



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Effect of aqueous extracts of *Datura stramonium* W. seeds, leaves and roots on mortality of second stage juveniles of *Globodera rostochiensis* in Kenya

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Article Info

Volume 2, Issue 4, October 2020

Received : 23 March 2020

Accepted : 01 July 2020

Published : 05 October 2020

doi: [10.33472/AFJBS.2.4.2020.99-106](https://doi.org/10.33472/AFJBS.2.4.2020.99-106)

Abstract

Experiments were carried out in the laboratory to evaluate the effects of aqueous extracts of leaves, seeds and roots of *Datura stramonium* at different concentrations on mortality of *Globodera rostochiensis* juveniles. Volumes of 5 mL of the extract at 100 (crude extract), 50, 25 and 12.5% dilutions were dispensed separately into Petri dishes containing 20 freshly hatched second stage juveniles (J2s) of *G. rostochiensis*. Juvenile mortality was recorded at 24, 48 and 72 h intervals. The treatments were replicated four times. Petri dishes were arranged in a Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was used to test for the differences (means) among the extracts and the dilutions. The results showed that the crude extracts (100%) of all extracts and 50% dilution of roots and seeds caused 100% mortality after 24 h exposure. This study established that aqueous extracts of *D. stramonium* has the potential for use as a bionematicide. Further experiments are required to confirm their effect under cropping conditions. There is also a need to analyze the phytochemicals of the plant which cause nematode mortality.

Keywords: *Datura stramonium*, *Globodera rostochiensis*, Nematode mortality, Phytochemicals, Second stage juveniles (J2s)

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1. Introduction

Plant Parasitic Nematodes (PPN) feed on roots and complete their life cycles in or on the root zone and in a few cases in the shoot. They affect both the quality and quantity of marketable yields of agricultural crops (Daramola et al., 2015). The *Globodera rostochiensis* also referred to as yellow/golden potato cyst, is a Potato Cyst Nematode (PCN) that causes serious yield loss in infested potato growing areas and can cause up to 80% yield loss under heavy infestation (CABI, 2019). The pest is also difficult to eradicate once it infests an area since the eggs are encased in cysts that can remain viable for many years in the soil without a host (Coyne et al., 2018). This poses a serious threat to potato production where the nematode is present. The PCN is a quarantine pest in most

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countries (EPPO, 2014). Eggs which are contained within the cysts are the persistent stage of the life cycle. Each cyst contains between 200 and 500 embryonated eggs. Eggs hatch to second stage juveniles (J2s) and the hatching increases rapidly after plant emergence (Devine and Jones, 2003). The PCN has a narrow host range (mainly the solanaceous hosts) and a few exceptions which produces secretions that stimulate eggs to hatch. The optimum soil temperature for hatching and development is 15 to 27 °C (Kaczmarek et al., 2014).

The J2s penetrate the root behind the root tip after which they move through the root feeding on the cortex, endodermis and pericycle. The J2s then evades the host immune system and establish a permanent feeding site consisting of 'transfer cells' known as syncytia, which provides nutrients to the nematode (EPPO, 2004; and Eves-van den Akker et al., 2016). The J2s undergo three moults before they reach the adult stage. Sedentary female adults enlarge and burst through the root with the posterior body portion facilitating mating. Mating occurs within 50 days of J2 root invasion and the females continue feeding and retain their eggs within their bodies (CABI, 2019). When the female dies, she becomes a cyst changing the color from white to yellow or gold and finally to brown. Cysts remain attached on roots or in the soil. The eggs can remain viable within the cyst for 30 years (Winslow and Willis, 1972).

G. rostochiensis was first reported in Kenya, in 2015 (Mwangi et al., 2015). Hence, it is a new pest in the country, and there are yet no established management practices carried out to manage the nematode. However, farmers are advised on general management practices like crop rotation, use of resistant varieties and use of clean and certified potato seeds (NPCK, 2017).

Use of plant phytochemicals to manage PNN including *G. rostochiensis* has been studied in many countries as discussed herein, with several plants showing nematicidal effects on various nematodes. The secondary metabolites produced by these plants as defense chemicals include alkaloids, flavonoids, essential oils, phenols and saponins. The extracts from plants, are being studied extensively so as to evaluate their effectiveness in management of diseases (Chitwood 2002; and Padmarathy and Mekala, 2013).

A study that was carried out to evaluate the effect of crude neem seed extract and neem seed concentrate on the mortality rate of J2s of *Heterodera glycine*, showed high effectiveness (Silva et al., 2008). The water extracts had up to 98% mortality, methanolic extracts had up to 48.13% while the neem concentrate had 92.4%. Extracts of *Medicago sativa* have also been tested against *M. incognita*, *G. rostochiensis* and *X. index* nematode species (Argentieri et al., 2008; and D'Addabbo et al., 2009). The chemical compounds present were toxic to the nematodes, resulting in 23-62% mortality depending on the concentration, time of exposure and nematode species. This is in agreement with another study carried out by D'Addabbo et al. (2010). The effects of leaf ethanoic extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* were studied against *M. incognita*. The extracts were found to possess phytochemicals with nematicidal activity against *M. incognita* (Nandakumar et al., 2017). Castor bean extracts have been tested against root knot nematodes where they resulted in 3.75-4.88 mean number of dead of J2s within 24 h exposure, with the crude extract showing a significant difference from the control and the other dilutions of the extract (Adomako and Kwoseh, 2013).

Adegbite and Adesiyan (2005) studied the root extracts of plants to control root knot nematodes on edible soyabeans. A 100% concentration extracts of Siam weed and Neem showed 100% mortality after 12 h exposure time. While 100% concentration of lemon grass and castor bean showed 75 and 62.1% mortality respectively after 12 h exposure time. From the above studies, it is evident that the secondary metabolites in plants have the potential to be utilized for management of PPN. The products are also environmentally friendly and have minimal effects on living organisms (Ntouden et al., 2017). This advocates for use of such materials towards eco-friendly crop protection.

Datura stramonium (Jimson weed) belongs to the Solanaceae family and grows wildly in temperate and sub-tropical regions (Ganesan et al., 2016). The plant is believed to be have originated from South America. *D. stramonium* contains different phytochemicals which include saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides (Jakabova et al., 2012; Sayyed and Shah, 2014; and Ganesan et al., 2016). In Kenya, the plant grows extensively as a weed in cultivated fields, roadsides and also wastelands. *D. stramonium* leaf powder and extract have been evaluated against *M. javanica* on sweet melon. Juvenile mortality was as high as 97% by the crude extract (Umar and Ngwamdai, 2015).

The use of phytochemicals to control PPN, motivated this study. The aim was to evaluate the effect of different *D. stramonium* organs extracts on the mortality of J2s of the PCN *G. rostochiensis*.

2. Materials and methods

2.1. Study area

The study was carried out in the department of Biological Sciences Laboratory at Egerton University. Egerton University is located in Njoro Sub County with coordinates as 0° 23' south, 35° 35' and altitude of 2000 m above sea level. Temperatures range between 17-22 °C while the average annual rainfall is 1000 mm. The laboratory conditions were not regulated, and all experiments were carried out on the laboratory benches at ambient temperatures.

2.2. Collection and preparation of plant material

Fresh whole Jimson weed (*D. stramonium*) plants (including root, leaves and stems) were collected from agricultural field 3 of Egerton University, Njoro Campus, Nakuru County in Kenya. The plants were thoroughly washed in running tap water and then finally washed with distilled water (Nandakumar et al., 2017). The seeds were collected from dry fruits in field 3 of Egerton University. The plant materials were separated into leaves, roots and seeds and each part treated as described below.

2.3. Leaves extract

The leaf extract was prepared using a modified method described by Umar and Ngwamdai (2015). 100 g of fresh *D. stramonium* leaves was cut and blended using a normal blender in 300 mL of distilled water and then allowed to settle in the refrigerator for 24 h. The extract was centrifuged at 5,000 rpm for 5 min after which the supernatant was passed through a filter paper (Whatman No 1) to obtain a clean extract. The filtrate was designated as crude extract (100%) and stored at 4 °C for not more than one week before use. During juvenile mortality test the crude extract was diluted into 50, 25 and 12.5% using distilled water.

2.4. Roots extract

The root extract was obtained using a modified method as described by Doughari et al. (2007). 300 g of medium sized roots of *D. stramonium* cut into small pieces and blended in 500 mL of water. The extract was centrifuged at 3,500 rpm for 5 min and then filtered using Whatman No. 1 filter paper. The filtrate was designated as the crude extract (100%) and stored in refrigerator at 4 °C for not more than a week prior to use. During the juvenile mortality test the crude extract was diluted into 50, 25 and 12.5% using distilled water.

2.5. Seeds extract

The dry *D. stramonium* seeds (10 g) were blended in 100 mL of water to obtain the aqueous extract. The extract was filtered using Whatman No 1 filter paper, after which it was centrifuged at 3,500 rpm for 5 min and the supernatant collected. This was stored at 4 °C as crude extract used for evaluation within one week.

2.6. Hatching *G. rostochiensis* eggs

Cysts were extracted from soil collected from PCN infested farms in Njoro and Molo sub-counties, Nakuru County, Kenya, using the Fenwick floatation method at ICIPE, Nairobi (Fenwick, 1940). Root exudate of *Solanum scabrum* (African nightshade) was used to stimulate hatching of the eggs. The exudate was obtained by first saturating pots containing one-month old *S. scabrum* with distilled water. Then about 200 mL of distilled water was passed through the pots and the leachate collected in a container (Fenwick, 1949; and Scholte, 2000). The leachate was centrifuged at 3,500 rpm for 5 min and then used to stimulate hatching. Fifty cysts were pre-soaked for one week in distilled water after which they were exposed to the exudates. The hatched juveniles were collected after five days and prepared for the juvenile mortality test.

2.7. Juvenile mortality tests

The seed, leaf and root extracts at 12.5%, 25%, 50%, 75% and 100% concentrations were dispensed in volumes of 5 mL in separate Petri dishes, using a 5 mL syringe. A control containing distilled water was included (Umar and Ngwamdai, 2015). Approximately twenty (20) freshly hatched *G. rostochiensis* J2s suspension were added to each of the Petri dishes containing the extracts using a micro pipette. Each dilution level had four replicates arranged in Completely Randomized Design (CRD) on the laboratory benches. The number of dead J2s were counted at 24, 48 and 72 h intervals (Table 1). The dead J2s were identified by assumption of straight (I) shape and lack of motility after probing with a needle.

2.8. Statistical analysis

The mortality data was corrected for natural mortality using Schneider-Orelli's formula (Püntener, 1981). Comparison of mortality rates of the juveniles in different extracts was analyzed using ANOVA and the means compared using Least Significant Difference (LSD). The mean mortality in percentage for each extract per day were represented graphically. All the hypotheses were tested at 95% level of significance.

3. Results

3.1. Nematicidal activity of extract of *D. stramonium* at 12.5, 25, 50 and 100% dilutions

The mortality rate of J2s at the different dilutions are summarized in Table 1. The control mortality was recorded as 2.5%, 12.5% and 30% after 24, 48 and 72 h of exposure respectively, these results were used to correct the mortality data.

At the lowest dilution of 12.5% root extract had the highest effect causing 64% mortality ($M = 64.10 \pm 13.24$) while seed and leaf had a mean mortality of $M = 29.49$ and 16.67% respectively (Table 1). A similar pattern was observed after 48 h of exposure with root having the highest effect on mortality ($M = 72.60 \pm 7.75$) followed by seed extract at ($M = 53.43 \pm 13.04$) while leaf extract had the least effect on mortality on J2s ($M = 27.40 \pm 9.36\%$). No significant differences were found among the three extracts at 12.5% after 72 h exposure ($F(2, 9) = 1.909$, $p = 0.204$).

At the dilution of 25% LSD procedure indicated that root and seed extract had same effect on mortality of J2s ($p = 0.390$), and the leaf extract had significantly lower effect on mortality of J2s after 24 h of exposure. Similarly, the difference in the effects of root and seed extracts on mortality were not significant at 95% after 48 h of exposure ($p = 0.418$) and the leaf extract had significantly lower effect ($M = 31.51 \pm 9.49$). The effects of the three extracts after 72 h of exposure were not significantly different. It thus shows that period of exposure is a critical variable influencing the nematicidal effect of plant extracts on J2s.

At the higher dilution of 50%, root and seed extracts resulted in higher mortalities than leaf extract. Root and seed resulted in 100% mortality after 24 h exposure. Leaf extract had mean mortality of 42.31, 78.08 and 97.05% after 24, 48 and 72 h exposure respectively (Table 1). A small proportion of J2s (2.85%) survived even after being exposure to 50% leaf extract for 72 h as opposed to root and seed extracts which eliminated all the J2s after 24 h exposure at 50% dilution.

At 100% concentration (crude extract), all the extracts resulted in 100% mortality of J2s after 24 h of exposure. The findings suggest that, type of extract, concentration of the extracts and period of exposure had significant effect on mortality of J2s even after adjusting for natural mortality.

Dilution	Extract	24 h	48 h	72 h
12.5%	Seed	29.49 ± 8.76 ^a	53.43 ± 13.04 ^a	85.72 ± 7.38
	Root	64.10 ± 13.24 ^b	72.60 ± 7.75 ^b	77.14 ± 4.67
	Leaf	16.67 ± 2.56 ^a	27.40 ± 9.36 ^c	82.86 ± 6.60
		$F(2,9) = 27.940$, $p = 0.000$	$F(2,9) = 19.441$, $p = 0.001$	$F(2,9) = 1.909$, $p = 0.204$
25%	Seed	82.05 ± 8.88 ^a	89.04 ± 7.75 ^a	94.29 ± 8.08
	Root	75.64 ± 7.70 ^a	83.56 ± 10.01 ^a	95.72 ± 8.57
	Leaf	29.49 ± 12.82 ^b	31.51 ± 9.49 ^b	88.57 ± 4.67
		$F(2,9) = 32.631$, $p = 0.000$	$F(2,9) = 48.341$, $p = 0.000$	$F(2,9) = 1.068$, $p = 0.384$

Dilution	Extract	24 h	48 h	72 h
50%	Seed	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	100.00 ± 0.00
	Root	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	100.00 ± 0.00
	Leaf	42.31 ± 4.91 ^b	78.08 ± 7.75 ^b	97.05 ± 3.30
		$F(2,9) = 551.890,$ $p = 0.000$	$F(2,9) = 32.000,$ $p = 0.000$	$F(2,9) = 3.000,$ $p = 0.100$

Note: Values are expressed as % mean mortality ± SD of four replicates mean values with common letter within a column are not significantly different ($p = 0.05$) according to LSD test.

4. Comparison of effectiveness of seed, root and leaf extracts of *D. stramonium* on mortality of J2s of *G. rostochiensis* over time

The two-way analysis of variance (ANOVA) conducted indicated that the three extracts were significantly different in their effect on mortality of J2s ($F(2, 45) = 111.032, p = 0.000$) (Figure 1). Among the three extracts, there was a significant difference in mortality of J2s observed after 24 and 48 h of exposure to the extracts ($p = 0.000$ and $p = 0.001$ respectively). However, after 72 h, the three extracts did not have any significant difference ($p = 0.204$ and $F = 1.909$). The tests further showed significant difference in the effect of various dilution levels on the mortality of J2s ($F(4, 45) = 888.439, p = 0.000$). The crude extracts from all parts (roots, leaves and seeds) had the best performance causing 100% mortality within 24 h of exposure. In addition, there was significant interaction between type of extract and the level of dilution with respect to their effect on the mortality of J2s ($F(16, 90) = 14.540, p = 0.000$). The study established that the effect of the various extracts and levels of dilution on mortality of J2s varied with the period of exposure. During the 24 h period, the three extracts had significantly different effect on the mortality of J2s ($F(2, 11) = 27.940, p = 0.000$).

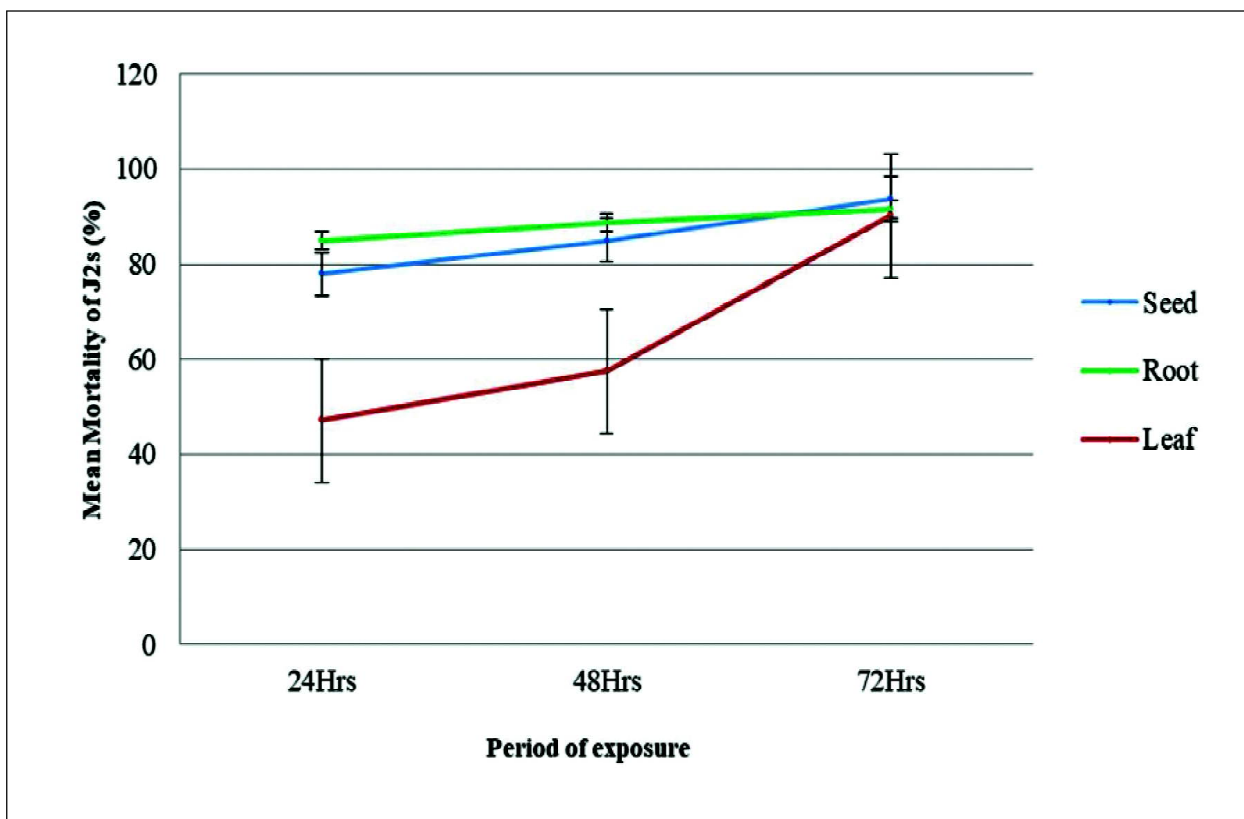


Figure 1: Comparison of effectiveness of seed, root and leaf extracts on J2s of *G. rostochiensis*

5. Discussion

This study has established that the extracts of leaves, seeds and roots of *D. stramonium* have nematicidal effects on the J2s of *G. rostochiensis*. The mortality effect of all the extracts varied at the different levels of dilutions, with the highest mortality recorded in the crude extract and the lowest in the control. This is in agreement with a study by Umar and Ngwamdai (2015), where the crude extract of *D. stramonium* recorded the highest mortality (97%) on *Meloidogyne javanica* juveniles followed by 5 mL dilution (92%) and the least was control at 0%. Adomako and Kwoseh (2013) reported that the crude extract of castor beans caused the highest mortality of *Meloidogyne spp.* juveniles as compared to the lower dilutions.

The J2s mortality also increased with increase in time of exposure. Similarly, in a study by Chaudhary et al. (2013), *D. stramonium* was reported to cause mortality on *Meloidogyne incognita* J2s. The mortality caused by the extracts was at $71.5 \pm 1.4\%$ at 50 mg/mL and $88.7 \pm 2.6\%$ at 100 mg/mL after 24 h exposure. A mortality of 100% was recorded after 72 h exposure at 50 mg/mL and 100 mg/mL. Previous work by Oplos et al. (2018) also show that time of exposure to *D. stramonium* extracts affected the mortality of *Meloidogyne javanica* and *Meloidogyne incognita*. The mortality of J2s in the present study can possibly be due to the presence of metabolites in *D. stramonium*, such as alkaloids, saponins, phenols, steroids flavonoids and tannins (Sayyed and Shah, 2014; and Girmay, 2015). While this study does not identify the active components in the extracts that caused the J2s mortality, the metabolites have been reported to have negative effects on the body systems of organisms (Carpa et al., 2017). Alkaloids such as atropine, hyoscyamine and scopolamine affect the nervous system with atropines depressing the smooth muscles and secretory glands (Carpa et al., 2017). The saponins have been reported to interact with the collagen protein in the cuticle of nematodes (Argentieri et al., 2008). They also cause changes in the permeability of the cell membrane which leads to the mortality of the J2s (Ibrahim and Srour, 2013). Similarly, Adegbite and Adesiyani (2005) reported that the presence of tannins, alkaloids and flavonoids in *D. stramonium* leaves are known to kill nematodes. The complexity of the biological interactions among the chemical constituents of the plant may also be contributing to synergistic performance of the extracts.

The performance of the three extracts evaluated on the mortality of the *G. rostochiensis* J2s was significantly different at 24 h and 48 h. The root extract caused the highest mortality, followed by the seed and lastly the leaf extract. Interestingly, the performance of the leaf extract increased exponentially between 48 h and 72 h of exposure. Without biochemical analysis of the extracts, there are limitations in identifying the specific chemicals involved in the J2 mortality. The difference in performance, however, can be attributed to the difference in the levels of the metabolites in different plant parts at different times of their growth (Iranbakhsh et al., 2006). The leaves contain slight amounts of alkaloids, moderate amounts of tannins and a high amount of saponins and flavonoids (Umar and Ngwamdai, 2015). The seeds have many amino acids and high levels of atropines while the roots have low levels of scopolamine and moderate amounts of atropines (Al-Snafi, 2017).

6. Conclusion

Since *D. stramonium* is a widespread weed in Kenya and its parts have the ability to reduce the population of J2s *in vitro*, it is worthy of testing further under cropping conditions as an alternative tool to control these nematodes, and other PPN such as *Meloidogyne spp.* Hence, this plant can be exploited as a potential eco- friendly bionematicide against *G. rostochiensis* in potatoes. There is need to carry out further studies to identify the mode of action of the phytochemicals present in *D. stramonium* against the PCN. In the long-term this may lead to development of new and low cost nematicidal and nematostatic formulations from this plant.

Acknowledgment

I would like to acknowledge the Department of Biological Sciences (Egerton University, Njoro) and International Centre of Insect Physiology and Ecology, Plant Health, Nematode Research, Nairobi, for providing necessary facilities and assistance during the study. Also, my family for the continuous support that made it possible to carry out this work.

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Cite this article as: Naomi Waithira Kamau, Japhet Mburugu Muthamia, Daniel Otieno Otaye and Solveig Haukeland (2020). Effect of aqueous extracts of *Datura stramonium* W. seeds, leaves and roots on mortality of second stage juveniles of *Globodera rostochiensis* in Kenya. *African Journal of Biological Sciences*. 2(4), 99-106. doi: 10.33472/AFJBS.2.4.2020.99-106.