. senegalensis leaf powder 80.0% and water extracts of Annona senegalensis leaf powder 66.6%. There were no significant differences among the rest treatments in RF (Table 1). In the field experiments, there were significant (P≤0.05) differences in RF between water (control) and the other treatments but there were no were no significant (P≤0.05) differences in RF between the dry powders of C. odorata 88.5% and A. senegalensis 91.2%, water extracts of C. odorata and A. senegalensis 88.5% and uninoculated control (Table 2). Carbofuran-treated plants had the lowest RF with 91.9% reduction in RF, followed by A. senegalensis leaf powder 91.2% reduction in RF.

In the pot experiments, there significant (P≤0.05) differences in the root damage (GI) on this cultivar among water (control) treatments and carbofuran 37.1%, C. odorata leaf powder, water extracts of C. odorata roots, A. senegalensis leaf powder 37.1% each, and water extract of A. senegalensis leaf 57.1% (Table 1). In the field experiments, there were significant (p≤0.05) differences in the root damage between control (water) and the other treatments, and no significant differences among the rest treatments (Table 2). Root damage was least in carbofuran treated plants with reduction of 51.1% in GI. Other reductions in GI were C. odorata leaf powder 44.4%, C. odorata root powder and A. senegalensis leaf powder 40.0% each.

In the yield parameters of fresh shoot weight, dry shoot weight, fresh root weight, number of fruits and fresh fruit weight there were no significant differences among the treatments. In pot trial (Table 1) there were significant differences in fresh shoot weight among the treatments but *C.odorata* and *A. senegalensis* leaf powder and carbofuran treatments resulted in greater values than water treatment. Such observations were noticed in the other parameters. In field trial (Table 2) there were no significant differences among the growth parameters due to the treatments, though some individual differences were noticed.

1.1.2 Discussion

Organic amendments in forms of dry powders and water extracts have been used in the management of the root knot nematode, *Meloidogyne* spp., on other crops (Abbasi *et al*., 2008; Olabiyi and Oyedunmade, 2008; Nwanguma *et al*., 2011). The differences in nematicidal activity of the test plants against *M. incognita* on pepper could be explained on the basis of the nematicidal principles they contained which varied quantitatively (Radwan *et al*., 2007). The powder extract of *Annona senegalensis* performed better than the other extracts in the control of *M. incognita* on pepper, the aqueous extracts of *A. senegalensis* leaves performed better than the aqueous extracts of *Chromolaena odorata* leaves and roots and *A. senegalensis* leaves powder which was surpassed only by carbofuran. All the powdered extracts were superior in performance of control of *M. incognita* to the aqueous extracts. This finding was similar to the findings of Ogwulumba *et al*. (2011) who reported that powdered extracts of leaves of *Carica papaya, Vernonia amygdalina, Gongronema latifolia, Azadirachta indica* and *Ocimum basilium* proved better on gall reduction than aqueous extracts except that of *A. indica* which proved superior to the other extracts and powdered *O. basilium* proved better than the other powders on tomato. The findings by the study was similar to the findings of Shaukat *et al*. (2002) who reported that decomposing soil amendments of *Argemone mexicana* in the soil was more effective in the control of *M. javanica* on tomato than its aqueous extracts, presumably because of more effective and continuous release of the toxic principles or some agents that were not water soluble.

The nematicidal activity of organic amendments in the soil can be attributed to chemical mineralization with the ultimate release of ammonia, increased nitrogen and carbon dioxide levels (Abbas *et al*., 2009). Organic amendments with very low C/N or high content of ammonia will either result to plasmolysis of nematodes or proliferation of nematophagous fungi (Anaya, 2006). The mechanisms of plant extracts was suggested to be a direct effect on the second-stage juveniles in the eggstage (Kienick and Sikora, 2006) or by denaturing and degrading of proteins, inhibition of enzymes and interfering with the election flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al*., 1994).

The application of the botanical to the pepper resulted into improved growth parameters such as plant height, number of leaves, fresh shoot weight, dry shoot weight, fresh root weight number of fruits produced and fresh fruit weights per plant. This finding was similar to findings of Shaukat *et al*. (2002), Shaukat *et al*. (2004) and Abbasi *et al*. (2008). The findings that pepper plants treated with *Chromolaena odorata* extracts produced more fruits and fresh fruit weight per plant than those treated with *Annona senegalensis* extracts, carbofuran and water (control) was in contrast to the findings by Nwanguma *et al*. (2011) who reported that pepper treated with Pacesetter and *C. odorata* based compost had significantly lower number of fruits when compared to those treated with poultry manures, also recorded significantly lowest fruit weight.

The management of the *Meloidogyne incognita* on the pepper cultivar with the botanicals might be due to single or mixture effects of the phytochemicals they contained. The water (control) treatments that contained no phytochemicals had the highest final nematode population, highest value of Reproductive Factor and highest root damage as compared to the botanicals that contained phenols, alkaloids, flavonoids, saponins, tannins, and anthraquinones and cardenolides (Agaba, 2014; Agaba *et al*., 2015). It was most probably that the management of root knot nematode on the pepper cultivar was due to the various phytochemicals contained in the botanicals used as organic amendments. The effectiveness of the botanicals in the management of the nematode was only surpassed by the synthetic nematicide carbofuran. Therefore, these botanicals can be used as alternatives to the use of the synthetic nematicides for the management of plant-parasitic nematodes on crops. The dry powders were more effective than the water extracts in the management of the root-knot nematode on pepper.

This paper calls for more studies into the use of botanicals for the management of nematodes on crops in Nigeria as the country is rich in flora diversity.

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