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N Ashokkumar
Ph. D Scholar, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

A Shanthi
Professor, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Sivakumar
Professor, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

K Rajamani
Professor and Head, Department of Medicinal and Aromatic Plants, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
N Ashokkumar
Ph. D Scholar, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Studies on antibacterial activity of different plant parts of glory lily (*Gloriosa superba* L.) against bacterial wilt pathogen, *Ralstonia solanacearum*

N Ashokkumar, A Shanthi, M Sivakumar and K Rajamani

Abstract
The methanol extracts from different plant parts of *G. superba viz.*, rind, rhizomes, leaves flower and seeds were evaluated by *in vitro* against antibacterial activity of *Ralstonia solanacearum*. In different methanol extracts tested, seeds (1.93 mm) and rhizomes (1.93 mm) extract was found to possess the highest *R. solanacearum* growth inhibition at 100 per cent concentration when compared to the others followed by leaves (1.70 mm), flowers (1.66 mm) and rind (1.63 mm) methanol extract was also inhibit the *R. solanacearum* growth. At 25 per cent concentration seed extract was showed highest growth inhibition followed by leaves, rhizomes, Flowers and Rind Extracts derived from different plant parts of *G.superba viz.*, rind, rhizomes, leaves, flowers and seeds inhibited the growth of bacterial wilt pathogen, *R. solanacearum* with highest inhibition using seed and rhizome extracts.

Keywords: *Gloriosa superba*, antibacterial, growth inhibition, concentration *Ralstonia solanacearum*

1. Introduction
Glory lily (*G. 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 the 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. It is native of South Africa and is widely distributed across the tropical and subtropical countries [8]. Its natural distribution spreads mainly in tropical Asia, *viz.*, India, Sri Lanka, Malaysia and Myanmar. In India, it is commonly found in Himalayan foot-hills of Central India, Tamil Nadu, Andhra Pradesh, Karnataka and West Bengal. 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 [3]. 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 [4]. Different parts of the plant have a wide variety of uses especially in traditional system of medicine.

2. Materials and methods
2.1 Maintenance of pure culture of root knot nematode, *Meloidogyne incognita*
2.2 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 a stock culture in the Nematology glasshouse. The nematodes required for the experimental purpose were collected from this culture.

2.3 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.

2.4 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 [9].

2.5 Effect of crude extracts on growth inhibition of bacterial wilt pathogen, *Ralstonia solanacearum*
The crude extracts of rhizomes, rind, flowers, leaves and seed for antibacterial effect was carried out by determining the zone of inhibition using disc diffusion method [10]. Sterile nutrient agar plates were prepared and inoculated with bacterium by spread plate method under aseptic conditions. The filter paper discs of 6mm diameter (Whatman's No.1 filter paper) were prepared and sterilized. The crude extract tested were prepared with different concentrations of 100, 75, 50 and 25 % and were added to each disc of holding the capacity of 10 microlitres. The sterile impregnated discs with crude extracts were placed on the agar surface with framed foreceps and gently pressed down to ensure complete contact of the disc. Control discs of methanol were prepared and placed on the agar surface. All the plates were incubated at 37 ºC for 24 hours. After incubation, the size (diameter) of the inhibition zone was measured.

2.6 Statistical analysis
The data generated from various experiments in the present study were subjected to statistical analysis following the method of [7]. The package used for analysis was IRRISAT version 92-1 developed by International Rice Research Institute, Biometrics Unit, Manila, Philippines.

3. Experimental results
3.1 Effect of extract of different plant parts of *G. superba* on growth inhibition of bacterial wilt pathogen, *Ralstonia solanacearum*
The results showed that, the different plant parts of *G. supeba* showed antibacterial activity against *R. solanacearum* (Plate 1). The results presented in Table 1, showed that the maximum growth inhibition was observed in seed and rhizome extract with 1.93 and 1.93 mm at 100 per cent concentration where 1.80,1.60, 1.26 mm growth inhibition was observed at 75, 50, 25 per cent concentrations in seed extract and 1.66, 1.46, 1.13 mm growth inhibition at 75, 50, 25 per cent concentrations in rhizome extract. The minimum inhibition was recorded in rind extract at 100 per cent concentration with 1.63 mm where 1.43, 1.26, 1.10 mm growth inhibition in 75, 50, 25 per cent concentrations (Fig 1). The leaves extract inhibit the growth of 1.70, 1.46, 1.33 and 1.20 mm at 100, 75, 50 and 25 per cent concentrations. Flower extract at 100, 75, 50 and 25 per cent concentrations recorded the growth inhibition of 1.66, 1.50, 1.36 and 1.13 mm. Among the different extracts seed and rhizome extracts were found effective in inhibiting the growth of *R. solanacearum* followed by leaves, flowers and rind (Table 1)

4. Discussion
4.1 Efficacy of *G. superba* extracts on *Ralstonia solanacearum*
The antibacterial activity of *G.superba* has been well documented. It is highly effective against gram positive *Streptococcus faecalis*, *Enterococcus faecalis*, gram negative *Klebsiella pneumontae*, *Proteus mirabilis*, [7] gram positive *Bacillus cereus*, *B. subtilis*, *Streptococcus cremoris*, *S. faecalis*, *S.aureus* and gram negative *E. coli*, *Pseudomonas auriginosa*, *Salmonalla typhi*, *Klebsiella pneumonia* and *Proteus vulgaris* [5] Value addition of *G. superba* rind by fortification with FYM and their decomposed products showed antinemic, antifungal, antibacterial, action besides increased nutrient contents by value addition [2]. 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 [1]. In the present study aqueous extract of seeds, rhizomes, rind, leaves and flowers of *G. superba* showed significant antibacterial activity against *R. solanacearum* a gram negative bacterium.

Table 1: Effect of extract of different plant parts of *G. superba* on growth inhibition of bacterial wilt pathogen, *Ralstonia solanacearum*

Plant parts	Zone of inhibition (mm) / Concentration of the extract (%)					SEd	CD (P=0.05)
	Control*	25*	50*	75*	100*		
Seeds	0	1.26	1.60	1.80	1.93	0.07	0.17
Rhizomes	0	1.13	1.46	1.66	1.93	0.08	0.18
Rind	0	1.10	1.26	1.43	1.63	0.04	0.10
Leaves	0	1.20	1.33	1.46	1.70	0.05	0.12
Flowers	0	1.13	1.36	1.50	1.66	0.06	0.13

*Mean of four replications

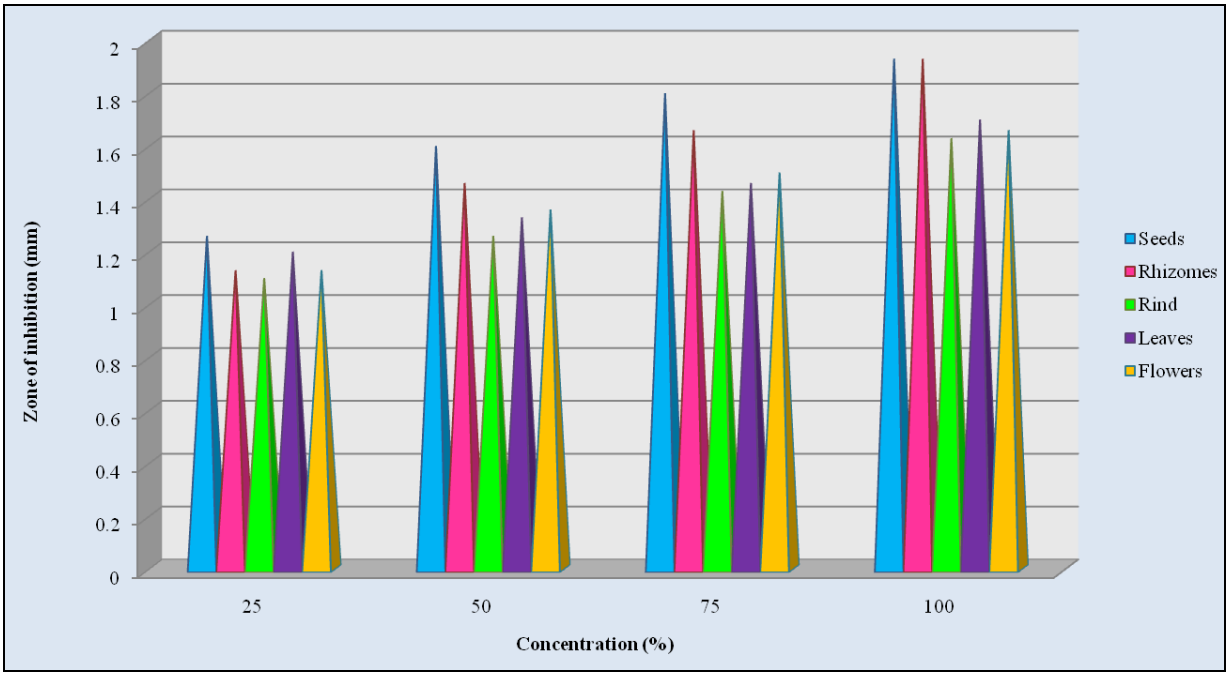

Fig 1: Effect extract of different plant parts of G superba on growth in hibition of bacterial wilt pathogen, Ralstonia solanacearum

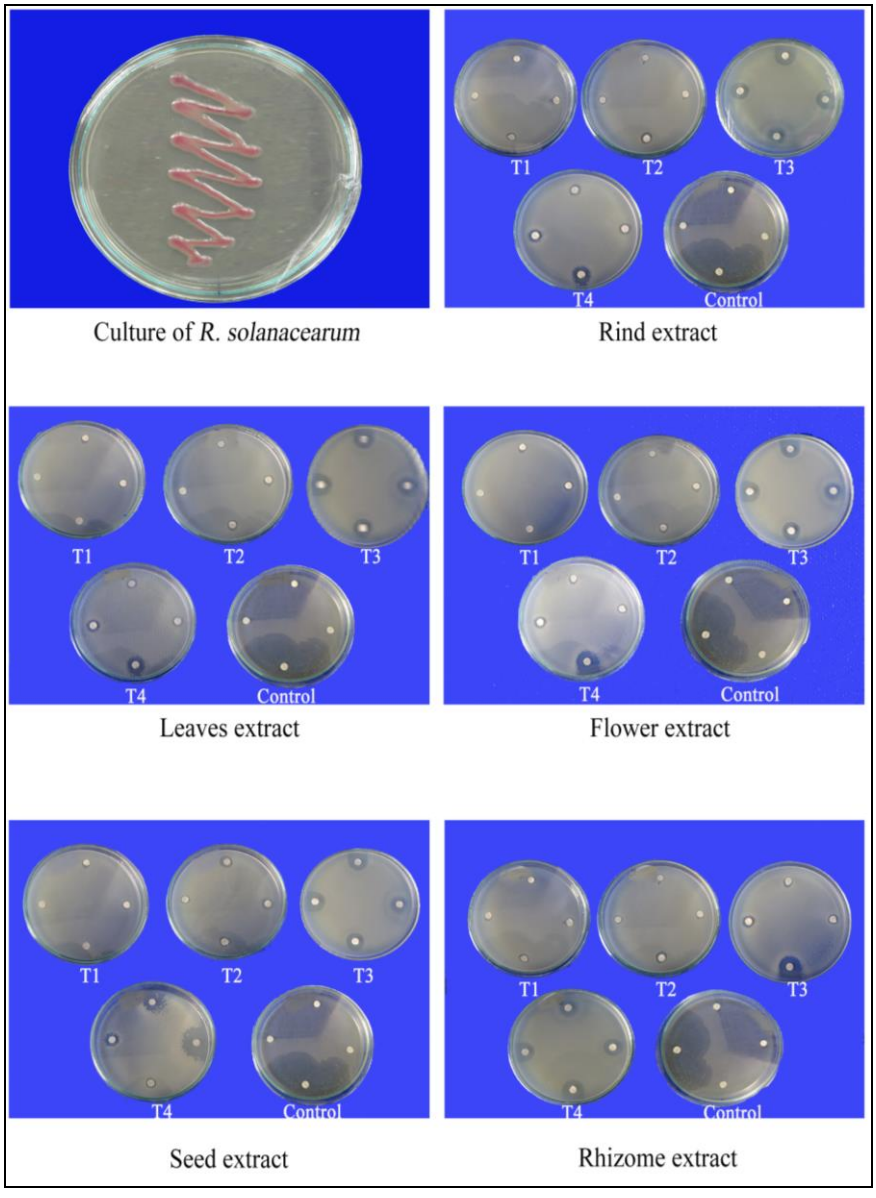

Plate 1: *in vivo* experiments using different Extract of G. Superba for bacterial Wilt Pathogen Ralstonia Solanacearum

5. Conclusion

The results of the present study undertaken on the *in vitro* bioassay of methanol extract of different plant parts of *G. superba* against *Ralstonia solanacearum*. Extracts derived from different plant parts of *G. superba viz.*, rind, rhizomes, leaves, flowers and seeds inhibited the growth of bacterial wilt pathogen, *Ralstonia solanacearum* with highest inhibition using seed and rhizome extracts.

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