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Management of root rot disease of bael (*Aegle marmelos* Correa) with *Trichoderma* spp. *invitro*

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Abstract: The bael (*Aegle marmelos* Correa) is an important indigenous arid zone fruitbelonging to family rutaceae often termed as underutilized minor fruit. The population of *F. solani* 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.* 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* grow 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: *Aegle marmelos* Correa, Root rot, *F. solani, Trichoderma harzianum, T. virens* and *T. viride*.

Introduction

The beal (*Aegle marmelos* Correa) is an important indigenous arid zone fruit belonging to family rutaceae often termed as underutilized minor fruit. 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.In the ancient medical Aurvedic treatise 'CharakaSamhita' every part of this tree, stem, bark, root, leaves and fruit at all stages of maturity have medicinal merits and have been used as remedy for a long time. Bael is valued for its high nutritional and medicinal properties and recognized as one of the most important medicinal tree species. The leaf extract of bael completely inhibit the growth of *Penicillum oxalicum* and *P. italicum* a storage fungi (Tiwari *et al.*, 1987). The unripe fruit is regarded as astringent, digestive and stomachic and is usually prescribed for diarrhoea and dysentery. Anti-diarrhoetic activity of bael root was studied by Pitre and Srivastava (1987).

The fruits are found in plenty in the wild state in U.P., Orissa, Bihar, West Bengal and Madhya Pradesh, etc. it is being cultivated in limited areas in Gonda, Basti, Deoria, Mirzapurand Etawa districts of Uttar Pradesh and Sewan district of Bihar. Gond*et al.,* (2007)isolated endophytic fungi from healthy, living, and symptomless tissues of inner bark of leaf and roots of *Aeglemarmelos*, a well-known medicinal plant, growing in different parts of India including Varanasi. A total of 79 isolates of endophytic fungi representing 21 genera were isolated, adopting a standard isolation protocol. Nursery plants suffer from a number of fungal diseases namely root rot, collar rot and wilt caused by *F. solani*, leaf spot caused by *Myrothecium roridum* and *Alternria alternata*, die back and leaf spot caused by *Fusarium pallidoroseum* (Anonymous, 2010). Owing to expansion of bael orchards, working out of management strategies is also equally important aonla fruits and hence the present study was conducted to test the effect of different *Trichoderma* spp.The present study was undertaken with the following objectives: Isolation of root rot pathogen, identification of root rot pathogen, management of root rot pathogen through *Trichoderma* species.

Materials and Methods

To test the efficacy of *T. harzianum, T. viride* and *T. virens* against the pathogen *Fusarium* (Kuhn) was assessed by using dual culture technique (Morton and Strouble, 1955). Biological agents that control fungal diseases of plants in the field include antagonists such as *Trichoderma harzianum, T. viride and T. virins* are parasitic organisms. Twenty ml of sterilized melted PDA was aseptically poured in to sterilized petri dishes (90 mm diameter) and then allowed to solidify. Five mm disc of each of the antagonists and *F. solani* were cut with the help of sterilized cork borer from the edge of the three days old cultures and were placed over solidified PDA in Petri dishes at 60 mm apart from each other. In control set, only a disc of *F. solani* was placed over the PDA plate. The Petri dishes were incubated in BOD incubator at 26 ± 2^0C. The radial growth of the fungal colonies was recorded every day at 24 h interval till the colonies of *Trichoderma* species moved the colonies of *F. solani.* Fives replications for each treatment were maintained.

The fungal antagonistic effects of putative biocontrol fungi such as *T. harzianum, T. viride, T. koningi* and *Gliocladium virens* were tested *in vitro* for their bio-efficacy in inhibiting the putative pathogens using the dual culture technique (Dennis and Webster, 1971). *Trichoderma* and *Gliocladium* gave best control of pathogens of pea mainly *R. solani, F. solani*and *S. sclerotiorum* (Lacicowa and Pieta, 1994).The antagonistic activity of *T. virens, T. viride, T. harzianum, T. koningii, Pseudomonas fluorescens*and *Bacillus subtilis*were evaluated against *F. solani*and *R. solani*causing root rot of sage (Mallesh *et al.,* 2009).

Results and Discussion

Although research on exploitation of bio control agents in the management of major plant diseases has advanced considerably but the limited work has been done on soil borne diseases of bael (*Aegle marmelos* Correa). In dual culture test the highest growth after 24 h of incubation was recorded with *T. virens* (1.58 cm) followed by *T. viride* (1.17 cm) and *T. harzianum* (1.06). During this period the colonies of *Trichoderma* species and *Fusarium solani* did not touch with each other and the *Trichoderma* species also did not develop any pigment. Next day the same trend was also maintained but differences in the size of colonies between *T. harzianum* and *T. viride* was reduced to insignificant level. After 72 h the colonies of *Trichoderma* species and *F.solani* in between gap and touched each other. The center portion of all the *Trichoderma* colonies developed their characteristics green colour with white spreading mycelia around the green center by this time size of the colony of *T. virens* become maximum (1.98 cm). The second big colony size was recorded with *T. harzianum* (1.58) while minimum *T. viride* (1.52 cm) upto 5th day (Table-1, 2, 3).

The same trend was maintained although the difference between *T. harzianum* and *T. viride* was reduced to the insignificant level. However, on the 6th day the highest growth was recorded with *T. harzianum*(2.82 cm) andminimum *T. viride* (1.67 cm) up to the 8th day the same trend was maintained. During this period *T. harzianum* was found to engulf the entire colony of *F. solani*. Hence, further recording of colony size of *T. harzianum* was stopped. *T. virens* become successful to over grow the colony of *F. solani* on 14th day. *T. viride* took one day more time to completely smooth the colony of *F. solani*. The rate of increase of colony size of three *Trichoderma* species as well as rate of reduction of the size of the colonies of *F. solani* by different antagonists, it is apparent from the table that at the initial 24 h the growth rate of *T. harzianum* was highest (41.50%) followed by *T. virens* (23.41%)while minimum in *T. viride* (20.51%) the same trend was maintained upto next 24 h (Table- 4). However on the 4th day, there was considerable reduction in the growth rate of all the three *Trichoderma* species. This is the time when the colonies of *Trichoderma* species and *F. solani* touched with each other but on the next day. *T. harzianum* resumed its high growth rate and maintained the same for next three days, while the growth rate of other two species remained low upto 10th day. From 11th day and onwards they recovered from the setback and resumed high growth rate. *F. solani* registered high growth rate during the initial two days i.e. before touching its colony

Table-1: Interaction between *F. solani* and *T. harzianum* in dual culture test *in vitro*

Date of observation	*T. harzianum* size of colony (cm)	% Interaction of *T. harzianum* colony (cm)	*F. solani* size of colony (cm)	% decrease of *F. solani* colony/day
27-04-13	1.06	-	2.80	-
28-04-13	1.50	41.50	2.35	16.07
29-04-13	1.58	5.33	2.27	3.40
30-04-13	2.02	27.84	1.83	19.38
01-05-13	2.28	39.60	1.04	43.17
02-05-13	3.45	22.34	0.60	61.54
03-05-13	3.70	7.24	0.15	62.50
04-05-13	3.85	4.04	0.00	100
CD at 5%	0.36	-	0.15	-
C.V.	8.33	-	6.45	-

Table-2: Interaction between *F. solani* and *T.viride* in dual culture test *in vitro*

Date of observation	*T. viride* size of colony (cm)	% Interaction of *T. viride* colony (cm)	*F. solani* size of colony (cm)	% decrease of *F. solani* colony/day
27-04-13	1.17	-	2.68	-
28-04-13	1.41	20.51	2.44	8.96
29-04-13	1.52	7.80	2.35	3.46
30-04-13	1.61	5.92	2.24	4.68
01-05-13	1.67	3.73	2.18	2.68
02-05-13	1.69	1.19	2.16	0.92
03-05-13	1.78	5.32	2.07	4.17
04-05-13	1.80	1.12	2.05	0.96
05-05-13	1.83	1.67-	2.02	1.46
06-05-13	2.24	-22.40	1.61	-20.29
07-05-13	2.64	17.86	1.21	24.84
08-05-13	3.26	23.48	0.60	50.41
09-05-13	3.59	10.12	0.40	33.33
10-05-13	3.85	7.24	0.00	100
CD at 5%	0.24		0.25	-
CV	6.79		8.60	-

Table-3: Interaction between *F. solani* and *T.virens* in dual culture test *in vitro*

Date of observation	*T. virens* size of colony (cm)	% Interaction of *T. virens* colony (cm)	*F. solani* size of colony (cm)	% decrease of *F. solani* colony/day
27-04-13	1.58	-	2.27	-
28-04-13	1.95	23.41	1.89	16.74
29-04-13	1.98	1.54	1.87	1.06
30-04-13	2.05	3.54	1.80	3.74
01-05-13	2.07	0.98	1.78	1.11
02-05-13	2.09	0.96	1.76	1.12
03-05-13	2.11	0.95	1.74	1.15
04-05-13	2.12	0.47	1.73	0.57
05-05-13	2.13	0.47	1.72	0.58
06-05-13	2.53	18.74	1.32	23.26
07-05-13	2.82	11.46	1.03	21.97
08-05-13	3.40	20.56	0.46	55.33
09-05-13	3.85	13.20	0.00	100
CD at 5%	0.32		0.19	
CV	8.02		7.51	

in table 1 to 3: Date of inoculation 26-04-2013, Disk size 0.3 cm

Table-4: Rate of increase of colony size three *Trichoderma* species as well as rate of reduction of decrease of *F. solani* in dual culture test *in vitro*

Date of observation	% increase of colony/day of *Trichoderma species*			% decrease of colony /day of *F. solani* cultured with *Trichoderma* species		
	T. harzianum	*T. viride*	*T. virens*	*T. harzianum*	*T. viride*	*T. virens*
27-04-13	-	-	-	-	-	-
28-04-13	41.50	20.51	23.41	16.07	8.96	16.74
29-04-13	5.33	7.80	1.54	3.40	3.46	1.06
30-04-13	27.84	5.92	3.54	19.38	4.68	3.74
01-05-13	39.60	3.73	0.98	43.17	2.68	1.11
02-05-13	22.34	1.19	0.96	61.54	0.92	1.12
03-05-13	7.24	5.32	0.95	62.50	4.17	1.15
04-05-13	4.00	1.12	0.47	100.00	0.96	0.57
05-05-13	-	1.67	0.47	-	1.46	0.58
06-05-13	-	22.40	18.74	-	20.29	23.26
07-05-13	-	17.86	11.46	-	24.84	21.97
08-05-13	-	23.48	20.56	-	50.41	55.33
09-05-13	-	10.12	13.24	-	33.33	100.00
10-05-13	-	7.24	-	-	100.00	-

Not recorded as the *Trichoderma species* totally over growth the colony of *F. solani*

with those of *Trichoderma* sp. Thereafter, it started declining from 6th day and onwards the decline was sleep. The first declining rate of *F. solani* colony with *T. viride* and *T. virens* was noticed from 11th day interaction between *T. harzianum, T. virens* and *T. viride* with *F. solaniin vitro* over growth of colonies of *T. harzianum* and *T. virens*the colony of *F. solani*. A yellow pigmentation zone formed surrounding the colony of *T. viride*with proceeding of the yellow zone towards the colony of *F. solani*. Mycelia of *F. solani* disintegrated and the size of colony gradually reduced. A clear zone free from mycelia either of the pathogen and the antagonist was formed.

In dual culture test the highest growth after 24 h of incubation was recorded with *T. virens* followed by *T. viride* and *T. harzianum*. The population of *T. virens* registered a gradual decline, *T. harzianum* showed the capacity to reduce the colony of *F. solani* within the first week (Khan and Sinha, 2007) while *T. viride* caused maximum reduction at the 2nd week (Jain *et al*., 2008) and *T. virens* caused maximum damage to *F. solani* twenty one days after. Similar findings were observed by Dennis and Webester (1971) found that many isolates of *Trichoderma* spp. produced volatile and nonvolatile antibiotics active against wide range of fungi. After 72 h *T. virens* become maximum and then *T. harzianum*and *T. viride.* Similar study was also conducted by Raziq, 2005. Besides its parasitized the hyphae of *F. solani*, *T. virens* grew very fast and left on space for the pathogen to be established there. Similar findings were recorded by Lacicowa and Pieta (1994) against *R. solani, F. solani*and *S. sclerotiorum.* The *F. solani* registered high growth rate during the initial two days i.e. before touching its colony with those of *Trichoderma* sp. Thereafter, it started declining from 6th day and onwards the decline was sleep. The first declining rate of *F. solani* colony with *T. viride* and *T. virens* was noticed from 11th day.*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*. The *T. harzianum*has been recognized as a strong mycoparasite against soil borne pathogens such as *R. solani, S. rolfsii* and *F. oxysporum* (Papavizas, 1985).

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