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Evaluation of local isolates of *Trichoderm*a spp. against *Macrophomina phaseolina*

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ABSTRACT

Throughout the world, charcoal rot, caused by *Macrophomina phaseolina*, is one of the most destructive and widespread disease of field crops.. In the present study, evaluation of the antagonistic activity of isolates of *Trichoderma* by direct confrontation method was used. Twenty five promising isolates of *Trichoderma* spp. were obtained from different farmers' field of Pune District. Isolations were carried out on Trichoderma Selective Medium. These different isolates of Trichoderma were *in vitro* confronted to *Macrophomina phaseolina*. Efficacy of isolates of *Trichoderma* inhibited growth of the *Macrophomina phaseolina* as compared to control. Highest inhibition was observed in Isolate 2 (82.22 per cent) followed by Isolate 13 (81.48 per cent).The growth of Trichoderma isolates was significantly different in all isolates at all the observation periods.

Key words:, *Macrophomina phaseolina, Trichoderma* spp, Dual culture, Inhibition of growth

INTRODUCTION

Charcoal rot of various crops such as sorghum, bean, cotton, sesame, sunflower, melon, tobacco, corn, soybean and safflower has been reported to be due to *Macrophomina phaseolina*. It is one of the most important soil borne disease, which causes significant yield losses. Charcoal root rot may be a difficult disease to control because of the nature of causal pathogen. The disease is favored by hot, dry conditions. The fungus has a very wide host range, with more than 500 crop and weed hosts. *Macrophomina phaseolina* invades the roots of a host at an early stage, but the symptoms appear only in mature plants (Pearson *et al.* 1984). Chemical fungicides are being replaced with biocontrol agents because of the emergence of fungicide-resistant fungal isolates and public concerns regarding the health and environmental impacts of these chemicals.

During the past few decades, several potential biocontrol organisms have been isolated, characterized, and commercialized. Thus, biocontrol of plant diseases has received more consideration in disease management strategies (Shali *et al.* 2010). Biological control of phytopathogens by microorganisms is a method of plant disease management. It has been used as an alternative in the fight against pathogens in plants (Baker *et.al.*1996).

Biological control is a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment. *Trichoderma* spp. actively suppresses the growth of plant pathogens. The bio-control activity of

Trichoderma is of immense importance not only to agriculture and its crops but also the environment as it does not accumulate in the food chain and thus does no harm to the plants, animals and humans. The genus *Trichoderma* includes the most common saprophytic fungi in the rhizosphere and occurs worldwide and are commonly associated with roots, soil, and plant debris (Howell *et al*., 2003). Several *Trichoderma* spp. reduce the incidence of soil borne plant pathogenic fungi under natural conditions (Benitez *et.al,* 2004). Recently there have been numerous attempts to use *Trichoderma* spp. against soil borne pathogens. In the present study attempt has been made to isolate local *Trichoderma* isolates from different fields of farmers in Pune region and evaluate their efficacy in *in vitro* to find out effective isolate to be advocated against this disease.

MATERIAL AND METHODS

Isolation

The soil samples were collected from seven Tehsil's of different fields of farmers of Pune District during 2016-17. Isolations of *Trichoderma* spp. were carried out by dilution and pour plate method on *Trichoderma* selective medium under aseptic condition. The plates were incubated in the laboratory at 25 $\pm$ 1^{O}C.Growth of fungus on these plates was watched daily. Soon as growth was noticed, the fungal colony was transferred on Potato Dextrose Agar (PDA) medium slants. The isolated fungi were purified by hyphal tip method described by Dohroo and Sharma, (1992). Isolated fungal organisms i.e *Trichoderma* isolates were identified based on colony characters (Gams and Bisset, 1998) for confirmation.

Fungal pathogen

Fungal pathogen *Macrophomina phaseolina* was obtained from Plant Pathology Section, College of Agriculture, Pune-05.

Dual culture technique

The antagonistic potential of *Trichoderma* isolates were assessed *Macrophomina phaseolina* by dual culture technique on Potato Dextrose Agar (PDA) medium as per procedure described by Stack *et al*., (1986).

For this, 20 ml of sterilized and cooled medium (PDA) was poured in each petri plate allowed to solidify. Cut discs of pathogen and *Trichoderma* isolates with the help of cork borer. A 5 mm disc of pathogens was placed at one end of the medium with the help of sterilized inoculating needle. Just opposite to it, 5 mm disc of *Trichoderma* isolate was placed. Control i.e. without inoculation of the *Trichoderma* isolates fungus were maintained. Petri plates were incubated at 25 ±1 ºC temperature.

Observation on per cent inhibition and colony radius of pathogen and *Trichoderma* were recorded after 6 and 9 days after inoculation. The Per cent inhibition of the pathogen were calculated by using following formula (Vincent, 1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I- Per cent inhibition of fungal growth

C- Growth or colony diameter (mm) of the fungus in control plate

T- Growth or colony diameter (mm) of the fungus in treatment plate

Statistical analysis:

The data obtained from different observations was statistically analyzed following Completely Randomized Block Design (CRD) as per procedure suggested by Panse and Sukhatme (1969).

RESULTS AND DISCUSSION

Results presented in Table.1 indicated that different twenty five isolates of *Trichoderma* have significantly influenced growth of *Macrophomina phaseolina* at all the observation periods.

Perusal of results from Table 1, the results indicated that growth in all the isolates of *Trichoderma* significantly reduced growth of *Macrophomina phaseolina* than control. At 6 days after incubation, the results indicated that growth in all isolates of Trichoderma significantly reduced growth of *Macrophomina phaseolina* than control. Growth in control was on par with the isolates 14, 22 and 21. While, other isolates of Trichoderma had significantly less growth of Macrophomina phaseolina than control. Minimum growth of pathogen was observed in Isolates 10 and 2 i.e. 12.00 mm and which were at par with Isolates 7 and 13. Isolate 1 (15.00 mm) which was on par with Isolates 6, 9 and 24. Rest of the isolates ranged between 18.00 to 36.66 mm as compared the growth of pathogen in control (39.00 mm).

At 9 days after incubation, minimum growth of pathogen was observed in Isolate 2 (16.00 mm) and it was at par with isolates 13 and 10. Isolates 7, 1, 6, 19, 4, 3, 23, 25, 9 and 5 had growth ranged between 20.00 to 30.00 mm. Rest of the isolates ranged between 31.00 to 66.66 mm as compare to the growth of pathogen in control (90.00 mm). Trichoderma isolates have effectively inhibited growth of *Macrophomina phaseolina* as compared to control. Inhibition of growth varied from 25.93 to 82.22 per cent. Highest inhibition was observed in Isolate 2 (82.22 per cent) followed by Isolate 13 (81.48 per cent).

The growth of Trichoderma isolates were significantly different in all isolates at all the observations. At 6 days after incubation, maximum growth was observed in Isolate 5 (68.33 mm) and it was significantly superior over all and also it was at par with isolate 13. Rest of the isolates ranged between 13.00 mm to 63.66 mm.

At 9 days after incubation, Isolate 25 (74.00 mm) showed the maximum growth and it was significantly superior over all the isolates. It was par with Isolates 13 and 10. Isolates 7, 5, 1, 6, 19, 4, 3, 23, 25, 9, 24, 11, and 15 had growth ranged between 52.33 to 70.66 mm. Rest of the isolates ranged between 23.33 to 48.00 mm.

The results showed that the interaction between these two fungi appears to increase defensive and offensive mechanisms of each. Indeed, the reason for the inhibition zone between the two organizations made it clear that *Trichoderma* and *Macrophomina phaseolina* are secreted metabolites The potential of Trichoderma spp. to produce many volatile and non-volatile secondary metabolites has been reviewed by Reino et al. (2008). It is evident from aforesaid results that local isolates of *Trichoderma* had inhibitory effect against the pathogen. Inhibition of growth of *M. phaseolina* and *Rhizoctonia* species due to Trichoderma spp. has been observed by several workers (Patel and Annahusar, 2001, Anthan *et.al* 2003, Gayethri *et.al.* 2003, Tapwal and associates (2011)). It is also evident from the results that few *Trichoderma* isolates were fast growing while others had moderate growth. In this context mechanism of action of *Trichoderma* isolates can be antibiosis and competition as suggested (Papavizas and Lumseden, 1980 and Cook and Baker, 1983) also mentioned that

Trichoderma isolates inhibit fungal pathogen by way of antibiosis and competition mechanism which support finding of present study.

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Table 1: Influenced *Trichoderma* isolates on *Macrophomina phaseolina*
DAI- Days After Incubation *- Mean of three replications

Growth of *Macrophomina phaseolina* (mm)				**Growth of *Trichoderma* isolates (mm)**	
***Trichoderma* isolate No.**	**6 DAI***	**9 DAI***	**Per cent inhibition**	**6 DAI***	**9 DAI***
Isolate-1	15.00	22.66	74.82	60.33	67.33
Isolate -2	12.00	16.00	82.22	52.33	74.00
Isolate -3	20.66	27.33	69.63	59.00	63.33
Isolate -4	18.33	26.00	71.11	58.66	64.00
Isolate -5	22.66	29.66	67.04	68.33	70.33
Isolate -6	15.33	23.00	74.44	51.66	67.00
Isolate -7	12.33	19.33	78.52	60.66	70.66
Isolate -8	35.66	66.66	25.93	36.33	23.33
Isolate -9	15.66	29.66	67.04	22.66	60.33
Isolate -10	12.00	18.00	80.00	63.66	72.00
Isolate -11	24.33	34.66	61.48	43.33	55.33
Isolate -12	31.00	52.66	41.48	21.33	37.33
Isolate -13	13.66	16.66	81.48	68.00	73.33
Isolate -14	36.33	52.00	42.22	15.00	37.66
Isolate -15	33.66	37.66	58.15	23.66	52.33
Isolate -16	27.33	42.00	53.33	32.00	48.00
Isolate -17	26.00	44.00	51.11	42.00	46.00
Isolate -18	34.66	45.33	49.63	37.66	44.66
Isolate -19	18.00	25.66	71.48	45.66	64.33
Isolate -20	31.33	50.33	44.07	32.33	39.66
Isolate -21	36.66	60.33	32.96	26.33	29.66
Isolate -22	36.66	53.00	41.11	13.00	37.00
Isolate -23	25.33	29.00	67.77	49.00	61.00
Isolate -24	17.66	31.00	65.55	52.66	59.00
Isolate -25	18.00	29.33	67.40	42.00	60.66
Control	39.00	90.00	-	-	-
S.E.± **C.D. 0.01**	**0.72** **2.69**	**0.57** **2.15**	**-**	**0.76** **2.85**	**0.58** **2.17**