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Identification on nematicidal compound in Glory lily (*Gloriosa superba* L.) and their different plant parts against, hatching of *Meloidogyne incognita* eggs

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Abstract
Studies were carried out to identify a nematicidal compound from different plant parts *viz.,* rind, rhizomes, leaves, flowers and seeds of *Gloriosa superba* and the efficacy of nematicidal activity was tested against root knot nematode, *Meloidogyne incognita in vitro* also tested for its nematicidal property against *M. incognita* eggs. The results of *in vitro* study revealed that, the methanol extract of rind, rhizomes, leaves, flowers and seeds of *G. superba* inhibited egg hatching of *M. incognita* eggs. Its effect on inhibition of hatching showed directly proportional to their concentrations and time of exposure. Among the different plant parts tested, rhizome extract was found effective followed by seeds in inhibition of egg hatching of *M. incognita* at 25, 50, 75 and 100 per cent concentrations and different time of exposure. Total inhibition was noticed at higher concentrations of 75 and 100 per cent after 48h of exposure and at 72h total inhibition was noticed in all the concentrations. The rind was slightly less effective as compared to rhizome and seed extracts. The per cent inhibition in hatching was less than 50 per cent after 12h and its efficacy increased after 48 and 72h of exposure. Total inhibition in hatching was not noticed even after 72h of exposure in higher concentrations. The leaves and flower extracts were found less effective in inhibition of hatching at different concentrations and time of exposure.

Keywords: *Gloriosa superba*, nematicidal, hatching, *Meloidogyne incognita*.

Introduction
Glory lily (*Gloriosa superba* L.) is an important medicinal plant belonging to the family Liliaceae. It is a high value medicinal crop, commercially cultivated in India, particularly in Tamil Nadu. It is recognized as state flower of Tamil Nadu. The name *Gloriosa* is said to be derived from the word 'glorious' meaning handsome and *superba* from the word 'superb' meaning splendid or majestic kind. In Tamil Nadu, it holds a monopoly in the production with an annual production of 600-700 tonnes and productivity of 1.04 tonnes/ha grown in an area of 6,000 acres (Padmapriya *et al*., 2015) [6]. The flower has analgesic, anti inflammatory, anti microbial, larvicidal, antipoxviral, antithrombotic, antitumor, enzyme inhibition potential and used in the treatment of snake bite, skin disease and respiratory disorders (Babu Rao *et al*., 2014) [1]. The seeds and tubers have been exploited for the extraction of alkaloids mainly colchicines ($C_{22}H_{25}O_6N$) and colchicoside ($C_{27}H_{33}O_{11}H$) which is used traditionally for the treatment of bruises and sprains, colic, chronic ulcers, hemorrhoids, cancer, leprosy and also for inducing labour pains and gout. Paste of the tuber is externally applied for parasitic skin diseases (Deepak Acharya *et al.,* 2006) [3]. Many chemical compounds have now been withdrawn from use promoting the need for new, safe and effective options (Zuckerman and Esnard, 1994) [8]. There is an increasing interest in discovering nematicidal compounds in plants (Chitwood, 2002) [2]. The use of plant products is one of the most promising alternatives to observe the possibility of their nematicidal / nematostatic properties for the management of nematodes.

Materials and Methods
Pure culture of root knot nematode, *M. incognita*
Pure culture of root knot nematode, *M. incognita* required for the studies was maintained on tomato cv. CO 1 in earthern pots containing steam sterilized pot mixture (1:1:2 red earth, sand and farm yard manure). The egg masses required for the experiments were collected from the

roots by carefully uprooting the plants and roots with conspicuous galls were washed gently in water and the egg masses were then handpicked under the stereozoom microscope and allowed to hatch by placing the egg masses in 100 ml beaker containing distilled water and incubated at room temperature. Then the hatched out second stage juveniles (J_2) of *M. incognita* obtained from the egg masses were inoculated at 1 J_2 / g of soil in the tomato rhizosphere at two weeks after transplanting and covered with sterilized pot mixture soil. The nematodes were multiplied and maintained separately as stock culture in the Nematology glasshouse. The nematodes required for the experimental purpose were collected from this culture.

Collection of plant parts of *G. superba*

The plant parts *viz.*, rind, rhizomes, flowers, leaves and seeds of *G. superba* were collected from the farmer field at Dharapuram, Tirupur District, Tamil Nadu.

Preparation of crude extracts of *G. superba*

Soxhlet apparatus was used for extraction purpose. Twenty five gram of the powdered plant parts of *G. superba viz.*, rind, rhizomes, flowers, leaves and seeds were weighed separately into 200 ml methanol and percolated for 24 hours. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to electric heating mantle with soxhlet unit, filled with 240 ml of methanol and temperature 64.6 ºC was maintained. The unit was regulated with water to give a slow controlled flow of the solvent through the compressed sample. The filtrate was collected in a rained bottom flask. The residual extract was collected in a flask and transferred to a rotary flask vacuum evaporator for evaporation of the solvent. The residue thus obtained was stored at 4 ^{0}C in airtight bottles for future use (Keita *et al.*, 2001) [5].

Identification of nematicidal compound in *G.superba*

The nematicidal compound was identified from different plant parts of *G.superba* by using HPLC (High Performance Liquid Chromatography) analysis.

Purification of nematicidal compound through HPLC (High Performance Liquid Chromatography)

Analysis of the active bands was done in HPLC (Agilent technologies 1200 series) equipped with LC8A pump, SPD-M 10A γp photo diode array (PDA) detector in combination with class LC 10A software and Beckman Ultrasphere supelco ODS column (250 x 4.6 mm).

Chromatographic conditions

Mobile phase	:	Methanol:Water (75:25)
Column	:	C18, 5µ size, 250 x 4.6mm (Supelco)
Detector	:	SPD-M 10 A γp photo diode array detector (PDA).
Wave length	:	245nm
Flow rate	:	1.00 mL/min
Injection volume	:	30µL

Standardization of analytical technique for quantification of Colchicine

To determine colchicine content in methanol extract of rind, rhizome, leaves, seeds and flowers the method was standardized as described below.

Chemicals and Reagents

HPLC grade of water and methanol, analytical standard of colchicine (98.00% purity)

Preparation of standard stock solution

Fifty mg of the analytical standard of colchicine (98.00% purity) was mixed to 1 ml HPLC methanol for HPLC determination.

Working out standard solution

From the stock solution, working standard solution of 30µl was taken. This working standard was used to find out the retention time and quantitative determination of colchicine content in methanol extract.

Sample Preparation

Preparation of methanol extract of rind, rhizome, leaves, seeds and flowers

Methanol extract of 0.5 µl was diluted with 450 µl of methanol HPLC grade and 30 µl was taken from the sample and injected into HPLC.

Effect of crude extract on inhibition in hatching of *M. incognita* eggs

For hatching test, egg masses of *M. incognita* were obtained from the pure culture maintained in the glasshouse on tomato plants. One ml of crude extract of different concentrations (100, 75, 50 and 25%) was transformed to 6.0cm diameter Petri dishes and one egg mass of root knot nematode were placed in each Petri dish and incubated at room temperature. The egg mass placed in distilled water served as control. Each treatment was replicated five times. The number of hatched juveniles was counted after 24, 48 and 72 hours incubation.

Treatment details

T_1- 25% concentration
T_2- 50% concentration
T_3- 75% concentration
T_4- 100% concentration
T_5- Distilled water (Control)

Statistical analysis

The data generated from various experiments in the present study were subjected to statistical analysis following the method of Gomez and Gomez (2010) [4]. The package used for analysis was IRRISAT version 92-1 developed by International Rice Research Institute, Biometrics Unit, Manila, Philippines

Results

Identification of nematicidal compound in *G.superba* by HPLC

The nematicidal compound has been identified as colchicine by subjecting the methanol extract of *G.superba* rind, rhizome, seed, leaves and flower to HPLC.

The Retention Time (RT in minutes) of different plant parts of *G.superba* is as follows

Colchicine standard	rind	rhizome	leaves	flowers	seeds
1.71	1.71	1.78	1.70	1.73	1.73

The major peak was obtained at retention time of 1.71 min, 1.78 min, 1.70 min, 1.73 min, 1.73 min of *G. superba* rind extract, rhizome extract, leaves extract, flowers and seed extract respectively. The result showed the presence of colchicine in different plant parts of *G. superba*

Effect of different extracts of rhizomes, seeds, rind, leaves, and flowers of *G. superba* against *M. incognita* eggs

Effect of rhizome extract of *G. superba* on hatching of *M. incognita* eggs

The count on inhibition of hatching of *M. incognita* was recorded at 12, 24, 48 and 72 h of exposure. Hundred eggs were exposed uniformly at concentrations of 25, 50, 75 and 100per cent. After 12h exposure, an inhibition of 41.20 per cent was recorded at the highest concentration of 100 per cent followed by 28.36 per cent in 75 per cent concentration with total inhibition of 96.21 and 90.00 per cent of inhibition was noticed at 100 and 75 per cent concentration after 24h of exposure. At 48h exposure, all the concentrations recorded significant inhibition in hatching of eggs with 84.24 and 90.32 per cent inhibition even at lower concentration of 25 and 50 per cent and total inhibition was noticed at 75 and 100 per cent. Complete inhibition was noticed in all the four concentrations whereas in the control, only negligible inhibition of 10.00 and 16.00 per cent noticed after 48 and 72 h (Table 1).

Effect of seed extract of *G. superba* on hatching of *M. incognita* eggs

The seed extract of *G. superba* showed significant inhibition in hatching of eggs only after 24h of exposure. After 12h, the inhibition ranged from 6.52 to 16.00 per cent at concentrations of 25 to 100 per cent near total inhibition of 95.10 and 81.32 per cent were noticed at concentrations of 100, 75 and 50 per cent after 24 h of exposure. Total inhibition in 100 and 75 per cent concentration and 89.12 and 83.23 per cent were noticed at 50 and 25 per cent concentrations after 48h of exposure and cent per cent inhibition was noticed at all the concentrations tried after 72h of exposure. In the untreated control no inhibition was noticed up to 48h and 6.4 per cent inhibition was noticed after 72h (Table 1).

Effect of rind extract of *G. superba* on hatching of *M. incognita* eggs

The methanol extract of rind of *G. superba* showed significant inhibition in the hatching of *M. incognita* eggs only after 24h of exposure. After 12h, the inhibition of hatching was less (15.23 per cent) at 100 per cent concentration followed by 10.00, 6.42 and 5.31 per cent at 75, 50 and 25 per cent concentrations. Nearly, 50 per cent inhibition was noticed at 24h of exposure with highest value of 64.00 at highest concentration after 24h, significant reduction in the inhibitions were noticed at 48 and 72h of exposure. However, not much of differences in the inhibition was noticed between these two periods. Total inhibition was not noticed even with highest concentration of 100 per cent after 72h of exposure, the inhibition was ranged from 80.10 to 97.12 per cent and 88.32 to 98.00per cent at the four concentration levels (Table 1).

Effect of leaves extract of *G. superba* on hatching of *M. incognita* eggs

The leaf extracts of *G. superba* was found to inhibit the hatching of *M. incognita* eggs significantly even at 12h of exposure. At higher concentration of 100 per cent, the inhibition was 84.6 per cent followed by 59.70, 48.62 and 42.50 at concentrations of 75, 50 and 25. After 24h, total inhibition was noticed at highest concentration followed by 81.30, 75.01 and 60.02 per cent respectively at 75, 50 and 25 per cent concentrations, total inhibition was noticed at 100 and 75 per cent concentrations, after 48hwhere as in 50 and 25 per cent concentrations, the inhibition was 83.12 and 78.34 per cent. After 72h of exposure, except in lowest concentration of 25 per cent all the concentrations recorded cent per cent inhibition. At 25 per cent concentration, 94 per cent inhibition was noticed. In the untreated control, up to 48h no inhibition was noticed and only after 72h negligible inhibition (6.50) was noticed (Table 1).

Effect of flower extract of *G. superba* on hatching of *M. incognita* eggs

The data appended in the (Table 1) showed that, the inhibition of hatching was less with flower extract of *G. superba*. After 12h of exposure, only the highest concentration of 100 per cent recorded 64.22 per cent inhibition rest of the concentrations *viz.*, 75, 50 and 25 per cent recorded only 32.04, 21.00 and 11.80 per cent inhibition. After 24h of exposure the highest concentration of 100 per cent recorded 73.66 and 58.64 per cent in 75 per cent concentration. The other two concentrations recorded less than 50 per cent inhibition. There was no much variation between 48 and 72h of exposure, where in 93.01, 88.43 and 81.00 per cent was recorded with the concentrations of 100, 75 and 50 and 45.12 per cent was recorded in 25 per cent concentration after 48h. Even after 72h cent per cent inhibition was noticed in none of the treatments the inhibition was ranged from 81.22 to 96.21 per cent in the four concentrations and there was no inhibition in the control was after 72h of exposure (Table 1).

Table 1: Effect of different extract of *G. superba* on hatching of *M. incognita* eggs

Reatments	Percent inhibition on hatching of juveniles at different periods of exposure																			
	Rhizome				Seed				Rind				Leaves				Flower			
	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h
T_1- 25% concentration	12.16	12.16	12.16	12.16	6.52	74.14	83.23	100	5.31	42.00	80.10	88.32	42.50	60.02	78.34	94.00	11.80	26.40	45.12	81.22
T_2 - 50% concentration	21.16	21.16	21.16	21.16	7.12	81.32	89.12	100	6.42	49.43	86.34	91.00	48.62	75.01	83.12	100.00	21.00	46.40	81.00	84.14
T_3 - 75% concentration	28.36	28.36	28.36	28.36	11.12	89.25	100.00	100	10.00	57.00	93.12	96.21	59.70	81.30	100.00	100.00	32.04	58.64	88.43	91.00
T_4 - 100% concentration	41.20	41.20	41.20	41.20	16.00	95.10	100	100	15.23	64.00	97.12	98.00	84.60	100.00	100.00	100.00	64.22	73.66	93.01	96.21
T_5 - Distilled water (Untreated control)	0	0	0	0	0	0	0	6.40	0	0	0	0	0	0	0	6.50	0	0	0	0
SEd	0.09	0.09	0.09	0.09	0.03	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.02
CD (P=0.05)	0.19	0.19	0.19	0.19	0.06	0.05	0.05	0.07	0.06	0.05	0.06	0.05	0.06	0.06	0.08	0.07	0.07	0.07	0.06	0.05

Discussion

Efficacy of different extracts of *G. superb*a on *M. incognita* eggs

In the present study rhizome extract of *G. superba* was tried against root knot nematode to test its efficacy on inhibition of egg hatching at four concentrations *viz.*, 25, 50, 75 and 100

per cent. The results on per cent inhibition in hatching of *M. incognita* eggs showed that the efficacy in inhibition of egg hatch increased with increased period of exposure from12hr to 72hr. Significant inhibition was noticed after 24hr of exposure and near total inhibition was noticed at higher concentrations. Total inhibition was noticed at higher concentrations after 48hr of exposure and at 72hr total inhibition was noticed in all the concentrations. In the present study, aqueous extracts of the seeds of *G. superba* was tested for their efficacy in inhibition of hatching of the *M. incognita* eggs at four concentrations and four exposure period *viz.*, 12, 24, 48 and 72hr.The seed extracts of *G. superba* was also found as effective as the rhizome extract as shown by its efficacy in the inhibition hatching of the *M. incognita* eggs. Significant inhibition in hatching was noticed at higher concentrations there after cent per cent inhibition was noticed at all the concentrations.

The rind is the outer cover of the seeds of *G. superba* and after collection of seeds the rind goes as waste. In order to study the nematicidal value of rind the aqueous extract of the same was studied against *M. incognita*. Methanol extract of *G. superba* rind at 25, 50, 75 and 100 per cent concentration was tested for its efficacy on hatching inhibition of *M. incognita*. Comparing the rhizome and seeds of *G. superba*, the rind was slightly less effective as shown by the results. The per cent inhibition in hatching was less than 50 per cent after 12hr and its efficacy increased after 48 and 72hr of exposure. Total inhibition in hatching was not noticed even after 72hr of exposure in higher concentrations.

This an interested findings that a material goes as a waste is having nematicidal properties and which can be utilized as an amendment as such or it can be made in to compost and applied to soil.

The present study was on the efficacy of leaves of *G. superba* which has not so far been tried against nematodes. The aqueous extracts of leaves of *G. superba* at four concentrations *viz.*, 25, 50, 75 and 100 per cents showed significantly effective in inhibition of hatching of *M. incognita* eggs even at 12 hr of exposure, and total inhibition was achieved at 100 per cent concentration at 24 hr of exposure and above.

The present study showed that more than 50 per cent inhibition in hatching has been noticed even at 12 hr of exposure at highest concentration and near total inhibition of egg hatch was noticed at 48 hr of interval which increased slightly at 72 hr of exposure.

In summarising up of the findings on the nematicidal properties of all the plant parts *viz.*, rhizome, seed, rind, leaves and flowers exhibit nematicidal properties as evidenced by the hatching inhibition and juvenile mortality. Similar finding was documented with species of marigold *viz.*, African marigold *Tagetes erecta* cv. Atlantis, *T. erecta* cv Single orange and Indian yellow, *T. patula*, the French marigold, *T. minuta* where in the whole plant parts like leaves, flowers, root and stem showed nematicidal properties (Sankarimeena *et al.*, 2010) [7]. The present finding is most significant one that all the plant parts have nematicidal properties and no work has been done on this earlier on *G. superba* on their nematicidal properties.

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