Comparative evaluation of powdered and aqueous extract of citrus peels and carbofuran in the management of root-knot nematode on infected cowpea field

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Summary

Two years of experimental trials were conducted in Nigeria to investigate the effect of powdered and aqueous extract of citrus (sweet orange) peel in the management of root-knot nematodes of cowpea cultivar 'IT96D-610'. Different concentrations of aqueous citrus (sweet orange) peel extracts were used as well as different amount of citrus (Sweet Orange) peel powder. As a standard check carbofuran was used. Furthermore, phytochemical screening was carried out to determine the presence of secondary metabolites, while GC-MS was used to identify the presence of some chemical compounds. Results in the present study showed, that the treated plants performed significantly higher than the untreated counterparts in terms of growth, yield and reduction of soil nematode population. Phytochemical screening of the secondary metabolites revealed the presence of tannins, saponins, flavonoids, glycosides, sterols and alkaloids while the analysis by GC-MS revealed the presence of citral and β-caryophyllene to be the prevalent compounds. These compounds might be responsible for the control of root-knot nematode infecting cowpea since they exhibited nematicidal potential in their activities.

Key words

root-knot nematode, bio-active ingredients, citrus peel, GC-MS, allelopathy

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Introduction

Cowpea (*Vigna unguiculata* (L) Walp) that belongs to the family Leguminosae is an annual legumes of nutritional importance. Cowpea forms an important part in Nigerian diet mainly due to its high protein content. It is cheap and contains two or three times more proteins than cereal crops. The legumes produce a cheap principal source of dietary protein ranging from 18 to 20% (Anon, 1982) and account for up to 80% of dietary protein intake. Cowpea is a promising multi-purpose legume in many parts of the world, being the only available high quality legume hay for livestock feed. Cowpea leaves are richer protein source than cowpea seed. Cowpea is also used as a nitrogen fixing crop or for erosion control among others. However, the protein in the cowpea seed is rich in amino acids, lysine and tryptophan, compared to cereal grains but it is deficient in methionine and cystine when compared to animal protein (Olowe, 1981).

Over the years, there has been serious decline in the production of cowpea due to challenges posed by pests and pathogens. These include insect pests, fungal, viral, bacterial and nematode infestations. Root-knot nematode can be grossly harmful to cowpea as they cause root galls, surface necrosis or reduction in total volume by causing stubby roots. Affected plants often wilt because the root system is incapable of absorbing adequate amount of water and nutrients. The injuries sustained also make the plant more susceptible to secondary invaders.

Effective management of this deleterious pathogen will improve the quantity and quality of cowpea. Several methods that have been adopted for nematode control include the use of synthetic nematicides (Oyedunmade, 1998), organic amendments (Nwanguma and Fawole, 2004), botanicals (Izuogu et al., 2011; Olabiyi et al., 2013), use of bio-agents (Izuogu, 2013; Izuogu and Abiri, 2015) and resistant varieties (Izuogu et al., 2015a) etc.

Although synthetic chemicals are useful and effective, their adverse effect on humans and the environment, high cost and non-availability at the time of need discourage most potential users in Nigeria. This necessitates the search for pollution free and cheaper alternative control measures that are appropriate for the resource poor farmers in Africa. Plant extracts and amendments used in control of plant parasitic nematodes have advantage of availability and cheapness. They also increase soil fertility as they act as manure. Their environmental safety further contributes to their acceptability and use by resource poor farmers. This study was therefore aimed at determining the effectiveness of powdered and aqueous extract of sweet orange peels in the control of root-knot nematodes (*Meloidogyne incognita*) infection and consequent growth and yield improvement in cowpea cultivar 'IT96D-610'.

Material and methods

Pot experiment

This trial was conducted between December, 2013 and March, 2014 in the crop pavilion of the Department of Crop Protection, Faculty of Agriculture, University of Ilorin, Nigeria. The experimental design used was a Completely Randomized Design (CRD). Thirty were nematode inoculated per each pot. Five treatments used included: 100%, 75%, 50% and 0% (control) citrus peel aqueous extracts and 0.75 g of carbofuran that served as a standard check. Each treatment was replicated six times. One kilogram of ground air-dried citrus peel was soaked in four liters of boiled water for 24 hours to obtain the 100% aqueous extract. A portion of this was further serially diluted to obtained 75% and 50% levels. Using the methods of Guatam and Goswami (2002), sandy loamy soil was sterilized and allowed to cool for 48 hours before pouring into the sixty perforated pots. Planting of three seeds per hole was done and germination took place between 5 and 7days. Seedlings were thinned to one vigorous plant per pot on the 10^{th} day and approximately 1000 newly hatched juveniles were inoculated to each pot. One week after nematode inoculation, different levels of citrus peel extracts were applied at the rate of 50 ml per plant, while 0.75 g or carbofuran was added per plant as a standard check. Control pots did not receive any treatment. Plants were sprayed with cypermethrine 500 EC insecticide after emergence of first flowers on weekly basis until full podding.

Data were collected on plant height, number of leaves and number of flowers. Postharvest parameters such as number of pods, seed weight, final soil nematodes and root gall indices were accessed. Soil nematode extraction was done using the modified Baerman's tray method described by Whitehead and Hemming (1965). Roots were accessed for galling using the method described by Taylor and Sasser (1978).

Field experiment

This trial was carried out between July and November, 2012 at the University of Ilorin Teaching and Research Farm. The field measuring 70 m x 7 m (490 m^2) was divided into two: 70 m x 4 m (280 m^2) and 70 m x 1 m (70 m^2), with 2 m alley in between the two plots. While the larger plot served as the treated experimental plot, the second untreated plot served as the control. The experimental design used was Randomized Complete Block Design (RCBD) where each treatment was replicated four times.

Soil samples were randomly collected in a zig-zag pattern from the two plots to estimate the initial soil nematode population before evenly incorporating 100 kg of galled roots of *Celosia argentea* infected with *M. incognita* to the plots to increase the inoculum population.

Three cowpea seeds were planted at 5 cm depth with a spacing of 40 cm in between the ridges. Seedlings were thinned down to one vigorous plant per hole. Treatments that included 60 g, 40 g, and 20 g of ground citrus peel powder and 0.75 g of carbofuran were applied to the plants on the treated plots. The second plot which received only the inoculum without treatment (0%) served as control. Root-nematodes present in the soil were extracted using the modified Baermann's method of extraction described by Whitehead and Hemming (1965) and identified and counted using a stereo microscope at International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. Cultural practices such as weeding and watering were done when needed. After emergence of the first flowers, cypermethrin 500 EC was used on weekly basis until the plants attained full podding. Data of results collected followed similar trend as in pot experiment.

Preparation of plant material

The peel from sweet orange was collected in large quantity, air-dried for ten days and ground to powder. For the pot trial, 4 kg of ground powder was soaked in boiled water for 24 hours and sieved to obtained aqueous extract. For the field trial, the grounded peel was applied as powder.

The chemical constituent of the fresh and ground citrus peel were determined using hydrostillation method and later subjected to GC-MS for the chemical analysis at the Department of Chemistry, University of Ilorin.

Extraction of compounds

Extraction of saponins

Conventional methods of extraction and purification of chemical constituents from plant tissues was carried out according to Chen et al. (2007).

Extraction of tannins

Extraction of tannins from the citrus peel was carried out according to Yuliana et al. (2014).

Extraction of alkaloids

Extraction of alkaloids from the citrus peel was carried out using Anju et al. (2005) method.

Extraction of Flavonoids

Extraction of alkaloids from the citrus peel was carried out using Mooza et al. (2014) method.

Results and discussion

Results from the two trials show that the main effect of root-knot nematode inoculation and treatments with carbofuran, aqueous and powdered extracts of citrus peels on plant height, number of leaves and pod and seed weight were significantly higher in treated plants than in the untreated plants (Tables 1-3). Significant difference was also observed in soil nematode population and root gall indices. The inoculated untreated plants recorded significantly higher soil nematode and root gall indices than the treated plants. Generally, treatment level of 100% and 75% aqueous citrus peel extract and 60 g and 40 g citrus peel powder compared favorably with carbofuran, a synthetic nematicide which served as a standard check (Table 4).

Our investigation also revealed that the amendment of soil with aqueous and powdered citrus peel extracts were not phytotoxic to cowpea plants but rather nematicidal thereby improving growth and yield of cowpea. The superior performance of the treated plants implicates nematodes, especially *Meloidogyne incognita* that is capable of distorting the normal physiology of the plants resulting into poor nutrient uptake, reduced growth and consequently low quality and quantity of the yield. Fifty percent flowering was recorded 3-8 days earlier in treated plants than in the untreated control. Consequently, the treated plants recorded higher number of pods and seed weight than the control. Late flowering and poor fruiting recorded in the untreated plants might be linked to nematode activities and their effect on nutrients available for seed formation resulting to improper filling of the pods in control plants. This corroborates the findings by Afolabi (1980) that the yield of cowpea is directly related to pod filling.

The effect of amendments on plants does not only indicate the fact that they release nutrients for plant growth but includes release of pathogen killing/immobilization gas from the citrus peel powder and aqueous extract thus, possibly making them nematotoxic or nematostatic. This process of gas release is termed Bio-fumigation. However, in a broader sense where the effects

Table 1. Main effects of root-knot nematode and treatment with carbofuran (0.75 g) and powdered and aqueous extract of citrus peels on the height (cm) of treated cowpea plant

POT					
Treatment	5WAP*	6WAP	7WAP	8WAP	9WAP
Inoculated and untreated	16.46^{b**}	17.99^{b}	19.01^{b}	18.29^{b}	20.33^{b}
Treated	19.33^{a}	20.7^{a}	21.67^{a}	23.61^{a}	24.73^{a}
S.E	0.71	0.68	0.83	1.10	1.01
Citrus extract					
100%	18.37^{a}	19.72^{a}	41.40^{a}	21.20	22.97^{a}
75%	17.77^{ab}	19.65^{a}	20.28^{ab}	21.08	22.83^{a}
50%	17.67^{ab}	19.52^{a}	20.20^{ab}	20.62	22.43^{a}
0%	16.18^{b}	17.63^{ab}	18.90^{ab}	20.45	22.20^{ab}
Carbofuran	19.13^{a}	20.20^{a}	20.82^{a}	21.38	23.20^{a}
S.E	1.13	1.07	1.31	1.74	1
N.S.				N.S.	
FIELD					
Citrus peel powder					
60g	19^{ab}	22.3^{a}	33.3^{a}	37.1^{ab}	41.5^{ab}
40g	18.6^{abc}	21.6^{ab}	30.5^{ab}	34.?^{abc}	40.3^{ab}
20g	17.8^{bc}	20.4^{abc}	25.9^{bc}	30.0^{bcd}	37.6^{c}
0g	16.6^{c}	18.6^{abcd}	22.1^{c}	29.3^{ef}	33.5^{d}
Carbofuran	21^{a}	23.3^{a}	35.6^{a}	38^{a}	43.5^{a}
S.E	0.1	0.1	3.0	3.32	1.40

* WAP= weeks after planting

** Means followed by the same letters in the same column do not differ significantly according to Duncan's Multiple Range Test ($P<0.05$).

Table 2. Main effects of root-knot nematode and treatment with carbofuran (0.75 g) and powdered and aqueous extract of citrus peels on the number of leaves of treated cowpea plant.

POT					
Treatment	5WAP*	6WAP	7WAP	8WAP	9WAP
Inoculated and untreated	15.87^{b**}	17.60^{b}	17.90^{b}	16.10^{b}	18.27^{b}
Treated	16.13^{a}	19.00^{a}	20.70^{a}	20.90^{a}	20.00^{a}
S.E	1.08	0.93	2.40	2.91	1.03
Citrus extract					
100%	16.67^{a}	19.00^{a}	20.00^{a}	20.20^{a}	19.50^{a}
75%	16.00^{a}	18.18^{ab}	19.70^{ab}	18.20^{ab}	19.00^{ab}
50%	15.83^{ab}	17.83^{ab}	19.00^{ab}	16.80^{b}	17.83^{b}
0%	14.67^{b}	17.33^{b}	17.80^{b}	16.80^{b}	17.83^{b}
Carbofuran	16.83^{a}	19.17^{a}	20.20^{a}	20.30^{a}	20.33^{a}
S.E	1.67	1.46	3.23	4.60	1.64
FIELD					
Citrus peel powder					
60g	19^{ab}	36.3^{ab}	45.3^{b}	48^{a}	47^{ab}
40g	17^{abc}	36^{ab}	43.3^{b}	46.5^{ab}	44^{b}
20g	15.6^{bc}	34b^{c}	37.3^{c}	40.6^{b}	37^{c}
0g	14^{c}	33^{c}	36.3^{c}	37.3^{bc}	33^{cd}
Carbofuran	20^{a}	38.3^{a}	51^{a}	51.3^{a}	49^{a}
S.E	1.3	0.6	1.7	3.3	4.1

* WAP= weeks after planting

** Means followed by the same letters in the same column do not differ significantly according to Duncan's Multiple Range Test (P<0.05).

Table 3. Main effects of root-knot nematode and treatment with carbofuran (0.75 g) and powdered and aqueous extract of citrus peels on the mean number of pods and seed weight per plant.

POT					
Treatment	Total No. of pods per plant	Total weight of seed per plant		Total No. of pods per plant	Total weight of seed per plant
Inoculated and untreated	12.28^{b*}	4.13^{b}		19.04^{b}	5.35^{b}
Treated	14.74^{a}	5.90^{a}		22.66^{a}	7.39^{a}
S.E	8.60	0.64		5.58	0.77
Citrus extract			*Citrus peel powder*		
100%	15.66^{a}	5.47^{ab}	*60g*	26.16^{a}	5.92^{b}
75%	14.17^{a}	4.32^{b}	*40g*	15.52^{a}	5.62^{b}
50%	10.60^{bc}	4.21^{b}	*20g*	16.07^{bc}	5.07^{bc}
0%	8.67^{c}	3.14^{c}	*0g*	13.34^{c}	4.35^{c}
Carbofuran	18.49^{a}	6.43^{a}	*Carbofuran*	30.17^{a}	8.00^{a}
S.E	7.28	1.01		8.87	1.21

** Means followed by the same letters in the same column do not differ significantly according to Duncan's Multiple Range Test (P<0.05).

Table 4. Main effects of root-knot nematode and treatment with carbofuran (0.75 g) and powdered and aqueous extract of citrus peels on root gall index and nematode population in 100 ml soil

	Root gall index							Nematode population	
Pot		Field			Pot				Field
Citrus extract		Citrus powder		Extract	Initial (egg)	Final	Citrus powder	Initial	Final
100%	1.10^{a*}	60g	0.40^{a}	100%	1000	17^{a}	60g	252.20	17.40^{b}
75%	1.80^{ab}	40g	0.62^{ab}	75%	1000	28^{b}	40g	265.73	22.56^{c}
50%	2.50^{b}	20g	0.57^{b}	50%	1000	35^{b}	20g	257.44	22.89^{c}
0%	4.00^{c}	0g	0.98^{c}	0%	1000	147^{c}	0g	249.72	197.21^{d}
Carbofuran	0.80^{a}	Carbofuran		Carbofuran	1000	10^{a}	Carbofuran	260.56	14.39^{a}
S.E	0.03		0.22			8.71		10.59	4.82
								N.S	

** Means followed by the same letters in the same column do not differ significantly according to Duncan's Multiple Range Test (P<0.05).

of amendments would also entail nutrients release for plant growth, allelopathic effect becomes more appropriate. Allelopathy in itself is defined as a biological phenomenon by which an organism produces one or more biochemicals (allelochemicals) that influence the growth, survival and reproduction of other organisms. The ability of the citrus peel amendment to release plant nutrients and biochemicals that reduced soil nematode portrays it as an allelopathic plant. This can also be linked to the results obtained in the phytochemical screening of the peel for secondary metabolites that revealed the presence of tannins, saponins, flavonoids, sterols and alkaloids (Table 5). These bio-active ingredients have been reported to be toxic to pathogenic nematodes, inhibiting their activities (Oluremi et al., 2006; Izuogu et al., 2012, 2015b). Confirmation of the allelochemicals present in the essential oils from citrus powder was further elucidated from the chemical constituents characterized by GC-MS; these include citral and β-caryophyllene as prevalent compounds. However, Stamp (2003) described allelochemicals as subset of secondary metabolites that are not required for metabolism (growth, development and reproduction) of allelopathic organism. This being the case, most of the chemicals released from the citrus peel were probably channeled against the insidious pathogens attacking the cowpea, thus killing or immobilizing these nematodes and improving the growth and yield of the treated plant.

Table 5. Results of the phytochemical screening of the citrus peels extract

Constituents	Hexane Extracts	Methanol Extracts
Tannins	_*	+
Saponins	+	+
Flavonoids	+	+
Glycosides	-	_
Sterols	+	+
Alkaloids	+	+

*- = absent, + = present

Conclusion

Citrus peel is generally considered as agricultural waste. Our investigation has shown that the use of powder or aqueous extracts of citrus peels integrated as soil amendment in the management of plant parasitic nematodes reduced the soil nematode population and provided healthier conducive environment for the growth and production of cowpea cultivar 'IT96D-610' that is susceptible to root-knot nematode *M. incognita* infection.

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