



The nematicidal effect of aqueous leaf extract of *Crotalaria juncea* L. in the control of *Meloidogyne* species infecting two varieties of *Solanum tuberosum* L. (potato) in Jos, Nigeria

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Abstract

The presence of bioactive substances in plant extract with nematicidal activity on *Meloidogyne* spp. to reduce crop damage, minimize chemical pesticide toxicity to man and safety of the environment. *Solanum tuberosum* L. (Potato) is one of the world's most valuable and widely grown food crops, because of its nutritional values, high yield and is ranked sixth with cereals. The study investigated the nematicidal efficacy of aqueous leaf extract of *Crotalaria juncea* L. in the control of *Meloidogyne* species infecting potato varieties. The nematodes were isolated by modified funnel Baerman method and identified by comparative morphology, descriptions and lattice keys. The nematode suspension was prepared by taken 4 ml of the homogenized suspension (108 juveniles) was subjected to the leaf extract at varied concentrations of (0.75, 1.5, 3.0, 6.0 mg/mls). The set up was replicated three times and observed daily for live and immobilized nematodes for four days. The setup was arranged in a complete randomized design with five replicates and the two potato varieties (Caruso and Marabel) roots were inoculated with second stage juvenile root-knot nematodes at 2 weeks after planting (2WAP). The pots were drenched with the leaf extract solutions at concentrations of (1.5, 3.0, 6.0 mg/mills) at 4 weeks after planting (4WAP). Three controls (Furadan, inoculated + unamended, un-inoculated + unamended) were used. The phytochemical analysis indicated the presence of alkaloids, saponins, tannins, flavonoids, steroids and cardiac glycosides. The results revealed that the higher the concentration of the extract, the higher the mortality of root-knot nematodes. For instance, 6.0 mg/mls resulted in (100±00%) mortality by extract of *C. juncea*. The *in vivo* results showed that at ($p \leq 0.05$) level of significance, highest tuber weight was obtained from the plants treated with extract of *C. juncea*. The test plant extract can be used to control root-knot nematodes at a concentration of 6 mg/ml which had the highest mortality effect on the nematodes, which suggested that it had potential of being formulated into a biological nematicide.

Keywords: Nematicidal, *Crotalaria juncea*, *Meloidogyne* species, *Solanum tuberosum*

Introduction

Solanum tuberosum L. (Potato) is one of the world's most important and widely grown food crops save maize because of its richness in nutritional values and has high yielding potential as maize (Anderson, 2008) [2]. It is an important cash crop in many countries (Tolno *et al.*, 2016). In most African countries including Nigeria, potato is ranked sixth after rice, maize, sorghum and millet as the most valuable food crop (Horton, 2006) [13]. Potato production in Africa tripled between 1994 and 2011 from 8 to 24 million metric tons, largely due to increase in the cropping area. Half of this production comes from sub-Saharan Africa (CIP, 2018) [8]. Potato production in Nigeria is faced with a lot of problems among which but not limited to infestation of crop by pathogen, chief of which is root-knot nematode. This parasite reduces the yield and the quality of the crop (CIP, 2018) [8]. International Potato Centre (CIP) and its partners from 2013 to 2016 in ten sub-Saharan African countries, including Nigeria, carried out study which showed that farmers are getting only one-third of what they could produce on the same piece of land (8 tons/ha instead of 24 tons/ha) and that the current level of production could be increased by 140% if identified problems were addressed. The identified limitations were inadequate quality seeds and pests and/or diseases (CIP, 2018) [8]. Plant parasitic nematodes have been identified as a limiting factor in potato production leading to decreased yield, poor tuber quality and deformations, which make them unmarketable (Medina *et al.*, 2016) [18] root-knot nematodes, of all the nematodes parasitizing potato, are the most aggressive, damaging, and economically important (Jones *et al.*, 2016). Also,

nematodes have developed some level of resistance thereby rendering the nematicides impotent (Ojo, 2016). *Meloidogyne* species (one of the parasitic nematodes), have been identified as a limiting factor in potato production, leading to decreased yield, poor tuber quality and deformations, which makes them unmarketable (Medina *et al.*, 2016) [18]. Of all the nematodes parasitizing potato, root-knot nematodes (RKNs) are the most aggressive, damaging and economically important (Jones *et al.*, 2013) [15]. Root-knot nematodes make host plants vulnerable to infections caused by other disease agents (Ekpenyong *et al.*, 2016) [11]. Nematodes have been reported to cause an estimated loss of about \$157 billion annually to world agriculture (Singh *et al.*, 2015). Loss of potato produce can be mitigated if these parasites are successfully combated (Tibugari *et al.*, 2012). Due to hazardous effects of nematicides and pesticides even though they are the most effective in the control of nematodes (Ojo, 2016), the use of plant extracts with nematicidal properties have been considered effective, cheap and safe alternative organic methods of control of nematode (Ekpenyong *et al.*, 2016) [11]. This research is therefore aimed at determining the efficacy of aqueous leaf extract of *Crotalaria juncea* in the control of *Meloidogyne* species infecting potato in Jos.

Materials and Methods

Experimental site

The research work was carried out in the Department of Plant Science and Biotechnology, Laboratory University of Jos, and at the plant nursery of Federal college of forestry, Jos, Nigeria.

Collection of test plants

Young and fresh leaves of *Crotalaria juncea* L. were collected at Lamingo, Jos, Plateau State during the rainy season in polyethylene bags and brought to the Biology Laboratory for identification by a taxonomist. Subsequently identification of the plants based on Voucher number and Binomial naming was accomplished using the herbarium of Federal College of Forestry, Jos, Plateau State, Nigeria.

Preparation of the test plant extract

The fresh leaves of the test plant were washed under running tap water and air-dried on a laboratory bench at room temperature of 25 - 27°C. Cold maceration technique was used for the extraction. Fifty (50) g of *Crotalaria juncea* leaf powder was added to 500 ml of distilled water in separate, well labelled 1000 ml conical flasks and placed in a laboratory shaker at 60 rpm for 24 hours in the laboratory. The resulting mixture was filtered with a muslin bag and re-filtered through a Whatman No 1 filter paper. The filtrate was retained and the residues discarded. The filtrate was then evaporated to dryness in crucibles, over a water bath, at 100°C.

Phytochemical analysis of the test plant

The plant was kept on Laboratory Bench to dry under room temperature (25 - 27°C) for one month (Salako, Valencia and Oyabanji, 1997) and dried to constant weight in the oven for 12 hours at a temperature of 35°C and crushed into a fine powder for the purpose of extraction and phytochemical analyses. The phytochemical analysis for qualitative detection of flavonoids, tannins, alkaloids, saponins, cardiac glycosides, steroids, carbohydrates and anthraquinone was done on the plant extract as described by AOAC (2007) [3].

Isolation and identification of root-knot nematodes

Root-knot nematodes were obtained from galled roots of infected tomato plants at the Fadama farmland bordering Rock Haven and ECWA Staff Area of Jos. Root-knot nematodes from the infected tomato roots were isolated using the modified Baermann Funnel method of nematode extraction as described by Sato *et al.* (2009). Galls were excised from the infected roots, dissected transversely and placed in Petri dishes containing about 10 ml distilled water. Using sterile inoculating needles, the galls were then teased to release the root-knot nematodes from the plant tissue. The Baermann funnel is a regular laboratory funnel with a piece of rubber tube about 25-30 cm long attached to the stem of the funnel. The tube is in turn connected to a test tube tightly held together with the aid of a masking tape. The set-up was kept in an upright position using a table stand with small regular holes and was filled with distilled water to the brim of the funnel's stem. Cotton wool was placed in the funnel to assume the shape of the funnel so that the water slightly covered the wool before the teased root samples for were placed on the cotton wool and covered with water. The set-up was allowed to stand for 24 hours. The active nematode juveniles readily passed through the cotton wool down the funnel stem and were collected at the bottom of the test tube. Twenty sets of the set-up were used so as to obtain sufficient quantities of the inoculum. The test tube was then carefully removed and its content centrifuged at 2,000 rpm (revolutions per minute) for 5 minutes to

concentrate the nematode juveniles at the bottom of the test tube.

Root-knot nematodes were identified by infection often identified by swelling in roots that look like knots or galls that are large and easy to see with naked eye and microscopic identification of the nematodes was by comparative morphology, descriptions and lattice keys. Female shapes are oval while male characters are stylet length and shape, stylet cone shape, head shape and distance of esophageal gland outlet from stylet base (Jepson, 2024).

Preparation of extract concentrations for nematocidal and pot experiments

Various concentrations of the aqueous leaf extract of the test plant were prepared as follows: 1.5 g of each extract was dissolved in 250 ml of distilled water to yield a stock solution of 6.0 mg/ml. Other concentrations were separately prepared from the stock solution of each extract. Using the stock as one of the concentrations, the following concentrations were obtained: 6.0, 3.0, 1.5 and 0.75 mg/ml.

Determination of Nematicidal Activity

1ml of each concentration of extract was added to 4 ml of homogenised suspension of root-knot nematode suspension (108 juveniles). It was replicated three times for each concentration and examined at 24, 48, 72 and 96 hours for live and immobilized nematodes. Four concentrations of each extract, a control (Nematode suspension + distilled water without extract) and 4 replicates for each treatment was prepared. The number of immobilized nematodes were counted and expressed as percentage of mortality and recorded. Nematodes that appear stiff and straight and do not move when probed with fine needle were counted as dead nematodes (Muhammad *et al.*, 2004).

Sourcing of potato seed tubers for polyethylene bag experiment

Egg-size seed tubers of Caruso and Marabel varieties of potato were obtained from National Root Crop Research Institute (NRCRI), Kuru, Jos South LGA of Plateau State.

Polyethylene bag experiment

A completely randomised design was used in the pot experiment. Two potato varieties (Caruso and Marabel), three concentrations each of aqueous leaf extracts of *Carica papaya* L. and *Crotalaria juncea* L. (1.5 mg/ml, 3 mg/ml and 6 mg/ml) and three controls, that is, a nematicide (Furadan at an application rate of 4 kg/ha), inoculated + un-amended and un-inoculated + un-amended control were used. All treatments were replicated five times to give a plant population of 90. The potato plants were raised in steam-sterilized loamy soil in polyethylene pots. The plants were inoculated with 1,000 second-stage larvae of *Meloidogyne* species at 2 weeks after planting (2 WAP). This was done by injecting 50 ml of the nematode solution into the polyethylene bag using syringe.

The various treatments; 1.5 mg/ml, 3 mg/ml and 6 mg/ml and the controls, that is, (Furadan, inoculated + un-amended and un-inoculated + un-amended) were administered at 4 WAP by drenching each polyethylene bag with 500 ml of the appropriate treatment. The plants were carefully uprooted after 84 days (i.e., 12 WAP) to determine shoot height, fresh tuber weight and the number of galls per plant.

The roots were rated for galling index on a 0-5 scale (Taylor and Sasser, 1978), where 0 = no gall/plant, 1 = 1-2 galls/plant, 2 = 3-9 galls/plant, 3 = 10-30 galls/plant, 4 = 31-100 galls/plant and 5 = > 100 galls/plant.

Results

The result of the phytochemical analysis of the test plant *Crotalaria juncea* extract on table 1 shows that bioactive substances such as alkaloids and saponins are moderately high in the extract of *Crotalaria juncea*. Tannins is moderately high in the extract of *C. juncea*. Flavonoids is very high in test plant' extract anthraquinone was absent while cardiac glycosides were moderately high-test plant' extract. Steroid was present. The results of various concentration of *Crotalaria juncea* leaf extracts on root-knot nematodes second stage juveniles indicated that each of the concentration of *C. juncea* had effect on the nematodes as shown in table 2. The mortality of the nematodes increases with increase in concentrations and time of exposure of the nematodes.

Result on table 3 showed the effects of various concentrations of *Carica papaya* extract on root-knot nematodes second stage juveniles. There is a significant difference at ($P \leq 0.05$) on effects of the various concentrations of the aqueous extract on the root-knot nematodes just as in the case of *C. juncea* extract. The concentration of 6 mg/ml significantly ($P \leq 0.05$) showed highest level of effect as 100 per cent mortality was observed and recorded within 24 hours of exposure. The effect of the extract on the root-knot nematodes increases as the time of exposure also increases.

Result on table 4 showed effects of various concentrations of aqueous extracts of *Crotalaria juncea* and *Carica papaya*

and the controls on stem height of the two varieties of *Solanum tuberosum* (Caruso and Marabel). Plants in pots treated with Furadan control at ($p < 0.05$) level of significant, exhibited highest stem height compared to others followed by plants in pots treated with extract of *Crotalaria juncea* with concentration, of 6 mg/ml, 3 mg/ml, 1.5 mg/ml. Result on table 5 showed effects of various concentrations of aqueous extracts of *Crotalaria juncea* and the controls on leaf count of the two varieties of *Solanum tuberosum* (Caruso and Marabel). Leaf count value recorded for each treatment were significant at ($p < 0.05$). The potato plant treated with the aqueous extract of *Crotalaria juncea* at concentration of 6 mg/ml had highest number of leave count followed by plants treated with extract of *C. juncea* at concentration of 3 mg/ml and by furadan.

The effect of the treatments on leaf count of marabel variety of *Solanum tuberosum* L were also significant at ($p < 0.05$) with the extract of *C. juncea* at concentration of 6 mg/ml and Furadan treatments exhibiting highest overall value.

Table 1: The Result of the Phytochemical Analysis of the test plant (*Crotalaria juncea*) extract for the following Constituents

Constituents <i>C. juncea</i>	Constituents <i>C. juncea</i>
Alkaloids	++
Saponins	++
Tannins	++
Flavonoids	+++
Carbohydrates	+++
Anthraquinones	—
Steroids	++
Cardiac glycosides	++

Key: — absent; + present; ++ moderately high; +++ very high

Table 2: Effect of Various Concentrations of *C. juncea* Aqueous Extract on Root-Knot Nematodes Second Stage Juveniles.

Concentration of Extract	Time of Exposure (Hours)			
	24	48	72	96
0.75 mg/ml	55.56±6.4 ^b	62.96± 6.42 ^b	71.61±7.5 ^b	80.25± 4.45 ^b
1.5 mg/ml	66.67±4.2 ^b	70.37± 4.28 ^b	80.25±3.2 ^b	87.65± 3.26 ^a
3 mg/ml	66.67±2.1 ^b	74.07± 4.28 ^b	100.00±0.0 ^a	100.00±0.0 ^a
6 mg/ml	100.00±0. ^a	100.00±0.0 ^a	100.00±0. ^a	100.00±0.0 ^a
Control(water)	00.00±0.0 ^c	3.01± 0.32 ^c	5.24± 0.26 ^c	8.10± 0.33 ^c
L.S. D	10.94			
P-value	<0.0001			

Means followed by the same letter within a column are not significantly different from each other at $P < 0.05\%$ (DMRT)

Table 3: Effect of Various Concentrations of *C. juncea* Aqueous Extract on Second Stage Juveniles of root-knot nematodes.

Concentration of Extract	Test Plant	Time of Exposure (Hours)			
		24	48	72	96
0.75	Cj	55.56 ± 6.42a	2.96 ± 7.51a	71.61 ± 7.51a	80.25 ± 4.45a
1.5	Cj	66.67 ± 4.28a	70.37 ± 4.28a	80.25 ± 3.27a	87.65 ± 3.26a
3	Cj	66.27 ± 2.14a	74.07 ± 4.28a	100.00 ± 0.00a	100.00 ± 0.00a
6	Cj	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
L.S. D	-	-	-	-	-
P-value	-	<0.0001	-	-	-

Means followed by the same letter within a column are not significantly different from each other at $P < 0.05\%$ (DMRT) Duncan Multiple Range Test.

Table 4: Effect of different levels of treatments on stem height of *Solanum tuberosum* L. treatment stem height (cm)

	4WAP	6WAP	8WAP	10WAP	12WAP
Caruso					
Cj 1.5	13.33d	15.33e	23.33ed	41.33d	51.33e
Cj 3	18.33b	21.33a	28.33ab	43.33c	52.33c
Cj 6	16.33c	20.33ab	29.33a	48.33a	56.33b

Furadan	19.33ab	19.33ab	29.33a	46.33b	58.33a
Unamended + Inoculated	21.33a	21.33a	22.33d	23.33g	24.33g
Unamended + Uninoculated	20.33ab	21.33a	27.33ab	30.00f	31.33f
SE±	0.648	0.629	0.648	0.648	0.648
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Marabel					
Cj 1.5	18.33bc	21.33b	33.00b	39.33d	49.33cde
Cj 3	18.33bc	20.33bc	34.33ab	41.33cd	51.33c
Cj 6	21.33a	23.00a	36.33a	45.33a	55.33a
Furadan	19.33ab	20.00bc	34.00ab	44.00ab	56.33a
Unamended + Inoculated	18.33bc	19.67bc	22.33d	39.33d	36.33f
Unamended + Uninoculated	17.33bc	18.33cd	29.33c	41.33cd	48.33ed
SE±	0.667	0.638	0.667	0.711	0.667
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Independent T-test (Caruso × Marabel)	0.276ns	0.111ns	0.000**	0.125ns	0.148ns

Values (in the same column) with the same subscript letters do not differ significantly from each other according to the Duncan Multiple range test Where: ** = significant; ns=not significant Cj 1.5, 3, 6 = concentration of test plant.

Table 5: Effect of Different levels of treatments on leave count of *Solanum tuberosum* L.

	Treatment Leave count				
	4WAP	6WAP	8WAP	10WAP	12WAP
Caruso					
Cj 1.5	18.33c	22.00c	37.33c	51.00d	59.00c
Cj 3	22.33ab	27.33ab	41.33b	53.33d	67.33ab
Cj 6	23.33ab	28.33a	42.33b	64.33a	68.33a
Furadan	21.33b	28.33a	46.33a	61.33b	67.33ab
Unamended + Inoculated	23.33ab	25.33	32.33d	38.33f	65.00b
Unamended + Uninoculated	22.33ab	26.33ab	36.33c	57.33c	58.00
SE±	0.648	0.648	0.648	0.648	0.648
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Marabel					
Cj 1.5	21.33abc	31.00b	43.00bc	53.33cd	59.00b
Cj 3	22.33ab	31.00b	44.33bc	54.00bc	62.00ab
Cj 6	23.33a	34.00b	47.00a	57.33a	65.00a
Furadan	23.33abc	31.00b	45.33ab	57.33a	65.00a
Unamended + Inoculated	23.33ab	24.00	27.67d	31.00e	29.33c
Unamended + Uninoculated	20.00bcd	29.00bc	42.33c	51.00d	41.00d
SE±	0.667	0.667	0.667	0.667	0.667
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Independent T-test (Caruso × Marabel)	0.324ns	0.000**	0.059ns	0.482ns	0.736ns

Values (in the same column) with the same subscript letters do not differ significantly from each other according to the Duncan Multiple range test Where: ** = significant; ns=not significant Cj 1.5, 3, 6 = concentration of test plant.

Discussion

The leaf extract of the test plant (*C. juncea*) exhibits a very high nematicidal effect on *Meloidogyne* species, which justifies the suggestions of Baskaran *et al.* (2012) [7] who mentioned that extracts from other plant parts could be prospective to suffice in the management of *Meloidogyne* species. The effect of *C. juncea* in this present study also corroborates with the findings done by Danahap and Wonang (2016) [9] who affirmed the nematicidal activities of the root exudates of *Crotalaria juncea* as observed on nematodes isolation infected tomato plant studied. The study carried out by Marla *et al.* (2008) on the ability of *C. juncea* extract to suppress the southern root-knot nematode. The effect of *C. juncea* on *Meloidogyne* species in the present findings may be attributed to the presence of bioactive compounds such as alkaloids, saponins, tannins, flavonoids, steroids and Cardiac glycosides. Al-Snafi (2016) [1] preliminary phytochemical screening on leaves of *Crotalaria juncea* suggested antimicrobial activities which he mentioned was effective due to the presence of phytochemicals such as, carbohydrates, steroids, triterpenes,

phenolics, flavonoids, alkaloids, amino acids, saponins, glycosides, tannins and volatile oils. However, Rocha *et al.* (2017) [23] noted that phytochemical compounds have anti-helminthic effect against plant parasites and are more active and eco-friendlier, particularly those screened from medicinal and aromatic plants such as serpentine, saponins, phenols, alkaloids, tannins, flavonoids, steroids, and cysteine proteinases. The effectiveness of the leaf extract of *C. juncea* increases with increase in concentration and in the period of interface with the nematode in the suspension. This goes in tandem with the research carried out by Okechalu *et al.* (2020) [22] which showed that the nematicidal activities of the leaf extracts increased with concentration and time of exposure. Significant effects of the different concentrations tested on plant stem height, stem girth, leave counts and tuber weight. Results from this present study revealed that for both varieties of potato evaluated the Furadan treatments and leaf extracts of *Crotalaria juncea* at a concentration of 6mg/ml (Cj6) used in the study had a similar effect on stem height as the plant age progresses. This suggests that the plant extract

at a concentration of 6 mg/ml can achieve the same nematocidal effect on root-knot nematodes to enhance plant growth. This corroborates with the findings of Kepenekci *et al.* (2016) [17] who reported same in tomato? where the leaf extract had significant effect on morphological traits compared to the synthetic treatments induced. Similar results were observed on the effect of the various concentration of plant extracts and other treatments against root-knot nematodes on stem girth for both varieties of potatoes evaluated. The leaf extracts of *Crotalaria juncea* was significantly effective on the leave count of Marabel variety at a concentration of 6 mg/ml (Table 5). *Crotalaria juncea* as suggested by Al-Snafi (2016) [1] and Rocha *et al.* (2017) [23].

The leaf extract of *Crotalaria juncea* at a concentration of 6 mg/ml (Cj6) had a significant effect on the tuber weight (yield) of both Caruso and Marabel varieties of potato evaluated in this study. This agrees with the suggestion of Danahap and Wonang (2016) [9] whose findings on the root extracts of *Crotalaria retusa* suggested plant exudates from *Crotalaria* spp. could be sources of affordable and efficient nematicides of *Meloidogyne incognita*. Similar result reported by Kepenekci *et al.* (2016) [17] on the effectiveness of extracts from *Melia azedarach* against root-knot nematodes suggest the prospect leaf extract have in contributing to crop yield, which is evidence in this present study.

Generally, the difference in the responses of the potato varieties to the different concentrations of the treatments used in this study against *Meloidogyne* spp. was evidence from the results shown in this study. The concentration of the aqueous leaf extract applied was more effective at higher concentration in Marabel than in Caruso. This could be attributable to the differences in the biochemical composition and genetic constituents of the potato varieties. This is in alignment to the findings of Danladi *et al.* (2023) [10] who reported that fungi infestation is more in Marabel than in Caruso owing to the difference in dry matter and moisture content. Marabel was reported to have higher occurrences of fungi than Caruso due to low dry matter content and higher water content (Danladi *et al.*, 2023) [10]. This could be said for same in nematode infestation in potato plants as reported by Figueiredo *et al.*, (2022) [12] who mentioned that moisture could trigger the incubation period of nematodes.

Conclusion

The study has shown that aqueous leaf extracts of *Crotalaria juncea* was effective in the management of *Meloidogyne* sp. in potato. Therefore, the extract of *C. juncea* is recommended in the control of root-knot nematodes.

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