Nematol. medit. (1994), 22: 37-40

Indian Institute of Horticultural Research, Hessaraghatta Lake P. O., Bangalore-560 089, India

IN VITRO NEMATICIDAL ACTIVITY OF EXTRACTS OF BULBS AND SEEDS OF ONION AGAINST ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*[1]

by
E. S. J. NIDIRY, M. V. CHANDRAVADANA, R. M. KHAN[2] and M. S. RAO

Summary. Among the various solvent extracts of the bulbs and seeds of onion assayed for *in vitro* nematicidal activity against root-knot nematode, *Meloidogyne incognita*, hot methanol extract of defatted seeds exhibited significant activity. The active extract contained a compound which fluoresced in ultra violet light; it coloured pink when sprayed with diazotized sulphanilic acid followed by sodium hydroxide on a TLC plate. Free amino acids viz. leucine (isoleucine), aspartic acid, methionine and threonine each exhibited nematicidal activity, but to a much lesser extent than the extract itself.

The essential oil of garlic (*Allium sativa*) has been reported to possess nematicidal activity (Nath *et al.*, 1982). However, Tarjan (1990) reported that garlic and onion slurries do not possess *in vivo* activity against *Meloidogyne incognita*. The present study investigated the *in vitro* nematicidal activity of the various extracts of the bulbs and seeds of onion (*Allium cepa* L.) against *M. incognita* (Kofoid *et* White) Chitw. and the chemical nature of the active fractions.

Materials and methods

Commercially available bulbs and seeds (cv. Nasik red) were used for the preparation of the extracts. To obtain the essential oil 1 kg of sliced onion was mixed with 500 ml of distilled water in a two litre flask and steam distilled. Approximately 50 mg of essential oil were obtained. After steam distillation, the solution in the flask was filtered and concentrated in vacuum to obtain an aqueous extract of the bulbs. One kg of onion bulbs was separately sliced and dried at 60 °C for 48 hrs, powdered and 20 g of this was extracted with hexane in a Soxhlet apparatus. The hexane extract was concentrated in vacuum. The hexane-extracted onion bulb powder was further Soxhlet extracted with methanol, which was also concentrated.

Seeds (20 g) were washed in mild soap solution followed by tap water and then distilled water. They were dried in oven at 60 °C for 18 hrs, then powdered in a grinder and Soxhlet extracted with hexane. The defatted seed material was further Soxhlet extracted with methanol, which on concentration under vacuum gave hot methanol extract 1.

Because the methanol extract showed activity, the extraction was done on a larger scale. One kg of cleaned powdered onion seeds was extracted with hexane in an aspirator bottle by hot percolation. By continuous extraction, the seed material was completely defatted and then extracted with methanol by cold percolation. This methanol extract after concentration gave cold methanol extract. After exhaustive extraction with cold methanol, hot water (95 °C) was added to the aspirator bottle and the water extract was collected and concentrated to dryness under vacuum to provide the aqueous extract. The dried aqueous extract, was re-extracted with hot methanol in a conical flask which on concentration gave hot methanol extract 2.

Two dimensional paper chromatography was carried out using Whatman No. 1 paper with butanol-acetic acid-water (4:1:5) system in the horizontal direction and butanol-phenol-acetic acid-water (5:5:2:10) system in the vertical direction. For the identification of amino acids, co-chromatography was done with authentic samples. Detection was done by spraying with ninhydrin in alcohol and warming the chromatograms at 80 °C for 20 minutes.

One ml each of the extracts or compounds with concentrations adequate to get the final required concentrations given in Tables I and II was taken for nematicidal bio-

[1] Contribution No. 43/93.

[2] Present address: Central Horticultural Experimental Station, Indian Lac Research Institute Campus, Namkum, Ranchi-834 010, Bihar, India.

assay. One hundred freshly hatched second stage juveniles of the root knot nematode *M. incognita* suspended in one ml distilled water were added to a Petri dish containing the extract solution which was covered with a lid and kept under room temperature. Mortality of the nematodes was observed after 24 and 48 hrs of incubation in the case of extracts. With pure compounds observations were made 96 hrs after incubation also. Thus 1 ml of 2% solution of the extract when mixed with 1 ml of distilled water containing the nematodes gave 1% solution. Extracts of other concentrations were obtained in a similar way. Since the hexane extract and the essential oil did not dissolve in distilled water, 0.3% Tween 20 was used instead of distilled water. Two controls, one with distilled water and the other with 0.3% Tween 20 in distilled water were maintained. Independently we had observed that 0.3% Tween 20 was not toxic to *M. incognita*. The concentrations of different extracts and compounds given in Tables I and II have been adjusted depending on their solubility and availability.

Nematicidal assay was carried out with different extracts in two replications.

Results and discussion

The hexane extracts of onion bulbs and seeds and the essential oil of onion bulb did not show nematicidal activity (Table I). The methanol extract of onion bulbs showed moderate nematicidal activity. But the methanol extract obtained by direct hot percolation of defatted seeds (hot methanol extract 1) as well as that obtained by re-extraction of the dried aqueous extract (hot methanol extract 2) showed significantly high activity, with the latter producing 96% mortality at a concentration of 0.5% (w/v) after 24 hrs. The aqueous extract was also nematicidal especially after an incubation of 48 hrs. Although the cold methanol extract showed only 1% mortality after 24 hrs, nematostatis affected 52% of the nematodes. Similarly aqueous extract at a concentration of 1% level had no nematicidal effect after 24 hrs, but nematostatis affected 40% of the nematodes. Mortality of the nematodes was ascertained by individually touching immobile nematodes with a needle; those which responded to the touch were judged as inactive and those which did not respond to the touch were ascertained as dead. The substantial difference in the activity of the methanol extracts obtained by hot and cold percolations suggests that the active principles are more soluble in hot methanol.

The active fractions viz. hot methanol extract 1, hot methanol extract 2 and aqueous extract showed the following characteristics: 1) they gave deep violet colour with ninhydrin showing the presence of amino acids; ii) they showed strong fluorescence under UV light; iii) they coloured pink with Pauly's diazo reagent (spraying with diazotised sulphanilic acid followed by sodium hydroxide on a TLC plate).

Paper chromatography of the three active fractions with butanol-acetic acid-water (4:1:5) system and thin layer chromatography by elution with methanol were

TABLE I - *Nematicidal activity of onion extracts against* Meloidogyne incognita.

Bulbs/ Seeds	Extract	Per cent conc. (w/v)	Per cent mortality after 24 hrs	after 48 hrs
	Control 1		Nil	Nil
	Control 2	–	Nil	Nil
Bulbs	Essential oil	0.1	Nil	5
Bulbs	Hexane	1.0	Nil	Nil
Bulbs	Methanol	1.0	10	15
Bulbs	Aqueous	1.0	2	2
Seeds	Hexane	1.0	Nil	Nil
Seeds	Cold methanol	1.0	1	12
Seeds	Hot methanol ext. 1	1.0	81	90
Seeds	Hot methanol ext. 2	1.0	97	100
Seeds	Hot methanol ext. 2	0.5	96	98
Seeds	Aqueous	1.0	Nil	61
Seeds	Aqueous	2.5	11	80

done to compare the chemical nature of the fractions. Observation under UV light and treatments with ninhydrin and Pauly's diazo reagent revealed that the same compounds are present in each of the three fractions.

Paper co-chromatography of the hot methanol extract 2 with authentic samples of various amino acids revealed the presence of leucine (isoleucine), aspartic acid, methionine and threonine. Nematicidal activity of commercially available amino acids is presented in Table II. They all show mild activity after an incubation of 48 hrs but the activity of methionine and aspartic acid after an incubation of 96 hrs is more conspicuous. Since leucine and isoleucine could not be distinguished on the paper chromatogram, both were assayed for nematicidal activity. While all these amino acids respond to ninhydrin test, they are not fluorescent under UV light and do not respond to Pauly's diazo reagent.

Column chromatography of hot methanol extract 2 on cellulose and elution with ethyl acetate gave a fluorescent compound devoid of amino acids. The compound coloured pink with Pauly's diazo reagent. Pauly's diazo reagent gives colour with phenols, aromatic amines and related compounds (Kirchner, 1978). Nematicidal activity of the fluorescent compound is reported in Table II. The detailed chemistry and elucidation of the structure of the fluorescent compound is being investigated (Nidiry *et al.*, unpublished). Table II shows that the amino acids and the fluorescent compound do possess nematicidal activity, but to a much lesser extent compared to the active fraction in which they are present. Therefore it is concluded that a combination of the amino acids and the fluorescent compound is responsible for the high activity of the hot methanol extracts. The possibility of any unidentified compound in the active fraction is also not ruled out. Recently Kintya and Degtyareva (1989) have isolated a steroidal glycoside from onion seeds. However, the chemical structure of the steroidal glycoside suggests it to be nonfluorescent in UV light and therefore different from the fluorescent compound mentioned in this paper. Moreover, the active fraction did not give characteristic colour reaction for steroids (Liebermann-Buchard reaction which involves treatment of the compound with acetic anhydride and conc. sulphuric acid).

Tanta *et al.* (1989) have reported the effect of sesame root exudates and its amino acids on inhibition of egg hatching of *M. incognita*. They also reported that DL-amino acids except DL-valine have some contact toxicity to root-knot second stage juveniles.

The inactivity of the essential oil of onion also deserves special mention especially in view of the fact that the essential oil of garlic is reported to possess significant nematicidal activity (Nath *et al.*, 1982). It may be noted that while the chief chemical constituent of essential oil of garlic is diallyl disulphide that of the essential oil of onion is dipropyl disulphide (Block, 1985). The poor activity of the essential oil and the various extracts of onion bulbs is consistent with the observation of Tarjan (1990) for *in vivo* studies.

Acknowledgements. The authors express their gratitude to Director, I. I. H. R., Bangalore for providing facilities and to Dr. P. P. Reddy, Head of Division of Entomology and Nematology for encouragement and guidance. Technical assistance rendered by Mrs. H. Shanthamma and Mr. Bujji Babu is gratefully acknowledged.

TABLE II - *Nematicidal activity of the free amino acids and the fluorescent compound present in onion seeds against* Meloidogyne incognita.

Compound	Per cent conc. (w/v)	Per cent mortality observed after		
		24 hrs	48 hrs	96 hrs
Control		Nil	Nil	2
Leucine	0.5	1	4	6
Isoleucine	0.5	1	4	6
Aspartic acid	0.5	2	5	45
Methionine	0.5	1	9	42
Threonine	0.5	2	8	
Fluorescent compound	0.5	7	10	27
	1.0	12	21	41
	2.0	18	40	65

Literature cited

BLOCK E., 1985. The chemistry of garlic and onion, *Sci. Am.*, *252*: 94-99.

NATH A. S., SHARMA N. K., BHARDWAJ S. and THAPA C. D., 1982. Nematicidal properties of garlic. *Nematologica*, *28*: 253-255.

KINTYA P. K. and DEGTYAREVA L. P., 1989. Steroidal glycosides of onion seeds: Structure of ceposide D. *Khim. Prir. Soedin.*, *1*: 139-140.

KIRCHNER J. G., 1978. *Detection of colorless compounds, Thin layer chromatography*, John Wiley and Sons, Inc., 244 pp.

TANDA A. S., ATWAL A. S. and BAJAJ Y. P. S., 1989. *In vitro* inhibition of root knot nematode *Meloidogyne incognita* by sesame root exudates and its amino acids. *Nematologica*, *35*: 115-124.

TARJAN A. C., 1990. Tests with ubiquitous materials for biocontrols of *Meloidogyne incognita* infecting tomato plants. *Nematol. medit.*, *18*: 231-232.

Accepted for publication on 15 December 1993.