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Comparative ecological behaviour of some *pre-* and *post-tsunami* isolates of *Trichoderma harzianum* and *T. viride* from Andaman & Nicobar Islands

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A B S T R A C T

A comparative ecological study of some pre- and post *Tsunami* isolates of *Trichoderma harzianum* and *T. viride* from the Andaman and Nicobar Islands, India, was undertaken to assess the effect on some ecological behavoiur of these biocontrol agents. All the isolates showed better parasitic ability and rhizosphere colonization when the competition of other organisms withdrawn compared to natural and sun dried soil. Both competitive parasitic behaviour and rhizosphere colonization was low in post-Tsunami isolates of *Trichoderma*. The chlamydospore inoculum was found best in percentage colonization of sclerotia of boh *R. solani* and *S. rolfsii*, followed by mycelial and conidial inoculum. The isolate ThrAN-5 (*T. harzianum*) was most efficient in parasitizing the sclerotia of *S. rolfsii* and *R. solani*, followed by TvAN-3, TvAN-5 and ThrAN-7 (*Pre-Tsunami* isolates), whereas there was significant reduction in their parasitizing ability of post-Tsunami isolates. Similar results were also noted in their rhizosphere colonizing ability in rhizosphere of chick pea seedlings. All isolates of *Trichoderma* showed significantlty low competitive parasitic ability and rhizosphere colonization in Mohanpur (B.C.KV.) soil as compared to Port Blair (CARI) soil.

Introduction

The *Trichoderma* spp. as potential biological control agents against soilborne plant pathogens has been recognized for more than seven decades (Orr and Knudsen, 2004; Bhagat and Pan, 2007). Taking advantage of the natural competition between microorganisms for limited biological resources is the basis for biocontrolling plant pathogens. But for maximum effectiveness, fungal biocontrol agents should become established in soil and crop ecosystems and remain active against target pathogens. The parasitic or saprophytic ability of antagonists, *Trichoderma* spp. are two major attributes which make them a potential antagonist against many soil borne plant pathogens. The penetration and subsequent colonization by antagonists on the sclerotia of many phytopathogens obviously reflect much upon their parasitic ability rather than saprophytic attributes in the intensely competitive microbiotic environment in soil. *R. solani* and *S. rolfsii* are well known to produce sclerotia, the principal structure as the means of survival propagules under adverse environmental condition, sclerotia are known to survive for several years in soil (Young and Ashford, 1995; Coley Smith & Cook, 1971) and how they behave so has been a subject of research to find new methods of biological control.

Rhizosphere competence of antagonistic fungi in root zone of many crops is a vital area of research that requires elaborated exploration for successful management of plant diseases by biocontrol agents. It is expected that the *Trichoderma* spp. should come in contact and establish within the rhizosphere zone of plant earlier than any other micro organisms, thus they will provide a protective cover for root tips and hairs which otherwise vulnerable to attack by several plant pathogenic fungi. Interestingly, more aggregation of *Trichoderma* population have been observed near the stem base or collar region and root tip or root hairs than middle sections of root systems, which are the most vulnerable plant parts to be attacked by pathogens (McLean *et al.* 2005).

The change in soil microbial situation particularly after massive earth quake followed by deadly *Tsunami* on 26th December, 2004, appears to have immense scientific importance in the Bay Island. Therefore, present study was

undertaken to explore the native isolates of *Trichoderma* with respect to their competitive parasitic abiity to *R. solani* and *S. rolfsii* and rhizosphere colonization in chick pea at Port Blair (C.A.R.I.) and Mohanpur (B.C.K.V)

Materials and Methods

The pre- and post-*Tsunami* isolates of *Trichoderma* were isolated from the rhizosphere soil of by following soil dilution technique. The *Trichoderma* spp have been identified upto species level by following taxonomic keys of Rifai (1969) and Bissett (1991a-c). The pure culture of *T. harzianum* and *T. viride* were preserved at 4ºC in a refrigerator for subsequent use.

Preparation of mycelial, conidial and chlamydospore inocula

Mycelial plug of *Trichoderma* spp. of 6 mm dia. was aseptically inoculated into an Erlenmeyer flask(500 ml) containing 100 ml potato dextrose broth medium and incubated at 28 ± 1ºC for 3 - 4 days (mycelial inocula) and 9 days (conidial inocula) into BOD incubator. Both the mycelial and conidial inocula prepared by homogenizing the mycelial mat and conidia separately into a homogenizer after harvesting. The chlamydospore inocula were prepared by growing the *Trichoderma* isolates into a glucose tartarate (GT) medium for 21 days and final inocula prepared as stated above. The mycelial, conidial and chlamydospore inocula of *Trichoderma* isolates are tested immediately for their parasitic ability against sclerotia of *R. solani* and *S. rofsii*.

Competitive parasitic ability

100g air-dried soil (natural, sun dried and sterilized) was mixed thoroughly with mycelial, conidial and chlamydospore inocula of test isolates of *Trichoderma*, MHC was adjusted at 50 % and it was filled in plastic cup (100 ml). Ten sclerotia of *R. solani* and *S. rolfsii* were buried at 0.5 – 2.0 cm depth, covered with perforated aluminium foil and incubated at 28 ± 1ºC for one week. The sclerotia were harvested and plated onto modified TSM after surface sterilization. The number of sclerotia colonized by *Trichoderma* isolates was recorded in each case. The experiment was replicated into four times in each case with suitable control.

Rhizosphere colonization

Two kg potting mixture (natural, sun dried and sterilized) of soil and farm yard manure (2: 1, v/v) was mixed thouroughly with 10 g of WB + MC(20 %) formulation of *Trichoderma* isolates (x 10^8cfu/g potting mixture), MHC adjusted at 50 % and 25 seeds of gram (cv. *Mahamaya*) was sown per plot. The soil sample from the rhizosphere soil of gram was collected at 45 DAS and population (cfu/g soil) of *Trichoderma* isolates was estimated by soil dilution technique. The experiment was laid out in randomized block design (RBD) and replicated thrice with suitable control. Statistical analysis was done after angular transformation of the data.

Results and Discussion

Results presented in Table 1 and 2 revealed that all isolates of *Trichoderma* colonized and parasitized the sclerotia of *R. solani* and *S. rolfsii*, but sclerotia of *S. rolfsii* was less infected by *Trichoderma* isolates than sclerotia of *R. solani*. Irrespective of soil types and geographical locations, the pre- *Tsunami* isolates of *Trichoderma* i.e. ThrAN-5, ThrAN-7, TvAN-3 and TvAN-5, shown better parasitic ability than post-*Tsunami* isolates by comparatively high percentage colonization of sclerotia of test pathogens. The isolate Thr AN-5 of *T. harzianum*, irrespective of soil types (natural, sun dried and sterilized soil), geographic locations (Port Blair and Mohanpur) as well as antagonist inocula (mycelial, conidial and chlamydospores), was most effective in parasitizing the sclerotia of *R. solani* and *S. rolfsii*, Tv AN-3, Tv AN-5 and ThrAN-7 followed and extent was not statistically significant. Among the post-Tsunami isolates, ThrAN-13 of *T. harzianum* was superior than other isolates. In all isolates of *Trichoderma* chlamydospore form of inoculum was most effective in percentage colonization of sclerotia of test fungi. Sterilized soil supported better colonization of *Trichoderma* as compared to sun dried and natural soil.

Figure 1-3 indicate that irrespective of different locations, *Trichoderma* isolates have significantly high rhizosphere colonizing ability in the rhizosphere of chick pea, compared to non-treated control. The isolate Thr AN-5 of *T. harzianum* was most efficient in rhzosphere colonization of chick pea at both locations, viz., Port Blair and Mohanpur and in all three types of soil used. This was followed by Tv AN-3, TvAN-5 and ThrAN-7 (pre-*Tsunami* isolates) but significantly differed with post-*Tsunami* isolates viz., ThrAN-13, ThrAN-16, TvAN-8 and TvAN-11. Serilized soil supported better colonization of rhizosphere in gram plant than the sun dried and natural soil.

Existence of *Trichoderma* spp. in various soils, depends on their ability to degrade various organic substrates in soil, metabolic versatility, and resistance to microbial inhibitors. It suggests that they may possess the ability to survive in many ecological niches depending on the prevailing conditions and the species or strains involved. This assumption highlights the distinction between passive and active competitive saprophytic ability, including the dynamic colonization of plant

rhizosphere. Our present findings revealed that irrespective of geographical locations, soil types and the form of inoculum the competitive parasitic ability and rhizosphere colonization ability of pre-*Tsunami* isolates of *T. harzianum* and *T. viride* have significant edge over the post-*Tsunami* isolates. This may be due to the change in soil environmental conditions after sea water inundation, acute salinity development that might have weakened the *Trichoderma* spp.(Papavizas, 1985). Present findings suggested that the test isolates of *Trichoderma* varied in their ability to colonize the sclerotia of *R. solani* and *S. rolfsii*. These findings are in accordance with Hennis *et al.* (1983) who have shown that strains of *Trichoderma* spp. varied in their ability to colonize the sclerotia of *S. rolfsii*. The possible role of lectin in specific recognition between *Trichoderma* spp. and *S. rolfsii* and colonization of sclerotia has been highlighted (Barak *et al.* 1985). The results of present investigation also suggested that the per cent colonization of sclerotia of pathogens, *R. solani* and *S. rolfsii* increased with increasing concentration of inoculum regardless of nature of inocula and thereby corresponding decreased EID_{50} values were recorded. Indigenous isolates of *T. harzianum* and *T. viride* seemed to be more efficient than introduced one in relation to both competitive parasitic ability and rhizosphere colonization of *Trichoderma* spp. The possible explanation of this study could be attributed to the fact that the natural soil often ecologically does not allow introduced biocontrol agents to perform well due to some abiotic and biotic factors (Hubbard *et al.* 1983; Papavizas, 1985; Hadar *et al.* 1984; Knudsen and Bin, 1990; Bae and Knudsen, 2005). Soil solarization and sterilization upsets the ecosystem to the extent that it may allow proliferation of *Trichoderma* spp. More over form of inoculum may play an important role in parasitic ability and rhizosphere colonization. Present results corroborated with the findings of Lewis and Papavizas (1984) where they suggested that mycelial inoculum was better than conidial inoculum, for soil application and chlamydospres were less sensitive to biotic and abiotic stresses.

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Fig 1: Rhizosphere colonization of Trichoderma spp. in natural soil of gram

Fig 2: Rhizosphere colonization of Trichoderma spp in natural sun dried soil of gram

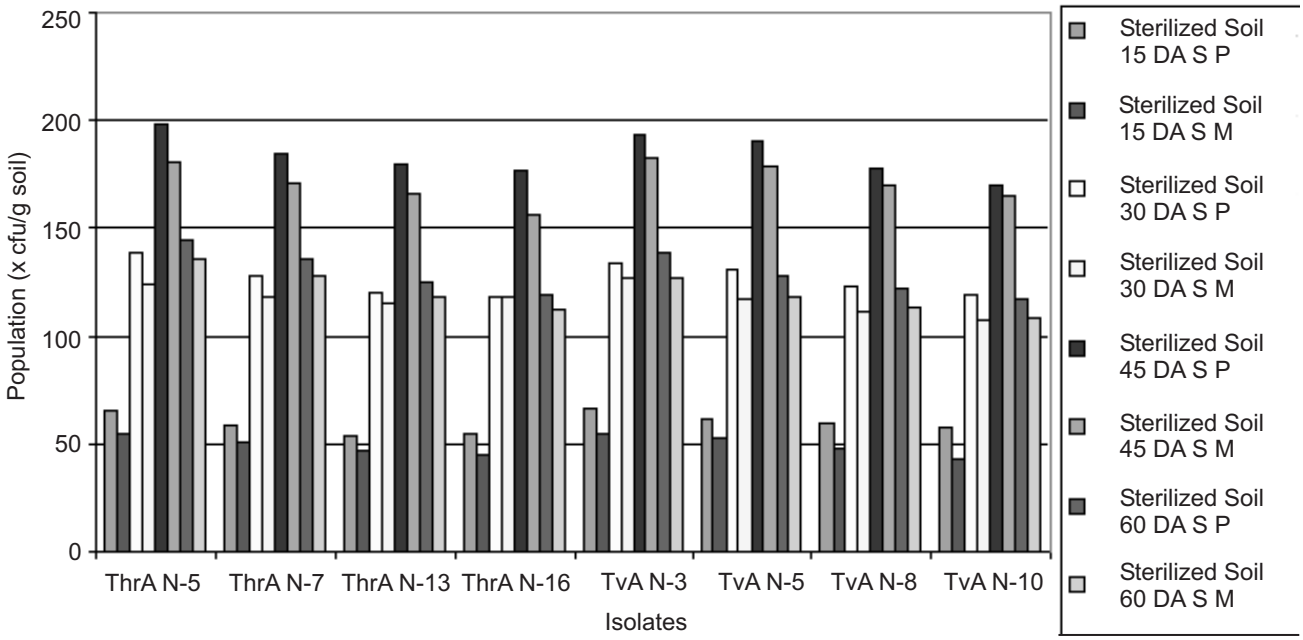

Fig 3: Rhizosphere colonization of Trichoderma spp. in sterilized soil of gram

Table 1:
Competitive parasitic ability of *Trichoderma* isolates against sclerotia of *S. rolfsii*

Percent Colonization of Trichoderma Isolates to Sclerotia of *S. rdfsii*																		
	Natural soil						Sun dried Soil						Sterlized soil					
Isolates	Mycelia		Conidia		Cllamyd Ospores		Mycelia		Conidia		Cllamyd Ospores		Mycelia		Conidia		Cllamyd Ospores	
	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M
Thran-5	16.6 (24.9)	13.4 (21.5)	12.4 (20.6)	10.5 (18.9)	20.2 (26.7)	17.5 (24.3)	19.2 (2.0)	17.1 (24.3)	15.4 (23.1)	13.2 (21.3)	35.9 (36.8)	30.6 (33.6)	31.4 (34.1)	28.4 (32.2)	23.7 (29.1)	21.1 (27.3)	41.2 (39.9)	38.7 (38.5)
Thran-7	24.0 (22.1)	12.7 (20.9)	11.2 (19.5)	10.2 (18.6)	18.5 (22.5)	16.4 (23.9)	16.1 (23.7)	14.7 (22.5)	12.4 (20.6)	11.2 (19.5)	29.3 (32.8)	25.5 (30.3)	27.2 (31.4)	24.5 (29.7)	19.4 (26.1)	17.9 (25.0)	38.6 (38.4)	36.1 (36.9)
Thran-13	11.5 (19.8)	10.4 (18.8)	9.3 (17.8)	8.5 (16.9)	17.4 (24.6)	15.1 (22.9)	15.5 (23.2)	13.0 (21.1)	9.8 (18.2)	8.2 (16.6)	25.8 (30.5)	23.1 (28.7)	25.4 (30.3)	22.1 (28.0)	17.2 (24.5)	16.5 (24.0)	36.4 (37.1)	33.4 (35.3)
Thran-16	11.3 (19.6)	10.2 (18.6)	7.1 (15.4)	6.1 (14.3)	17.4 (24.6)	15.3 (23.0)	14.2 (22.1)	12.3 (20.5)	10.1 (18.5)	8.9 (17.4)	27.2 (31.4)	24.3 (29.5)	20.4 (26.8)	16.4 (23.9)	15.2 (22.9)	13.4 (21.5)	30.1 (33.3)	27.8 (31.8)
Thran-3	16.4 (23.9)	14.5 (22.4)	14.1 (22.1)	11.7 (20.0)	19.8 (26.4)	16.9 (24.3)	21.7 (27.8)	18.7 (25.6)	14.5 (22.4)	12.1 (20.4)	34.8 (36.1)	29.5 (32.9)	29.7 (33.0)	26.3 (30.8)	25.3 (30.2)	22.6 (28.4)	43.6 (41.3)	40.2 (39.3)
Thran-5	14.7 (22.5)	12.3 (20.5)	13.2 (21.3)	11.0 (19.4)	18.8 (25.7)	16.5 (24.0)	17.8 (24.9)	14.6 (22.5)	13.3 (21.4)	11.9 (20.2)	32.1 (34.5)	27.3 (31.5)	28.5 (32.3)	23.6 (19.1)	21.5 (27.6)	19.6 (26.3)	39.9 (39.2)	36.5 (37.2)
Thran-10	12.5 (20.7)	10.2 (18.6)	9.2 (17.7)	7.5 (15.9)	18.0 (25.1)	15.7 (23.3)	12.2 (20.4)	10.4 (18.8)	11.7 (20.0)	10.2 (18.6)	28.1 (32.0)	24.2 (19.5)	27.0 (31.3)	25.3 (30.2)	20.0 (26.6)	18.2 (25.2)	33.4 (35.3)	31.2 (34.0)
Thran-8	11.0 (19.4)	9.9 (18.3)	9.6 (18.0)	7.5 (15.9)	18.0 (25.3)	16.0 (23.6)	11.9 (20.2)	9.5 (17.9)	12.5 (20.7)	10.2 (18.6)	31.9 (34.4)	26.4 (30.9)	25.4 (30.3)	22.5 (28.3)	18.6 (25.5)	16.8 (24.2)	32.5 (34.8)	28.9 (32.5)
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
CD (0.05)	1.84 3.90	1.76 3.73	2.63 5.53	1.61 3.40	2.12 4.63	1.07 3.52	1.99 4.35	19.3 4.10	20.6 5.29	3.63 7.91	3.63 4.89	2.66 4.92	2.68 4.92	3.60 7.63	3.15 6.69	3.10 6.57	2.66 8.56	4.89 7.42

Figures in parentheses are angular transformed values

Table 2:
Competitive parasitic ability of *Trichoderma* isolates against sclerotia of *R. solani*

Percent Colonization of Trichoderma Isolates to Sclerotia of *S. rolfsii*																		
	Natural soil						Sun dried Soil						Sterlized soil					
Isolates	Mycelia		Conidia		Cllamyd Ospores		Mycelia		Conidia		Cllamyd Ospores		Mycelia		Conidia		Cllamyd Ospores	
	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M
ThrAN-5	71.4 (57.7)	65.7 (54.1)	53.6 (47.1)	47.4 (43.5)	10.0 (88.4)	90.6 (72.1)	80.5 (63.8)	76.4 (60.9)	61.7 (51.8)	58.4 (49.8)	1.00 (88.4)	8.48 (74.4)	94.5 (76.4)	83.7 (66.2)	65.5 (54.0)	55.0 (47.9)	1.00 (88.4)	1.00 (88.4)
ThrAN-7	66.6 (54.8)	62.4 (52.2)	49.5 (44.7)	44.8 (42.0)	98.9 (84.0)	87.2 (69.0)	72.3 (58.2)	68.5 (55.9)	55.4 (48.1)	52.7 (46.5)	1.00 (88.4)	78.5 (62.4)	82.4 (65.2)	81.3 (64.4)	60.1 (50.8)	58.9 (50.1)	1.00 (88.4)	1.00 (88.4)
ThrAN-13	58.2 (49.7)	55.6 (48.2)	40.6 (36.6)	36.2 (37.0)	86.7 (68.6)	79.8 (63.3)	62.4 (52.2)	59.2 (50.3)	43.3 (41.1)	39.8 (39.1)	80.0 (88.4)	73.8 (59.2)	69.7 (56.6)	67.9 (55.5)	55.4 (48.1)	54.5 (47.6)	89.8 (71.4)	88.0 (69.7)
ThrAN-16	55.7 (48.3)	49.5 (44.7)	39.4 (38.9)	34.6 (36.0)	81.4 (64.4)	74.4 (59.6)	56.8 (48.9)	53.4 (46.9)	40.5 (39.5)	36.2 (37.0)	74.4 (59.6)	68.8 (56.1)	64.8 (53.6)	63.2 (52.6)	52.5 (46.4)	50.7 (45.4)	84.5 (66.8)	83.4 (66.0)
ThrAN-3	70.9 (57.3)	63.8 (53.0)	54.2 (47.4)	48.9 (44.4)	98.5 (83.0)	89.9 (71.5)	78.6 (62.4)	71.6 (57.8)	63.0 (52.5)	56.8 (48.9)	1.00 (88.4)	89.9 (71.5)	91.5 (73.0)	89.5 (71.0)	66.0 (54.3)	64.8 (53.6)	1.00 (88.4)	1.00 (88.4)
ThrAN-5	67.8 (55.4)	61.7 (51.8)	51.3 (45.7)	45.4 (42.4)	96.4 (79.1)	88.7 (70.4)	72.5 (58.4)	68.7 (56.0)	56.1 (48.5)	53.0 (46.7)	1.00 (88.4)	91.9 (73.5)	92.6 (74.2)	90.2 (71.8)	64.9 (53.7)	62.5 (52.2)	1.00 (88.4)	1.00 (88.4)
ThrAN-10	56.6 (48.8)	54.5 (47.6)	41.7 (40.2)	36.6 (37.2)	80.7 (63.9)	73.5 (59.0)	61.8 (51.80)	57.8 (49.5)	39.2 (38.8)	33.9 (35.6)	75.5 (60.3)	69.7 (56.6)	68.8 (56.1)	65.2 (53.8)	53.2 (46.8)	51.7 (46.0)	90.1 (71.7)	88.8 (70.4)
ThrAN-8	54.9 (47.8)	48.8 (44.3)	38.7 (38.5)	34.8 (36.1)	76.2 (60.8)	68.4 (55.8)	58.9 (50.1)	54.5 (47.6)	36.7 (37.3)	31.9 (34.4)	73.8 (59.2)	66.8 (54.8)	65.1 (53.8)	64.2 (53.2)	54.5 (47.6)	53.2 (46.8)	86.4 (68.4)	85.9 (67.9)
Control	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
SEM	2.35	2.98	3.05	3.51	2.31	3.47	2.56	1.96	2.85	4.31	3.11	2.15	3.49	5.21	4.26	3.57	4.58	4.51
CD (0.05)	7.89	8.46	7.53	9.76	8.52	10.6	8.24	6.57	9.36	10.8	9.66	6.59	8.46	11.9	9.96	9.45	12.6	10.5

Figures in parentheses are angular transformed values

Table 3:
Rhizosphere colonization of *Trichoderma* isolates in gram rhizosphere

Population (x 10^3cfu/g soil) of *Tricoderma* isolates																		
	Natural soil						Sun dried Soil						Sterlized soil					
Isolates	Port Blair			Mohanpur			Port Blair			Mohanpur			Port Blair			Mohanpur		
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
ThrAN-5	45	121	155	45	108	138	38	97	130	22	84	119	65	129	189	55	114	181
ThrAN-7	37	103	148	39	105	127	36	89	125	22	85	116	59	120	185	51	108	171
ThrAN-13	34	102	150	31	97	130	29	81	122	18	79	115	54	114	180	47	105	166
ThrAN-16	29	108	144	22	94	125	22	77	115	15	72	111	55	113	177	45	108	156
TvAN-3	41	114	145	32	99	138	31	92	124	16	79	120	62	125	190	54	120	183
TvAN-5	42	115	149	35	105	131	31	92	124	16	79	120	62	125	190	54	120	183
TvAN-10	39	105	143	28	96	122	30	86	120	19	81	118	66	134	193	55	118	181
TvAN-8	44	118	146	19	89	118	27	89	114	12	70	108	61	118	179	50	110	170
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SEM	3.65	5.37	4.65	2.55	6.5	5.47	4.68	4.79	5.58	3.98	4.62	5.53	5.12	6.01	5.99	5.04	4.24	5.88
CD(0.05)	7.58	9.86	11.35	6.78	10.98	14.52	9.88	8.88	12.89	7.14	8.23	9.63	9.87	10.45	11.8	8.66	9.66	12.86