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Variability of *Trichoderma* isolates against the Fusarium wilt of beal *in-vitro*

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Abstract
Efficacy of three *Trichoderma* species viz. *T. harzianum, T. virens* and *T. viride* against *F. solani* were tested by Dual Culture technique. *T. virens* and *T. viride* grew very fast before touching the colony of the *F. solani*. Thereafter, their growth was significantly slowed down. But *T. harzianum* grew very fast over the colony of *F. solani*: thus completely covering it within 8 days. *T. viride* secreted yellow pigment zone around its colony. The yellow pigment caused lysis of the colony of *F. solani*. Thereafter the mycelia of *T. viride* grew over this cleared zone. It took around 13 days to completely destroy the colony of *F. solani*, *T. virens* took larger period (about 14 days) to smoothed the colony of the pathogen.

Keywords: *Fusarium solani, Trichoderma*

1. Introduction
Bael is valued for its high nutritional and medicinal properties recognized as one of the most important medicinal tree species. The ripe fruit is a tonic, restorative, astringent, laxative and good for heart and brain. The unripe fruit is regarded as astringent, digestive and stomachic and is usually prescribed for diarrhoea and dysentery. Leaves of Bael are said to cause abortion and sterility in women, its leaves are extremely useful for treating diabetes, Jaundice, Cholera and Asthma. The beal fruit is very valuable in habitual constipation, chronic dysentery and dyspepsia. It is one of the ingredients in the 'Dashamul' or ten roots used in Ayurveda.
Trichoderma isolates were assessed for their ability to inhibit soil borne pathogenic fungi in *vitro* on PDA Medium 23±°C from 1994 to 1997. The highest inhibition on radial colony growth of *R. solani, Rosellinia necatrix, V. dahliae* was observed upon treatment with some isolates of *Trichoderma koningii*. The *Gliocladium virens* isolates recorded the great inhibition on radial growth, colour of *F. oxysporum* sp. *lycopersici, T. hamatum isolates* were most efficient in suppressing the radial colony growth of *Cylindrocarpon* sp. and *F. oxysporum* sp. *dianthi.* Many *Trichoderma* isolates completely colonized the mycelium of some pathogenic fungi [1].

2. Materials and Method
2.1 Bio-agents (*Trichoderma* species) tested against the pathogen were
1. *Trichoderma harzianum* (Rifai.)
2. *Trichoderma viride* (pers.)
3. *Trichoderma virens* (Rifai.)

2.2 Interaction between the pathogen and the antagonists *in vitro*
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). Twenty ml of sterilized melted PDA was aseptically poured in to sterilized Petri dishes (90 mm diameter) to which a pinch of streptomycine was added 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 colonized over the colonies of *F. solani.* Fives replications for each treatment were maintained.

3. Result
3.1 Interaction between *F. solani* and *Trichoderma* species in dual culture test *in vitro*:
In dual culture test the highest growth after 24 hours of incubation was recorded with *T. virens* (1.58 cm) followed by *T. viride* (1.17 cm) and *T. harzianum* (1.06) (Table 1, 2, 3).

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 *Fusarium solani* touched each other. The centre portion of all the *Trichoderma* colonies developed their characteristics green colour with white spreading mycelia around the green centre by this time size of the colony of *T. virens* become maximum (1.98 cm) (Table-3). The second big colony size was recorded with *T. harzianum* (1.58) while minimum *T. viride* (1.52 cm) up to 5th day. The same trend was maintained although the differences 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) and minimum *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 smoothed the colony of *F. solani*.

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

Date of observation	*T. harzianum* size of colony (cm)	% increase of *T. harzianum* Colony/day	*F. solani* size of colony (cm)	% decrease of *F. solani* colony/day
27-4-13	1.06	-	2.80	-
28-4-13	1.50	41.50	2.35	16.07
29-4-13	1.58	5.33	2.27	3.40
30-4-13	2.02	27.84	1.83	19.38
1-5-13	2.82	39.60	1.04	43.17
2-5-13	3.45	22.34	0.6	61.54
3-5-13	3.7	7.24	0.15	62.5
4-5-13	3.85	4.04	0	100
CD at 5%	0.36	-	0.15	-
C.V.	8.33	-	6.45	-

*Date of inoculation 26-04-2013, Disc size-0.3cm

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

Date of observation	*T. viride* size of colony (cm)	% increase of *T. viride* Colony/day	*F. solani* size of colony (cm)	% decrease of *F. solani* colony/day
27-4-13	1.17	-	2.68	-
28-4-13	1.41	20.51	2.44	8.96
29-4-13	1.52	7.80	2.35	3.46
30-4-13	1.61	5.92	2.24	4.68
1-5-13	1.67	3.73	2.18	2.68
2-5-13	1.69	1.19	2.16	0.92
3-5-13	1.78	5.32	2.07	4.17
4-5-13	1.8	1.12	2.05	0.96
5-5-13	1.83	1.67	2.02	1.46
6-5-13	2.24	22.40	1.61	20.29
7-5-13	2.64	17.86	1.21	24.84
8-5-13	3.26	23.48	0.6	50.41
9-5-13	3.59	10.12	0.4	33.33
10-5-13	3.85	7.24	0	100
CD at 5%	0.24		0.25	
CV	6.79		8.60	

*Date of inoculation 26-04-2013, Disc size-0.3cm

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

Date of observation	*T. virens* size of colony (cm)	% increase of *T. viride* Colony/day	*F. solani* size of colony (cm)	% decrease of *F. solani* colony/day
27-4-13	1.58	-	2.27	-
28-4-13	1.95	23.41	1.89	16.74
29-4-13	1.98	1.54	1.87	1.06
30-4-13	2.05	3.54	1.80	3.74
1-5-13	2.07	0.98	1.78	1.11
2-5-13	2.09	0.96	1.76	1.12
3-5-13	2.11	0.95	1.74	1.15
4-5-13	2.12	0.47	1.73	o.57
5-5-13	2.13	0.47	1.72	0.58
6-5-13	2.53	18.74	1.32	23.26
7-5-13	2.82	11.46	1.03	21.97
8-5-13	3.4	20.56	0.46	55.33
9-5-13	3.85	13.2	0	100
CD at 5%	0.32		0.19	
C.V.	8.02		7.51	

*Date of inoculation 26-04-2013, Disc

3.2 Rate of increase of colony size of three *Trichoderma* species and rate of reduction of *F. solani* in dual culture test *in vitro*

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 has been presented in the Table-4, 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. 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 up to 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 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. solani in vitro* over growth of colonies of *T. harzianum* (Fig. 4) and *T. virens* (Fig. 4) 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.

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.virens***	***T.viride***
27-4-13	-	-	-	-	-	-
28-4-13	41.50	20.51	23.41	16.07	16.74	8.96
29-4-13	5.33	7.80	1.54	3.40	1.06	3.46
30-4-13	27.84	5.92	3.54	19.38	3.74	4.68
1-5-13	39.60	3.73	0.98	43.17	1.11	2.68
2-5-13	22.34	1.19	0.96	61.54	1.12	0.92
3-5-13	7.24	5.32	0.95	62.5	1.15	4.17
4-5-13	4.00	1.12	0.47	100	o.57	0.96
5-5-13	-	1.67	0.47	-	0.58	1.46
6-5-13	-	22.40	18.74	-	23.26	20.29
7-5-13	-	17.86	11.46	-	21.97	24.84
8-5-13	-	23.48	20.56	-	55.33	50.41
9-5-13	-	10.12	13.24	-	100	33.33
10-5-13	-	7.24	-	-	-	100

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

Fig 1: Pure culture of *Fusarium solani*

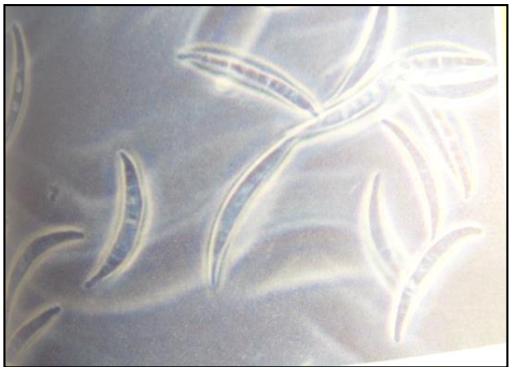
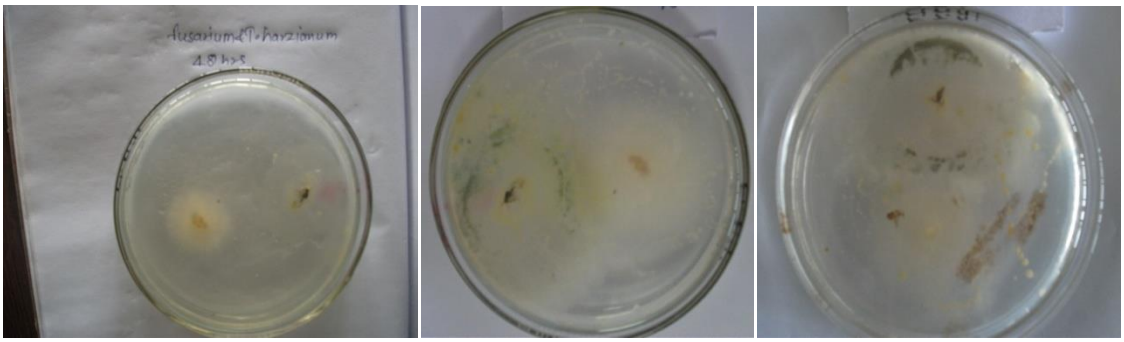


Fig 2: Conidia of *Fusarium solani*

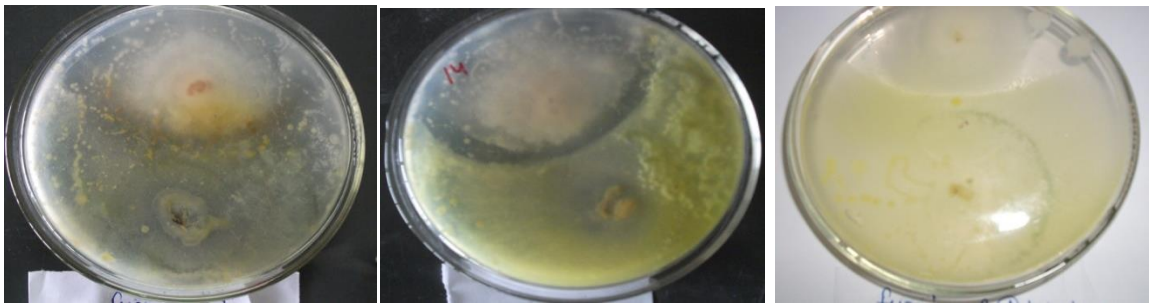
Fig 3: Clamydospore of *Fusarium solani*



One day after inoculation

Three days after inoculation

Five days after inoculation



Seven days after inoculation

Fig 4: Interaction between *F. solani* and *T. harzianum*, *T. viride* and *T. verens* in dual culture test *in vitro*, Each Fig. showed plate 1 *T. harzianum*, plate 2 *T. viride* and plate 3 *T. virens*

4. Conclusion

Trichoderma. harzianum almost constantly maintained a fairly good number of populations but *T. viride* required about three weeks' time to build up population. The population of *T. virens* registered a gradual decline, *T. harzianum* showed the maximum capacity to reduce the colony of *F. solani* within the first week while *T. viride* caused maximum reduction at the 2nd week and *T. virens* caused maximum damage to *F. solani* twenty-one days after. In the first week of the treatment *T. harzianum* followed by *T. virens* was most active against *F. solani*. But at later stage *T. viride* brought about maximum reduction in *F. solani* [2, 3].

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