

Studies on Antifungal Activity of Different Plant Parts of Glory Lily (*Gloriosa superba* L.) against Fungal Wilt Pathogen, *Fusarium oxysporum*

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ABSTRACT

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The antifungal activities of methanol extracts from different plant parts of *G. superba* viz., rind, rhizomes, leaves flower and seeds were evaluated by *in vitro*. In different extracts tested, seed extract (82.45%) was found to possess the highest mycelial inhibitory activity at 100 per cent concentration when compared to the others followed by rind (82.33%), rhizome (82.06%), leaves (77.45%) and flower (76.39%) extract was also inhibit the mycelial growth of *F. oxysporum*. At 25 per cent concentration flower extract was showed highest mycelial growth reduction followed by seed, rind, leaves and rhizomes. The results obtained in the present study indicate that seed and rhizome extracts are the greatest potential source as inhibit the mycelial growth for *F. oxysporum*.

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 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 (Deepak Acharya *et al.*, 2006). 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 (Padmapriya *et al.*, 2015). 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). Different parts of plant have

wide variety of uses especially in traditional system of medicine.

Materials and Methods

Maintenance of pure culture of root knot nematode, *Meloidogyne incognita*

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 earthen 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 °C 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 4⁰C in airtight bottles for future use (Keita *et al.*, 2001).

Effect of crude extracts on the mycelial growth of fungal wilt pathogen, *F. oxysporum in vitro* (Poisoned food technique - Schmitz, 1930)

The efficacy of different plant parts of *G. superba viz.*, rind, rhizomes, leaves, flowers and seeds at different concentrations *viz.*, 25, 50, 75, 100 % were tested against the growth of *F. oxysporum in vitro*. The required quantity of each extract was mixed with Potato Dextrose Agar (PDA) in order to get 25, 50, 75 and 100 per cent concentration of each extract separately and sterilized in an autoclave at 15lb pressure for 20 min and cooled. Twenty ml of each extract medium was immediately poured into sterilized Petri plates separately and allowed to solidify. A nine mm actively growing culture disc of *F. oxysporum* was placed at the centre of the each Petri plates separately. The Petri plates containing Potato Dextrose Agar (PDA) without extract served as control. Four replications were maintained for each treatment. It was carried out under aseptic conditions. The plates were incubated at room temperature (28 ± 2^0 C) for three days. The

diameter of the mycelial growth of the pathogen was measured in all the other treatments separately (Plate 1).

Results and Discussion

Effect of extract of different plant parts of *G. superba* on the mycelial growth of fungal wilt pathogen *F. oxysporum in vitro*

The effect of different plant parts of *G. superba* were tested against the mycelia growth of *F. oxysporum in vitro*. Among these, the seed and rind extract recorded the maximum mycelial growth reduction of 82.45, 82.33 per cent respectively over control followed by rhizome, leaves and flowers. The different concentrations of 25, 50, 75 and 100 per cent of extracts of different plant parts of *G. superba viz.*, rind, rhizomes, leaves, flowers and seed, were found significantly reduced the mycelial growth. The rhizome extract was at 100 per cent concentration recorded 10.44 mm and the

growth reduction was 82.06 per cent over control and also 75.85, 64.21 and 53.11 per cent at 75, 50 and 25 per cent respectively. The flower extract recorded less inhibition of mycelial growth at 100 per cent concentration with 14.42 mm and the growth reduction was 76.39 per cent over control and 70.49, 65.24, 60.0 per cent growth reduction at 75, 50, 25 per cent concentration. The rind extract at 100, 75, 50 and 25 per cent concentration the mycelial growth reduction was 82.33, 68.00, 67.33 and 54.33 per cent over control.

The seed extract at 100, 75, 50 and 25 per cent concentration the mycelia growth reduction has 82.45, 73.50, 67.54 and 57.28 over control. The leaves extract recorded 77.45, 67.64, 59.47 and 53.59 per cent mycelial growth reduction over control at 100, 75, 50 and 25 per cent concentrations. Among the different extracts of *G. superba*, the seed extract was effectively reduced the mycelial growth followed by rind, rhizome, leaves and flowers (Table 1; Fig. 1).

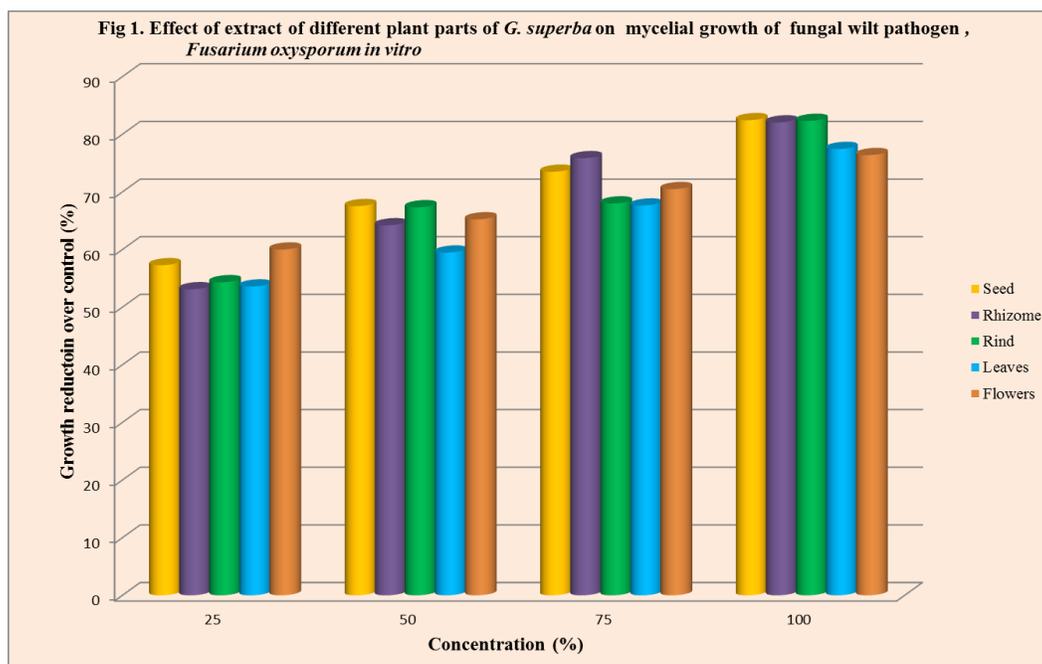


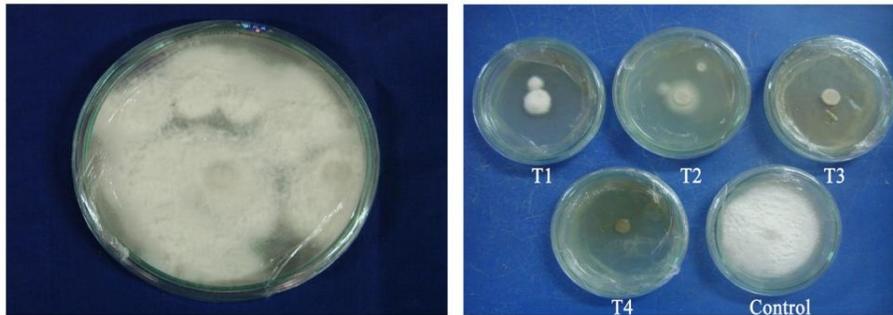
Table.1 Effect of extract of different plant parts of *G. superba* on mycelial growth of fungal wilt pathogen, *F. oxysporum in vitro*

| Treatments | Mycelial growth at 3 DAI (mm)* | | | | | | | | | |
|--|--------------------------------|-----------------------------------|---------|-----------------------------------|-------|-----------------------------------|--------|-----------------------------------|--------|-----------------------------------|
| | Seed | Growth reduction over control (%) | Rhizome | Growth reduction over control (%) | Rind | Growth reduction over control (%) | Leaves | Growth reduction over control (%) | Flower | Growth reduction over control (%) |
| T ₁ - 25% concentration | 25.84 | 57.28 | 27.82 | 53.11 | 27.46 | 54.33 | 28.40 | 53.59 | 24.46 | 60.00 |
| T ₂ -50% concentration | 19.62 | 67.54 | 21.44 | 64.21 | 19.62 | 67.33 | 24.82 | 59.47 | 21.24 | 65.24 |
| T ₃ -75% concentration | 16.00 | 73.50 | 14.60 | 75.85 | 19.24 | 68.00 | 19.86 | 67.64 | 18.00 | 70.49 |
| T ₄ -100% concentration | 10.68 | 82.45 | 10.44 | 82.06 | 10.66 | 82.33 | 13.80 | 77.45 | 14.42 | 76.39 |
| T ₅ - Distilled water (Untreated control) | 60.42 | - | 59.82 | - | 60.00 | - | 61.24 | - | 61.00 | - |
| SEd | 0.51 | - | 0.82 | - | 0.44 | - | 0.38 | - | 0.53 | - |
| CD (P=0.05) | 1.14 | - | 1.83 | - | 0.99 | - | 0.84 | - | 1.20 | - |

DAI-Days after inoculation

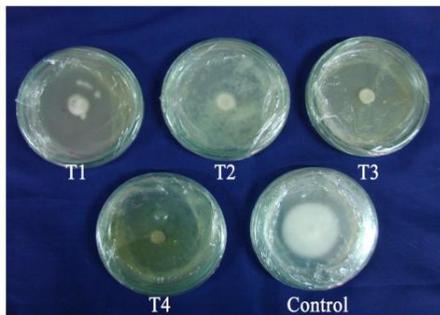
*Mean of four replications

Plate 10. *In vitro* experiments using different extract of *G.superba* for fungal wilt pathogen, *Fusarium oxysporum*

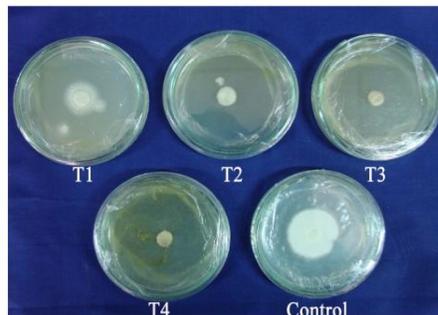


Culture of *F. oxysporum*

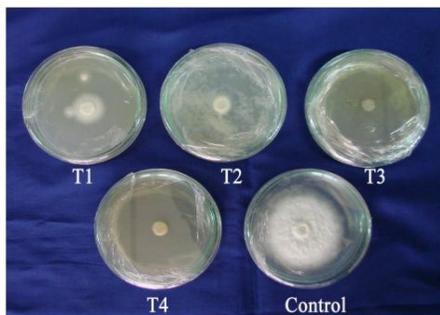
Rind extract



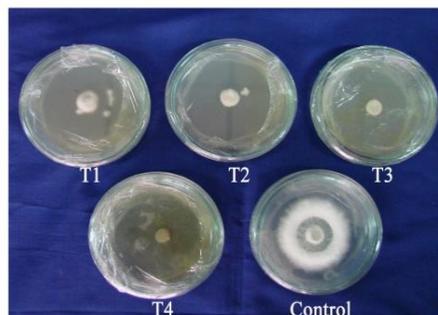
Leaves extract



Flower extract



Seed extract



Rhizome extract

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). The package used for analysis was IRRISAT version 92-1 developed by International Rice Research Institute, Biometrics Unit, Manila, Philippines.

Efficacy of *G. superba* extracts on *F. oxysporum*

Aqueous extract of several botanicals have been reported to be antifungal viz., *Thymbra spicata*, *Origanum syriacum*, *Rosemarinus officinalis*, *Foeniculum vulgare* have been reported to reduce the infestation of *Phytophthora infestans* (Soylu *et al.*,2006) *Nerium oleander* and *Pithecolobium dulce*

showed significant antifungal activity on *Bipolaris oryzae* in terms of inhibition of mycelial growth and spore germination (Joh *et al.*, 2013). A cursory perusal of literature revealed that *G. superba* has not been tested against fungal pathogens and probably the present investigations could be the first of its kind. The result of the present investigation showed that all the parts of *G. superba viz.*, seeds, rhizomes, rind, leaves and flowers exhibited antifungal activity against *F. oxysporum* by significantly reducing the mycelial growth *in vitro*. The seed extract resulted in more than 50per cent of reduction in the mycelial growth at a lower concentration of 25 per cent and the per cent reduction increase with increase in the concentration and the highest reduction was noticed in 100 per cent concentration. Among the plant parts used *viz.*, seeds, rhizomes, rind, leaves and flower extract were found to be equally effective in reducing the mycelial growth significantly and not much variation have been noticed. However, the per cent reduction was highest at 100per cent concentration. The findings are in line with that of Joh *et al.*, (2013) who obtained similar results with *Nerium oleander* and *Pithecelobium dulce* extract on *Bipolaris oryzae*.

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