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Biosuppression of Root Rot Disease of *Gloriosa superba* Caused by *Macrophomina phaseolina*

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ABSTRACT

The medicinal crop Gloriosa superba is severely affected by root rot disease caused by Macrophomina phaseolina as the pathogen is carried from soil to tubers at 30 days after planting, spreads rapidly under field conditions and later passed on to planting material during storage. Perusal of literature shows very limited research on the management of this disease and more over owing to the medicinal value of this crop, an experiment was planned with treatments including application of biocontrol agents viz., Trichoderma viride, Pseudomonas fluorescens and Bacillus subtilis in different combinations. The results showed that dipping tubers in talc based formulation of Bacillus subtilis @ 0.2% followed by drenching with B. subtilis @ 0.2% on 30 DAP was effective in managing the root rot disease which recorded root rot incidence of 15.5% with 45.7 percent reduction in disease over control with significant increase in number of pods/plant (42.5 and 38.3) and number of seeds / pod (62.7 and 56.6). Similar trend was observed in the treatment with dipping tubers in talc based formulation of P.fluorescens followed by drenching with B. subtilis @ 0.2% on 30 DAP . In both the treatments with B.subtilis and P.fluorescens, increased dry seed yield of 523.2 and 497.9 kg/ha respectively was recorded with B:C of 2.34 and 2.15 respectively compared to control (362.5 kg/ha).

Key words: *Gloriosa superba, Bacillus subtilis, Root rot, Macrophomina phaseolina.*

INTRODUCTION

The medicinal crop, *Gloriosa superba* (Glory Lily) cultivation in Tamil Nadu has extended up to 10,000 acres with major regions comprising of Tirupur, Erode, Dindugal, Karur, Ariyalur, Permabalur and Nagapattinam districts. This crop fetches high revenue and export of glory lily seeds is estimated to be around Rs.150 crores. However, lots of challenges are faced by farmers since the crop is affected by root rot pathogen *Macrophomina phaseolina* which survives in the soil as sclerotia for several years and causes disease in more than 400 hosts which makes it difficult to manage the pathogen. Under field conditions, symptoms of root rot disease is observed as sudden drying of plants accompanied with rotting of roots from 30 days after planting until harvest.

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The crop is cultivated from August to February month and after harvest the tubers are left in the field itself for the next crop. During this time, the pathogen *M.phaseolina* spreads from diseased tubers of previous crop to other tubers. More over, the field incidence is carried over to storage where the loss is high. Farmers taking up fresh planting of tubers every year purchase the tubers from other growers. Due to this, the pathogen spreads from infected tubers to unifested areas. Yield loss of 25 -30 per cent has been reported both in field and storage conditions. *M. phaseolina* a soil-inhabiting fungus, is an important root pathogen which causes dry root rot/stem canker, stalk rot or charcoal rot of over 400 plant species including *G. superba*. Management of root rot disease of *M. phaseolina* with biocontrol agents has been reported in many crops like *Coleus forskohlii* and Chickpea[9,10] In this context, the present study was formulated with an aim to use antagonistic microorganisms against *Macrophomina* root rot of *Gloriosa superba* in an ecofriendly manner.

MATERIAL AND METHODS

A field trial was taken up at Vellipalayam of Coimbatore district on the management of root rot disease of *Gloriosa superba* during 2013-2017 involving the treatments as T_1: Basal soil application of *Trichoderma asperellum* (2.5 kg/ha) + Dipping tubers in *Pseudomonas fluorescens* (0.2%) ,T_2: Basal Soil application of *T. asperellum* (2.5 kg/ha) + Drench with *P. fluorescens* (0.2%) on 30 DAP,T_3: Dipping tubers in *P. fluorescens* (0.2%) + Drench with *P. fluorescens* (0.2%) on 30 DAP, T_4: Dipping tubers in *Bacillus subtilis* 0.2% + Drench with *B. subtilis* 0.2% on 30 DAP,T_5: Dipping tubers in carbendazim (0.1%) + Drench with carbendazim (0.1%) on 30 DAP, T_6: Control. The treatments were replicated four times in Randomized Block Design. The growth parameters *viz.*, plant height, number of primary branches, number of secondary branches and number of leaves per plant were recorded on 60 DAP. The yield parameters *viz*., number of flowers per plant, number of pods per plant, number of seeds per pod, tuber yield per plant and seed yield per plant were also observed. The seed yield per hectare was recorded for each treatment and the data were statistically analyzed. Pooled data results of the four trials were analysed and presented.

RESULTS AND DISCUSSION

The results of the field experiment revealed that the treatment (T_4) dipping tubers in talc based formulation of *Bacillus subtilis* @ 0.2% followed by drenching with *B. subtilis* @ 0.2% on 30 DAP was effective in managing the root rot disease which recorded the root rot incidence of 15.5% with 45.7 percent reduction in disease over control.The treatment (T_3) with dipping of tubers in talc based formulation of *Pseudomonas fluorescens* followed by drenching with *P. fluorescens* @ 0.2% on 30 DAP also recorded lowest root rot incidence (18%) with 36.7 per cent reduction in disease over control. Treatment (T_5) with carbendazim recorded 18.9 % root rot incidence (34.9 % reduction in disease) next to biocontrol agents. (Table 1). Similarly the the treatment (T_3) dipping of tubers in talc based formulation of *Bacillus subtilis* @ 0.2% followed by drenching with *B. subtilis* @ 0.2% on 30 DAP and treatment (T_4) with dipping of tubers in talc based formulation of *P. fluorescens* followed by drenching with *P. fluorescens* @ 0.2% on 30 DAP promoted plant growth height (122.5 amd 118.9 cm respectively) that contributed for increased number of flowers per plant (51.5 and 48.8 numbers respectively) when compared to control (95 cm; 32.3 numbers of flowers respectively. Though carbendazim treatment (T_5) recorded 104. cm plant height, the number of flowers were comparatively less than the biocontrol treatments. This data clearly shows that *B. subtilis* and *P.fluorescens* is effective against root rot incidence as well promotes plant growth. Supporting the above results, the treatment (T_3) and (T_4) recorded significant increase in number of pods/plant (42.5 and 38.3) and number of seeds / pod (62.7 and 56.6). As a result, the dry seed yield recorded was maximum in treatment (T_3) dipping of tubers in talc based formulation of *Bacillus subtilis* @ 0.2% followed by drenching with *B. subtilis* @ 0.2% on 30 DAP and treatment (T_4) with dipping of tubers in talc based formulation of *P. fluorescens* followed by drenching with *P. fluorescens* @ 0.2% on 30 DAP (523.2 and 497.9 kg/ha respectively) with increased yield (30.7 % and 27.2% respectively) compared to control (362.5 kg/ha). This reflected in maximum B:C ratio in treatments T_3 and T_4 (B:C of 2.34 and 2.15 respectively). Though the fungicide treatment also gave good disease control (18.9%) this treatment recorded only 434.4 kg dry seed yield with B: C of 1.85.

The results imply that prophylactic treatment of dipping the with talc based formulation of *B. subtilis or P. fluorescens* is effective in managing the root rot incidence. Though the fungicide carbendazim gave good control over root rot disease initially, the root rot incidence higher than the bacterial biocontrol treatments. This clearly shows that both *Bacillus subtilis* and *Pseudomonas fluorescens* application as dipping of tubers responded for reduction in root rot incidence which might be due to multiplication of bioontrol agents in the tubers and in rhizopshere soil and further drenching at 30 DAP protected the crop against *Macrophomina* root rot incidence. Improved growth and yield parameters in these treatments further prove the plant growth promoting nature of *B. subtilis* and *P. fluorescens*

To combat this disease, biological control is a potential non chemical organic means for plant disease management in sustainable agriculture. Rhizosphere associated microorganisms with antifungal properties may be useful candidates as biological control agents as their root colonization depends on certain organic acids secreted by host plants. For the biological control of *M. phaseolina*, antagonistic bacteria and fungi have been investigated[2,8] where they reported that combined application of two bacilli isolates reduced the *Macrophomina* charcoal rot of maize by 54%. The members of the genus Bacillus are very promising against soil borne plant parasitic fungi as they are endospore forming ,wide spread in nature, saprophytic, quickly growing, easily culturable, can produce large amounts of cells with long life time with high population densities in soils .Strains from the genus Bacillus especially *B. subtilis* are ideal candidates for biological control strategies because they have properties consistent with commercial development of microbes for use in sustainable agriculture[4]. Since the disease is caused by soil borne fungus, endospore forming rhizosphere associated bacteria may play a significant role in reducing the inoculum level in the soil. They also secrete a variety of biologically active compounds for improving plant growth and a broad spectrum of antimicrobial compounds against phytopathogens to induce host systemic resistance[5,7]. Several strains of of *B. subtilis* have been reported to be capable of forming multicellular structures or biofilms in rhizosphere and roots to enhance the plant growth[3]. This mechanism would have protected the Glory lily crop against *M.phaseolina.* In our study, *P.fluorescens* also offered good crop protection against *M.phaseolina* which might be due to the efficient colonization in the rhizopshere followed by production of antibiotics, siderophores, volatile compounds like HCN, ammonia, induction of systemic resistance[6]. Basal application of *P. fluorescens* and *T. viride* combined with foliar spray of 0.5% zinc sulphate resulted in 60 per cent disease reduction of *M. phaseolina* incidence in Glory lily[1].

Glory lily being medicinal crop, certainly, inclusion of plant growth promoting rhizospheric biocontrol agents like endospore forming *B. subtilis* and siderophore producing *P.fluorescens* offers good scope for management of Glory lily and serves best in suppressing the inoculum levels of *M. phaseolina* in soil.

Table 1: Effect of treatments on disease incidence and yield parameters

Treatments	*Pooled mean of Disease incidence		*Pooled mean of growth parameters		*Pooled mean of yield parameters				B:C
	Incidence (%)	Per cent reduction over control	Plant height (cm)	No. of flowers/ plant	No. of pods/ plant	No. of seeds/ pod	Dry seed yield /ha	Increased yield over control (%)	
T_1	20.1 (19.3)	29.6	108.3	41.3	31.6	51.8	448.3	18.7	2.02
T_2	20.7 (20.3)	27.8	109.9	42.3	31.6	49.9	438.4	17.3	1.97
T_3	18.0 (18.5)	36.8	118.9	48.8	38.3	56.6	497.9	27.2	2.15
T_4	15.5 (16.5)	45.7	122.9	51.5	42.5	**62.7**	523.2	30.7	2.34
T_5	18.9 (18.6)	34.9	104.0	39.6	29.8	50.0	434.6	16.2	1.85
T_6	28.7 (25.7)	-	95.0	32.3	22.4	44.2	362.5	-	1.63
SEd	1.03		8.6	1.23	1.21	1.77	9.2		
CD (P=.05)	2.15		12.5	2.4	2.6	3.74	19.8		

*** Data represents pooled mean of the experiments conducted 2014-15, 2015-16, 2016-17 and 2017-2018**

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Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration between all authors. Authors GT, BM and KR designed the study, performed the experiments and analysed the results. Authors GT and KR wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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