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Management of Die back and Fruit Rot Disease of Chilli (*Capsicum annum* L.)

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Die-back and fruit rot diseases are major yield limiting factor in all chilli growing areas of India. *Trichoderma* species are commonly used as biological control agents against phytopathogenic fungi and represent differential capacity of antagonism. In the present investigation, the effect of seven native *Trichoderma* isolates from Madhya Pradesh was examined for management of chilli die back and fruit rot using seed treatment with three foliar sprays of respective isolate. Different isolates of *Trichoderma* spp. significantly inhibited the growth of *Colletotrichum capsici* causing die-back and fruit rot in chilli under confrontation assay and maximum inhibition of 39.29% was recorded by *Trichoderma* isolate T_2(REWA) after 96 hrs of incubation period. In field conditions, among treatments with different *Trichoderma* isolates, it was observed that minimum PDI of 19.40% was recorded in seed treatment and three foliar sprays with T_2 isolate of *Trichoderma* with maximum yield of 69.55q/ha. Among different fungicides evaluated, minimum PDI of 21.47% was recorded in foliar spray with propiconazole @0.1%. This was followed by foliar spray with Thiram + Carboxin @0.2% where 23.73 % PDI was recorded.

Introduction

Chilli (*Capsicum annum* L.) is an important spice cum vegetable crop, often referred to as Capsicum, hot pepper, sweet pepper or paparika. Chilli cultivation has existed for several hundred years as a sustainable form of agriculture in India and in many other countries. India stands 3rd in production of chillies (Saxena *et al.*, 2016) and *Capsicum annuum* is the widely cultivated species. Green chilli provides vitamin-C while, the red chilli provides vitamin-A (Martin *et al.*, 2004) in addition to iron, potassium and magnesium. The area and production of green chillies in India is 0.316 mha and 3.63 mt respectively

during the year, 2016-17 (Anonymous, 2017). The sustainability of chilli-based agriculture is threatened by a number of biotic and abiotic factors. The chilli suffers from more than 40 fungal species and of these *Colletotrichum capsici* is one of the most caustic species causing leaf spot or die back at different stages of crop growth and fruit rot or anthracnose at fruiting stage leading to reduced fruit yield and marketability. Although infected fruits are not toxic to humans or animals, severely affected fruits showing blemishes are generally considered unfit for human consumption. This is because the fruit rot causes an unpleasant colour and taste in it's products. Studies conducted on resistance aspect of this disease show very little resistance in chilli germplasm which indicate the presence of diverse population within the fruit rot/dieback or anthracnose causing fungus. Management of the disease under the prevailing farming systems in India has, thus, become a recurrent problem to chilli growers. The disease can be kept under check with chemical spray programme (Thind and Jhooty, 1987; Datar *et al*., 1990; Sharma and Thakore, 1999; Rathore, 2004) but the complete control is still intractable.

Trichoderma, a filamentous soil inhabiting mycoparasite, is used in commercial preparation for biological control of many fungal plant pathogens (Jash, 2006) and included the mechanisms like antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solublization and sequestration of inorganic nutrients and inactivation of pathogen enzymes (Harman, 2000). However, with the increasing interest in biological control, owing to environmental and economic concerns, and with the rapid development of biotechnology, several *Trichoderma* species were formulated in a commercial production for protection and growth enhancement of a number of crops in several countries (McSpadden and Fravel, 2002). It is always beneficial that the selected strain of *Trichoderma* should have the ability to compete with the native microflora, establish itself successfully in the crop rhizhoshere/ spermosphere and should have a wide array of mechanisms to inhibit several pathogens. Given these considerations, it is expected that the best method for obtaining a potential biocontrol agent might be to isolate *Trichoderma* strains originally from those areas where they are actually expected to function later as a biocontrol agent and where they are already growing under conditions of temperature, moisture etc. similar to those found in nature (Howell, 2003). Keeping this in view, the present investigation was envisaged to use the native isolate of *Trichoderma* in management of die back and fruit rot of chilli.

Materials and Methods

Trichoderma harzianum isolates

A set of seven *T. harzianum* isolates of were procured from Department of Plant Pathology, College of Agriculture, Rewa and further used in present investigation. The isolates were isolated from soil samples collected from Satna, Rewa (Kuthulia), Khargone, Indore, Umaria, Rewa (Birkham) and Sidhi locations of Madhya Pradesh and coded as T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. The procured isolates of *T. harzianum* were maintained throughout the study by periodical transfers on Potato dextrose agar (PDA) medium.

Dual culture experiment (Confrontation assay)

Antagonistic efficacy of different isolates of *Trichoderma* spp. was tested *C. capsici* by dual culture experiment using confrontation

assay (Kumar *et al.*, 2010). *Trichoderma* spp. and test fungus was inoculated at 7 cm apart. Four replicates were maintained for each treatment and incubated at 28 ± 2° C for 4 days. Monoculture plates of both served as control. Radial growth of test fungus and *Trichoderma* isolates were measured three and four days after inoculation (DAI). Radial growth of test fungus in dual culture plate was recorded and compared with control. The growth inhibition was calculated by using the formula: 100 X C - T / C, Where C = radial growth of test pathogen in control and T = radial growth of test pathogen in treatment (Vincent, 1947).

Management of die back and fruit rot of chilli

Experiment was conducted in experimental area of Department of Plant Pathology, J.N.K.V.V., College of Agriculture, Rewa. The seeds of chilli cv. Kohinoor special were sown in lines at a spacing of about 5 cm apart on raised beds of about 15 cm above ground level and covered with soil, thereafter, mulched with dry grasses. Appropriate moisture level was maintained for proper growth of the seedlings. Dry grass was removed to expose the seedlings to sunlight for better growth after germination. The seedlings were transplanted to individual plot size of 1.5 m × 2.5 m with row to row and plant to plant spacing of .75 m and 0.30 m respectively. For biological management of die back and fruit rot disease slurry of different isolates of *Trichoderma* was prepared separately for each isolate and seeds were treated @10 g/kg seed. Seeds were dipped in the slurry for 30 minutes and dried in shade before sowing. For foliar spray, the first spray was given 25 days after transplanting followed by two more sprays at 15 days interval. The spray solution was prepared by adding 2.5 g culture of *Trichoderma* separately in 1 litre of water. In total seven treatments were formulated using different isolates of *Trichoderma* (T_1 to T_7) as seed treatment followed by three foliar sprays of respective isolate.

Further, for chemical management of die back and fruit rot in chilli a set of five fungicides including systemic and non-systemic fungicides *viz.*, Propiconazole (Tilt 25 EC) @ 0.1%, Carboxin 37.5 per cent + Thiram 37.5 per cent (Vitavax power) @ 0.2%, Captan (Captaf 50WP) @ 0.2%, Thiram (Thiram 50 WP) @ 0.1% and Carbendazim (Bavistin 50% WP) @ 0.1% were evaluated for their efficacy under field conditions. Treatments were laid out in plots arranged in a Randomized Block Design (RBD). Four replications were maintained for each treatment and fungicides were applied as water-based spray liquid at specified concentrations using a hand-held low volume electric sprayer. The first spray of fungicides was applied after appearance of disease in field and two subsequent sprays were given at 20-day intervals. Disease incidence was assessed on 15 days after last spray based on a disease rating scale (Score 0, 1, 2, 3, 4 for respectively 0, 1–5%, 6–25%, 26–50% and 51–100% fruit area infected) as proposed by Bansal and Grover (1969). Per cent disease index (PDI) was calculated according to following formula given by Wheeler (1969) and data collected were subjected to Randomized Design for their significance (Gomez and Gomez, 1984).

PDI = Sum of all disease ratings x 100/Total no. of observations x Maximum disease rating

Results and Discussion

Confrontation assay

All the isolates of *Trichoderma* tested for antagonistic activity against *C. capsici* showed different degree of antagonism to the pathogen and inhibited the test pathogen by its mycoparasitic activity. Phenomenon of

inhibition could be noticed by growth check of the pathogen in treatment plate with *Trichoderma* under confrontation assay. However, mycoparasitic activity could be visualized by over growth of *Trichoderma* isolate above test pathogen. Under *in-vitro* conditions, radial growth of *C. capsici* ranged from 13.67 mm to 21 mm and 17 mm to 28 mm respectively after 72 hrs and 96 hrs of incubation period in different treatments with *Trichoderma* and control plate. After 96 hours of incubation, growth of test pathogen *C. capsici* was checked and *Trichoderma* starts overlapping the pathogen. This shows their mycoparasitic activity against the pathogen. After 72 hrs and 96 hrs of incubation period, maximum inhibition of respectively 34.92% and 39.29% was recorded by *Trichoderma* isolate T_2(REWA). Prolonged incubation of 96 hours also depicted same isolate T_2(REWA) inhibiting maximum growth of *R. solani* (54.2%). Detailed data for radial growth and per cent inhibition after 72 hours and 96 hours of incubation period of each isolate of *Trichoderma* are given in table 1.

Management of die back and fruit rot of chilli

Different isolates of *Trichoderma* spp. were applied as seed treatment and three foliar sprays at 15 days intervals to identify their role in die back and fruit rot management in chilli under field conditions and it was observed that all the seven isolates were able to control the disease. The maximum PDI of 32.49% was recorded in control. However, among treatments with different *Trichoderma* isolates, it was observed that minimum PDI of 19.40% was recorded in seed treatment and three foliar sprays with T_2 isolate of *Trichoderma*. Maximum per cent inhibition of 40.29% was recorded in treatment T_2 with maximum yield of 69.55 q/ha. However, in control plot minimum yield of 58.46 q/ha was recorded. This clearly demonstrated the disease inhibition and plant growth promotion activity of *Trichoderma*. The detailed data for different treatments for PDI and yield are presented in table 2.

Table.1 Table 1: Average radial growth and per cent inhibition in growth of *C. capsici* by isolates of *Trichoderma* after 72 and 96 hours incubation period

Treatment (*Trichoderma* isolate)	Incubation Period (72 hours)		Incubation Period (96 hours)	
	Avg. radial growth (mm) of *C. capsici*	Per cent inhibition	Avg. radial growth (mm) of *C. capsici*	Per cent inhibition
T_1	15.33	26.98	18.33	34.52
T_2	13.67	34.92	17.00	39.29
T_3	14.33	31.75	17.33	38.10
T_4	17.33	17.46	20.00	28.57
T_5	14.67	30.16	17.33	38.10
T_6	18.33	12.70	21.00	25.00
T_7	17.33	17.46	20.67	26.19
Control	21.00	0.00	28.00	0.00
S.Em±	0.43	-	0.51	-
C.D. (p=0.05)	1.29	-	1.55	-

Table.2 Effect of different isolates of *Trichoderma* in management of die back and fruit rot chilli under field conditions

Treatments	PDI*	Per cent disease reduction (%)	Yield (q/ha)
T_1:Seed treatment with *Trichoderma* isolate T_1 with its three foliar sprays	18.52 (25.42)	21.76	65.58
T_2:Seed treatment with *Trichoderma* isolate T_2 with its three foliar sprays	11.11 (19.40)	40.29	69.55
T_3:Seed treatment with *Trichoderma* isolate T_3 with its three foliar sprays	12.59 (20.66)	36.41	63.22
T_4:Seed treatment with *Trichoderma* isolate T_4 with its three foliar sprays	19.26 (25.92)	20.22	61.36
T_5:Seed treatment with *Trichoderma* isolate T_5 with its three foliar sprays	17.78 (24.90)	23.36	68.27
T_6:Seed treatment with *Trichoderma* isolate T_6 with its three foliar sprays	21.48 (27.58)	15.11	59.27
T_7:Seed treatment with *Trichoderma* isolate T_7 with its three foliar sprays	20.74 (27.07)	16.68	59.87
T_8:Control	28.89 (32.49)	0.00	58.46
S.Em±	1.09		2.52
C.D. (p=0.05)	3.34		8.79
CV %	7.44		6.92

Table 3: Effect of different fungicides in management of die back and fruit rot of chilli under field conditions

Fungicide	Dose (%)	PDI	Per cent disease reduction (%)
Propiconazole	0.1	13.43 (21.47)	40.51
Thiram + Carboxin	0.2	16.20 (23.73)	34.25
Captan	0.2	18.06 (25.18)	30.23
Thiram	0.1	20.83 (27.13)	24.83
Carbendazim	0.2	25.93 (30.59)	15.24
Control		34.72 (36.09)	0.00
C.D. (p=0.05)		2.22	
CV %		6.78	

Among different fungicides evaluated, it was observed that all the five fungicides were able to manage the disease but degree of management significantly varied among the treatments. Minimum PDI of 21.47% was recorded in foliar spray with propiconazole

@0.1%. This was followed by foliar spray with Thiram + Carboxin @0.2% where 23.73 % PDI was recorded. However, maximum PDI of 34.72% was recorded in control. Per cent inhibition in die back and fruit rot was calculated and it was observed that inhibition in PDI ranged from 15.24% to 40.51%. The data related to PDI in different fungicidal sprays are presented in table 3.

The control of chilli anthracnose fruit rot has, for many years, relied on chemicals and resulted in many undesirable problems. There is a need to incorporate alternative control components that are effective in field. Biological control of fruit rot and dieback of chilli with *Trichoderma* has been successfully used in present investigation using its seed treatment along with three foliar sprays. During last decade, species of *Trichoderma* have been identified as most powerful arsenal of bioprotectants for eco-friendly management of a wide variety of plant diseases. This is more accurate in the background of the fact that there is great social and environmental scientist's pressure to reduce emphasis on chemical protectants and increase the use of bioprotectants. The genus *Trichoderma* by virtue of its broad spectrum action against a number of plant diseases caused by fungi, bacteria and even nematodes, has occupied the top position among the bioprotectants developed for plant disease management (Kumar *et al.*, 2009; Srivastava *et al.*, 2009; Kumar *et al.*, 2014; Kumar *et al.*, 2015 and Jain *et al.*, 2016). Previous study reported inhibitory mechanisms by *Trichoderma virens* and *Trichoderma harzianum* to *C. truncatum* through competition, parasitism and antibiosis (Begum *et al.*, 2008). However, in this study, mycoparasitism by *Trichoderma* sp. was observed in confrontation assay with varied degree of inhibition in different isolates of *Trichoderma*. The *Trichoderma* sp. grew over the colony of *C. capsici* and at the point where the two fungi encountered, mycelia of *C. capsici* was found to change in turgidity. Amin *et al.*, (2010) reported to control mycelial growth of *C. capsici* by more than 50% and the results of present findings also suggested inhibition in growth of *C. capsici* during confrontation assay.

In this study, the antagonistic ability of the isolated *Trichoderma* sp. was also tested in the field conditions and isolate T_2 was most successful in management of disease under field conditions when applied as seed treatment and its three foliar sprays. The finding in the present study agree with the study conducted by Rahman *et al.*, (2012) who also found that application of culture filtrate of *T. harzianum* significantly decreased the disease severity caused by *C. capsici*. In addition, Rahman *et al.*, (2013) reported that 30 day old culture filtrates of all *Trichoderma* strains in their study significantly reduced percentage of anthracnose disease severity on chilli fruits. Further, among different tested fungicides propiconazole @ 0.1% was recorded most effective in management of fruit rot disease in chilli. Similar results were obtained by Gopinath *et al.*, (2006) who reported propiconazole (0.1%) as most effective against colony growth and sporulation of *C. capsici*. They also reported yield of chilli increased in range of 86 per cent and 60 per cent for Propiconazole and Carbendazim respectively. Combination of systemic and non-systemic fungicides like Vitavax power (Vitavax + Thiram) will be much cheaper and more effective management of anthracnose of chilli. Alternative and/or need based used of chemicals or/along with *Trichoderma* application will also reduce the chance of evolution of new races of *C. capsici* against the systemic fungicides and by using *Trichoderma* as seed treatment and foliar spray can manage the disease in eco-friendly manner.

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