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Biological control of *Fusarium oxysporum* f. sp. *lycopercici* on tomato with fungal antagonists

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ABSTRACT: Vegetables are important sources of minerals and vitamins. They are attacked by several fungal pathogens and these fungi cause severe losses to these vegetables. Therefore, a study was undertaken to evaluate the effect of biocontrol agents such as *Trichoderma viride, T. harzianum, Glomus mossae* and *G. fasciculatium* for the control of wilt disease caused by *Fusarium oxysporum* f. sp. *lycopercici* on tomato. There was a significant reduction in wilt severity and improvement in the plant growth parameters and chlorophyll content as a result of application of different doses of biocontrol agents. The growth characteristics also increased with the increase in the dose of biocontrol agents. Highest growth and chlorophyll content was found at higher doses per pot followed by lower doses of treatments as compared to untreated control which showed lowest plant growth.

KEY WORDS: Biocontrol agents, chlorophyll, *Fusarium oxysporum*, plant growth, tomato.

INTRODUCTION

Tomato is an essential vegetable. It is an important source of carbohydrates, vitamins and other nutrients. This vegetable is attacked by several pathogens such as fungi, bacteria and nematodes. The *Fusarium oxysporum* is known to cause wilting on tomato, brinjal and other plants (Carron *et al.,* 1986; Prasad *et al.,* 1952; Davis *et al.,* 1979; Fayaz *et al.,* 1994). This fungus initially infects the finer roots, enter the vascular system (Edward, 1960; Chattopadhyay and Bhattacharya, 1968; Chattopadhyay and Sengupta, 1955). Several methods employed for the control of fungi are: physical methods, chemical methods, cultural methods and biological methods. These methods have either one or other limitations, but biological methods of control being eco-friendly have been utilized for the control of several fungal pathogens. The control of several root-borne diseases caused by *Fusarium* species by the application of VAM fungi and other fungal biocontrol agents have been reported by several workers (Dehne and Shoenbeck, 1979; Caron *et al.,* 1986a,b; Jalali and Jalali, 1991). Therefore, present study was undertaken to assess the role of bio-control agents such as, *Trichoderma viride*, *T. harzianum*, *Glomus mossae* and *G. fasiculatum* on *Fusarium oxysporum f.* sp. *lycopercici* affecting tomato. The pure cultures of *T. viride* and *T. harzianum* was obtained from SKUAST, Shalimar, Srinagar, whereas inoculum of VAM fungi, *G. mossae* and *G. fasiculatum* were obtained from Central Sericulture Research and Training Institute, Barhampura, West Bengal.

MATERIALS AND METHODS

15cm clay pots were filled with 1kg autoclaved soil manure mixture in the ratio of 3: 1.

Then surface sterilized seeds (0.1% mercuric chloride) of tomato cv. Local were sown in these clay pots. After 20 days of sowing thinning was done so that only one seedling remained per pot. The fungi, *F. oxysporum, T. viride*, and *T. harzianum*, were cultured on potato dextrose agar (PDA). For mass production, the fungi were grown in Richards liquid medium (Riker and Riker, 1936) for 15 days at 28 ± 2°C to obtain mycelial mat. The mycelial mat obtained was dried in an oven at 65°C for 1 hour and then blended in mortor and pestle to get powder form of fungus. For *Fusarium oxysporum*, the mycelium suspension was prepared by blending 100g of mycelium in 1000ml of distilled water so that 10ml of suspension consisted of approximately 0Ig mycelium for application. The mycelium suspension was poured into depression made in the soil around the root system of the seedling at the rate of 2g per seedling. Different doses, *viz*., 0.5, 1.0, 1.5 and 2.0g of *T. viride* and *T. harzianum* were prepared and poured in the depression around the root system of tomato seedlings.

In case of vesicular arbuscular mycorrhizal fungi (VAM), different doses, viz. 5, 10 15 and 20g of soil root

inoculum of *G. mossae* and *G. fasciculatum* were prepared and dipped in the depression around in the root system of tomato.

There were five replicates for each treatment including the control. After two months of inoculation, experiment was terminated. Plants were uprooted, rinsed free of soil and different parameters such as wilt severity, plant length, fresh plant weight and chlorophyll content were determined. Chlorophyll content was determined by the method described by Hiscox and Israealstam, 1979. Wilt severity (%) was recorded on a 0 - 5 scale, whereas: 0 = no wilt; 1 = 1 - 20%; 2 = 21- 40%; 3 = 41 - 60%; 4 = 61 - 80% and 5 = 81 - 100%. The data was analysed statistically by the method described by Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

The results indicate (Table 1, 2) that biocontrol agents, *T. viride*, *T. harzianum* and VAM fungi, *G. mossae*, and *G. fasciculatum* caused significant reduction in the severity of wilt caused by *F. oxysporum f.* sp. *lycopercici* on tomato plants and thereby improved plant growth parameters and chlorophyll content. The highest reduction in wilt severity was obtained in plants treated with maximum dose of bioagents. It was followed by lower doses of bioagents respectively as compared to untreated control which showed highest wilt severity. Plant height of tomato plants improved considerably as a result of treatment with bio-agents, *T. viride* and *T. harzianum*. The highest plant length was found in plants grown in pots treated with 2g inoculum of bioagents, *T. viride* and *T. harzianum*. It was followed by plants treated with 1.5, l.0 and 0.5g of fungal inoculum, respectively, as compared to untreated control which showed lowest plant height. Similarly, plant weight also improved significantly as a result of treatment with fungal bio-agent. The highest plant weight was observed in plants grown in pots treated with 2g of inoculum followed by plants treated with 1.5, l.0 and 0.5g of inoculum respectively as compared to untreated plants which showed least plant weight. The chlorophyll content of the plants also increased with the increase in the doses of inoculum of fungal biocontrol agents as compared to untreated control. The highest chlorophyll content was observed in plants grown in pots treated with 2g of fungal inoculum followed by plants treated with lower doses of inoculums respectively.

The results also indicate (Table 2) that reduction in wilt severity caused by *F. oxysporum* f. sp. *lycopercici* was due to treatment of tomato plants with different doses of root inoculum VAM fungi, *G. mossae* and *G. fasciculatum* thereby significantly improved the plant growth and chlorophyll content as compared to untreated control. The highest suppression in wilt severity was observed in plants treated with highest doses (20g) of root inoculum. It was followed by plants treated with lower doses of inoculums, *viz*., 15, 10 and 5g respectively as compared to untreated control which showed highest wilt severity. Plant length and plant weight improved significantly as a result of different doses of root inoculum of VAM fungi. The highest plant length and plant weight was observed in plants grown in pots treated with 20g inoculum of *G. mossae* and *G. fasciculatum* followed by plants grown in pots treated with 15, 10 and 05g, respectively of soil root inoculum as compared to untreated control which showed lowest plant growth. Similarly, chlororphyll content of leaves of tomato also increased considerably as a result of treatments with different doses of root inoculum of VAM fungi. Highest increase in chlorophyll content was found in leaves of plants grown in pots treated with 20g inoculum of VAM fungi followed by plants treated with 15, 10 and 5g inoculum of VAM fungi as compared to untreated plants which showed lowest chlorophyll content.

The improvement in the plant growth might be due to decrease in the infection by *Fusarium* wilt fungus (Khan, *et al*., 2004). The improvement in plant growth and decrease in infection might also be due to release of certain antibiotic substances during the decomposition of fungal biocontrol agents which are inimical to pathogenic fungi or through lysis or death of pathogens or by indirect toxic effect on the pathogen by the volatile substances such as ethylene, released by the metabolic activates of the antagonist (Agrios, 1997). Bhat *et al*. (2003) also reported effect of *Trichoderma* species on the chickpea wilt caused by *F. oxysporum f.* sp. *ciceri*. The effect of *T. harzianum* might be due to putative-mycoparatism related proteins which they induce during growth on pathogenic fungi (Vasseur *et al.,* 1995). Likewise, there are several species of *Trichoderma* which caused suppressive effect on *Fusarium sp*. and other pathogenic fungi (Bedlam, 1988; Xue Baodi *et al.*, 1995).

Similarly, treatment of tomato with different doses of soil root inoculum of VAM fungi, *G. mossae* and *G. fasciculatum* caused significant reduction in the wilt severity by *Fusarium oxysporum*. The highest suppression in the wilt severity was observed in plants treated with higher doses of inoculum of bioagent. It was followed by plants treated with lower doses of inoculum of bioagents respectively. As a result of reduction in wilt severity there was found significant

Table 1. Effect of *Trichoderma viride* and *Trichoderma harzianum* on wilt severity caused by *Fusarium oxysporum f. sp. lycopercici* and plant growth of tomato

Treatment	Dose Pot / g	Plant weight (g)			Plant length (cm)			Chlorophyll (mg)		Total	Wilt severity (0 - 5 scale)
		Shoot	Root	Total	Shoot	Root	Total	Chl. a	Chl. b	a + b	
Trichoderma viride	0.0	24.5	13.5	38.0	35.6	12.0	47.6	1.035	0.635	1.670	5.0
	0.5	24.5	14.4	39.9	37.8	14.5	52.3	1.143	0.845	1.988	2.0
	1.0	26.8	15.5	42.3	38.6	14.8	53.4	1.154	0.940	2.094	1.8
	1.5	27.4	16.5	43.9	41.5	15.6	57.1	1.164	0.967	2.131	1.5
	2.0	28.8	17.5	46.2	42.5	16.8	58.3	1.176	0.988	2.164	0.0
Trichoderma harzianum	0.0	23.5	13.6	37.1	34.8	11.4	46.2	1.032	0.625	1.657	5.0
	0.5	24.6	16.4	40.0	36.2	13.4	49.6	1.140	0.840	1.980	1.8
	1.0	25.4	16.2	41.6	37.4	13.5	50.9	1.152	0.924	2.076	1.5
	1.5	26.8	16.8	43.6	39.5	14.6	53.1	1.158	0.935	2.093	1.2
	2.0	27.5	17.0	44.5	41.6	15.4	57.0	1.164	0.954	2.118	0.0
C.D. (P = 0.05)				1.72			1.92			0.254	
C.D. (P = 0.01)				1.84			2.23			0.261	

Each value is a mean of five replicates

improvement in the plant growth and chlorophyll content of tomato. This might be due to structural, physiological and biological changes in the host roots by VAM fungi. The development of endomycorrhizae results in loss of root hairs but relatively sparse hyphal mantle occur at the rhizoplane and extend to surrounding soil. Roots offer structural support to plant function in absorption of water and mineral salts and provide nutrients for a wide range of microorganism (Curl and Truelove, 1986; Rovira 1985). Dehine (1982) found that mycorrhizal fungi reduced disease response to plant pathogens due to some morphological changes in the plant. Phenolic compounds have been shown to be formed after mycorrhizal colonization (Sylvia and Sinclair, 1983) and have been thought to play a role in disease resistance (Goodman *et al.,* 1967). The production of phytoalexin is believed to play a major role in the host defence system against pathogen (Kaplan *et al.,* 1986) and production of phytoalexin was greater on mycorrhizal roots than on non-mycorrhizal roots. (Morandi, 1987). VAM fungi have also been found to enhance mineral uptake especially P, K and Zn thereby improved plant growth (Khaliq *et al.,* 2001) and that caused plants to escape root disease (Newsham *et al.,* 1995). Caron *et al* (1986) observed reduction of infection by *Fusarium* on tomato by application of *G. intradices*. Similarly, there are several reports of control of root borne diseases by *Fusarium* species through the application of VAM (Davis *et al.,* 1979; Zambolim and Schenck, 1983; Graham 1988; Chandra and Chatterjee, 1990; Dwivedi, 1996 and Srivastava *et al.,* 2001).

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Table 2. Effect of VAM fungi on wilt severity caused by *Fusarium oxysporum* f. sp. *lycopercici* and plant growth of tomato

Treatment	Dose Pot / g	Plant weight (g)			Plant length (cm)			Chlorophyll (mg)		Total	Wilt severity (0 - 5 scale)
		Shoot	Root	Total	Shoot	Root	Total	Chl. a	Chl. b	a + b	
Glomus mossae	0.0	31.8	13.0	44.8	23.2	9.8	33.0	1.012	0.621	1.633	5.0
	5.0	34.5	14.0	48.5	25.0	10.0	35.0	1.302	0.734	2.036	2.0
	10.0	36.4	14.5	50.9	26.2	10.5	36.7	1.324	0.789	2.113	1.8
	15.0	37.7	15.2	52.9	27.5	11.4	38.9	1.356	0.843	2.199	1.5
	20.0	39.5	16.3	55.8	28.0	12.2	40.2	1.367	0.895	2.252	0.0
Glomus fasciculatum	0.0	28.5	11.5	40.0	22.8	8.5	31.3	1.010	0.612	1.622	5.0
	5.0	31.4	12.0	43.4	23.5	9.5	33.0	1.232	0.730	1.962	1.8
	10.0	32.7	12.6	45.3	24.6	10.6	35.2	1.245	0.778	2.023	1.5
	15.0	33.4	13.5	46.9	26.5	10.5	37.0	1.264	0.831	2.095	1.2
	20.0	34.5	14.0	48.0	27.4	11.2	38.6	1.282	0.854	2.136	0.0
C.D. (P = 0.05)				1.94			1.89			0.272	
C.D. (P = 0.01)				2.25			1.94			0.285	

Each value is a mean of five replicates.

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