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Amelioration of root-knot disease of lady's finger plants by potentized *Cina* and *Santonin*

NC Sukul[1,*], S Ghosh[1], A Sukul[2] and SP Sinhababu[1]

[1]*Department of Zoology, Visva-Bharati University, Santiniketan 731235, West Bengal, India*
[2]*Sukul Institute of Homeopathic Research, Shyambati, Santiniketan 731235, West Bengal, India*

Lady's finger plants *(Hibiscus esculentus)*, grown in pots, were inoculated with the second-stage larvae (76±6) of root-knot nematodes *Meloidogyne incognita*, starting 7 days later they were treated with *Cina* 30c, *Santonin* 30c or *Ethanol* 30c by foliar spray for 10 consecutive days. The drugs in 90% ethanol were diluted with distilled water 1:1000 before application on plants. Thirty days after the last treatment the plants were uprooted. *Cina* 30c and *Santonin* 30c reduced nematode infestation of plants significantly in terms of root-gall number, root-protein content and nematode population in roots. *Santonin* 30c reduced root water content. *Santonin* 30c may have influenced the water channel proteins of root tissues thereby altering the water contents of roots. The reduced water content in roots might have adversely affected the root-knot nematodes and thus reduced nematode infestation. *Ethanol* 30c also has some effect on treated plants. *Homeopathy* (2006) **95**, 144–147.

Keywords: *Meloidogyne incognita*; homeopathic drugs; root water; root gall

Introduction

Plant parasitic nematodes are ubiquitous and cause damage to field and fruit crops as well as forest trees. *Meloidogyne incognita* and related species produce root-knot disease of vegetables, cereals, pulses and many other crops. These are sedentary endoparasites of roots and are one the most damaging crop pathogens.[1,2] Chemical nematicides cause environmental pollution, contaminate ground water, induce resistance in nematode parasites, cause chromosome aberrations in the root tips and affect germination and growth of plants.[3,4] Biological control of nematodes using fungi and other natural enemies of nematodes has not yet met with any degree of success.[5–9] Development of nematode-resistant crops is not feasible because of multispecies population of nematodes in the rhizospheric soil of plants.[4] Nematicidal plant products are effective and easily biodegradable,[3,10] but procuring them from natural resources for large-scale use is a big problem. Homeopathy holds promise in controlling nematode parasites without disturbing the agroecosystem.

We have already observed that *Cina* 200c and *Cina* 1000c reduced root-knot disease of tomato and cowpea plants.[11,12] The purpose of the present study is to determine whether *Cina* 30c and *Santonin* 30c could reduce root-knot disease of lady's finger and whether the treatment could influence the water content of root tissues.

Materials and methods

Host plants

Aseptically germinated seeds of lady's finger plants, *Hibiscus esculentus*, were sown, one seed/pot measuring 20 cm in diameter and 20 cm in depth. The earthen pots containing a mixture of sterile loamy soil and composted manure (2:1 v/v) were divided into five groups, each of 10 pots.

Inoculation

When the seedlings were at the 4-leaf stage they were inoculated with the second-stage larvae of *M. incognita*

*Correspondence: NC Sukul, Department of Zoology, Visva-Bharati University, Santiniketan 731235, West Bengal, India.
E-mail: ncsukul@rediffmail.com
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at the rate of 75 + 6 larvae/pot. Of the five groups, one group of pots remained uninoculated control.

Drugs

Flowering tops of *Artemisia nilagirica* (Clarke) Pamp were collected from Shillong, Meghalaya, India, dried in the shade and extracted with 90% ethanol at room temperature for 2 weeks. This mother tincture was diluted with 90% ethanol 1:100 and given 10 powerful downward strokes to prepare the first centesimal potency called *Cina* 1c. *Cina* 30c was prepared by successive dilution and succession of *Cina* 1c in 29 more steps. Pure Santonin, an active principle of *Artemisia*, was purchased from Sigma Chemical Company, USA. *Santonin* 30c was prepared the same way as *Cina* 30c. *Ethanol* 30c was prepared from 90% ethanol following the same procedure. All the three potencies were diluted with distilled water 1:1000 just before application to plants.

Treatment

Seven days after inoculation three groups of plants were treated by foliar spray with *Ethanol* 30c, *Cina* 30c and *Santonin* 30c. The treatment continued for 10 days. Foliar spray was started when the plants were 14-day old and at 4-leaf stage and finished at 8–10 leaf stage. Each plant received 5–10 ml of test agent depending on its number of leaves. Treatment was started on 21 July and continued until 3 August, 2004. The plants were kept outdoor at ambient atmospheric temperature 30 ± 3 C and irrigated when necessary.

Harvesting

All the plants were uprooted 30 days after the last treatment and the following parameters recorded: shoot length, shoot weight, root length, root weight, root gall number, nematode population/2.4 gm root and 200 gm soil, root and leaf protein content and water content in roots and leaves. Five samples of roots or leaves were taken at random from each group of plants and the total protein fraction in each sample was estimated by the folin-phenol method.[13,14] An aliquot of the same 5 samples of roots was weighed fresh, torn into fine pieces, kept in an incubator for 12 h at 90 C and weighed again. The difference in weight gave the water content in roots. Water content in the leaves was determined by the same procedure. The experiment was repeated twice and the data from the second experiment have been taken.

Results

Both *Cina* 30c and *Santonin* 30c reduced root-gall number, nematode population in roots, root-protein content and increased leaf protein content significantly ($P<0.05$, ANOVA, Table 1) as compared to the inoculated untreated group. Treatment with these two

Table 1 Reduction of root-knot nematode infestation of lady's finger plants by *Cina* 30c and *Satonin* 30c. Inoculum level 76 ± 6 larvae/plant. Each group consists of 10 plants

Treatment	*Shoot length (cm)*	*Shoot weight (g)*	*Root length (cm)*	*Root weight (g)*	*Number of leaves*	*Number of root galls*	*Nematode population/ 2.4 g root*	*Nematode population/ 200 g soil*	*Leaf protein content %*	*Root protein content %*	*Water content of leaf g/g dry wt*	*Water content of root g/g dry wt*
Un.un. con I	46.7 ± 2.2^{b}	48.1 ± 2.8^{a}	26.1 ± 0.8^{a}	12.1 ± 1.1^{a}	9.0 ± 0.3^{a}	nil	nil	nil	0.24 ± 0.0004^{a}	2.12 ± 0.26^{a}	3.10 ± 0.05^{a}	5.17 ± 0.07^{a}
In.un. con II	56.0 ± 2.5^{a}	49.9 ± 4.3^{a}	35.2 ± 1.4^{b}	21.75 ± 1.4^{b}	7.2 ± 0.5^{b}	138.1 ± 18.7^{a}	67.4 ± 1.4^{a}	15.0 ± 1.20^{a}	0.19 ± 0.0003^{b}	5.78 ± 0.25^{b}	3.50 ± 0.17^{a}	4.05 ± 0.15^{b}
Cina 30	51.5 ± 3.2^{a}	41.2 ± 2.8^{a}	23.6 ± 2.0^{a}	13.2 ± 1.0^{a}	6.2 ± 0.3^{c}	67.4 ± 8.8^{b}	53.4 ± 1.1^{b}	48.0 ± 2.15^{b}	0.27 ± 0.0009^{a}	2.58 ± 0.21^{a}	3.32 ± 0.17^{a}	4.52 ± 0.41^{a}
Santonin 30	46.6 ± 1.6^{b}	41.4 ± 3.5^{a}	23.5 ± 1.8^{a}	13.6 ± 0.7^{a}	7.1 ± 0.3^{b}	68.6 ± 8.9^{b}	54.2 ± 1.3^{b}	42.4 ± 1.68^{b}	0.21 ± 0.0005^{b}	2.96 ± 0.32^{a}	3.35 ± 0.12^{a}	3.14 ± 0.28^{c}
Ethanol 30	45.5 ± 1.9^{b}	42.1 ± 2.1^{a}	24.3 ± 0.9^{a}	20.5 ± 1.8^{b}	7.0 ± 0.4^{b}	126.2 ± 10.9^{a}	69.8 ± 3.5^{a}	18.4 ± 1.9^{a}	0.19 ± 0.0007^{b}	4.84 ± 0.15^{c}	3.39 ± 0.02^{a}	3.91 ± 0.02^{b}

^{a,b,c}Figures with different letters are significantly different ($P<0.05$ one way ANOVA) per column.

agents also resulted in a significant increase in nematode population in soil ($P<0.05$, ANOVA) as compared to the inoculated and untreated control (Table 1). Water content in roots decreased significantly in the inoculated untreated group as compared to the uninoculated untreated control ($P<0.05$, ANOVA, Table 1). *Santonin* 30c and *Ethanol* 30c reduced water content in roots significantly ($P<0.05$, ANOVA) as compared to the inoculated untreated group. Shoot length, root length and root weight increased significantly in the inoculated untreated group as compared to other groups ($P<0.05$, Table 1). *Ethanol* 30c did not show any significant difference from the inoculated untreated group with respect to nematode infestation parameters (Table 1).

Discussion

At low inoculum level, as in the present experiment, *M. incognita* does not inhibit plant growth and may even stimulate it.[15] This is evident with shoot length and root length (Table 1). *Cina* 30c and *Santonin* 30c showed similar effects with respect to the reduction of nematode-induced symptoms of host plant *Ethanol* 30c, the potentized vehicle control, produced significant changes in shoot length, root weight, leaf-protein and root-protein content and water content of roots (Table 1). Potencies like *Cina* 30c and *Santonin* 30c do not contain any drug molecules derived from their mother tinctures.

The potencies are thought to be specifically structured water bearing a relationship with the molecules of the mother tinctures from which they have been produced, perhaps due to a hydrogen-bond-mediated structure.[16]

The cells of the leaves have cell walls outside the plasma membrane. The cell wall is thick but porous allowing water and small molecules to pass readily through them.

All the cells are interconnected by plasmodesmata which provide small openings between adjacent cells through which electric currents, ions, small molecules and water can pass.[17] Water molecules covering all the cell surfaces maintain their normal structure which, in a diseased state as with nematode infection, may assume a different structure. During foliar spray the potentized drug or a specifically structured water comes in contact with the water covering the cell membrane and brings about a change in the water structure which may influence the passage of water through the aquaporins and also the function of other integral membrane proteins.[16] This might have resulted in the altered water content in the roots of plants treated with *Santonin* 30c. Nematode parasites, which induce formation of giant cells in the root tissues and derive their nourishment from those cells, are likely to face an adverse condition due to change in the water content of cells. This prevented further infection of roots by the nematodes, and as a result there was reduction in root-gall number, root-protein content and number of invading nematodes. An increase in the nematode population of the rhizospheric soil of *Cina*- and *Santonin*-treated plants might be due to the unfavorable condition of the root tissues. Nematode infestation decreased water absorption capacity of plants.[18]

Conclusion

Cina 30c and *Santonin* 30c reduced root-knot disease in experimental infected lady's finger plants. *Santonin* 30c and *Ethanol* 30c reduced water content of roots.

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