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

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Abstract
The present study was carried out in Department of Nematology, Tamil Nadu Agricultural University during March – June, 2016 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*. The results of *in vitro* study revealed that, the methanol extract of rind, rhizomes, leaves, flowers and seeds of *G. superba* caused juvenile mortality of *M. incognita*. Its effect of causing mortality showed directly proportional to their concentrations and time of exposure. Among the different plant parts tested, rhizome extract was found effective followed by seeds caused juvenile mortality of *M. incognita* at 25, 50, 75 and 100 percent concentrations and different time of exposure. The rhizome extract significantly caused mortality of J_2 of *M. incognita* even at 12h of exposure with 75 and 100 percent concentrations and total mortality was resulted after 24h of exposure at 100 percent concentrations and in all the concentrations after 48h of exposure. The rind was slightly less effective as compared to rhizome and seed extracts. The leaves and flower extracts were found less effective in caused juvenile mortality at different concentrations and time of exposure.

Keywords: *Gloriosa superba*, nematicidal, juvenile mortality, *Meloidogyne incognita*

1. Introduction
Glory lily (*Gloriosa superba* L.) is an important medicinal plant belonging to the family Liliaceae [1]. It is a high value medicinal crop, commercially cultivated in India, particularly in Tamil Nadu. It is recognized as state flower of Tamil Nadu [2]. 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 [3]. 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 [4]. 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 [5]. 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[6]. Paste of the tuber is externally applied for parasitic skin diseases [7]. The chemical approach for the management of these nematodes has been successful but has become less attractive due to increasing environmental concerns. In the recent years, these chemical nematicides are being gradually replaced by botanical nematicides. Many chemical compounds have now been withdrawn from use promoting the need for new, safe and effective options [8]. There is an increasing interest in discovering nematicidal compounds in plants [9]. 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. However, most of the researchers have investigated seeds, tubers, shoots and valuable constituents of the plants for their nematicidal activities. However, the present study is a first attempt to test the nematicidal activity from the rind part of the medicinal plant. Hence, the present investigation was undertaken with the following objective to identify a nematicidal compound in *G. superba* and to test its efficacy against mortality of root knot nematode, *Meloidogyne incognita* juveniles.

2. Materials and Methods

2.1 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.

2.2 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.

2.3 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 [10].

2.4 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.

2.4.1 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).

2.4.2 Chromatographic conditions

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

2.4.3 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.

2.4.4 Chemicals and Reagents

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

2.4.5 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.

2.4.6 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.

2.4.7 Sample Preparation

2.4.7.1 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.

2.5 *In vitro* bioassay of crude extracts of different plant parts against *M. incognita*

Crude extracts of rind, flowers, rhizomes, leaves and seeds were selected for assessing their efficacy against *M. incognita* by conducting hatching and mortality tests.

2.5.1 Effect of crude extract on mortality of *M. incognita* juveniles

One ml of crude extract of different concentrations (100, 75, 50 and 25%) was transformed to 6.0cm diameter Petri dishes. The juveniles of root knot nematode were placed as 100 J2 in each Petri dish and incubated at room temperature. Juveniles placed in dishes containing distilled water served as control. Five replications were maintained for each observation and mortality of juveniles after 24, 48 and 72 hr of incubation was made.

Treatment details

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

2.6 Statistical analysis

The data generated from various experiments in the present study were subjected to statistical analysis following the method of [11]. The package used for analysis was IRRISAT version 92-1 developed by International Rice Research Institute, Biometrics Unit, Manila, Philippines

3. Results

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

The nematicidal compound was 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*

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

3.2.1 Effect of rhizome extract of *G. superba* on mortality of juveniles of *M. incognita*

The data on the effect of *G. superba* rhizome extract on the mortality of *M. incognita* second stage juveniles (J_2) appended in Table (1) showed that at concentrations of 50 and 100 percent significantly caused 68.00 and 80.24 percent mortality of the juveniles of *M. incognita* after 12h of exposure. The concentrations of 25 and 50 percent showed only 29.00 and 39.13 percent mortality after 24h of exposure. Total mortality was observed with higher concentration of 100 percent followed by 85.14, 67.32, 46.21percent respectively at 75, 50 and 25 percent concentrations no juveniles were alive after 48 and 72h in all the four concentrations. The control recorded no mortality up to 48h and after 72h a minimum of 7.6 percent juvenile mortality was noticed. There was no mortality of juveniles in control up to 48h and after 72h minimum of 7.6 percent juvenile mortality was noticed (Table 1).

3.2.2 Effect of seed extract of *G. superba* on mortality of juveniles of *M. incognita*

The seed extract of *G. superba* after 12h of exposure caused 64.00, 38.70, 22.10 and 18.60 percent mortality of *M. incognita* juveniles respectively at concentrations of 100, 75, 50 and 25 percent. After 24h of exposure, the mortality was highest (79.00%) at 100 percent concentration followed by 64.41, 38.12 and 26.00 percent at 75, 50 and 25 percent concentration. Complete death of J_2 observed at 100 percent concentration after 48h of exposure followed by 83.23, 65.00 and 44.21 percent at 75, 50 and 25 percent concentrations. After 72h, in the lowest concentration of 25 percent recorded 98.20 percent mortality and in control up to 48h, no mortality was noticed and only 4.2 percent mortality was noticed after 72h of exposure (Table 1).

3.2.3 Effect of rind extract of *G. superba* on mortality of juveniles of *M. incognita*

The rind extract of *G. superba* showed comparatively less mortality of juveniles of *M. incognita*. After 12h of exposure in which less than 50 percent mortality was noticed. Among the concentrations, the highest concentration of 100 percent recorded 47.22 percent followed by 31.60 percent in 75 percent concentration. After 24h of exposure, the mortality of *M. incognita* was 77.20, 62.0, 37.02 and 24.21 percent respectively at 100, 75, 50 and 25 percent concentrations. Total death of J_2were noticed at highest concentration after 48h of exposure followed by 80.41 percent at 75 percent concentration. The high concentration of 100 and 75 percent resulted in total death of *M. incognita* followed by near total death of 99.00 and 96.00 percent at concentrations of 50 and 25 percent. Whereas in the control, a minimum of 2.60 mortality was noticed after 72h (Table 1)

3.2.4 Effect of leaf extract of *G. superba* on mortality of juveniles of *M. incognita*

The extract of leaves of *G. superba* significantly caused juvenile mortality even at 12h of exposure. The data showed a highest mortality of 74.00 percent was noticed at a concentration of 100 percent of the extract even after 12h of exposure followed by 59.25, 34.33 and 22.06 percent at concentrations of 75, 50 and 25. After 24h of exposure, highest mortality of 98.41 percent was noticed at highest concentration of 100 percent followed by 76.32, 60.00 and 39.14 respectively at 75, 50 and 25 percent concentrations. Total mortality and near total mortality of 99.00, 96.00 and 94.21 percent of *M. incognita* J_2 was noticed after 48h of exposure at concentrations of 100, 75, 50 and 25.At 72h exposure recorded cent percent mortality in all the concentrations whereas in the untreated control only 3.60 percent mortality was noticed that too after 72h of exposure (Table 1).

3.2.5 Effect of flower extract of *G. superba* on mortality of juveniles of *M. incognita*

Comparing the rhizomes, seeds and leaves of *G. superba*, the flower extract was found less effective in the mortality of *M. incognita* juveniles as shown in the data appended in (Table 1). The highest mortality of 46.00 percent of *M. incognita* J_2 at high concentration followed by 21.70, 13.20 and 8.60 percent respectively at 100, 75, 50 and 25 percent concentrations. After 24h of exposure, only the higher concentrations of 100 and 75 percent resulted in significant mortality of 71.00 and 57.00 percent whereas, the concentrations of 50 and 25 recorded only 32.22 and 20.21 percent mortality. After 48h of exposure only the highest concentration of 100 percent caused 97.00 percent mortality of *M. incognita* where as the other three concentrations of 75, 50 and 25 percent resulted in 74.12, 59.21 and 36.40 percent. After 72h, only the highest concentration recorded cent percent mortality followed by 96.22, 94.00 and 92.00 percent respectively at 75, 50 and 25 percent concentrations. The untreated control recorded minimum 1.26 percent mortality after 72h (Table 1).

Table 1: Effect of different extract of *G. superba* on mortality of *M. incognita* juvenile

Treatments (Concentration)	Percent mortality of juveniles at different period of exposure																			
	Rhizome				Seed				Rind				Leaves				Flower			
	12 h	24h	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%	29.0	46.2	100	100	18.6	26.0	44.2	98.2	8.6	24.2	41.0	96.0	22.0	39.1	94.2	100	8.6	20.2	36.4	92.0
T_2-50%	39.1	67.3	100	100	22.1	38.1	65.0	100	17.2	37.0	63.2	99.0	34.3	60.0	96.0	100	13.2	32.2	59.2	94.0
T_3-75%	68.0	85.1	100	100	38.7	64.4	83.2	100	31.6	62.0	80.4	100	59.2	76.3	99.0	100	21.7	57.0	74.1	96.2
T_4-100%	80.2	100	100	100	64.0	79.0	100	100	47.2	77.2	100	100	74.0	98.4	100	100	46.0	71.0	97.0	100
T_5-Control	0	0	0	7.6	0	0	0	4.2	0	0	0	2.6	0	0	0	3.6	0	0	0	1.26
SEd	0.8	0.4	0.2	0.8	0.6	0.3	0.4	0.32	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.03	0.03	0.03	0.02
CD (P=0.05)	1.7	1.0	0.4	1.7	1.4	0.6	1.0	0.67	0.5	0.	0.5	0.4	0.3	0.3	0.5	0.4	0.07	0.07	0.06	0.05

4. Discussion

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

In the present study different extract of *G. superba* was tried against root knot nematode to test its efficacy of juvenile mortality at four concentrations *viz.*, 25, 50, 75 and 100 percent. The rhizome extract of *G. superba* resulted in significantly caused mortality of J_2 of *M. incognita* even at 12hr of exposure with 75 and 100 percent concentrations and total mortality resulted after 24hr of exposure at 100 percent concentrations and all the four concentrations at 48hr of exposure. The results are in line with the findings of [12] with *Acorus calamus* rhizome extracts on *M. incognita* and [13] with the rhizome extracts of *Artemesia vulgaris* who found that the mortality increases with increase in concentration and time of exposure. However, in the present study total mortality of *M. incognita* was resulted even at 24h of exposure itself indicating that the active nematicidal principles in the rhizomes of *G. superba* is having a strong nematicidal actions. The seed extracts of *G. superba* was also found as effective as the rhizome extract as shown by its efficacy of juvenile mortality. Significant inhibition in hatching was noticed at higher concentrations there after cent percent inhibition was noticed at all the concentrations. Similar observation was also noticed in the juvenile mortality with the aqueous extract of seeds of *G. superba.* Total mortality of juveniles was noticed at 48hr and 72hr. Increased inhibition in hatching of eggs and juvenile mortality was noticed as the concentration of the extracts and the time of exposure. Similarly, the juvenile mortality of leaves and flowers extracts of *G. superba* significantly high at 12 hr of exposure itself which increased progressively and attained cent percent at 72 hr. There was not much differences between 48 hr and 72 hr of exposure as near total mortality was noticed on 48 hr of exposure in all the four concentrations tried. As in the other plant parts, the juvenile mortality increased with the increase in concentration and exposure time. 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 percent concentration was tested for its efficacy on juvenile mortality of *M. incognita*. Comparing the rhizome and seeds of *G. superba*, the rind was slightly less effective as shown by the results. After 12hr of exposure, less than 50 percent mortality was observed and total mortality was noticed at highest concentration after 48hr and 100 and 75 percent concentrations after 72hr of exposure. [14] obtained methanol extracts from defatted seeds of *G. superba* and tried at 0.45 percent concentration against *M. incognita*.

5. Conclusion

In summarizing up of the findings on the colchicine content was identified from different parts of *G. superba viz.*, rind, rhizomes, leaves, flowers and seeds by using High Performance Liquid Chromatography (HPLC) analysis. The methanol extract of rind, rhizomes, leaves, flowers and seeds of *G. superba* caused juvenile mortality of *M. incognita*. Its effect on causing mortality showed direct proportion to their concentrations and time of exposure. Rhizome extract was found effective followed by seeds in inhibiting egg hatching and causing juvenile mortality of *M. incognita* at 25, 50, 75 and 100 percent concentrations with different times of exposure. This was an interested finding 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.

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