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Biological Management of Chrysanthemum Wilt (*Fusarium oxysporum* f.sp. *chrysanthemi*)

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A B S T R A C T

Chrysanthemum flower (*Chrysanthemum morifolium* Ramat.) is popularly designated as "Queen of the east", or autumn queen (as its bloom in November-December) **(**Shibata, 2008**;** Teixeira *et al.,* 2013). In terms of global ornamental market value, its stands to the second position after rose. Vascular wilt caused by *Fusarium oxysporum* f. sp. *chrysanthemi* is one of the most devastating disease attacking all the growth stages from nursery to flowering(Pinto *et al.,* 2010).Bio-efficacy of five bio-control agents namely *Trichoderma harzianum, T. hamatum, T. viride, T. asperellum and T. virens* were evaluated *in-vitro* against *F.oxysporum* f.sp. *chrysanthemi*, using dual culture technique on PDA and also pot condition. However, *T. viride* was found to be most effective antagonist in lab followed by *T. harzianum, T.hamatum, T. virens* and *T. asperellum.* Highest percent growth inhibition of pathogen recorded by *T. viride* (69.53%) and *T. harzianum* (63.60%). Meanwhile *T. asperellum* (36.33%) showed least inhibition. Under greenhouse condition *T. viride* was found to be most effective and resulted the lowest disease severity (32.0 %) with highest inhibition(59.05 %) in compared to control, which was followed by *T. harzianum, T. virens* and *T. hamatum.* However, the soil treatment with *T. asperellum* was found to be least effective and resulted highest disease severity (60.0 %) of plants with lowest percent inhibition (29.04%).

Introduction

Chrysanthemum is one of the most leading commercial floriculture crop, grown for cut and loose flowers throughout the globe. The word chrysanthemum is derived from the Greek words 'chrysos' and 'anthemon' or 'anthos' (flower) (Alam *et al.,* 2020). This flower is botanically named as *Chrysanthemum morifolium* Ramat, which belongs to the family Asteraceae. In field the crop is challenged by a number of plant pathogens. Among all known pathogens, *Fusarium oxysporum* f. sp. *chrysanthimi* (F*oc*)causing wilt found to be the most destructive one in all growing regions

including India (Singh *et al.*, 2014). This facultative saprophyte can survive in soil up to six years in the absence of susceptible host, and can attack any growth stage of the plant(Pinto *et al.*, 2010).It produces chlamydospores in harsh condition which helps in to withstand prevailing unfavorable environmental conditions (Singh *et al.*, 2014, Booth 1971, Nash *et al.*, 1961). Eradication of this pathogen is nearly impossible if once established in the field, due to multiple reasons, as; wide host range, saprophytic survival, resistant resting structures, break of resistance in newly developed resistant varieties, etc. At the same time, injudicious use of chemicals in farming activities is adding a great surplus to global threat of pollution. Hence, there is a strong need to adopt an ecofriendly approach to manage this threat. Therefore, the present investigation has been undertaken to figure out the best possible biocontrol agent against this devastating pathogen.

Materials and Methods

The present studies were carried out under laboratory and pot conditions at during *kharif* season of 2017-18, at Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh (India).

Isolation and identification of the pathogen

A survey was conducted during *kharif* season (2017-18) in nearby areas of Aligarh Muslim University. The diseased plant showing typical wilting symptoms were collected in polythene bags and brought to the laboratory for further isolation and identification of pathogen. Collected plantswere washed thoroughly under the running tap water to remove the adhering soil and debris. The root and basal stem were cut into small piece and rinsed in distilled water.

These pieces were surface sterilized with sodium hypochloride (0.5 – 1.0 %) followed by 2-3 washing with distilled water to remove excess of NaOCL. Two to three surface sterilized pieces were placed on solidified potato dextrose agar (PDA) poured in previously sterilized petri plates of 90 mm diameter, aseptically in laminar flow. The inoculated petri plates were further incubated in the Biological Oxygen Demand(BOD) incubator at 25±2°C. These plates were further observed daily for fungal growth, if any, was repeatedly sub-cultured on PDA slants to obtain pure culture. Thereafter, isolated fungus was identified and confirmed on the basis of their cultural characteristics appeared in petri plates and morphological characteristics temporary slides under the compound microscope.

Pathogenicity detection

The pathogenic behavior of pathogen was tested in sterilized as well as unsterilized soil on local cultivar of chrysanthemum. The vegetative propagative material (rooted cuttings) was collected from local market and the inoculum of pathogen was prepared on Potato dextrose broth. Inoculation is done by cotton swab method on one-month old plants. The pathogen was re-isolate after 21 days of inoculation, from the inoculated plants.

Efficacy of *Trichoderma* spp. against *F. oxysporum* f. sp. *chrysanthemi*

In-vitro

Bio-efficacy of five different species of *Trichoderma* was evaluated *in-vitro* against pathogen. All the five species were provided by Department of Plant Protection, AMU (Aligarh). For identification of species, cultural and morphological studies were performed. Antagonistic activity of all *Trichoderma* was done against pathogen dual

culture techniques (Chet *et al.*, 1982; Bell *et al.*, 1982). 5 mm disc of pathogen and bio-agent were placed opposite to each other equidistantly from the periphery of 90 mm petri plate having solidified PDA. Petri plates were incubated in BOD incubator at 25±2°C, until the petri plate with control treatment covered with the mycelial growth. All the treatments were replicated thrice. The percent inhibition was calculated by using formula given by Singh & Vijay (2011) are as follows:

$$\% \text{ inhibition over control} = \frac{C-T}{C}$$

C = Growth of fungus in the control Petri dishes.
T = Growth of fungus in the Dual Culture Petri dishes.

Pot condition

Reevaluation of all the prior tested *Trichoderma* spp. (tested in dual culture) was done in pot condition under green house. In this experiment plastic pots of 9 cm were filled with the mixture of well sterilized soil and FYM in the ration of 9:1 and 10 days old rooted suckers from nursery were planted in the pots. Inoculation was done when the suckers attain an age of 35 days. To inoculate healthy plants inoculum of pathogen (spore suspension of 10^8 CFU) was applied at the basal part of plant @ 10 ml/kg of soil and then bio-agents were applied to the soil 10g/kg of soil one week after inoculation. Each replication was replicated thrice and watered daily. The observation of diseased plant showing yellowing, wilting mortality was recorded after 90 days of transplanting by using of disease severity scale ranging 0-5 (Lori *et al.*, 2008).

Disease scale

Disease symptoms using a wilt severity index based on the scale,

0 = no symptoms, healthy plant;
1 = up to 25% light chlorotic foliage;
2 =25-50% light chlorotic foliage;
3 = severe chlorotic foliage plus up 10% necrotic leaves on 51-75% of the plant;
4 = necrotic foliage on 11-50% of the plant;
5 = dead plant.

$$percentdiseaseindex = \frac{\text{Sum of all disease rating}}{\text{Total number of rating X Maximum disease grade}} \text{ X } 100$$

$$\% \text{ inhibition} = \frac{C-T}{C} \text{ X } 100$$

Where,
C= control plant.
T= Treatment plant.

Results and Discussion

In-vitro (in dual culture test)

Bio-efficacy of five bio-control agents namely *Trichoderma harzianum, T. hamatum, T. viride, T. asperellum and T. virens* were evaluated *in-vitro* against *Fusarium oxysporum* f.sp. *chrysanthemi*, using dual culture technique on PDA. The observations, thus, recorded on radial growth of antagonists and test fungus is represented in fig. 1. It is evident from the observations that all bio-control agents significantly inhibited the radial growth of pathogen in compared to control. However, *T. viride* was found to be most effective antagonist followed by *T. harzianum, T.hamatum, T. virens* and *T. asperellum* resulted in 21 mm, 24 mm, 29 mm, 32.33 mm and 35 mm growth of the test pathogen, respectively. Highest percent growth inhibition of pathogen recorded by *T. viride* (69.53%) and *T. harzianum* (63.60%) (fig. 1). Meanwhile *T. asperellum*(36.33%) showed least inhibition. Interestingly, the bio-efficacy of all these antagonists varied significantly in radial growth inhibition in of *F. oxysporum* f.sp.*chrysanthemi.*

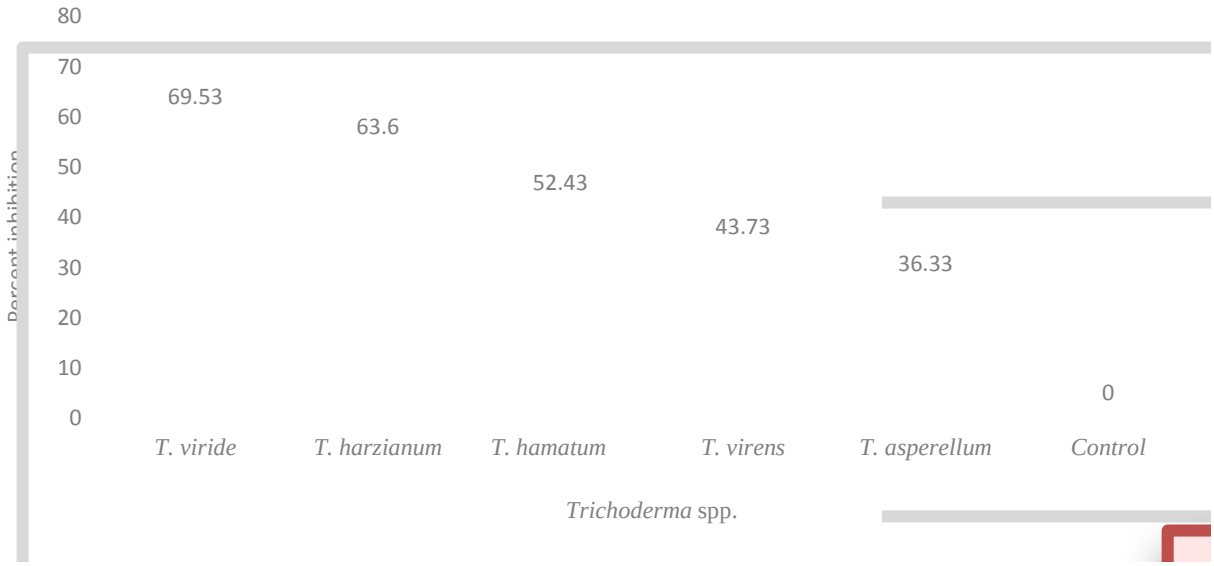

Fig.1 Efficacy of *Trichoderma* spp. against *F. oxysporum* f. sp. *chrysanthemi* in vitro condition

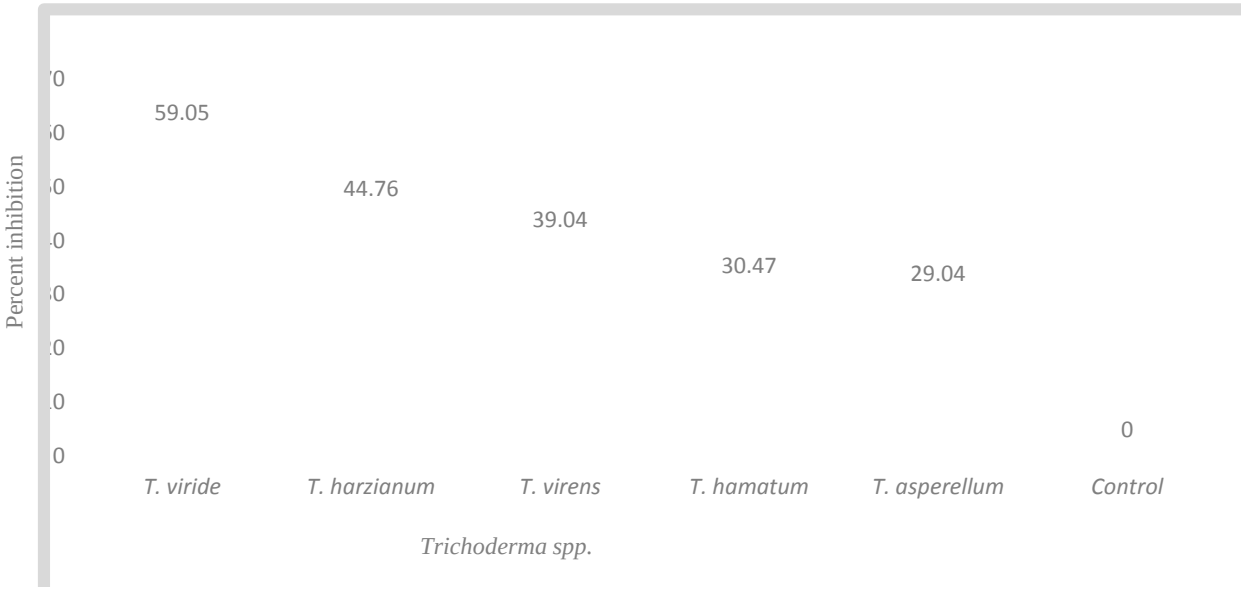

Fig.2 Efficacy of *Trichoderma* spp. against *F. oxysporum* f. sp. *chrysanthemi* in pot condition

Pot condition

Bio-efficacy of five *Trichoderma* sp. namely *T. harzianum*, *T. viride*, *T. asperellum*, *T. virens* and *T. hamatum*, were also evaluated in pots under greenhouse condition. All the *Trichoderma* spp. were applied in soil after one week of pathogen inoculation. A similar effectiveness of *T. viride* was recorded in term of reduced disease severity (32.0 %) with highest inhibition (59.05 %), i.e., lowest among all the tested species, which was followed by *T. harzianum*, *T. virens* and *T. hamatum*. However, the soil treatment with *T. asperellum* was found to be least effective and resulted highest disease severity (60.0 %) of plants with lowest percent inhibition (29.04%) (Fig. 2).

The present investigation, thus, suggests the antagonistic potential of *Trichoderma* spp. which could be exploited further for the management of *F. oxysporum* f. sp. *chrysanthemi.* Although, efficacy of *Trichoderma* spp. has earlier been reported against *F. oxysporum* by several other workers under in-vitro condition. Singh & Vijay (2011)were evaluated seven isolates of *T. harzianum*(Th) namely T1, T2, T3, T4, T5, T6 and T7 against *F. oxysporum*f. sp. *chrysanthemi*. The isolates effectively inhibited the mycelial growth of pathogen and recorded maximum inhibition with T3 isolate i.e. 66.0%.Bahatnagar H. (1986) were evaluated antagonist *Trichoderma harzianum, T. coningi* and *T. viride* were highly antagonistic to *F. udum* under in vitro. S. Sundaramoorthy and P. Balabaskar (2013) reported that ANR-1 inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* to an extent of 53.00 per cent over control. This was followed by KGI-3 (38.12 %), RTM-5 6 (31.11%) and KPI-9 (27.22 %).

The efficacy of *Trichoderma* spp. had earlier been also reported against *Fusarium oxysporum* under pot condition by Singh & Kumar (2011)Among the 3 isolates maximum disease control was recorded with T3 (91.0%) and minimum with T5 (81%). Disease control provided by T3 isolate was significantly higher among all the Trichoderma isolates, Locke *et al.,* (1985) a wild-type isolate of *Trichoderma viride* (T-l) and a benomyl-resistant biotype (T-I-R9), alone or in combination with *Aspergillus ochraceus*, reduced disease by at least 50% in vegetatively maintained plants. (Sivan *et al.*, 1984). Sivan and Chet (1986) reported that *T. harzianum* successfully controlled *Fusarium* spp. in cotton, wheat and musk melon in naturally infected soil. Muhukumar *et al.*, (2005), and Nikam *et al.*, (2007) also found *Trichoderma* spp. as a potential bio-control agents of *F. oxysporum* on various crops.

It is clear from the foregoing discussion that *Tricoderma* spp. is a good candidate for biological control due to the different modes of action the fungus employs in inhibiting the growth of *F. oxysporum* f. sp. *chrysanthemi*. The present evaluation thus gave clear indication that the*T. viride* and *T. harzianum* are strong and virulent antagonists, which can be effectively used in the management of chrysanthemum wilt. Combination of seedling dip and soil application appears to be most effective.

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