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ISOLATION OF ANTINEMATODE PROHIBITINS FROM *CASSIA OCCIDENTALIS* AND THEIR EFFECT ON HATCHING AND MORTALITY OF *MELOIDOGYNE INCOGNITA* (KOFOID & WHITE) CHITWOOD*

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Both aqueous and ethyl acetate extracts of roots, leaves and pods of *Cassia Occidentalis* showed nematicidal and hatch inhibitory effect on *Meloidogyne incognita*. Aqueous extracts were more nematicidal and hatch inhibitory than ethyl acetate extracts. Ethyl acetate extracts of roots showed the presence of 4 bands on TLC plates, two of which have been tentatively identified as Coumestan (Rf. value=0.07 with peak at 267 nm) and Flavone (Rf value=0.59 with peak at 260 nm).

Keywords : Antinematode prohibitins; *Cassia occidentalis;* Hatching; Mortality; *Meloidogyne;* Coumestan, Flavone.

Many plant species contain or secrete compounds of various nature which have nematicidal or nematostatic properties. In many cases, they inhibit nematode penetration and disease development indicating specificity of their action in certain instances (Gommers, 1973; Husain and Masood 1975 a, b; Husain *et al*; 1984). This paper deals with the isolation of nematode inactivating agents from *Cassia occidentalis* and their effect on mortality and hatching of root-knot nematodes.

The roots, leaves and pods of *Cassia occidentalis* were collected and their aqueous extracts prepared at 4°C by comminuting 1 g plant material in 10 ml of cold, distilled and sterilized water in a blender. The slurry thus obtained was squeezed through several layers of cheese cloth and the filtrate centrifuged at 10,000

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g for 10 minutes. The pellet was discarded and the supernatant (aqueous extract) used in experiments on the hatching and mortality of *Meloidogyne incognita* (Kofoid and White) Chitwood.

Ethyl acetate extracts (EAE) of roots, leaves and pods were also prepared by mixing equal amounts of the aqueous extract (AE) and ethyl acetate in a separatory funnel. Both the aqueous and ethyl acetate phases were separated. The aqueous phase was re-activated with an equal amount of ethyl acetate, the aqueous phase was then discarded and the organic portions combined. The ethyl acetate extract was evaporated in 40°C in a rotary evaporator. The residue was dissolved in 5 ml distilled water for use in the experiments.

Chromatographic separation of the chemical compounds from the ethyl acetate extracts was attempted using chloroform (95); Methanol (5) as a solvant system. The spots so obtained were detected under UV lamp some of which were flourescent but others not. Rf values were determined and the compounds identified on the basis of Rf values and their colour under UV light, wherever possible.

Five ml AE or EAE of individual plant part was separately poured in 40 mm diameter sterilized petri dishes into which 5 egg masses of nearly the same age and size were transferred for determining juvenile hatching. Egg masses were similarly transferred to the sterilized petri dishes containing distilled water to serve as control. Each treatment was replicated three times. The number of juveniles hatched in each petri dish was counted after 24 hours and the mean juvenile hatch for each treatment was calculated.

For mortality determinations *Meloidogyne* juveniles were obtained in a similar manner by allowing larger number of egg masses to hatch out distilled water. The juveniles were collected and standardized to contain approximately 100 per ml. One ml of this standardized nematode suspension was poured over 1 cm diam, 30 hm pore size sieve. The sieve was later inverted over sterilized cavity-block and the nematodes were washed down with 2 ml of active agent from the original aqueous extract or ethyl acetate fraction of each plant material separately. Nematodes were similarly transferred to cavity blocks containing distilled water to serve as control. The numbers of dead (immobile) and leaving nematodes were counted after 24 hours and per cent mortality was calculated.

The death of nematodes was asertained by giving 2-3 pushes to the apparently immobile nematodes

with a bamboo splinter and later transferring them to sterilized distilled water for an hour and again checking their mobility under the microscope. Nematodes which showed no movement were considered dead.

Both aqueous (AE) and ethyl acetate extracts (EAE) of roots, leaves and pods were significantly inhibitory to the juvenile emergence of *M. incognita*. Egg hatching was completely suppressed in the aqueous extracts whereas in the ethyl acetate extracts there was 56, 52 and 25% suppression respectively in extracts of pods, leaves and roots.

As were more nematicidal than EAE. There was 100 per cent mortality within 24 hours in AE of all three plant part extracts as compared to 47, 60 and 70% of root, leaf and pod respectively. The toxicity of EAE of pods was distinctly more than EAE of leaf or root.

Four separate bands with Rf values 0.07, 0.47, 0 72 and 0.59 were detected in the EAE of roots on thin layer chromatography (TLC) plates. The compounds with Rf values 0.07 and 0.59 have been tentatively identified as coumestans (with a peak at 267 nm) and Flavon (peak at 260 nm) on the basis of their UV spectral maxima. These groups of chemicals are reported toxic to many different types of parasitic organisms including nematodes. They are known to occur naturally in many different plant species.

Greater efficiency of aqueous extracts suggests greater water solubility of the antinematode prohibitins present in *C. occidentalis*. Significantly variable antinematode effect of extracts of different parts of the plant can be attributed to the variation in concentration and the nature and type of prohibitins present in different plant organs (Mahadevan, 1982).

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