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Antagonistic Effect of *Trichoderma* Isolates against the *Fusarium* Wilt of Bael under Soil Condition

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Abstract: ***The investigation entitled "Biological Management of Nursery Diseases (Root rot and wilt) of Bael (Aegle marmelos Correa)" was conducted in the Narendra University of Agriculture and Technology, Kumarganj, Faizabad during 2012-2013. At nursery stage about 35-40% seedlings of Bael (Aegle marmelos Correa) were died due to root rot and wilt. The disease is highly prevalent during rainy season under warm and high humidity as well as low sunshine hours (4-6 hrs/day) that further aggravated the situation. The affected seedlings may come out on soil surface but failed to grew further. The root rot affected seedlings remained alive for sometimes but under highly disease conducive condition none of the seedlings survived beyond one month. They either toppled over or become necrotic black and finally died. The population of F. solani infested soil could be minimized by treating the soil with T. harzianum, T. virens and T. viride. Trichodema harzianum and T. virens grew very fast. Beside T. harzianum parasitized over F. solani, resulted into lyses of its hyphae. The antagonistic activity of T. viride happened due to its yellow pigments secreted in the rhizosphere. The yellow coloured metabolite was toxic and killed the hyphae of F. solani. Thereafter, T. viride grew over the space cleared by its metabolite on the basis of the results of the present experiment. Application of T. viride and T. virens as preventive measures and that of T. harzianum as curative one may be suggested.***

Keywords:-Fusarium solani, Trichoderma

INTRODUCTION

The beal (*Aegle marmelos* Correa) is an important indigenous arid zone fruit belonging to family rutaceae often termed as underutilized minor fruit. The importance of bael is mentioned in ayurvedic and religious literatures since ancient times. It has been known in India from pre-historic times. It is also known as Bengal quince, Indian quince, golden apple, holy fruit, stone apple, Bel, Bela, Sriphal, Belger, Baelpatra, Bilva and maredoo. The bael has great mythological significance, it is found near every temple in India. According to Hindu customs, the leaves of the tree are traditionally used as scared offering to Lord Shiva. The history of the bael tree has been traced in Vedic times (2000 B.C.-800 B.C.). In the 'Ramayana' period, the bael fruit was known and bael trees were found growing in Chitrakut hills and Panchavati. Bael fruit has been portrayed in paintings of Ajanta Caves along with other fruits.

The effectiveness of *Trichoderma harzianum and T. viride* in the control of damping off affected pine seedlings caused by *Cylindro carpon destructrons, F. oxysporum and R. solani*. *Trichoderma* sp. were used in pine or beech sawdust substrate in the from of *T. harzianum* powder. The experiment was carried out in the glasshouse at 20-25/15-18 °C day/ night temp. over 4 weeks. *Trichoderma harzianum* used as both a water suspension and a powder as well as applied in the beech the sterile beech sawdust was also effective in protecting against *C. destructans and F. oxysporum.* The protective effect of *T. harzianum* depended on the amount of sawdust used with a reduction in damping off occurring only when large amount of sawdust were

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used there was no reduction in damping off caused by *R. solani* induced by *Trichoderma* sp. used either as sawdust or powder(2).

The effect of soil solorization alone and in combination with *Trichoderma* sp. (*T. harzianum & T. koningii*) on cucumber root rot pathogens (*Rhizoctonia solani & F. solani*). The maximum soil temperature was 43.2°C and 37.4°C at 10 cm and 20 cm depth respectively. The cucumber root rot disease ratio decreased in these treatments compared to the control. No difference was found among applications. The effect of treatments on yield was also determined yield value 43.9, 49.4, 46.7 and 41.2 in treatment of solarization alone(3).

The management of soil borne disease is difficult through application of fungicide and it was also observed that *F. solani* has been successfully controlled by using *Trichoderma* sp. causes root rot in acid lime(1).

MATERIAL AND METHOD

2.1Efficacy of the antagonists against the pathogen *in vivo*:

2.2 Soil inoculation with *F. solani*

To a mixture of garden soil and FYM in equal proportion, finely chopped tender bael shoots (50 gm./kg.) were added lightly irrigated; pot surface was covered with brown paper and sterilized in autoclave keeping for 20 minutes at 120^0C. After cooling of the soil, the inoculum (*F. solani*) grown in solid sorghum media was mixed in aseptic condition with the sterilized soil (@ 150 gm. inoculum for 1.5 kg sterilized soil). Surface of the pots were again covered with the brown paper and incubated BOD incubator (26 ± 2^0C) for five days.

2.3 Inoculation of the *F. solani*-infested soil with *Trichoderma* species:

When the inoculated pot soil was found to be totally infested with the mycelia growth of the fungus, *F. solani*, as it was evident by the growth of the mycelia over soil surface of the pots, *T. harzianum*, *T. viride* and *T. virens* grown over solid (sorghum) medium for 7 days at 26 ± 2^0C were mixed separately (@ 150g/pot) with the *F. solani*-infested soil thoroughly.

2.4 Estimation of moisture of the pot soil:

10 g soil from 10 cm depth were collected and dried in Hot Air Oven at 50^0C for one hour. Next day soil was again weighted and thus moisture content was determined. Thus, the moisture of the pot soil was continuously maintained (82%) by adding sterilized distilled water as the when required.

2.5 Population count of *F. solani* and *Trichoderma* species:

The experiment was conducted by soil plate dilution technique. After 7 days of inoculation, 200 mg sample from each experimental pot was taken from 10 cm depth with sterilized disk cutter. It was put in to 200 ml sterilized distilled water and shaken well, 0.5 ml of the suspension was put into sterilized Petri plate into which 30 ml molten and warm PDA and a pinch of Streptomycin were added. The whole mixture was thoroughly mixed and incubated 26 ± 2^0C in BOD incubator.

To investigate the increase or decrease of population of the *Trichoderma* spp. and *F. solani* with passing time, the experiment was repeated three times at 7 days interval. The colonies of *F. solani* and *Trichoderma* species in each plate were counted by colony counter. For each treatment five replications were maintained.

RESULT

3.1 Interaction between *Trichoderma* species and *F. solani* in soil:

3.2 *T. harzianum*:

It appears from the Table 5 that after seven days of treatment with *T. harzianum* the population *F. solani* was drastically reduced 27.5 x 10^3 colonies/g soil whereas the same in untreated soil was counted 109.5 x 10^3, number of *T. harzianum* colonies in the same soil was 65.5 x 10^3 colonies/g soil. After fourteen days of treatment there was a reduction in number of colonies of *T. harzianum*. But on twenty-one day after treatment the population of *T. harzianum* was again found to be increased. At this time it was almost at par with the number of colonies of *F. solani* in the treated soil showed a gradually decline. *F. solani* in the untreated control also recorded a similar gradual reduction. However, a number of *F. solani* in untreated soil throughout the experiment was substantially more than that of the treated soil.

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3.3*T. viride*:

The number of colonies of *T. viride* and *F. solani* both in the treated and control soil have been presented in Table-6. The number of colonies of *T. viride* in the treated soil seven days after the treatment was recorded as 51.2 x 10^3 colonies/g soil after fourteen days there was some reduction in its population. But within the next seven days the population was again built-up and it reached the peak. The population of *F. solani* both in treated and untreated soil recorded a gradual reduction from seven days after treatment upto the end of the experiment. However, rate of reduction of population of *F. solani* in treated soil was considerably higher but population level was just reverse.

3.4*T. virens*:

The results presented in the Table-3 shows the population of *T. virens* and *F. solani* both in treated and controlled soil. The population of *T. virens* from seven days to twenty one days of the treatment showed a marginal decline while that of *F. solani* in the treated soil reduced significantly. Contrarily, population of *F. solani* in untreated soil was considerably higher than the treated soil.

3.5 Efficacy of the *Trichoderma* species in reducing the population of *F. solani*

The percent reductions in *F. solani* colony over the control after the treatment with the three species of *Trichoderma* are presented in the Table-4 with *T. harzianum* the initial reduction was 74.88%. But in the next seven days the percent reduction was reduced to 65.64%. However at the end of experiment the percent reduction again increased and attended the level of 72.59% with *T. viride* the reduction after seven days of treatment was the minimum (69.68%) of all the three treatments. But, within the next seven days, *T. viride* increased its reduction percentage to 82.66%. *T. virens* showed the percent reduction of 72.42%, 67.33% and 74.57% at seven, fourteen and twenty one day after treatment, respectively. Thus according to the initial efficacy the *Trichoderma* species may be arrangement in the following sequence.

T. harzianum> *T. virens*> *T. viride*

But at the end of the experiment a reverse picture was noticed like this

T. viride> *T. virens*> *T. harzianum*

CONCLUSION

The infection of *F. solani* in the Bael nursery took heavy loss the symptoms of *F. solani* were manifested either in the form of pre-emergence rotting, root rot and wilt of young seedlings. In the disease conducive conditions about 28% seedlings were killed due to pre-emergence rotting while 72% seedlings that managed to survive and emerged out of soil died within a month by root rot.The population of *F. solani* in infested soil could be minimized by treating the soil with *T. harzianum*, *T. virens* and *T. viride. Trichoderma harzianum* and *T. virens* grew very fast and occupied the space and consumed nutrients in the root rhizosphere, thus, outwitted the pathogen. Besides *T. harzianum* parasitized over *F. solani*, resulting into lysis of its hyphac. The antagonistic activity of *T. viride* was due to its yellow pigments secreted in the rhizosphere. The yellow coloured metabolite was toxic and killed the hyphae of *F. solani*. Thereafter, *T. viride* grew over the space cleared by its metabolite.

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Table-1: Population of *F. solani* and *T. harzianum* in *F. Solani* infested soil (colonies/g soil) 7,14,21 days after amendment with *T. harzianum in vitro.*

No. of days after treatment	The fungal population in *T. harzianum* treated soil		Colonies of *F. solani* in untreated (control) soil	% decrease of colonies in *T. harzianum* amended soil over the control
	T. harzianum	*F. solani*		
7	$65.5x10^3$	$27.5 x10^3$	$109.5 x10^3$	74.88
14	$46.6 x10^3$	$22.4 x10^3$	$65.2 x10^3$	65.64
21	$62.4 x10^3$	$18.5 x10^3$	$67.5 x10^3$	72.59
CD at 5%	5560.41	20.99	7048.75	
C.V.	5.37	5.18	4.90	

Table-2: Population of *F. solani* and *T.viride* in *F. Solani* infested soil (colonies/g soil) 7,14,21 days after amendment with *T. viride in vitro.*

No. of days After Treatment	The fungal population in *T. Viride* Treated soil		Colonies of *F. solani* in untreated (control) soil	% decrease of colony in *T. viride*amended soil over the control
	T. viride	*F. solani*		
7	$51.2x10^3$	$33.2 x10^3$	$109.5 x10^3$	69.68
14	$46.4 x10^3$	$11.3 x10^3$	$65.2 x10^3$	82.66
21	$59.1 x10^3$	$15.1 x10^3$	$67.5 x10^3$	77.62
CD at 5%	4608.88	1775.94	7048.75	
C.V.	4.960	5.03	4.90	

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Table-3: Population of *F. solani* and *T. virens* in *F. Solani* infested soil (colonies/g soil) 7,14,21 days after amendment with *T. virens in vitro*

No. of days after treatment	The fungal population in *T. virens* Treated soil		Colonies of *F.solani* in untreated (control) soil	% decrease of colony in *T. Virens* amended soil over the control
	T. virens	*F. solani*		
7	$58.3x10^3$	$30.2x10^3$	$109.5\ x10^3$	72.42
14	$56.2\ x10^3$	$21.3\ x10^3$	$65.2\ x10^3$	67.33
21	$55.1\ x10^3$	$17.2\ x10^3$	$67.5\ x10^3$	74.51
CD at 5%	5711.94	1679.67	7048.75	
C.V.	5.67	4.12	4.90	

Table-4: % reduction of *F. Solani* colony over the control in *Trichoderma* amended soil.

No. of days after treatment	Percent reduction of *F. solani* colony over the control		
	T. harzianum	*T. viride*	*T. virens*
7	74.88	69.68	72.42
14	65.64	82.66	67.33
21	72.59	77.62	74.57

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Fig: 1 Healthy seedling of bael

Fig: 2 Root rot infected seedling of bael

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