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IN VITRO NEMATICIDAL ACTIVITY OF *GLORIOSA SUPERBA* SEED EXTRACT AGAINST *MELOIDOGYNE INCOGNITA*¹

by

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Summary. The methanol extract of defatted seeds of *Gloriosa superba* shows *in vitro* nematicidal activity against root knot nematode *Meloidogyne incognita*. One of the active principles has been identified as colchicine.

There is an increasing interest in the study of suppressive effects of plant extracts on parasitic nematodes and the identification of nematicidal principles in them (Munakata, 1978). We report here the nematicidal activity of the seed extract of *Gloriosa superba* L. against the root knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. and the identification of one of its active principles as colchicine. *G. superba* is a herbaceous climbing plant common in low jungles almost throughout India (Anonymous, 1956).

Materials and methods

Powdered seeds (30 g) were completely extracted with hexane and the hexane extract was concentrated under vacuum. Soxhlet extraction of the seed powder was made with methanol and the extract concentrated under vacuum to give 3.7 g of dry extract.

The efficacy of the extracts was tested against second stage juveniles of *M. incognita*. Both the hexane and methanol extracts were dissolved in 0.3% Tween 20 (Koch-Light, U.K.) in distilled water to get 10% (w/v) solutions. From these 4% and 2% solutions were made by dilutions. One ml of each of these solutions were taken for bioassay studies. One ml of 0.3% Tween 20 in distilled water was kept as a control. One hundred freshly hatched second stage juveniles of *M. incognita* in 1 ml of distilled water were added to each Petri dish, which was covered with a lid and kept at room temperature. Thus the final concentrations of solutions were 5%, 2% and 1% (w/v) respective-

ly. Each treatment was repeated twice. Observations were taken after 48 hrs. The average of the two observations is given in Table I.

TABLE I - *Nematicidal activity of Gloriosa superba seed extracts and colchicine against Meloidogyne incognita.*

Extract/ Compound	Concentration of extract/ compound in the medium (%)	Toxicity to nematodes		
		Nemati- cidal activity (a)	Nemato- static activity (b)	Nemato- toxicity (a+b)
Control	—	Nil	2	2
Hexane extract	1	Nil	2	2
	2	Nil	6	6
	5	Nil	8	8
Methanol extract	1	30	24	54
	2	33	31	64
	5	39	53	92
Colchicine*	1	18	14	32
	2	46	40	86

* Solubility of colchicine was insufficient to prepare a 5% solution.

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To ascertain whether colchicine is solely responsible for the nematocidal activity of methanol extract, quantitative estimation of colchicine in the methanol extract was done by preparative thin layer chromatography. One gram of methanol extract was spotted on pre-coated silica gel plates using pure colchicine always as a standard, and eluted with chloroform-methanol (19:1) mixture. After detection in the iodine chamber, the band corresponding to colchicine was scraped, extracted with methanol dried and weighed.

Results and discussion

Only the methanol extract showed significant nematocidal activity and the hexane extract was not effective. Nematocidal activity is the percentage of nematodes killed at a given concentration and nematostatic activity is the percentage of nematodes which were found to be immobile. An increase in concentration of the extract did not substantially increase the nematocidal activity, probably because the solubility of the active principles in the ambient medium had already reached a maximum at 1% concentration. However, substantial increase in nematostatic activity was observed when the concentration was increased to 5%.

It is known that *Gloriosa superba* contains 22 alkaloids of which colchicine is the most important one (Dvovackova *et al.*, 1984). Co-chromatography of the methanol extract with an authentic sample of colchicine on silica gel plates [eluent, chloroform-methanol (19:1); detection in iodine chamber] showed that colchicine is

one of the active principles present in the methanol extract. Colchicine is an alkaloid used in plant genetic research for doubling chromosomes and in the treatment of gout (The Merck Index, 1968). But its nematocidal property has not been reported so far. Nematocidal activity of commercially available colchicine also was ascertained and is reported in Table I.

Colchicine content of the methanol extract was about 4.5% which is equivalent to 0.55% of the dry seed. Since the percentage of colchicine in the methanol extract is 4.5, a concentration of 1% methanol extract which showed 30% nematocidal activity corresponds to 0.45% colchicine. On the other hand, pure colchicine even at 1% level shows only 18% nematocidal activity. From this it is clear that colchicine is only one of the active principles present in the methanol extract of the seeds of *G. superba*.

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