BENZYL ISOTHIOCYANATE FROM *CARICA PAPAYA* SEEDS - A POTENTIAL NEMATICIDE AGAINST *MELOIDOGYNE INCOGNITA*

M. Nagesh*, M.V. Chandravadana, V.G. Sreeja and C.S. Bujji Babu

Division of Entomology and Nematology, Indian Institute of Horticultural Research Hessaraghatta Lake (P.O.), Bangalore - 560 089, India

Summary. Steam distilled oil from fresh/dry seeds of *Carica papaya* elicited nematicidal property *in vitro* against *Caenorhabditis elegans* and *Meloidogyne incognita*. Bioassay directed fractionation and GC-MS, IR and UV spectral data led to the identification of benzyl isothiocyanate (BITC) as the compound responsible for the activity. A total mortality at 25 ppm was evident within 150 and 210 minutes of exposure to BITC towards C. *elegans* and *M incognita,* respectively. BITC showed higher nematicidal activity *in vitro* than carbofuran (a commercially used nematicide).

The seeds of *Carica papaya* (Caricaceae) are used in India as an anthelmentic for children. Recently there has been a growing interest in using plant constituents for the environmentally acceptable nematodes. Plant-borne nematicides have been the subject of several investigations (Gommers, 1981). In the course of screening of plants for nematicidal products against *Meloidogyne incognita,* the extract of seeds of C. *papaya* was found to be active. This paper reports bioassay directed isolation of the active compound from *C. papaya* seeds using *Caenorhabditis elegans* as a test organism. Nematode toxicity *in vitro* was then studied on a plant parasitic nematode, *Meloidogyne incognita.*

MATERIALS AND METHODS

Fresh seeds of *Carica papaya* L. were collected from ripe fruits, washed free from pulp material, shade dried and ground to a fine powder. Dry seed powder (100 g) was soaked in petroleum ether (hexane fraction) at 27 ± 1°C overnight, extracted repeatedly and the solution concentrated *in vacuo.* The defatted seed powder was further extracted with 80% MeOH at ambient temperature and the MeOH solution concentrated under *vacuo.*

Fresh seeds were also homogenized with distilled water in a waring blender and hydro-distilled for 2 to 3 hrs. The aqueous fraction was extracted with 98% Et_2O, dried over anhydrous Na_2S0_4 powder and evaporated to leave a yellow oil. Dry seed powder was also hydro-distilled and extracted in the same way.

Second stage juveniles of *Meloidogyne incognita* (Kofoid *et* White) Chitw. were collected from naturally infected tomato plants. They were induced to hatch from eggs placed on filters in contact with sterile distilled water. A pure culture of *Caenorhabditis elegans* Oshe, was obtained from the Department of Biology, Mcgill University, Canada and maintained 12 °C on a lawn of *Escherichia coli* Migula, grown on an NGM medium (Sulton and Hodgkin, 1988). After the nematodes had multiplied to a considerable extent they were extracted with cold phosphate buffer 0.025 M (pH 6.0) containing 0.001 M $CaCl_2$, 0.001 M $MgSO_4$ and 0.3% NaCl and the suspension inverted over tissue paper to enable the live nematodes to migrate. Nematodes were collected by centrifugation and washed with cold water and suspended in distilled water to obtain 300 nematodes/ml.

The *in vitro* bioassay was carried out by using an immersion test (Kogiso *et al.*, 1976). The selected amount of test material was suspended in 1.5 ml of 0.1% Triton X-100 and placed in a 5 cm Petri dish together with 1.5 ml nematode suspension containing 100 ppm streptocycline. All treatments had five replications. The Petri dishes were incubated at 27 ± 1 °C for *M. incognita* and 22 °C for *C. elegans.* Observed by light microscopy, a nematode was considered dead if it was immobile, straight, did not react to stimuli caused by pricking with a microscopic needle and failed to regain mobility when placed in water for 2 hrs. A further check was failure of the nematodes to migrate in water when inverted over tissue paper. A suitable control was maintained in all experiments in which 1.5 ml of Triton-X was used instead of test solution, The nematicidal activity was expressed as per cent mortality:

$$\text{Mortality \%} = \frac{\text{Number of dead nematodes}}{\text{Total number of nematodes}} \times 100$$

Steam distilled oil from fresh/dry seed was fractionated by column chromatograph over silica gel using 5% EtOAC in petroleum ether followed by preparative

*Corresponding author, Project Directorate of Biological Control, Post Bag No. 2491, H. A. Farm P. O., Bellary Road, Bangalore 560 024, India. E-mail: nagesh55@yahoo.com; pdblc@kar.nic.in

TLC (silica gel 0.5 mm) in hexane: MeOH (99:1). The compounds were detected by exposing the reference chromatogram to iodine vapours. The corresponding bands from the other set was extracted using a mixture of MeOH:Et_20 at 1:1. The active compound was analyzed by GC on PE-Wax and PE-l capillary columns programmed at 60 °C for 4 min, 60 °C to 200 °C at 5 °C min-l, N_2 at 10 psi, detector-FID at 250 °C, injection temperature, 200 °C, split ratio 1:100, injection volume 0.2 to 0.5 µl. Its UV.IR and GCMS data was recorded. It appeared as a pale ye11ow oi1 with UV max -255 nm, GCMS (70 ev) M/Z 149 (m+), 91 (base peak), and FTIR V^{kor}_{max} $^{cm-l}$, 3032, 2175, 2093, 1508, 1497, 1455, 1437, 1348, 1300, 1200, 1028, 726, 700, 575, 450.

RESULTS AND DISCUSSION

Nematicidal activity of methanol and petroleum ether extracts and steam distilled oil from fresh and dry seeds of C. *papaya* was assessed at different concentrations ranging from 50 to 3000 ppm by *in vitro* bioassay using an immersion test (Table I). Methanol extracts of the seeds were found to display higher nematicidal activity than petroleum ether extract but the activity of steam distilled oil from both fresh and dry seeds was by far the highest. Steam distilled oil was 100% lethal to *C. elegans* at 50 ppm when the mortality was recorded after 24 hrs as against a mortality of 5.2% in untreated control. The steam distilled oil constituted 0.2 to 0.3% of the dry seeds. Bioassay guided

fractionation of this oil by column chromatography and preparative TLC led to the isolation of a compound which revealed a single peak on GC (both on polar and nonpolar capillary columns) with an Rt (retention time) of 29.4 and 17.5 min., respectively. The compound was a pale yellow liquid with a pungent, characteristic odour and was identified as benzyl isothiocyanate (BITC) based on its GC-MS, UV and IR spectral data in comparison with literature data (Spencer and Daxenbichler, 1980). Benzyl isothiocyanate has been reported to be a normal constituent of papaya seeds and fruits (Tang, 1971; Tang and Syed, 1972).

The activity of BITC isolated from seeds of *C. papaya* at different concentrations (5 to 50 ppm) after 24 hrs towards juveniles of *C. elegans* and *M. incognita* is presented in Table II; its activity was also compared with carbofuran (25 and 50 ppm), a commercially used synthetic nematicide. Results revealed that the extent of mortality varied with the concentration of BITC and 25 ppm was completely lethal to both species of nematodes. Its LD 50 value at 24 hrs was estimated to be 17 ppm from these data. In comparison, carbofuran at 25 ppm elicited low mortality. However, at 50 ppm it caused 100% and 75% mortality of *M. incognita* and *C. elegans* juveniles, respectively. It is evident from the above results that BITC is a more potent nematicide *in vitro* than carbofuran, against both nematode species.

Table I. Nematicidal activity of different extracts of seeds of *Carica papaya* against *Caenorhabitis elegans* after 24 hrs.

Sample tested		Mortality % (X ± SE)
Methanol extract		
i)	100 ppm	20 ± 1.5
ii)	200 ppm	80 ± 3.7
iii)	400 ppm	100
iv)	1000 ppm	100
Petroleum ether extract		
i)	1000 ppm	0
ii)	2000 ppm	47 ± 3.63
iii)	3000 ppm	100
Steam distilled oil from fresh/dry seeds		
i)	50 ppm	100
ii)	100 ppm	100
Control		5.2 ± 1.04

Table II. Nematicidal effect of benzyl isothiocyanate (BITC) and carbofuran at different concentrations after 24 hrs.

Treatment	Concentration in ppm	Mortality % (X ± SE)	
		C. elegans	M. incognita
BITC	5	4.69 ± 0.95	13.24 ± 1.01
	10	4.87 ± 0.97	27.42 ± 1.34
	15	42.09 ± 2.17	46.13 ± 2.28
	20	83.01 ± 2.84	100
	25	100	100
Carbofuran	5	2.60 ± 0.45	4.80 ± 0.96
	10	3.04 ± 052	5.10 ± 0.94
	15	3.20 ± 045	5.90 ± 1.02
	20	3.84 ± 0.78	6.20 ± 1.24
	25	10.00 ± 1.07	12.00 ± 1.70
	50	75.42 ± 2.84	100
Control	Untreated	2.52 ± 0.37	5.80 ± 0.86

The activity of BITC at 25 and 50 ppm and carbofuran at 50, 100 and 200 ppm as a function of their exposure periods was studied and the nematode mortality was ascertained every half a hour after treatment up to 5 hrs and then at 24 hrs and 48 hrs. BITC required 30 min. at 50 ppm and 2.5 hrs at 25 ppm for 100% kill of *C. elegans*, but it required slightly longer exposure periods of 2 hrs and 3.5 hrs at 50 ppm and 25 ppm, respectively, for 100% kill of *M. incognita.* In comparison carbofuran at 50 ppm required 24 hrs and 48 hrs exposure time to cause total mortality of *M. incognita* and *C. elegans* respectively. Even at a concentration as high as 200 ppm carbofuran needed 4 hrs exposure time to cause 100% mortality of *M. incognita.* These results corroborate the conclusion that BITC is a more potent nematicidal compound than carbofuran *in vitro.* It was noted with carbofuran treatment that in its early exposure there was a loss of motility but the nematodes remained coiled. Thus with carbofuran mortality was a gradual process treatment unlike with BITC where the mortality was sudden as indicated by nematodes becoming straight instantaneously.

The results of the present findings indicate that the seeds of *C. papaya* possesses excellent nematicidal activity towards both saprophytic and plant nematodes which is due to benzyl isothiocyanate. Isothiocyanates in plants are normally derived from the hydrolysis of glucosinolates (Daxenbichler *et al,* 1991). The nematicidal activity of certain glucosinolates from cruciferae was studied towards the sugarbeet cyst nematode, *Heterodera schachtii* (Gommers, 1981) and gluctoropeolin (a glucoside of benzyl isothiocyanate) from *L. sativum,* was found toxic to these nematodes after 48 hrs at 5000 ppm in presence of enzyme myrosinase. However, the present results show that BITC is 200 times more toxic towards *M. incognita* than reported for gluctoropeolin against *H. schachtii.* Papaya seed is reported to constitute 22% of the waste product from the papaya puree industry (Chan *et al.,* 1978). Based on the present study a profitable utilization of these seeds as a source of plant-borne nematicide against root-knot nematodes could be attempted.

Accepted for publication on 5 April 2002

ACKNOWLEDGEMENT

We are thankful to ICAR for granting funds for this project. We express our thanks to Dr. Y. Selvaraj, Head, Division of Plant Physiology & Biochemistry, and the Director, I.I.H.R., for their interest in this work and providing the facilities. We thank Dr. M.R. Dinesh, Senior Scientist for providing the papaya seed.

LITERATURE CITED

Chan H.T., Heu R.A., Tang C.S., Okazaki E.N. and Ishizaki S.M., 1978. Composition of papaya seeds. *Journal of Food Science,* 43: 255-256.

Cremlyn R., 1978. Pesticides. Preparation and mode of action. John Wiley and Sons, Chuchester, UK, 240 pp.

Daxenbichler M.E., Spencer G.F., Carlson D.G., Rose G.B., Brinker A.M. and Powell, R.G., 1991. Glusinolate composition of seeds from 297 species of wild plants. *Phytochemistry,* 30 : 2623-2638.

Gommers F.J., 1981. Biochemical interactions between nematodes and plants, and their relevance to control. *Helminthological Abstracts* (B), 50: 9-24.

Kogiso S., Wada K. and Munakata K., 1976. Isolation of nematicidal polyacetylenes from *Carthamus tinctorius* L. *Agricultural and Biological Chemistry,* 40; 2085-2089.

Spencer G.F. and Daxenbichler M.E., 1980. Gas chromatography-Mass spectrometry of nitriles, isothiocyanates and oxazolidinethiones derived from cruciferous glucosinolates. *Journal of Science of Food and Agriculture, 31:* 359-367.

Sulston J. and Hodgkin J., 1988. The Nematode, *Caenorhabditis elegans,* (Ed. W.B. Wood.) Cold Spring Herbor, NY, 587 pp.

Tang C.S., 1971. Benzyl isothiocyanate of papaya fruit. *Phytochemistry,* 10: 117-121.

Tang C.S. and Syed M.M., 1972. Benzyl isothiocyanate in the Caricaceae. *Phytochemistry, 11:* 2531-2533.