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SYMPTOMATOLOGY, CHARACTERISATION AND MANAGEMENT OF ROOT ROT DISEASE OF GERBERA

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ABSTRACT
A sampling survey conducted in Thrissur district of Kerala on the occurrence of root rot disease in gerbera revealed that the disease was confined only to protected conditions. The disease was observed in a hydroponic unit which was more pronounced in July-August with a percent disease intensity of 69.44. Symptoms like stem or collar rot resulted in fatal infection with complete death of the plant. Pathogenicity of the isolate was carried out by soil inoculation and Microdroplet inoculation technique (MDIT). Cultural, morphological and molecular characteristion along with the description of symptom of root rot disease confirmed the identity of the pathogen as *Phytophthora cryptogea.* Sensitivity of the pathogen with contact, systemic and combination fungicides *viz.,* Bordeaux mixture, copper hydroxide 77WP (Kocide), propineb 70WP (Antracol), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), carbendazim (12%) + mancozeb (63%) (Saaf), tebuconazole 250EC (Folicur) revealed cent percent inhibition with all the three concentrations tested. Among the bioagents tested against *Phytophthora cryptogea*, a percent inhibition of 68.44 percent was observed with *Trichoderma viride* and 44.4% with *P. fluorescens. In vivo* study revealed that various treatments like propineb, cympxanil + mancozeb, carbendazim + mancozeb and copper hydroxide could reduce the disease incidence compared to the treatment with hexaconazole and *T. viride.*

KEYWORDS: Gerbera, *Phytophthora cryptogea*, MDIT, *T. viride, P. fluorescens.*

INTRODUCTION

Cut flowers, one of the most profitable crops, is nowadays emerging as an important industry which furnish the need of the demand in the overseas market. Among them, *Gerbera jamesonii* Bolus, isa very attractive, commercial cut flower crop which ranks fourth in the international cut flower trade (Sujatha *et al.,* 2002). Gerbera grows well in open tropical and subtropical condition as it requires partial shade and hence flourishes well under poly houses (Singh, 2006). However, under protected conditions, fungal diseases including root rots were more prevalent which restricts the commercial cut flower production especially in Kerala where the climatic condition is highly congenial for the occurrence of the disease. Hence, the present objective of the work is to characterize and identify the root rot disease of gerbera and study its management.

MATERIALS & METHODS

Survey and collection of diseased samples

A purposive sampling survey was conducted in different regions of Thrissur district *viz.,* Vellanikkara, Madakkathara and Chalakudy during rainy (July-August), winter (November- December) and summer (March-April) for occurrence of root rot disease in gerbera. During the survey, per cent disease incidence was recorded and the diseased samples collected were subjected to isolation of root rot pathogen under aseptic condition.

Symptomatology studies

Symptomatology of root rot disease was studied under natural and artificial condition. Sampling survey conducted in different areas aided in observing symptom development of the root rot disease under artificial condition whereas Mycelial Droplet Inoculation Technique (MDIT) (Munaut *et al.,* 1997) was used to study the symptom development under artificial condition. An inoculum density of 10^5 spores/ml of spore suspension was prepared and inoculated into the rhizosphere and phylloplane region of the three month old gerbera plants and the plants were observed for the symptom development.

Isolation and pathogenicity studies

Standard isolation procedure was used for isolation of pathogen from diseased samples collected during the survey. The collected samples brought into laboratory were washed in running tap water and small bits were cut consisting of both healthy and infected portion using sterile blade. The bits were surface sterilised using sodium hypochlorite (1%) and rinsed with sterile water thrice under aseptic condition. Later, the blot dried bits were inoculated in Petri plate containing sterilised, solidified Potato Dextrose Agar (PDA) media and kept for incubation at room temperature (26 ± 2^oC).

Mycelial Droplet Inoculation technique (MDIT) and soil inoculation method was performed simultaneously to carry out pathogenicity test of the isolate against three month old gerbera seedlings. In MDIT, Petri plate containing ten day old isolates were used to prepare 10^5 cfu ml^{-1} of spore suspension and sprayed onto foliage of the seedlings. In the case of soil inoculation method, the isolate was mass multiplied in autoclaved carrot discs (100g) and the inoculum was added @ 5g in the root zone after giving an injury and incubated for symptom development (Sumbula, 2015). The pathogen was re-isolated from artificially inoculated plant parts and the cultural and morphological characters were compared with the original one to prove the pathogenicity of root rot pathogen.

Characterisation and identification of pathogen

The root rot pathogen isolated through standard protocol was subjected to cultural, morphological and molecular characterisation. Cultural characters of the isolate was studied after aseptically growing in PDA. Variations in colony characteristics, growth pattern and growth rate were the cultural characters recorded. Morphological characters *viz.*, type of mycelium, branching pattern, type of spores, their shape, size, L/B ratio, septal distance and presence of sexual structures of the pathogen were observed under microscope. Microphotographs and measurements of fungal structures were taken with the assistance of Ultrascope software.

Cultural and morphological characters recorded were compared with the description given in CMI Descriptions of Pathogenic Fungi and Bacteria and the isolate was tentatively identified upto genus level. Identity of the pathogen was further confirmed upto species level from National Centre for Fungal Taxonomy (NCFT), New Delhi. Moreover, the isolate was subjected to molecular characterisation at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing. Sequence analysis and nucleotide homology of pathogen was analysed through the BLASTn programme of NCBI (http:// ncbi.nlm.nhm.gov/blast) and confirmed the identity of the pathogen upto species level.

Sensitivity of the pathogen with fungicides and biocontrol agents

Efficacy of fungicides and bioagents were tested against root rot pathogen under aseptic conditions. Poison food technique (Zentmeyer, 1955) and dual culture method (Skidmore and Dickinson, 1976) was used for testing the efficacy of chemical fungicides and biocontrol agents respectively. The per cent inhibition of mycelial growth was calculated in both the technique by using the formula suggested by Vincent (1927).

$$\text{Per cent inhibition of growth} = \frac{C - T}{C} x\, 100$$

C= Growth of fungus in control (mm), T= Growth of fungus in treatment (mm)

***In vivo* management of root rot disease**

Promising treatments of chemicals and bioagents obtained under *in vitro* screening against root rot pathogen were used for management of root rot disease under pot culture conditions. Tissue culture plants of gerbera variety, Natasha were raised in growbags of size 35×20×20 cm which consisted of 1:1:1 mixture of soil, sand and cowdung. CRD design with 7 treatments and 5 replications were followed for the experiment.

Three month old gerbera plants grown in net house were challenge inoculated with the pathogen by Micro Droplet Inoculation Technique (MDIT) and by soil inoculation with mass multiplied carrot disc as described in pathogenicity test. Immediately after symptom development, PDI and PDS were recorded and treatments were given as soil drench as well as foliar spray. First treatment was given on symptom appearance and subsequently the second at ten days after first application. However, in the case of biocontrol agent, prophylactic application of *Trichoderma viride* in the respective treatments was given ten days prior to challenge inoculation of the pathogen. Observations on disease incidence and disease severity were also recorded at 10 days interval after each treatment.

RESULTS & DISCUSSION

Survey and collection of diseased samples

The purposive sampling survey on the occurrence of root rot of gerbera conducted in three regions *viz.*, Vellanikkara, Madakkathara and Chalakudy region of Thrissur district revealed that only in Vellanikkara region, the crop was maintained in a hydroponic system where the disease incidence was more pronounced in July-August with a per cent disease intensity of 69.44. Recent evidences suggest that *Phytophthora* rot is the most widespread and destructive disease of many ornamental crops (Padghan and Gade, 2006; Ampuero *et al.,* 2008 and Rajendran *et al.,* 2014). In this study, it was found that the disease occurred during monsoon season in Thrissur district and the results thus obtained are in line with the report by Ampuero *et al.* (2008) where they stated that weather conditions like temperature ranging from 15-30^{o}C and long, frequent soil saturation periods as in the case of hydroponics, favour the development of *Phytophthora* rot.

Symptomatology of root rots disease

Symptoms like stem or collar rot resulted in fatal infection with complete death of the plant. The disease was initiated as dark brown water soaked lesion on the stem and later spread through collar portion extending upto root hairs. Foliar yellowing and defoliation were the general aerial symptoms noticed in the affected plants (Plate 1). These findings are in agreement with Hyeong *et al.* (1996) who detailed the similar symptoms of root rot disease in gerbera.

PLATE 1. Root rot symptom

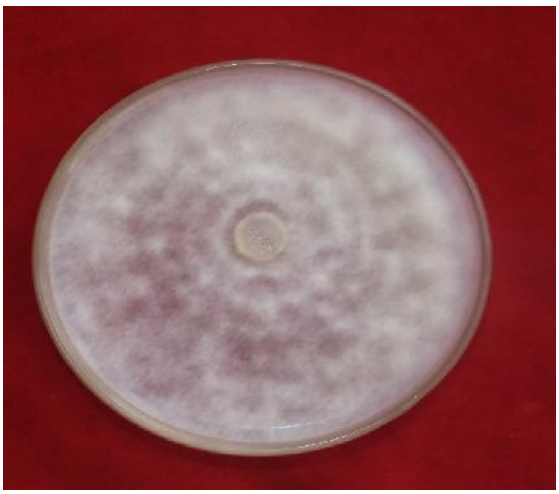


PLATE 2. *Phytophthora cryptogea* grown in PDA

Isolation and pathogenicity studies

The pathogen was isolated by standard isolation protocol and grown in PDA media and for testing pathogenicity, soil inoculation technique and MDIT was employed. Artificial inoculation of spore suspension (10^5 cfu/ml) aroused symptom development of root rot disease when applied into rhizosphere. The pathogenic nature of the isolate was further proved by re-isolating the isolate and by comparing with the cultural and morphological characteristics. Similar to that of the present study, Ampeuro *et al.* (2008) observed isolates of *Phytophthora cryptogea* pathogenic on petunia after crown and root inoculation with mycelial fragments and after soil inoculation with zoospores.

Characterisation and identification of pathogen

The isolate produced uniformly dense white cottony growth on PDA (Plate 2). The hypha was branched, hyaline, coenocytic with oval to obpyriform sporangia, nonpapillate borne either terminally or laterally on the sporangiophores in a simple sympodial fashion. Dimension of sporangia ranged from 32.5-57.5 x 25-35 µm (Plate 3). These characters are in agreement with that reported by Erwin and Ribeiro (1996) in gerbera. Cultural and morphological characters of the pathogen revealed the identity of the pathogen upto genus level as *Phytophthora* sp. Confirmation about the identity of pathogen upto species level was proved from National Centre for Fungal Taxonomy (NCFT), New Delhi. Moreover, Molecular characterization of the pathogen outsourced by Rajiv Gandhi center for Biotechnology (RGCB), Thiruvananthapuram putforth the final confirmation regarding the identity of the pathogen. Sequence homology observed for *Phytophthora* isolate in BLASTn analysis revealed that the isolate showed cent per cent homology with strains of *Phytophthora cryptogea* and *Phytophthora drechsleri* (Fig.1.). However, based on morphotaxonomy and molecular characterization, the isolate was finally confirmed as *Phytophthora cryptogea* and the ITS sequence of the isolate was deposited in the Gen Bank with accession no. KY285081.1.

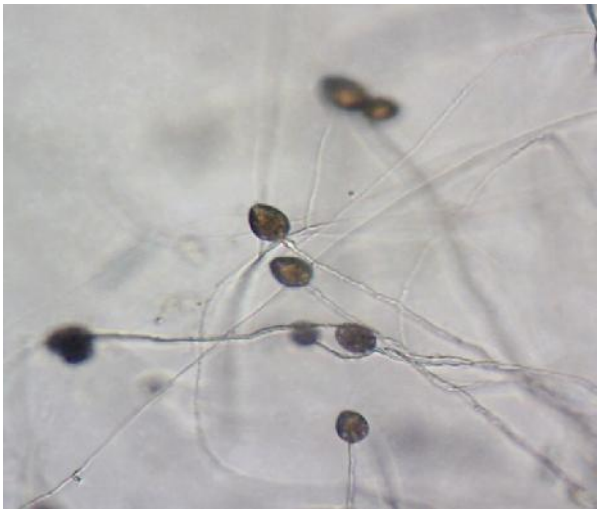


PLATE 3. Sporangiospores of *P. cryptogea* (100x)

PLATE 4. A view of the experimental plot

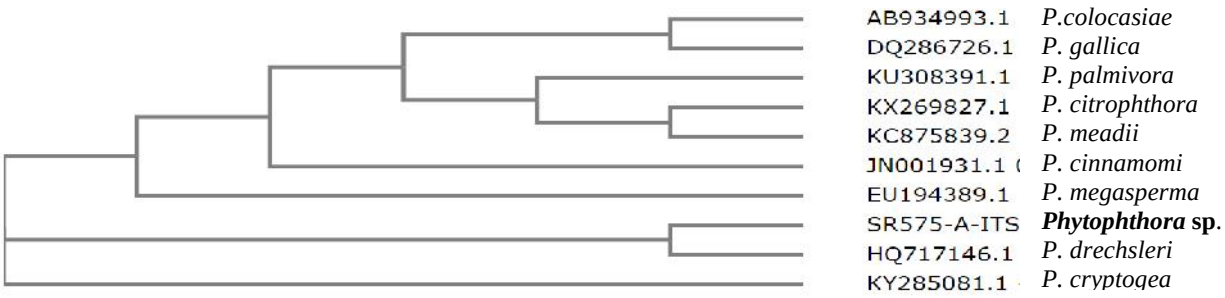


FIGURE 1. ITS sequence based tree of 10 different species of *Phytophthora* constructed using Neighbour joining method

Sensitivity of *P. cyrptogea* with fungicides and biocontrol agents

In vitro evaluation of nine chemicals against *Phytophthora cryptogea* recorded cent per cent inhibition with six chemicals *viz.*, propineb 70WP (Antracol), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), carbendazim (12%) + mancozeb (63%) (Saaf), copper hydroxide 77WP (Kocide), tebuconazole 250EC (Folicur) and Bordeaux mixture at all the three concentrations tested (Table 1). The above findings are in validation with Shashidhara *et al.* (2008) and Kaur *et al.* (2009) where they observed the efficiency of Bordeaux mixture and cymoxanil + mancozeb on *Phytophthora* sp. Cent per cent inhibition of carbendazim (12%) + mancozeb (63%) (Saaf) was also confirmed by the study of Mtasa *et al.* (2014). Moreover, cent per cent inhibition was also recorded with 0.1 and 0.15 % of hexaconazole 5EC (Mega master) and at the highest concentration of pyraclostrobin 20WG (Headline) whereas difenoconazole 25EC (Score) showed least inhibition of the pathogen. According to Lebrun (2002), chemicals preferred to *Phytophthora* root rot in gerbera were grouped under phenyl amides like furalaxyl, fosetyl Al, combination of oxadixyl and cymoxanil.

TABLE 1. *In vitro* evaluation of fungicides against *Phytopthora solani*

Sl. No.	Fungicide	Conc. (%)	*Per cent Inhibition
1.	Carbendazim 12% +Mancozeb 63% (Saaf)	0.15	100 (10)[a]
		0.2	100 (10)[a]
		0.25	100 (10)[a]
2.	Cymoxanil 8% +Mancozeb 64% (Curzate M-8)	0.15	100 (10)[a]
		0.2	100 (10)[a]
		0.25	100 (10)[a]
3.	Propineb (Antracol 70WP)	0.25	100 (10)[a]
		0.3	100 (10)[a]
		0.35	100 (10)[a]
4.	Pyraclostrobin (Headline 20WG)	0.05	55.33 (7.44)[e]
		0.1	71.56 (8.46)[b]
		0.15	100 (10)[a]
5.	Copper hydroxide (Kocide 77WP)	0.15	100 (10)[a]
		0.2	100 (10)[a]
		0.25	100 (10)[a]
6.	Hexaconazole (Mega master 5EC)	0.05	63.23 (7.95)[c]
		0.1	100 (10)[a]
		0.15	100 (10)[a]
7.	Tebuconazole (Folicur 250EC)	0.1	100 (10)[a]
		0.15	100 (10)[a]
		0.2	100 (10)[a]
8.	Difenoconazole (Score 25EC)	0.02	39.16 (6.26)[f]
		0.05	46.44 (6.82)[e]
		0.1	60.55 (7.78)[d]
9.	Bordeaux mixture	0.5	100 (10)[a]
		1	100 (10)[a]
		1.5	100 (10)[a]
	CD		0.525

***** Mean of the three replications
In each column figure followed by same letter do not differ significantly according to DMRT
√x + 0.5 transformed values are given in parantheses

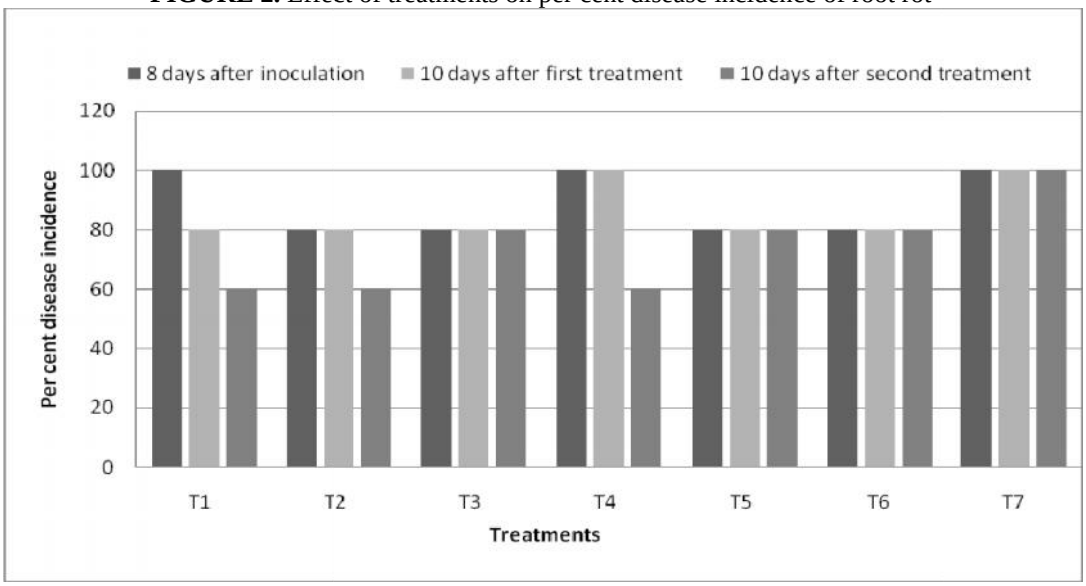
Phytophthora cryptogea when evaluated against the biocontrol agents recorded a per cent inhibition of 68.8 and 44.4 with *Trichoderma viride* and *Pseudomonas fluorescens.* Perusal of the literature suggests that the present findings are

in agreement with the reports putforth by the other researchers. Shashidhara *et al.* (2008) and Mir *et al.* (2011) elucidated the efficacy of different strains of *Trichoderma* spp against *Phytophthora* by overgrowth mechanism of antagonism. Anith *et al.* (2002) and Paul and Sarma (2006) confirmed the efficacy of different strains of *P. fluorescens* against *Phytophthora capsici.* Similar results of inhibition by different strains of *P. fluorescens* and *Trichoderma* sp. were previously reported by several authors (Zhang *et al.*, 2010; Hernandez *et al.*, 2011; Jagtap *et al.*, 2012 and Sumbula, 2015).

***In vivo* management of root rot disease**

The results of the *in vivo* experiment against *Phytophthora cryptogea* after challenge inoculation revealed that, among all the treatments, cent percent disease incidence was noticed in treatments T1 (propineb 70WP), T4 (copper hydroxide 77WP) and T7 (control) while the other treatments recorded a PDI of 80 per cent (Fig. 2.) (Plate 4). However, after ten days of first and second spray, a comparative reduction in the disease incidence was noticed after two treatment sprays. The data revealed that treatments T1 (propineb 70WP) (0.3%), T2 (cymoxanil (8%) + mancozeb (64%) (0.2%), T3 (carbendazim (12%) + mancozeb (63%)) (0.2%) and T4 (copper hydroxide 77WP) (0.2%) could reduce the incidence to some extent whereas T5 (hexaconazole 5EC) (0.1%) and T6 (*Trichoderma viride*) (2%) were incompetent in the management of the disease. With regards to the ineffectiveness of *T. viride* under field conditions, the main reasons attributed is that, under *in vivo* condition, the establishment of the antagonists might have been affected by competition with the other soil pathogens. It is well known that biological control depends on the establishment and maintenance of a threshold population of antagonists in soil. Hence, more care should be taken to develop better delivery techniques and to understand more about the soil ecology so that the antagonist activity can be enhanced. Contradictory to the above study Fravel (2005) reviewed several biocontrol products against different ornamental diseases under green houses and nurseries. Similarly, Grasso *et al.* (2003) and Gade (2012) evaluated the efficiency of *Trichoderma viride* against *Phytophthora* root rot in gerbera and citrus respectively. The present finding is in agreement with the studies of previous workers who reported the efficacy of chemical fungicides in the management of *Phytophthora* foot rot (Kaur *et al.*, 2009; Meyer and Hausbeck, 2013; Sumbula and Mathew, 2015).

FIGURE 2. Effect of treatments on per cent disease incidence of root rot



T1- Propineb 70WP (0.3%); T2- cymoxanil 8% + mancozeb 64% (0.2%);
T3- carbendazim 12% + mancozeb 64% (0.2%); T4- copper hydroxide 77WP (0.2%);
T5- hexaconazole 5EC (0.1%); T6- *Trichoderma viride* (2%); T7- Control

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