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Efficacy of various fungicides and nano-chemicals against Fusarium wilt of Chrysanthemum

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Abstract
Chrysanthemum flower (*Chrysanthemum indicum* Ramat) is popularly designated as "Queen of the east", or autumn queen (as its bloom in November-December) (Shibata, 2008; Teixeira *et al*., 2013). *Fusarium oxysporum* f. sp. *chrysanthemi* (*Foc*) causing vascular wilt is one of the most devastating pathogens attacking Chrysanthemum. This pathogen bears a great ability to attack all the growth stages, ranging from nursery to flowering stages (Pinto *et al.,* 2010). In this experiment, an attempt was made to manage this theat (*Foc*) by means of three systemic (Propiconazole, Hexaconazole, Vitavax) and two non-systemic fungicides (Mancozeb, Thiram) at four different concentrations (100, 200, 300 and 400ppm), both in lab (*in-vitro*) and in pot conditions. And the observations reflected that Mancozeb was the most effective in terms of radial growth inhibition and disease severity, followed by Propiconazole for all the tested fungicides at all the concentrations in both experiments. A significant effectiveness over control was also noted for Hexaconazole and Vitavax, but only at their higher concentrations in poison food technique. While in pot experiments, Hexaconazole and Vitavax produced quite similar effects. Among all tested fungicides, Thiram stands to be least effective at all the concentrations. An attempt was made to compare the effectiveness of four selected nano-chemicals namely, Copper, Molybdenum, Cobalt and Magnesium, over above mentioned traditional chemicals at the same concentration, both *in vitro* and in pot condition. Interestingly, a superiority of traditional fungicides was recorded in both *in vitro* and pot experiments. Among all the nano-particles under evaluation Cu NP was the most effective, followed by Molybdenum. Magnesium nanoparticles were found to be the least effective among all the tested nanoparticles. It was noteworthy that all the nanoparticles were effective, only at there higher concentrations.

Keywords: *Fusarium oxysporum* f.sp. *chrysanthemi*, Chrysanthemum, fungicide, nano-chemicals

1. Introduction
Chrysanthemum {Chrysos = gold + anthemon or anthos = flower}, is a partly woody, erect, perennial herb or sub-shrub (up to 1 m in height). Botanically recoginised as *Chrysanthemum morifolium* Ramat. and shares its roots to botanical family Asteraceae (Compositae). This beautiful flower is recognized as national flower of Japan, with several other popular names as, "Queen of the east", or autumn queen (as its bloom in November-December) **(**Shibata, 2008**;** Teixeira *et al*., 2013) [17]. Chrysanthemum is commercially grown for its cut and loose flowers throughout the globe. This beautiful flower occupies the second position after rose in terms of global ornamental market value (Xia *et al.,* 2006; Li *et al.,* 2017) [19, 7]. This floral crop is challenged by a vast range of botic stresses such as, insects, fungus, bacteria, virus and viroid. But, among all the known diseases fusarium wilt caused by *Fusarium oxysporum* f. sp. *chrysanthimi* (*Foc*) is one of the most devastrating disease. It is found to be a serious threat to commercial chrysanthemum cultivation around the globe including India (Singh *et al.,* 2014) [15]. All the growth stages ranging form nursery to flowering stage are vulnerable to *Foc* attack (Pinto *et al.,* 2010). This facultative saprophyte can survive in soil up to six years in the absence of susceptible host. Survival of *Fusarium* spp. in the soil is generally by chlamydospores, which have the increased capability to endure harsh environmental conditions (Singh *et al.,* 2014, Booth 1971, Nash *et al.,* 1961) [15, 3, 10]. Hsieh (1985) [5] reported that the pathogen was present in soil mainly from the surface to a depth of 20 cm and was detectable down to 30 cm. Considering the nature of damage and survival ability of the fungus, use of resistant varieties is the only economical and practical solution. But unfortnatily, most of the resistant varieties have been found to be susceptible after some years because of breakdown in their resistance and evolution of variability in the pathogen. Hence, use of fungicides is the only available solution to cope up with this distructive pathogen. Keeping the importance of the pathogen and economic value of this crop in mind, present investigation carried out to find out best chemical fungicides and effect of different nano chemicals against this pathogen.

2. Material and Methods

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

2.1 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 isolation of pathogen. Collected plants were washed thoroughly under the running tap water to remove the adhering soil. The root and basal stem were cut into small piece and rinsed in distilled water. These pieces were sterilized with NaOCl (0.5 – 1.0%) for 10-20 minutes followed by 2-3 washing with distilled water. Two to three surface sterilized pieces were placed on solidified potato dextrose agar medium, aseptically in laminar flow. The 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, were repeatedly sub-cultured on PDA slants for obtaining pure culture. The isolated fungus was identified and confirmed on the basis of their cultural characteristics appeared in petri plates and morphological characteristics under the compound microscope.

2.2 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. One month old lnts were inoculated by means of cotton swab method. The inoculated plants reexhibited the typical wilting symptoms (same as in field condition). The pathogen was reisolated for further conformation.

2.3 Testing efficacy of various fungicides against *F. oxysporum* f.sp. *chrysanthemi*

2.3.1 *In-vitro* efficacy of various fungicides against *F. oxysporum* f.sp. *chrysanthemi*

In-vitro evaluation of three systemic and one contact fungicides were evaluated by Poison food technique at four different concentrations of 100 ppm, 200 ppm, 300 ppm and 400 ppm. A 5 mm diameter mycelial plug of FOC was placed in each Petri Plates poisoned with fungicides at given concentrations. The culture was further incubated in BOD incubator at 25±2°c for 10 days.

Each treatment along with control (petri plates without fungicides) were replicated trice. Observation of radial growth inhibition was taken when the control petri plates completely covered with mycelial growth. Fungicidal toxicity was recorded in terms of percentage colony inhibition and calculated Percentage of Inhibition of Radial Growth (PIRG %) by using the following formula:

$$\text{PIRG} = \frac{R1-R2}{R1} \text{ x } 100$$

Where

R1: Radial growth of test pathogen in control plate.

R2: Radial growth of test pathogen in the treated plate.

2.3.2 Efficacy of various fungicides against *F. oxysporum* f.sp. *Chrysanthemi* in pot condition

To assess the effectiveness of fungicides, 10 days old rooted suckers were transplanted into 9 cm-diameter plastic pots containing sterile soil, inoculation of *FOC* was done after 35 days of transplanting @ of 10^8 CFU/g soil. Soil application of four systemic and one contact fungicide *viz.* Mancozeb, Propiconazole, Hexaconazole, Thiram and Vitavax at four concentrations i.e. 100 ppm, 200 ppm, 300 ppm and 400 ppm was done one week after inoculation. Control pots (without fungicides) were similarly treated with sterile distilled water. Experiment had three replicate of each treatment arranged in a completely randomized design. Observation was made after 90 days of plant stand, assessment of Fusarium wilt severity was conducted using the disease scale ranging 0-5 (Lori *et al.*, 2008) [8].

2.4 Efficacy of nano-chemicals against *F. oxysporum* f.sp. *chrysanthemi., in vitro* and pot condition

Four different nano-chemicals *viz*. Copper (Cu), Molybdenum (Mo), Cobalt (Co) and Magnesium (Mg) were tested against pathogen in both *in-vitro* (Poison Food Method) and *in-vivo* (in pots).

2.4.1 *In-vitro* efficacy of nano-chemicals against *F. oxysporum* f.sp. *chrysanthemi.*

Efficacy of 4 nano-chemicals on radial growth inhibition of FOC was done by poison food method, at four differents concentrations (i.e., 100, 200, 300 and 400 ppm). These poisoned petri plat were further inoculated with FOC (5mm mycelial disc) and incubated at 25±2°c for 10 days. Each treatment along with control replicated thrice. Observation of radial growth inhibition was taken when the control petri plates completely covered with mycelial growth and percent inhibition were calculated as above.

2.4.2 Efficacy of nano-chemicals against *F. oxysporum* f.sp. *Chrysanthemi* in Pot condition:

To assess the effectiveness of nano-chemicals, 10 days old rooted suckers were transplanted into 9 cm-diameter plastic pots containing sterile soil, inoculation of *FOC* was done after 35 days of transplanting @ of 10^8 CFU/g soil. Soil application of four nano-chemicals *viz.* Copper(Cu), Molybdenum(Mo), Cobalt(Co) and Magnesium(Mg) at four concentrations i.e. 100 ppm, 200 ppm, 300 ppm and 400 ppm was done one week after inoculation. Control pots (without nano-chemicals) were similarly treated with sterile distilled water. Experiment was maintained in CRD and observations were made after 90 days of plant stand, assessment of Fusarium wilt severity was conducted using the disease scale ranging 0-5 (Lori *et al.*, 2008) [8].

2.4.3 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.

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

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

Where
C= control plant.
T= Treatment plant.

3. Results

3.1 Testing efficacy of various fungicides against *F. oxysporum* f.sp. *chrysanthemi*

3.1.1 *In – vitro* efficacy of various fungicides against *F. oxysporum* f.sp. *chrysanthemi*

All fungicides exhibited a significant reduction of radial growth over control at every tested concentration. It has been noted that at every increasing concentration of each fungicide resulted in a proportionate reduction in radial growth of *Fusarium oxysporum* f.sp. *chrysanthemi.* Among these fungicides, Mancozeb was found to be most effective at all four concentrations followed by Propiconazole. Mancozeb recorded 100 percent inhibition at 300 ppm and 400 ppm concentrations while at 100 ppm and 200 ppm, this fungicides recorded 80.74% and 90.00% inhibition with radial growth of 17.33 mm and 9.0 mm, respectively. While, Hexaconazole showed highest growth reduction of 77.8% at 200 ppm. Whereas, vitavax was highly effective at their highest concentration i.e. 300 ppm and 400 ppm with percent inhibition of 91.00 and 94.44% respectively, while as, efficacy was significantly low (29.00 and 68.9%) at lower concentrations of 100 ppm and 200 ppm. Thiram was found to be least effective in compare to others fungicides which showed 23.66, 71.10, 78.66 and 82.20% radial growth inhibition at 100 ppm, 200 ppm, 300 ppm and 400 ppm concentrations respectively. The percent growth inhibition of *F. oxysporum* f. sp. *chrysanthemi* at various concentration of fungicide was depicted in fig. 1.

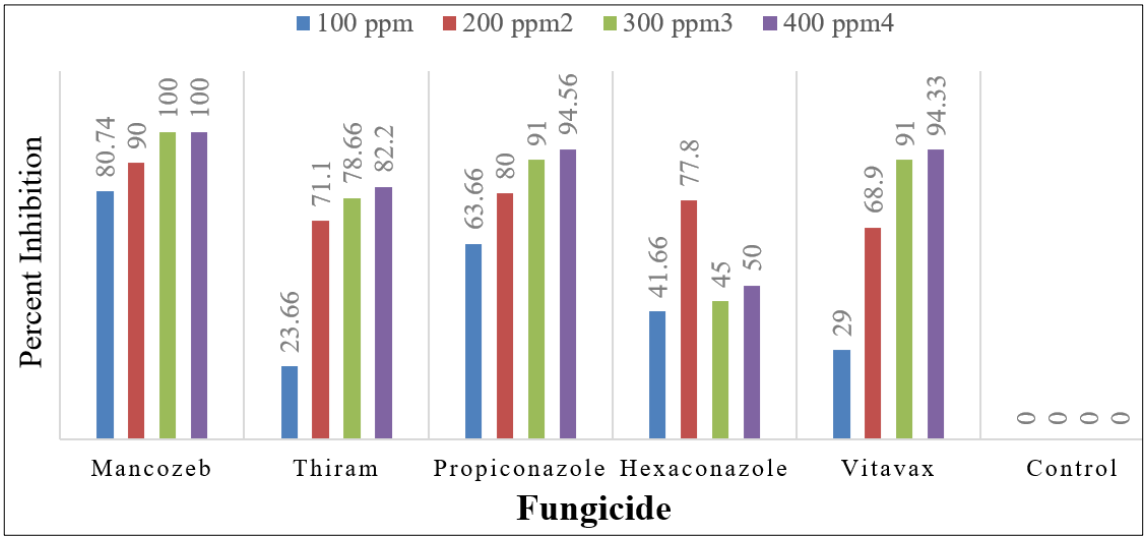

Fig 1: Effect of different Fungicides in radial growth of *Fusarium oxysporum* f. sp. *chrysanthemi*

3.1.2 Efficacy of various fungicides against *F. oxysporum* f. sp. *chrysanthem* in pot conditions

All the four fungicides (prior tested *in-vitro*) were again tested in pot conditions. All the tested fungicides exhibited superiority over control pots. Interestingly it has been noted that every increasing concentration of all fungicides resulted in significant decrease in disease severity. Among all the tested fungicides, Mancozeb stood to be the best one at all its concentrations. All the concentrations of mancozeb were found to be superior over every tested concentration of all other fungicides. The trend of decreasing disease severity at increasing concentration for mancozeb was 22.66, 15.33, 6.66, and 6.66%. Effectiveness of Mancozeb is followed by Propiconazole at all concentrations, while Hexaconazole and Vitavax produces quite similar effect with 27.33% disease severity and 64.04% percent inhibition at 300 ppm. At 400 ppm concentration, Vitavax found to be second most effective after Mancozeb and Propiconazole while Thiram observed to be the least effective fungicides at all the concentrations. The percent growth inhibition of *F. oxysporum* f. sp. *chrysanthemi* at various concentration of fungicide was depicted in fig. 2.

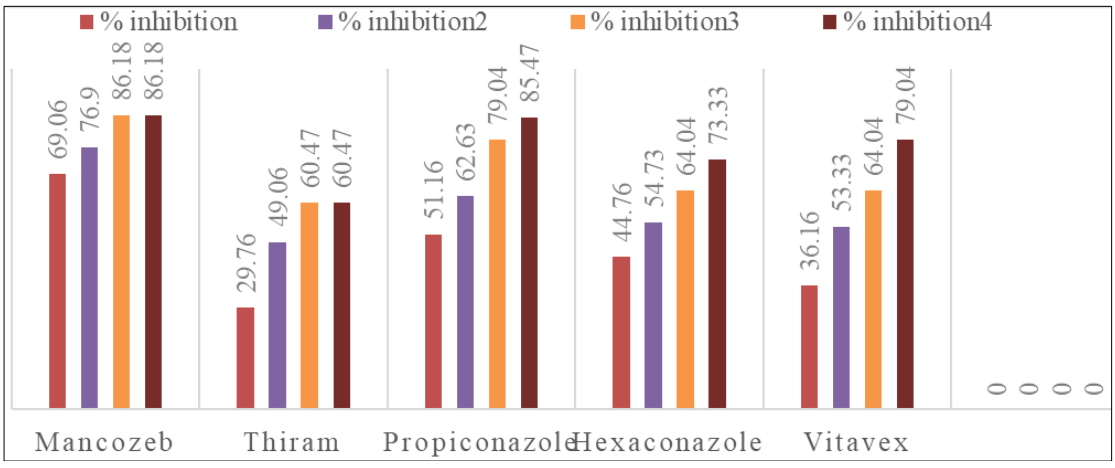

Fig 2: Efficacy of various fungicides against *F. oxysporum* f.sp. *chrysanthem* in pot conditions

3.2 Efficacy of nano-chemicals against *F. oxysporum* f. sp. *chrysanthemi., in vitro* and pot condition:

3.2.1 *In-vitro* efficacy of nano-chemicals against *F. oxysporum* f.sp. *chrysanthemi.*

The results exhibited that all nano-chemicals had significantly inhibited the radial growth of the pathogen. However, interestingly it has been observed that the nanochemicals were effective only at their higher concentrations. The Copper nano-particles found to be the best among all tested concentration with highest inhibition of 38.89 and 82.22 percent at concentrations of 300 ppm and 400 ppm. Molybdenum was found to be second most effective nano-chemical after Copper. Magnesium nano-particles found to be least effective at all concentration in this study (fig. 3).

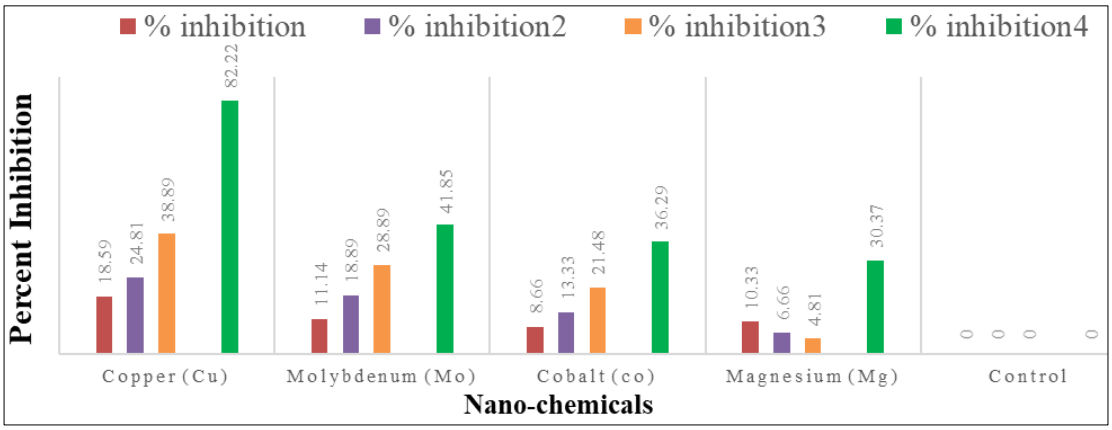

Fig 3: Magnesium nano-particles found to be least effective at all concentration in this study

3.2.2 Efficacy of nano-chemicals against *F. oxysporum* f.sp. *chrysanthemi* in Pot condition:

The copper nanoparticles again exhibited its superiority over all other nano particles under study at all its tested concentration same as its *in-vitro* experiment. Copper nanoparticles notebly reduced the disease severity up to 6.66% at 400ppm. Superiorty of Copper nano-particle was followed by Molybdenum nano-particles at all concentration i.e. 100 ppm, 200 ppm, 300 ppm, 400 ppm. While, Magnesium and cobalt nano-particles were the least effective with disease severity of 20.00 and 27.33% at 400 ppm (fig. 4).

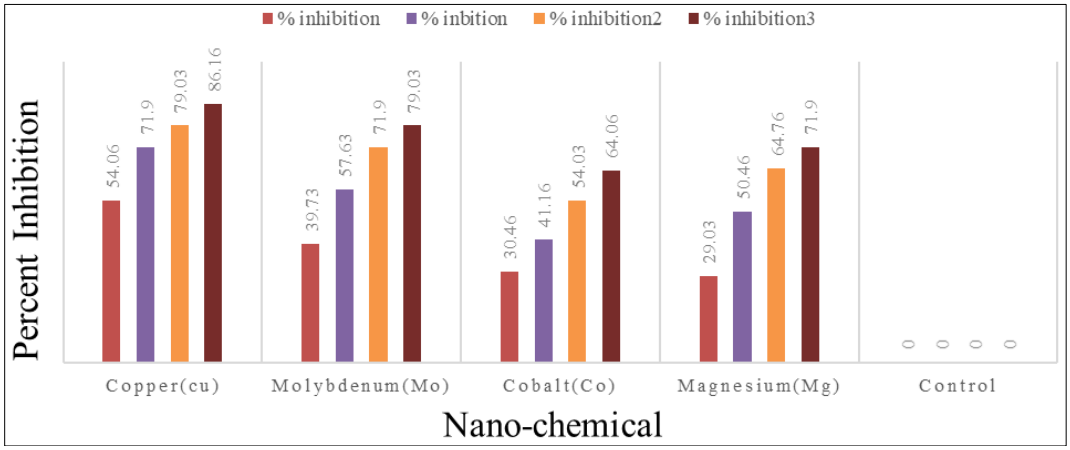

Fig 4: Effect of nano-chemicals against *F. oxysporum* f.sp. *chrysanthemi* under pot condition

4. Discussion

The efficacy of these fungicides had also been documented against *Fusarium oxysporum* f. sp. *ciceri* in earlier reports. Poddar *et al.*, (2004) [13] evaluated four systemic fungicides *viz*. Carbendazim, Propiconazole, Thiophanate-methyl and Tebuconazole were and Carbendazim caused maximum growth inhibition of the pathogen *in-vitro*. Maitlo *et al.*, (2014) [9] evaluated fourteen fungicides against wilt pathogen *in vitro* with five different concentrations ranging from 1-10000 ppm. Among these, only Carbendazim and Thiophanate-methyl was found as the most effective at all used concentrations. Amini, J. & Sidovich D. (2010) [2] studied the efficacy of six fungicides; Benomyl, Carbendazim, Prochloraz, Fludioxonil, Bromuconazole and Azoxystrobin against *Fusarium oxysporum* in *in-vitro*. He found that Prochloraz and Bromuconazole were the most effective fungicides against the pathogen *in vitro*. Song *et al.*, (2004) [16] tested seven fungicides *viz.*, Prochloraz, Carbendazim, Thiram, Toclofos-methyl, Hymexazol, Azoxystrobin and Carboxin *in-vitro* against *Fusarium oxysporum* and found Prochloraz and Carbendazim were the most effective fungicides in inhibiting mycelial growth.

Zhao *et al.*, (2016) [20] reported the carbendazim (MBC) and soil fumigant dazomet (DAZ) treatments were effective in suppressing the disease in pot condition, Engelhard *et al.*, (1971) reported Benomyl 50W and BAS 3201-F 50W drenched on potted chrysanthemums grown on a high-lime, all nitrate-nitrogen cultural regime, provided complete control of Fusarium wilt on higly susceptible cultivar of chrysanthemum. Amini, J. & Sidovich, D. (2010) [2], Trophy, R. (1985) [18], also worked earlier on the similar experiment.

The efficacy of these nano-particles in pot condition had also been documented against this pathogen in earlier reported Kim, S. W. *et al.* (2012) [6] reported that treatment with WA-

CV-WB13R AgNPs resulted in maximum inhibition of most fungi inhibition of plant pathogenic fungi was observed on PDA and 100 ppm of AgNPs, Parizi, *et al.,* (2014) [11] reported magnesium oxide nanoparticles have considerable impact on F*ol* such that controlling effect of nanoparticles improves in both solid and liquid media, Ahmed, *et al.,* (2016) [1] Nickel nanoparticles at 100 ppm concentration inhibited the mycelial growth of F. *oxysporum* f. sp. *lactucae* and F. *oxysporum* f. sp. *lycopersici* by 60.23 and 59.77%, respectively compared with control. In the liquid media, the fresh mycelial weight of pathogens decreased significantly and the reduction was more than 50% with the use of nickel nanoparticles at the concentration of 100 ppm. Although the best effect of nanoparticles was observed at the concentration of 100 ppm in *in vitro* concentration, but in greenhouse NiNPs at 50 ppm was better than 100 ppm of NiNPs in relation to plant health measured in terms of growth parameters.

5. Conclusion

Chrysanthemum flower (*Chrysanthemum morifolium* Ramat.,) is popularly commercially grown cut flower crop around the globe. Fusarium wilt is one of the most threat of this commercially grown crop. According to the experiment conducted fungicides were found to be most effective over others. Mancozeb was found to be most effective at all four concentrations followed by Propiconazole, Thiram was found to be least effective in compare to others fungicides both *in-vitro* and pot condition. The Copper nano-particles found to be the best among all tested concentration with highest inhibition. Suggest copper based chemical are very useful for control of fusarium wilt disease. but due to theres ill effect on environment leads to development of such package and practices which are based on integration of different management practices (i.e. cultural, biological and chemicals). *Trichoderma* spp. can be utilized as commercial biocontrol agent against *F. oxysporum* f. sp. *chrysanthemi* which can be recommended to the farmers for integrated and sole application in field.

6. References

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