Fundam. appl. Nematol., 1994, **17** (2), 185-192

Short notes

NEMATICIDAL ACTIVITY OF SERPENTINE AGAINST *MELOIDOGYNE INCOGNITA* [1]

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Accepted for publication 30 July 1993.

Key-words : Nematodes, nematicide, serpentine, *Meloidogyne incognita.*

Catharanthus roseus G. Don (Apocyanaceae), a medicinal plant, is a source of several indole alkaloids. The aqueous extracts of leaf, stem, flowers and root of *C. roseus* were reported to inhibit hatching of eggs of *Meloidogyne incognita* Chitwood (Patel *et al.,* 1987 *a*). Also the use of dried leaves of *C. roseus* mixed with soil was found to reduce root knot nematode infestation of okra (Patel *et al.,* 1987 *b*). The active nematicidal principle has not been identified so far. This paper presents the nematicidal activity of serpentine, a major component of the root alkaloid of *C. roseus* against *M. incognita.*

Materials and methods

ISOLATION OF SERPENTINE

Root material was collected from six-to eight-month-old plants of *C. roseus.* They were washed with distilled water and oven dried at 60 °C. The powdered root was extracted with hot methanol three to four times. The methanol layer was collected, concentrated by drying, extracted with water and filtered. The aqueous extract was brought to pH 9.0 and precipitated alkaloids were filtered out. The extract was lyophyllized and chromatographed on acidic alumina (35 g) and eluted with chloroform : methanol (95:5). The fractions were monitored by UV fluorescence and those which showed blue fluorescence were combined. This fraction was concentrated, dissolved in absolute ethanol and allowed to crystallize. The crystalline compound was air dried. Its melting point and UV spectrum were recorded and compared with those of standard serpentine. Its identification was further confirmed by thin layer chromatography on silicagel G (0.25 nm) with authentic serpentine using solvent systems (a) chloroform-methanol (5:1) and (b) chloroform-methanol-ethyl acetate-acetone (10:8:4:1).

IN-VITRO BIOASSAY

Bioassay was carried out by using an immersion test (Kogiso *et al.,* 1976). Freshly hatched and surface sterilized second stage juveniles of *M. incognita* at a concentration of 200 nematodes/ml was used. One ml of serpentine solution at different concentrations in 0.3 % triton X-100 was transferred to 50 mm diameter Petri dishes followed by addition of 1 ml of water. To this solution, nematode suspension (1 ml) and three drops of 0.25 % streptocycline (to avoid fungal growth) were added. Final concentrations of serpentine were 1 %, 0.5 %, 0.35 % and 0.2 %. The trials were replicated five times and the samples were incubated for 48 h at 25 ± 1 °C along with controls consisting of 1 ml of 0.3 % triton X-100, 1 ml of distilled water, 1 ml of nematode solution and three drops of streptocycline. After 48 h nematicidal activity was ascertained by taking observations on the number of nematodes dead. The mortality was further confirmed, when the juveniles failed to revive after subsequent transfer to water.

EFFECT OF SERPENTINE ON HATCHING OF *M. INCOGNITA*

Three egg masses of *M. incognita* collected from tomato roots were suspended in 3 ml of 0.2 % and 0.5 % aqueous solution of serpentine in three replications and incubated up to 5 days at ambient temperature along with control consisting of egg masses suspended in 3 ml of distilled water. Observation on the number of hatched larvae in each treatment were recorded under a microscope.

Results and discussion

Serpentine caused 10, 25, 40 and 100 % mortality of *M. incognita* juveniles after 48 h with increasing concentrations of serpentine (Table 1). Serpentine is a β-carboline quaternary indole alkaloidal base and is reported to be the major root alkaloid of *C. roseus* (Shimizu & Uchimara, 1958). Serpentine at 0.2 and 0.5 % concentrations showed 16 and 37 % inhibition of larval hatching after 5 days as compared to control (Table 2). However, 77 and 84 % of the larvae that emerged in 0.2 and

(1) Contribution No. 38/93 IIHR.