

## ORIGINAL PAPER

# Effects of *Cina* on root-knot disease of mulberry

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Root-knot disease of mulberry is caused by the nematode *Meloidogyne incognita*. It has important economic implications for sericulture. The homeopathic medicines, *Cina* mother tincture (MT) and potentised *Cina 200C*, prepared from the flowering meristems of *Artemisia nilagirica* (Clarke) Pamp, were applied by foliar spray on mulberry (*Morus alba* L.) infected with *M. incognita* juveniles (J<sub>2</sub>). Pretreatment (ending 6 days before inoculation) and post-treatment (starting 6 days after inoculation) schedules were tested. The two uninoculated control batches were treated with the same procedure with *Cina MT* and *Cina 200C*.

Both pre- and post-treatment significantly reduced nematode infection in terms of root gall number and nematode population in root. All the treated plants showed improved growth in terms of fresh biomass of shoot and root, length of shoot and root, number of leaves, leaf surface area, root and leaf-protein content. *Cina 200C* is more effective than *Cina MT* in all respects of nematode control as well as growth of the test plants. Pretreatments show slightly better effects than the post-treatments. It is interesting that inoculated and treated plants not only are less affected by nematodes but also have a better growth than uninoculated, untreated control. *Homeopathy* (2006) 95, 98–102.

**Keywords:** *Cina*; *Artemisia nilagirica*; mulberry; *Meloidogyne incognita*; control

## Introduction

Root-knot disease of mulberry, caused by the nematode *Meloidogyne incognita* (Kofoid and White) Chitwood, is very widespread affecting more than 80% of plantations in parts of India.<sup>1</sup> The indiscriminate use of pest control has already created problems for biodiversity conservation in tropical regions.<sup>2</sup> The use of chemical pesticides such as the nematicide Furadane is associated with problems including pollution of the natural environment and reduced palatability of the leaves to the feeding silkworm larvae, leading to reduction in growth of the larvae and silk production.<sup>3</sup>

The crude 90% ethanolic extract of the flowering meristems of *Artemisia nilagirica* (1 mg/ml concentration) and potencies prepared from it are referred to in homeopathy as *Cina*. It has been observed that the use of an extremely low dose of *Cina 200C* and *Cina 1M* in tomato plants reduced root-knot disease.<sup>4</sup> I investi-

gated the effects of *Cina* by foliar spray on root-knot disease caused by *M. incognita* of mulberry plants (*Morus alba* L., cv. S<sub>1</sub>).

## Materials and methods

### Preparation of crude extract

Air-dried and powdered flowering meristems of *A. nilagirica* (Clarke) Pamp were extracted with 90% ethanol at room temperature (25±2°C) for 15 days and were filtered to collect the extract. The ethanol from the extract was removed by evaporation at room temperature (25±2°C). The residue was dried in a dessicator over anhydrous calcium chloride. This crude residue was dissolved in 90% ethanol at 1 mg/ml concentration and formed the ethanolic extract of *A. nilagirica* called *Cina MT* (mother tincture).

### Preparation of potentised drug

*Cina MT* was diluted with 90% ethanol (1:100) in a vial. The vial was filled up to two-thirds, tightly corked and given 10 powerful downward strokes of the arm. This process of mechanical agitation is called

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succussion. This produces the first centesimal potency known in homeopathy as *Cina 1C*. All the subsequent potencies were prepared by further dilution with 90% ethanol in the same proportion (1:100) and given 10 powerful downward strokes.<sup>4,5</sup> *Cina MT* and potentised *Cina 200C*, used in this experiment were purchased from King & Co., Kolkata. *Cina MT* 0.25 ml was diluted with 9.75 ml distilled water and potentised *Cina 200C* 0.50 ml was diluted with 9.50 ml distilled water, giving 10 ml test solution for foliar spray treatment. The control solutions were similarly prepared with 90% ethanol.

#### Mortality test

Four sets of cavity blocks containing 1 ml distilled water containing 50  $J_2$  larvae were taken; two sets were treated as control and other two were treated as treatment sets. To assess the direct effect of *Cina*, the water was removed by pipette and immediately replaced by 1 ml *Cina* (0.25 ml drug in 9.75 ml distilled water) and *Cina 200C* (0.50 ml in 9.50 ml distilled water). Other two control sets received 1 ml of control solutions each (0.25 ml and 0.50 ml with 90% ethanol diluted with 9.75 ml and 9.50 ml distilled water, respectively) and observed at 1 h intervals for 12 h at room temperature ( $25 \pm 2^\circ\text{C}$ ).<sup>6-8</sup>

#### Schedule

After the preparation and denematisation of the soil-filled pots, the following events occurred:

- Day 0: planting of mature mulberry cutting in the denematised soil-filled pots (one cutting/pot).
- Day 15, 19, 23 and 27: four pretreatments (pretreatment groups) with *Cina*, ending 6 days before inoculation with *M. incognita* juveniles ( $J_2$ ).
- Day 34: inoculation of *M. incognita* juveniles ( $J_2$ ).
- Day 41, 45, 49 and 53: four post-treatments (post-treatment groups) with *Cina* and starting 6 days after inoculation of *M. incognita* juveniles ( $J_2$ ).
- Day 57: collection of mulberry leaves for thin layer chromatography (TLC).
- Day 0 to 113: plant growth period.
- Day 114: harvesting of all plants 60 days after the last treatment of inoculated post-treated *Cina* batches.

#### Planting and inoculation

Earthen-ware pots of 22.5 cm diameter and 22.5 cm depth were divided in to eight batches of 20 pots each; each pot contained 12.5 kg of clay soil and compost manure in the proportion of 2:1v/v, and after denematisation of the soil in the pots, they were planted with a mulberry cutting *Morus alba* L. Cv. 'S<sub>1</sub>' cutting (10 in length and 12 g fresh weight—one mulberry cutting/pot) at day 0. Cuttings were provided by the Sericultural Composite Unit, Sriniketan, Birbhum, West Bengal, India. The date of planting is called day-0.

The groups were:

- uninoculated untreated (control),
- uninoculated *Cina MT* treated (control),
- uninoculated *Cina 200C* treated (control),
- inoculated untreated,
- inoculated pretreated *Cina MT*,
- inoculated pretreated *Cina 200C*,
- inoculated post-treated *Cina MT*
- inoculated post-treated *Cina 200C*

The pots were denematised by boiling water five times daily for 3 consecutive days before planting. Five batches of pots were inoculated at day 34 after the planting of mulberry cutting with *M. incognita*  $J_2$  larvae (@ 3955  $\pm$  55 larvae/pot).<sup>6</sup> The remaining three batches served as controls: uninoculated untreated (control), *Cina MT* treated (control) and *Cina 200C* treated (control). The treatments were replicated in three batches in each experiment. Nematode population in soil was monitored 1 month after inoculation, then at 2 weekly intervals. The experiment was done outdoors in the garden of the Department of Zoology, Visva-Bharati University, Santiniketan 731235, West Bengal, India at an ambient temperature of  $30 \pm 4^\circ\text{C}$  and relative humidity  $75 \pm 5\%$  with irrigation and manuring when necessary.

#### Treatment

All treatments were given by foliar spray (@ of 10 ml/plant four times at an interval of 3 days. The drugs (test solution) and control solutions were sprayed on leaves in five instalments at intervals of 2.5 h every treatment day. Treatments were done; either before inoculation with *M. incognita* juveniles (pretreated batches) or without inoculation (uninoculated control batches). All the pretreatments were started at day 15, ending 6 days before inoculation of *M. incognita* at day 27. The treatments of the post-treated batches started 6 days after inoculation of nematodes on day 41.

All the *Cina MT* treated control batches were given as foliar spray with *Cina MT* (@ 10 ml/plant containing 0.25 ml crude ethanolic extract; the *Cina 200C* treated batches plants were given foliar spray with freshly potentised *Cina 200C* (@ 10 ml/plant containing 0.50 ml of *Cina 200C*). The uninoculated untreated (control) and inoculated untreated batches were treated with control solutions containing 0.25 and 0.50 ml 90% ethanol in distilled water, respectively, by foliar spray @ 10 ml/plant. During spraying, the soil surface underneath each plant was covered with polyethylene sheet.

#### Harvesting

Sixty days after the last treatment (d 114) of the post-treated batches, all the plants were uprooted and the following parameters recorded; shoot length and fresh weight, root length and fresh weight, number of leaves/plant, surface area of leaves, number of root

galls/plant, nematode population in root (2 g) and soil (200 g)<sup>7,9</sup> and leaf and root-protein content. For protein estimation, three samples of leaves and roots were taken at random from each batch of plants and the total protein fraction in each sample was estimated by the Folin-Phenol method.<sup>10,11</sup> All the data were analysed by one way analysis of variance (ANOVA).

### Analysis of residues

Mulberry leaves, collected 3 days after the last treatments, were homogenised in a blender and extracted with ethanol. The residue was run in a TLC plate with the standard of the test substance. The test substances were *Cina MT* and *Cina 200C*.

## Results

The effects of treatment with the test solutions *Cina* (*MT* and *200C*) on root-knot nematode infection of mulberry are given in Tables 1 and 2. *Cina* treated plants showed higher protein content in leaves and roots ( $P < 0.01$ , ANOVA). Nematode population and root gall number were significantly lower in the roots of plants treated with both of the test substances (*Cina MT* and *Cina 200C*) than in the untreated plants ( $P < 0.01$ , ANOVA). Nematode populations in the rhizospheric soil in all *Cina*-treated batches were significantly higher than in the untreated groups whilst the population in the roots were significantly lower in the treated groups compared to the untreated groups ( $P < 0.01$ , ANOVA). Length and fresh biomass of all the treated shoot and roots including both *Cina* treated control plants, increased more than that of inoculated untreated and uninoculated control batches ( $P < 0.01$ , ANOVA). Number of leaves and leaf surface area were also significantly higher in all *Cina* treated plants than in both the inoculated untreated and uninoculated control plants ( $P < 0.01$ , ANOVA). Nematode population in all the *Cina* treated batches were significantly higher in rhizospheric soil than in root ( $P < 0.01$ , ANOVA).

*Cina* had no direct effect on nematode mortality within the exposure period of 12h and *Cina* left no toxic residues of the test substance (*Cina MT* and *Cina 200C*) in the treated mulberry plants, collected 3 days after last treatment, by TLC. This experiment was repeated thrice with similar results, all the data are reproduced here (see Tables 1 and 2).

## Discussion

The flowering tops and leaves of *Artemisia* have long been used against human intestinal nematodes.<sup>5,12</sup> Species of *Artemisia* contain various essential oils and sesquiterpene lactones including santonin,<sup>13-16</sup> known to possess nematicidal properties.<sup>17</sup> However, *Cina 200C* is too dilute to contain any drug molecules.<sup>18,19</sup>

Table 1 Growth of mulberry following treatment by foliar spray of *CinaMT* and *Cina 200C*

| Treatment (20 plants/batch)           | Length (cm)     |                | Fresh biomass (g) |                 | No. of leaves/plant | Surface area of leaves (cm <sup>2</sup> ) |                   |                   |
|---------------------------------------|-----------------|----------------|-------------------|-----------------|---------------------|---|-------------------|-------------------|
|                                       | Shoot           | Root           | Shoot             | Root            |                     | Apical                                    | Middle            | Base              |
| 1. Uninoculated (control)             | 136.83 c ± 2.28 | 73.90 d ± 1.23 | 181.16 b ± 1.51   | 169.34 c ± 1.41 | 57.06 c ± 0.47      | 23321 cm ± 194.34                         | 23234 dm ± 193.61 | 18061 dn ± 150.51 |
| 2. <i>Cina MT</i> treated (control)   | 148.33a ± 2.47  | 85.33 b ± 1.42 | 188.00 a ± 1.57   | 181.00 b ± 1.51 | 70.67 a ± 0.59      | 25286 am ± 210.72                         | 24606 cn ± 205.00 | 25135 am ± 209.45 |
| 3. <i>Cina 200C</i> treated (control) | 152.33a ± 2.53  | 94.66 a ± 1.58 | 188.70 a ± 1.56   | 191.67 a ± 1.60 | 75.66 a ± 0.63      | 25966 am ± 216.38                         | 26902 an ± 224.20 | 23762 bo ± 198.00 |
| 4. Inoculated                         | 93.66 d ± 1.56  | 41.70 e ± 0.70 | 91.00 c ± 0.76    | 96.5 d ± 0.80   | 40.65d ± 0.39       | 6647dm ± 55.38                            | 6570 em ± 54.75   | 3578 en ± 29.82   |
| 5. Pretreated <i>Cina MT</i>          | 143.67b ± 2.39  | 83.34 c ± 1.38 | 187.67a ± 3.12    | 175.67 b ± 1.46 | 68.00 b ± 0.56      | 24502 bm ± 201.55                         | 23507 dn ± 195.89 | 23871 bm ± 198.92 |
| 6. Pretreated <i>Cina 200C</i>        | 147.00 a ± 2.45 | 92.34 a ± 1.53 | 189.00 a ± 3.15   | 193.34 a ± 1.58 | 72.34 a ± 0.60      | 24991am ± 208.26                          | 25035 bn ± 208.62 | 23083 bo ± 192.36 |
| 7. Post-treated <i>Cina MT</i>        | 140.66 b ± 2.34 | 80.63 c ± 1.34 | 187.33 a ± 1.56   | 177.33 b ± 0.67 | 66.92 b ± 0.55      | 24185 bm ± 201.55                         | 24419 cm ± 203.61 | 21163 cn ± 176.36 |
| 8. Post-treated <i>Cina 200C</i>      | 141.93 b ± 2.36 | 91.46 a ± 1.52 | 188.33 a ± 1.56   | 188.34 a ± 1.57 | 69.97 a ± 0.58      | 24866 am ± 207.23                         | 24404 cn ± 203.40 | 21823 co ± 181.86 |

Mean and standard error of mean, three experiments, each three batches per treatment. Means carrying same letters are not significantly different by ANOVA.

**Table 2** Effects of *Cina*MT and *Cina* 200C on *Meloidogyne incognita* infection of mulberry plants

| Treatment (20 plants/ batch)          | No. of root galls/ plants | Nematode population |                   |                | Protein content (%) of leaf |                |               |  |
|---------------------------------------|---------------------------|---------------------|-------------------|----------------|-----------------------------|----------------|---------------|--|
|                                       |                           | Root (2g)           | Soil (200g)       | Apical         | Middle                      | Base           | Root          |  |
| 1. Uninoculated (control)             | NIL                       | NIL                 | NIL               | 7.82 dm ± 0.03 | 8.98 bn ± 0.04              | 8.56 do ± 0.03 | 8.03 c ± 0.03 |  |
| 2. <i>Cina</i> MT treated (control)   | NIL                       | NIL                 | NIL               | 8.27 am ± 0.03 | 8.96 bn ± 0.03              | 8.98 an ± 0.03 | 8.98 b ± 0.04 |  |
| 3. <i>Cina</i> 200C treated (control) | NIL                       | NIL                 | NIL               | 8.35 am ± 0.03 | 9.19 an ± 0.04              | 9.00 ao ± 0.03 | 9.22 a ± 0.04 |  |
| 4. Inoculated (untreated)             | 922.47 a ± 15.38          | 1154.00 am ± 0.05   | 125.00 dn ± 4.16  | 2.85 em ± 0.01 | 3.00 en ± 0.01              | 3.01 en ± 0.01 | 4.31 d ± 0.02 |  |
| 5. Pretreated <i>Cina</i> MT          | 18.67 c ± 0.31            | 41.66 dm ± 0.69     | 179.00 cn ± 8.98  | 8.06 cm ± 0.03 | 8.99 bn ± 0.03              | 8.89 bo ± 0.04 | 8.94 b ± 0.04 |  |
| 6. Pretreated <i>Cina</i> 200C        | 9.00 d ± 0.45             | 29.00 cm ± 0.48     | 184.00 cn ± 9.23  | 8.17 bm ± 0.03 | 9.13 an ± 0.03              | 8.76 co ± 0.03 | 9.19 a ± 0.04 |  |
| 7. Post-treated <i>Cina</i> MT        | 28.29 b ± 0.47            | 85.00 bm ± 1.43     | 373.00 bn ± 12.45 | 7.91 cm ± 0.03 | 8.96 bn ± 0.05              | 8.86 bo ± 0.03 | 8.88 b ± 0.04 |  |
| 8. Post-treated <i>Cina</i> 200C      | 16.20 c ± 0.27            | 51.00 cm ± 0.85     | 399.00 an ± 13.12 | 7.97 cm ± 0.03 | 9.12 an ± 0.04              | 8.64 do ± 0.03 | 9.10 a ± 0.04 |  |

Mean of three experiments, each of three batches, with standard error of mean. Means carrying same letters are not significantly different by ANOVA.

It is surprising that inoculated and treated plants not only are less affected by nematodes but also have a better growth in all respects than uninoculated controls. It is also interesting that *Cina* treatment effects were more pronounced with *Cina* 200C than with *Cina* MT. Pretreatment was generally more effective than post-treatment. The effective compounds did not effect the nematodes directly, and mulberry leaves did not contain any residues of the test substance. *Cina* MT and *Cina* 200C not only reduced root-knot disease significantly but also improved the nutritive value of leaves.

This study shows that *Cina* homoeopathic treatments stimulate resistance against nematode-pathogen and that the pretreatments are more effective than the post-treatments. Large numbers of *M. incognita* larvae were present in the rhizospheric soil in all *Cina* treated plants. It may be that *Cina* can induce synthesis of antagonistic substances in the treated plants.<sup>20, 26</sup> *M. incognita* is known to share common antigens with its host plants.<sup>27</sup> It appears that during natural infection with the nematode, the host plant shows minimal defence responses to the nematodes because of this antigenic similarity. *Cina* MT and *Cina* 200C may induce natural defense response in plants against nematode parasites.

## Conclusions

The potentised homoeopathic drug *Cina* at an ultra high dilution is effective against root-knot disease and improves the growth of host plants. The higher nutritive value of the treated plants contributes to higher growth of larvae and silk production. These results suggest that plant diseases may be effectively controlled by the potentised drug without disturbing the biosphere and we can conserve our biodiversity with cost effective potentised homoeopathic medicines. Further research should be conducted.

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