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Management of wilt disease of chrysanthemum caused by *Fusarium oxysporum* f sp. *chrysanthemi*

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Abstract
Chrysanthemum (*Chrysanthemum morifolium*) is one of the important flower crops grown in Tamil Nadu, India. Fusarium wilt of chrysanthemum is causing damage in both green house and field conditions. The twelve isolates of *Trichoderma viride* were screened against *Fusarium oxysporum* f.sp *chrysanthemi* by dual culture method. A pot culture experiment with nine treatments was conducted using the promising *Trichoderma viride* of Tv-NK in the glass house at Agricultural College and Research Institute, Madurai, to assess the efficacy of isolate and the time of application of antagonist in managing wilt disease of chrysanthemum caused by *Fusarium oxysporum* f.sp *chrysanthemi.* The experimental results revealed that the treatment T_6 comprising of soil application of Tv-NK (2.5 kg talc-based formulation/ha) at 30, 60 and 90 DAP with root dipping of Tv-NK (0.2%) (T_2) recorded the least disease incidence of 12.58 PDI as against 88.88 PDI in the pathogen inoculated control and thus accounted for the highest disease reduction of 85.04 per cent over control. It was followed by the treatments T_7-soil application of Tv- (2.5 kg talc-based formulation/ha) at 30,60,90 and 120 DAP with root dipping of Tv-NK (0.2%) by recording 15.03 PDI which accounted for 82.13 and 79.15 per cent disease reduction over control respectively. The comparative check T_8 comprising standard control (Carbendazim 0.1% soil drenching) was found to reduce the disease intensity by recording 7.40 PDI which accounted for 91.66 per cent disease reduction over control.

Keywords: chrysanthemum**,** *Trichoderma viride***,** *Fusarium oxysporum* f. sp *chrysanthemi*

Introduction
The chrysanthemum (*Chrysanthemum morifolium*) is one of the important flower crop grown in Tamil Nadu, India. Like other flower crops chrysanthemum is being attacked by several fungal and bacterial diseases. Fusarium wilt of Chrysanthemum is caused by *Fusarium oxysporum* f sp. *chrysanthemi* (Singh and Kumar, 2011) [16] Fusarium wilt of chrysanthemum is causing damage in both green house and field conditions. Several scientists reported about the loss caused by Fusarium wilt disease in chrysanthemum (Garibaldi *et al.*, 2009; Minuto *et al.*, 2007; Murkar *et al*., 1994) [3, 9] Fusarium wilt is a serious disease in all chrysanthemum cultivated areas (Singh *et al*., 2014) [15]. Chemical control is a widely followed method to control the soil borne diseases. Now, bio control agents are tried as a safe eco-friendly method to manage this soil borne disease instead of synthetic fungicides. The root colonization with *Trichoderma* strains can increase the levels of defense related plant enzymes and pathogenesis related proteins (PR). β 1,3 – glucanse (PR -2) is one of the most important PR proteins which causes a direct inhibition of growth of several plant pathogens (Kauffmann *et al*., 1987) [5]. Due to the ecological importance of Trichoderma *spp* and its application as a biocontrol agent in the field, it is important to identify strains of *Trichoderma* spp that can be used for controlling plant diseases. The present investigation was under taken to study the effect of *Trichoderma viride* on the incidence of Fusarium wilt of Chrysanthemum caused by *Fusarium oxysporum* f sp. *chrysanthemi*.

Materials and Methods
The investigations were carried out under laboratory and pot culture conditions at the Department of Plant Pathology, Agricultural College and Research Institute, Madurai - 625104, Tamil Nadu India during the year 2015 -18.

Isolation of pathogen
The pathogen was isolated from the affected portion of the diseased plants collected from chrysanthemum growing areas separately by the tissue segment method (Rangaswami and Mahadevan, 1999) [13] on sterile Potato Dextrose Agar (PDA) medium. The infected plants were pulled out with intact root showing the presence of white mycelial mat with small round brown sclerotia near the collar region and gently tapped to remove the soil and dirt particle.

The infected portions of diseased plants collected from different places were cut into small pieces of 1 to 1.5 cm separately using sterilized scalpel and these were surface sterilized with 0.1 percent mercuric chloride for thirty seconds and washed in sterile distilled water thrice and then placed in a petri plates at equidistance separately into previously poured and solidified petri plates containing potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28±2 ºC) for five days and observed for the growth of the fungus. The hyphal tips of fungus grown from the pieces of plants collected from each area were transferred separately under aseptic conditions to PDA slants for maintenance of the culture.

Isolation of antagonists

Trichoderma viride isolates were isolated from the rhizosphere soil collected from the twelve Chrysanthemum growing areas of Tamil Nadu. The plants were pulled out gently and the excess soil adhering on roots was removed gently. 10 g of rhizosphere soil was transferred to 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate method (Pramer and Schmidt, 1956) [12]. From the final dilutions of 10^{-3}, 10^{-5} and 10^{-6} one ml of each aliquot was pipetted out, poured in sterilized Petri plates containing *Trichoderma* selective medium (TSM) and they were gently rotated clockwise and anti-clockwise for uniform distribution and incubated at room temperature (28±2 ºC) for 24 hours. The pure cultures were maintained on respective agar slants at 4 ºC.

Effect of biocontrol agents

The twelve isolates of *Trichoderma viride* were screened against *Fusarium oxysporum* f.sp *chrysanthemi* by dual culture method. A nine mm mycelial disc of *Fusarium oxysporum* f.sp *chrysanthemi* and the test antagonists were placed opposite to each other near the periphery of the petri plate separately and incubated at room temperature (28±2 ºC). The petri plates containing the medium inoculated with the pathogen alone were served as control. When the control plate showed full growth of the fungus the radial growth of the pathogen was measured in all the other treatments. The results were expressed as per cent inhibition over control by using the formula of Pandey and Upadhyay (2000) [11].

$$PI = \frac{DC - DT}{Dc} \times 100$$

Dc = average diameter of fungal growth (cm) in control
Dt = average diameter of fungal growth (cm) in treatment.
PI = Percent inhibition over control

A pot culture experiment with nine treatments was conducted using the promising isolate of *Trichoderma viride* (Tv-NK) in the glass house at Agricultural College and Research Institute, Madurai, to assess the efficacy of *Trichoderma viride* and time of application of antagonist against the wilt disease of Chrysanthemum caused by *Fusarium oxysporum* f.sp *chrysanthemi.* These treatments were replicated three times in a completely randomized design. One chrysanthemum seedling was planted in each pot containing sterile potting medium (red soil: sand: FYM at 1:1:1 w/w/w). The seedlings were dipped in the solution of talc-based formulation of antagonist for 15 minutes separately as shown in the treatment schedule. The pathogen was multiplied in sand maize medium and incorporated @ 5g per pot. The method of application of biocontrol agent included root dipping and soil applications of talc formulation of *Trichoderma viride*. Plants inoculated with pathogen alone were served as control. The effect of each treatment on the occurrence of wilt disease were recorded separately. The treatments for the pot culture experiment are listed in table 1.

Statistical analysis

Data was analyzed by statistical variance (ANOVA). The mean values of tested treatments were compared according to the procedures. The least significant differences (LSD) at $P \leq 0.05$ were used to test the significance of the differences among the mean values of tested treatments.

Results

The effect of twelve isolates of *Trichoderma viride* collected from different chrysanthemum growing areas were tested against the growth of *Fusarium oxysporum* f.sp *chysanthemi* by dual culture experiment. Colony morphology of each *Trichoderma viride* isolates, were recorded as white mycelial growth at initial, which later turned as greenish growth due to the production of spores. Among the antagonists tested, *Trichoderma viride* (Tv-NK) was found effective by recording the maximum mycelial growth reduction of 74.44 per cent over control which was followed by *Trichoderma viride* (Tv-NY) with 71.11 per cent growth reduction over control. The least mycelial growth reduction was recorded by *Trichoderma viride* (Tv-MP) with 42.22 per cent. (Table 2).

The effect of promising isolate of *viz*., *Trichoderma viride* (Tv-NK) was tested against the wilt disease of chrysanthemum plants under pot culture experiment. Carbendazim (0.1%) was maintained as standard chemical check. The experimental results revealed that the treatment T_6 comprising of soil application of effective antagonist(s) (Tv-NK) (2.5 kg talc-based formulation/ha) at 30 DAP, 60 DAP and 90 DAP + T_2 recorded the least disease incidence of 12.58 PDI as against 88.88 PDI in the pathogen alone inoculated control and thus accounted for the highest disease reduction of 85.04 per cent over control. It was followed by the treatments T_7 and T_5 by recording 15.03 and 18.51 PDI which accounted for 82.13 and 79.15 per cent disease reduction over control respectively. The minimum disease reduction (66.64%) was observed in soil application of effective antagonist (Tv-NK) (2.5 kg talc-based formulation/ha) at the time of planting + T_1.

The comparative check T_8 comprising standard control (Carbendazim 0.1% soil drenching) + T_1 was found to reduce the disease intensity by recording 7.40 PDI which accounted for 91.66 per cent disease reduction over control (Table 3).

Discussion

In the present investigation,among the isolates of *Trichoderma viride*, the isolate collected from Nilakottai (Tv-NK) was found effective by recording the maximum mycelial growth reduction of 74.44 per cent over control which was followed by *Trichoderma viride* (Tv-NY) collected from Nariyuthu village of Didigul District with 71.11 per cent growth reduction over control. Thiruvudainambi *et al*., (2010) [17] reported that MNT 7 isolate of *Trichoderma viride* significantly reduced the mycelial growth of *S. rolfsii* to an extent of 76.30 per cent over control under *in vitro* conditions. Manu *et al.*, (2012) [6] reported maximum inhibition of mycelial growth (61.88%) was observed in *Trichoderma harzianum* which was followed by *Trichoderma viride*

(57.77%) against *S. rolfsii.* Jabbar *et al.*, (2014) [4] reported that maximum inhibition of mycelial growth (66%) was recorded in *T. harzianum*-55 IIHR that was superior over all other bioagents. The results, thus obtained revealed that, maximum inhibition of mycelial growth (77%) recorded was due to the production of antibiotics, which diffused, air filled pores, which are detrimental to the growth of *S. rolfsii* and also may be due to higher competitive ability of *Trichoderma* spp. (Sahana *et al*., 2017) [14].

In the pot culture experiment, the incidence of wilt disease in chrysanthemum was reduced by 85.04% due to root dipping and the soil application of *Trichoderma viride* talc formulation. Anahosur (2001) [1] reported that the antagonist *T. harzianum* recorded least *Sclerotium* wilt incidence in potato (10%) followed by *T. viride* (14%) and maximum yield in *T. viride* (25 t/ha) and *T. harzianum* (23 t/ha), whereas the least yield of (4 and 6 t/ha) was observed in control under field condition. Ganesan *et al.*, (2007) [2] indicated that combined application of selected symbiotic N_2 fixing and antagonistic *Rhizobium* isolates and bio control agent *T. harzianum* decreases the stem rot incidence and also increases the growth of the groundnut plants under glasshouse condition. Mishra *et al.*, (2011) [8] reported that the combination of *T. harzianum* and *P. fluorescens* against many soil-borne plant pathogens *viz*., *R. solani*, *S. rolfsii* and *M. phaseolina* responsible for root and stem rot disease of soybean under glass-house condition. Nashwa *et al.*, *(*2019) [10] concluded that *Trichoderma* has been considered as an internationally important biocontrol fungus due to its significant effect on the wilt diseases in plants.

Table 1: Effect of antagonist on wilt disease incidence in Chrysanthemum under pot culture experiment

T. No.	Treatments
T_1	*Fusarium oxysporum* f.sp *chrysanthemi* alone
T_2	Root dipping of Tv-NK) (0.2%) + T_1
T_3	Soil application of (Tv-NK) (2.5 kg talc based formulation/ha) at the time of planting + T_1
T_4	Soil application (Tv-NK) (2.5 kg talc based formulation/ha) at 30 DAP + T_2
T_5	Soil application of (Tv-NK) (2.5 kg talc based formulation/ha) at 30 DAP and 60 DAP + T_2
T_6	Soil application of (Tv-NK) (2.5 kg talc based formulation/ha) at 30 DAP, 60 DAP and 90 DAP + T_2
T_7	Soil application of (Tv-NK) (2.5 kg talc based formulation/ha) at 30 DAP, 60 DAP, 90 DAP and 120 DAP + T_2
T_8	Standard control (Carbendazim 0.1% soil drenching) + T_1

Table 2: Antifungal activity of *Trichoderma* sp isolates against the mycelial growth of *Fusarium oxysporum* f.sp *chrysanthemi* (I_4) *in vitro*

S. No	Treatments	Mycelial growth (cm)*	Per cent reduction over control (%)*
1.	Tv-NK	2.3	74.44
2.	Tv-BG	4.5	50.00
3.	Tv-PK	5.1	43.33
4.	Tv-KM	3.2	64.44
5.	Tv-RP	3.9	56.68
6.	Tv-KK	4.8	46.70
7.	Tv-SP	2.9	67.80
8.	Tv-PM	3.4	62.22
9.	Tv-KT	3.9	56.68
10.	Tv-MP	5.2	42.22
11.	Tv-NY	2.6	71.11
12.	Tv-AN	4.3	52.22
13.	Control	9.0	-
	CD (P=0.05)	0.16	
	CV %	2.29	

*Mean of three replications

Table 3: Effect of antagonist on wilt disease incidence in Chrysanthemum under Pot culture experiment

T. No.	Treatments	Disease incidence (%)*	Disease reduction over control (%)*
T_1	*Fusarium oxysporum* f.sp *chrysanthemi* alone	88.88	-
T_2	Root dipping of Tv-NK (0.2%) + T_1	25.92	70.81
T_3	Soil application of Tv-NK (2.5 kg talc based formulation/ha) at the time of planting + T_1	29.62	66.64
T_4	Soil application of Tv-NK (2.5 kg talc based formulation/ha) at 30 DAP + T_2	22.23	74.96
T_5	Soil application of Tv-NK (2.5 kg talc based formulation/ha) at 30 DAP and 60 DAP + T_2	18.51	79.15
T_6	Soil application of Tv-NK (2.5 kg talc based formulation/ha) at 30 DAP, 60 DAP and 90 DAP + T_2	12.58	85.04
T_7	Soil application of Tv-NK (2.5 kg talc based formulation/ha) at 30 DAP, 60 DAP, 90 DAP and 120 DAP + T_2	15.03	82.13
T_8	Standard control (Carbendazim 0.1% soil drenching) + T_1	7.40	91.66
T_9	Uninoculated control	0.00	-
	CD (P=0.05)	0.72	
	CV %	1.51	

*Mean of three replications

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