NEMATICIDAL ACTIVITIES IN ARTEMISININ ANALOGUES[1]

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Summary. Different artemisinin analogues, viz. α and β-artemether, β-artepropylether, β-artebutylether, β-artebenzylether, artesunic acid, artepropionate, artechloroacetate and artebenzoate, were tested at the rate of 1000 ppm for their nematicidal and hatching inhibitory activities against *Meloidogyne incognita*. The maximum nematicidal activity was recorded in artechloroacetate, followed by β-artepropylether, α and β-artemether, β-artebutylether, β-artebenzylether, and the least in artepropionate and artesunic acid. Maximum egg hatching inhibitory activity was recorded in β-artepropylether followed by other analogues. This is the first experimental demonstration of the nematicidal effects of these compounds.

Nematodes are economically important plant pathogens. The root knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. is one of the most damaging pests of world agricultural crops and responsible for more than $100 billion of crop loss every year. Although various chemical nematicides have a potential for the management of this noxious pest, these chemicals lead to contamination of terrestrial and aquatic environments, cause harm to beneficial organisms, and are very highly expensive. These concerns have accelerated the search for new, plant-based, environmentally and toxicologically safe compounds that are inhibitory to nematodes and a considerable amount of work has been done (Allen and Feldmesser, 1970; Miller, 1979; Scramin *et al.*, 1987; Ghosh and Sukul, 1992; Sasanelli, 1992; Jatala *et al.*, 1995; Basu and Majumdar, 1998; Chitwood, 2002; Pandey *et al.*, 2000, 2001; Williams *et al.*, 2003).

There is great scope to isolate, characterise and identify compounds that are nematicidal/nematostatic in nature. In order to search for new nematicidal compounds, we screened different analogues of artemisinin for their *in vitro* effects on hatching and mortality of the root-knot nematode, *M. incognita*.

MATERIALS AND METHODS

The dried aerial parts of *Artemisia annua* L. were collected from the experimental farms of the Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The dried plant material (50 kg) was processed in a pilot plant at the Institute. Plant material was extracted with n-hexane and concentrated under vacuum pressure, and the concentrated extract (4 kg) was subjected to column chromatography over silica gel by eluting with hexane. The 5th, 10th and 15th fractions were rich in artemisinic acid and artemisinin (Tandon *et al.*, 2003). All these fractions, on crystallization, gave pure crystalline compounds, which were characterized by m.p., mmp, IR, NMR and MS. Later, the artemisinin was reduced to dihydroxy-artemisinin by chemical reaction with sodium borohydride. The dihydroxy-artemisinin was dissolved in aprotic solvent such as tetra hydrofuran, and, by adding methanol, α- and β-artemether were obtained. Similarly, other artemisinin analogues were obtained by the addition of isopropanoal, benzyl ether and benzyl alcohol. Further compounds were obtained and other artemisinin analogues were also prepared by dissolving dihydroxy-artemisinin in pyridine and benzoylchloride. All derivatives were purified in a chromatography columns and characterized by 1H(NMR), 13C(NMR) and FAB-MS.

Egg masses of *M. incognita* were obtained from stock culture maintained on tomato in a glasshouse. These egg masses were kept on paper tissues supported on wire gauge in a funnel containing water at 25 °C. Stock solutions of 2,000 ppm of the test analogues (α- and β-artemether, β-artepropylether, β-artebutylether, β-artebenzylether, artesunic acid, artepropionate, artechloroacetate and artebenzoate) were prepared by dissolving the different artemisinin analogues in 0.5 ml dimethyl sulphoxide (DMSO) or acetone, made up to 5 ml with distilled water. Then, 5 ml of nematode suspension, containing around 300 second stage juveniles, were placed in a vial and 5 ml of the stock solution (concentration of 2,000 ppm) were added to obtain a final concentrations of 1,000 ppm. The loosely capped vials were stored at 25 °C. Vials containing 0.5 ml DMSO/acetone and 9.5 ml distilled water with nematodes served as controls. After a 24-hour incubation period the nematodes were transferred to tap water and kept overnight

[1] Work done in CIMAP, Lucknow, India.

at 25 °C. If nematode juveniles did not resume motility they were assumed to be dead. There were five replicates for each treatment. The number of dead and surviving juveniles was counted after 24 hours and the mean percent mortality was calculated.

The efficacy of these extracts on hatching of *M. incognita* egg masses was also determined by adding five fresh egg masses of uniform size into each vial. The vials were incubated at 25 °C and the total numbers of emerged juveniles in each vial were counted after 120 hours. Each experiment was replicated five times and repeated twice for reconfirmation of the data.

RESULTS AND DISCUSSION

The results presented in Tables I and II indicate that different analogues of artemisinin possess strong to mild nematicidal and nematode hatching inhibitory activities. The maximum nematicidal activity (Tables I and II) was recorded for artechloroacetate (93.3%), followed by β-artepropylether (92.7%), α-artemether (91.4%), β-artebutylether (77%), and β-artebenzylether (74.1%). The least nematicidal activity was recorded in artepropionate (11.6%) and artesunic acid (18.9%). Nematode hatching inhibitory activity was greatest in β-artepropylether and least in β-artebenzylether (Table II). These results are the first experimental demonstration of the nematicidal effects of these compounds and thus may be useful in the further development and understanding of plant-based nematicides.

Similar results have been obtained earlier with extracts or oils obtained from leaves and roots of various plant species at similar and different concentrations (Allen and Feldmesser, 1970; Miller, 1979; Ghosh and Sukul, 1992; Jatala *et al.*, 1995; Basu and Majumdar, 1998; Pandey *et al.*, 2000, 2001) against *M. incognita* and *Panagrellus redivivus*. There is, however, a need to semi-synthesize and develop artemisinin analogues, which could be exploited as potent commercial nematicides especially in the absence of any effective synthetic nematicides in the world market. The present findings help in the search for plant-based, environmentally safe nematicides and provide clues for the synthesis of analogues based on plant compounds that will provide effective plant protection and higher crop yields.

Table I. Efficacy of artemisinin analogues (soluble in acetone) on mortality and hatching of *Meloidogyne incognita* at 1,000 ppm.

Treatment	% juvenile mortality (after 24 hrs.)	Total hatched eggs (after 120 hrs.)
Water-Acetone control	2.0	141
Artemether (α-AME)	91.4	26 (81.6)[x]
Artemether (β-AME)	87.8	17 (87.9)
Artesunic acid (ASA)	18.9	47 (66.7)
C.D. (P = 0.05)	4.017	7.132

Each value is the average of five replicates
[x] The % reduction in hatching as compared to the control is in parentheses

Table II. Efficacy of artemisinin analogues (soluble in DMSO) on juvenile mortality and egg hatching of *M. incognita* at 1,000 ppm.

Treatment	% juvenile mortality (after 24 hrs.)	Total hatched eggs (after 120 hrs.)
Water- DMSO control	3.0	147
β-Artepropylether (β-ARP)	92.7	12 (91.8)[x]
Artechloroacetate (ACA)	93.3	21 (86.7)
β-Artebenzylether (β-ARBZ)	74.1	64 (56.5)
β-Artebutylether (β-ARBU)	77.0	43 (68.7)
Artepropionate (ARPO)	11.6	35 (76.2)
C.D. (P = 0.05)	3.141	5.807

Each value is an average of five replicates
[x] The % reduction in hatching as compared to the control is in parentheses

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