

Effect of Lac Based Chemicals on Inhibition of Egg Hatching of Root-Knot Nematode (*Meloidogyne incognita*)

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Efficacy of lac based chemicals on egg hatching of root-knot nematode (*Meloidogyne incognita*) under *in vitro* condition is reported. Two lac based chemicals viz. 9-hydroxy Δ^2 -nonenoic acid and its methyl ester when evaluated against juveniles (J_2) of *M. incognita* at different concentrations [T_1 (1000ppm), T_2 (500ppm), T_3 (250ppm), T_4 (125ppm) and T_5 (62.5ppm)] showed marked suppression over control. There was a significant difference among the concentrations tested. Highest inhibition of egg hatching (100%) was recorded in T_1 with both the chemicals, while other concentrations showed decrease in percentage inhibition of egg hatch with time.

Key words: Lac, root-knot nematode

Root-knot nematodes (*Meloidogyne* sp.) enter roots as juveniles, select a feeding site of three to eight cells, and swell up in their chosen spot as they progress towards adulthood. They introduce hormone-like substances into the plant cells, causing them to swell in the area producing galls, or root-knots.

Males retain their slender profiles and leave the root at adulthood, but the fattened adult females remain inside. They lay eggs into the soil, and after the juveniles hatch, the cycle begins again. Roots infested with root-knot nematodes usually have visible galls and may exhibit excessive branching. Parasitized plants may be weak and stunted. Root systems get deformed, and underground parts such as potato tubers and carrot tap roots also get damaged and unmarketable⁵.

The root-knot nematodes are sedentary endo-parasites of underground plant parts. The eggs are retained in a gelatinous matrix, which normally protrudes out of the host tissues. About 200 to 300 oval eggs are found in a single egg mass which makes its size larger than the female body.

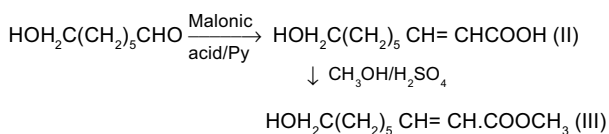
The life cycle starts from the egg usually in the one-celled stage deposited by the female. The embryo and the first stage larvae move within the egg but are not very active. The emerging second stage larvae are found free in the soil. They attack new host root tissue in the region behind the root tip (meristamatic zone).

MATERIALS AND METHODS

Two lac based chemicals viz 9-hydroxy Δ^2 -nonenoic acid (synthesized from aleuritic acid, a major component of lac) and its methyl ester were evaluated against juveniles (J_2) of *Meloidogyne incognita*. 9-hydroxy Δ^2 -nonenoic acid was synthesized from aleuritic acid adopting simple reaction sequence as under:

7-Hydroxy heptanal (3g), one of the periodate oxidation products of aleuritic acid, when heated with malonic acid (3g) in the presence of dry pyridine (5 ml) on steam bath for 4 hrs, resulted in 2.8 g of an unsaturated hydroxyl acid, 9-hydroxy Δ^2 -nonenoic acid (II) [$\text{HOH}_2\text{C}(\text{CH}_2)_5 \text{CH}=\text{CHCOOH}$], IR(cm^{-1}): 3250 (OH), 1700 (C=O), 970 (trans C=C) MS (m/z 0 172 (M+); M.F.- $\text{C}_9\text{H}_{16}\text{O}_3$

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Scheme

Methyl 9-hydroxy nonenoate (III) (4.5 g) was synthesized from 9-hydroxy Δ^2 -nonenoic acid (1.0 g) by its methylation with 10 ml CH_3OH and 2-3 drops of H_2SO_4 . The data for $\text{HOH}_2\text{C}(\text{CH}_2)_5\text{CH}=\text{CH.COOCH}_3$ was: IR (cm^{-1}): 3300 (OH), 1730 (C=O), 970 (trans C=C), MS (m/z 0 186 (M+); M.F.- $\text{C}_{10}\text{H}_{18}\text{O}_3$).

The presence of long chain (C 16) in addition to the adjacent hydroxyl groups at 9, 10 position makes this compound a potential source to synthesize bio-nematicides and their analogue. Since the nematicidal activity is generally shown by long chain fatty acids, an attempt has been made to synthesize potential nematicides from lac resin³. Per cent egg inhibition of hatching of (J_2) root knot nematode (*Meloidogyne incognita*) was recorded after 24, 48 and 72 hrs of exposure. For laboratory screening, stock solution of 2000 ppm of each compound (I) 9-hydroxy Δ^2 -nonenoic

acid (HNA) and its methyl ester [chemical-II (MENA)] were prepared in diethyl ether (1:1), two drops of Tween 80 were added as emulsifier and the volume was made up to 25 ml with the distilled water.

Five concentrations each of the two compounds were prepared from stock solution. Five ml of each test concentration of 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 62.5 ppm were added over 5 ml egg/egg mass suspension containing about 200 egg/egg mass of *Meloidogyne incognita* placed in sterile 5 cm diameter Petri dishes for hatching studies. The treatment was replicated thrice and incubated at 30+ 1°C. A control containing only diethyl ether and Tween 80 was similarly maintained. After the desired period of incubation, number of juveniles hatched out in each Petridish was counted with the aid of a stereoscopic binocular microscope at every 24 h interval, upto 7 days. Percentage mortality was calculated from the average of three replications.

RESULTS AND DISCUSSION

The *in vitro* study for inhibition of hatching of *M. incognita* with two lac based chemicals at different concentrations

Table 1. Effect of Lac based chemicals on per cent inhibition of egg hatching of root-knot nematode (*Meloidogyne incognita*) at different concentrations

Treatment	% Mortality at							Treatment Mean
	1 DAY	2 DAYS	3DAYS	4DAYS	5DAYS	6DAYS	7DAYS	
Compound-I								
T ₁	100	100	100	100	100	100	100	100.00
T ₂	100	98.8	97.1	95.8	94.2	93.7	93.2	96.11
T ₃	100	96.2	95.6	93.1	91.7	90.3	89.1	93.71
T ₄	100	94.1	92.4	91.0	89.4	86.1	84.2	91.02
T ₅	100	91.5	90.1	88.4	85.1	82.3	79.2	88.08
CD at 5% =6.1862							GRAND MEAN	93.78
T0	95.1	81.2	64.4	37.2	20.3	14.4	8.9	45.93
Compound-II								
T ₁	100	100	100	100	100	100	100	100.00
T ₂	100	99.6	99.0	98.6	98.1	97.7	97.2	98.06
T ₃	100	99.0	98.5	97.2	96.6	96.1	96.0	97.62
T ₄	100	97.8	97.3	96.1	95.8	95.5	95.3	96.82
T ₅	100	95.8	95.4	95.0	94.7	94.1	93.8	95.54
CD at 5% = 3.6874							GRAND MEAN	97.71
T0	94.1	80	62.5	34.4	19.6	13.2	8.1	44.56

Compound-I = Lac based chemical-I {9-hydroxy Δ^2 -nonenoic acid (HNA)}

Compound-II = Lac based chemical-II {methyl ester of 9-hydroxy Δ^2 -nonenoic acid (MENA)}

T₁ = 1000ppm, T₂ = 500ppm, T₃ = 250ppm, T₄ = 125ppm, T₅ = 62.5ppm, T₀ = Control

(T₁-T₅) caused marked suppression when compared with control. There was a significant difference among all the concentration tested.

Compound-I: Treatment 1 (T₁) showed the highest inhibition of egg hatching to the tune of 100% which was maintained with as the increase in number of days (1-7) while other concentrations showed decreasing trend in percentage inhibition of egg hatch as the number of days increased (Table 1). At the first day, there was no hatching recorded among all the treated chemical concentration while in control 5% eggs hatched out. Mean inhibition of egg hatching after 7 days of exposure recorded as T₂ (96.11%), T3 (93.71%), T4 (91.02%), T5 (88.08) while in control, it was 45.93%.

Compound chemical-II: Treatment 1 (T₁) also showed the highest inhibition of egg hatching to the tune of 100%. (Table 1). Mean inhibition of egg hatching after 7 days of exposure recorded as T₂ (98.06%), T3 (97.62%), T4 (96.8%), T5 (95.54) while in control, it was 44.56%. Observation on treatment mean reveals that Compound-II showed apparently higher inhibition of egg hatching as compared to Compound-I. As the number of days increased the comparative inhibition of egg hatch increased. It was also observed that inhibition of egg hatching increased as the concentration of chemicals increased.

Sarvanapriya et al.¹ also screened out the nematicidal properties of fifteen plant products against root-knot

nematode (*Meloidogyne incognita*) for egg hatching. Among all, latex of *Carica papaya* caused 98.2 % and 100% inhibition of hatching at 0.1 and 10.0% concentrations respectively. Srivastava et al.⁴ also supported these findings with the results on nematicidal activity of two lac based chemicals i.e, 16-OH-(E)-9-hexa decanoate(I) and methyl undecanoate(II) against the 2nd stage juvenile of root knot nematode (*Meloidogyne incognita*) infesting brinjal which were tested *in vitro* at 1000ppm, 500ppm, 250ppm, 125ppm and 62.5ppm.

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