

Management of Basal Stem Rot Disease in Coconut through Bio-inoculants and Chemicals

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ABSTRACT: In the present investigation, field experiments were initiated in two farmer's holding at Thennankudi village of Peravurani taluk, Thanjavur district and Coconut Research Station, Veppankulam for the management of basal stem rot disease using different bio-formulations. Talc based formulation of following bio-agent Trichoderma viride– 200gm/palm/year and talc based formulation of TNAU microbial consortia like Super Pseudomonas– 200 gm/palm/year, Bacillus subtilis (B.S. mixture) – 300 gm/palm/year applied along with 5 kg neemcake/year and AMF mass culture and Soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval were designated as treatments for the management of basal stem rot pathogen Ganoderma lucidum in the coconut.

Application of talc formulation of Trichoderma viride and TNAU microbial consortia along with neem cake were equally effective with soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval found to be the promising technologies in inhibiting the growth of basal stem rot pathogen Ganoderma lucidum in the field trials and these can be included for the integrated management of ever dreadful disease like Basal stem rot in coconut.

INTRODUCTION

Basal Stem Rot (BSR) disease in coconut is a major disease and a limiting factor in coconut cultivation in Tamil Nadu, Andhra Pradesh, Karnataka and parts of other states in India where coconut is grown (Bhaskaran *et.al.*, 1989). The disease was first reported from Thanjavur district (Tamil Nadu) and hence called as Thanjavur wilt. This disease is also called as Bole rot, Anabe or Ganoderma disease. Incidence of the disease was recorded as high as 40% in some of the coconut gardens in Thanjavur district (Bhaskaran et.al., 1984). Exudation of reddish brown viscous fluid from the basal portion of the stem (referred as bleeding) is the first sign of the disease which progresses upwards. As the disease advances drooping, drying and falling of leaves, extensive root rot and death of the palms are the characteristic symptoms of tha basal stem rot (Bhaskaran, et.al., 1989). All the three species of *Trichoderma* namely T.viride, T.harzianum and T.hamatum multiplied rapidly in neem cake as well as wheat grains and

moderate growth was observed in farm yard manure. In the field trials, conducted at Veppankulam for the management of BSR with the antagonistic fungus *T.harzianum* either applied alone or in combination with neem cake, significant disease suppression was observed in neem cake+T.harzianum applied coconut trees and the population of fungi, bacteria, actinomycetes and Trichoderma increased significantly (Bhaskaran et al., 1984). Among the bacterial antagonists Pseudomonas fluorescens and Bacillus subtilis were found effective against Ganoderma under laboratory conditions. In the field trials, there was a significant reduction in disease intensity in P.fluorescens treated palms. Root colonization by ECM and VAM fungi can provide protection from parasitic fungi and nematodes. Mycorrhizal fungus hyphae are important source for controlling the entry of pathogens at the root level and will not allow them to proliferate much on the host.

Rhizospheric soil sample analysis showed an abundance of Arbuscular Mycorrhizal Fungal (AMF)

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species was found to be the predominant genus in both coconut and arecanut cropping systems. *Glomus spp.* Such as *Glomus gieosporum*, *G.claridoneum and G.fasciculatum* could be identified in the root zones of coconut. (CPCRI Research highlights, 2009-2010).

The coconut-based cropping system with coconut as main crop and pepper, banana and pineapple as component crops showed an abundance of *Gigaspora* species, *Gigaspora* decipens and G.gigantea in coconut rhizosphere. In India , a number of fungi belonging to four genera viz., *Glomus*, *Gigaspora*, *Sclerocystis and Acaulospora* have been found to form mycorrhizal association with coconut (Thomas *et. al.*, 1991).

In views of the above facts and as the causal organism of the disease is soil borne having a very wide host range with disease expression talking a long time after infection, the management of the disease needs to be addressed by integrating different bio-control options and these by exploiting their consistency and efficacy in controlling BSR disease for the successful basal stem rot management strategy.

MATERIAL AND METHODS

Field Trial

Field experiments were initiated in two farmer's holding at Thennankudi village of Peravurani taluk, Thanjavur district and Coconut Research Station, Veppankulam for the management of basal stem rot disease using different bio-formulations viz., *T-viride, Pseudomonas*, Super *Pseudomonas* (PF1) and BS mixture along with neem cake and AMF + Vemicompost and chemical control comprising of Bordeaux mixture 1% (soil drench), Hexaconazole 2ml and Tridemorph (2ml) root feeding thrice at quarterly interval. palms with apparently uniform disease intensity were selected. The following treatments with four replications were randomized at the respective fields by adopting RBD design.

Treatments

- T₁ = *Trichoderma viride* Talc based formulation 200gm/palm/year + 5 kg neemcake/year
- T₂ = *Pseudomonas fluorescens* Talc based formulation - 200 gm/palm/year + 5 kg neemcake/year
- T₃ = *Bacillus subtilis* (B.S. mixture) Talc based formulation- 300 gm/palm/year + 5 kg neemcake/year
- T₄ = *Super Pseudomonas* Talc based formulation-200 gm/palm/year + 5 kg neemcake/year

- T₅ = Arbuscular Mycorrhizal Fungi* (AMF) (0.5kg) – 500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture
- T₆ = Soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval
- T₇ = Root feeding with Tridemorph (2ml in 100ml of water) at quarterly interval
- T₈ = Root feeding with Hexaconazole (2ml in 100ml of water) at quarterly interval
- T_{9} = Control
- Composition of microbial super consortia is super *Pseudomonas fluorescens* Strains - (TDK-1 + P.f-1 + PY-15)
- Composition of microbial super consortia is Bacillus subtilis, Pseudomonas fluorescens (B.S. mixture)
- * Mass multiplied in vermiculite based maize and sorghum roots

Disease Indexing Method

A disease indexing method was developed at Veppankulam in 1993 for assessing the severity of the disease. Disease index (DI)=23.6+17.7h+3.6r-0.6l, where h is the height in meters up to which bleeding symptom has spread in the stem, l is the number of functional leaves in the crown and r is the score for reduction in leaf size in 0 to 4 scale. According to this formula, an index score of 15 and below can be considered as mild, 15 to 40 as moderate and above 40 as severely diseased.

Staining Method for Mycorrhizal Infection

In the field trial, fifty root bits were stained from mycorrhizal assigned treatment before imposing the same treatment and assessed for the colonization of AMF. Root were cleared by heating (90°C for 1 hr) in 10% KOH, then rinsed and acidified with dilute HC1 and stained by simmering (5 min) in 0.05% trypan blue in lactophenol. Pigmented roots were cleared in 10% KOH for at least 2 hr, washed in fresh KOH and bleached in alkaline H_2O_2 (10 vol for 10 min-1 hr at 20°), then acidified and stained as before.

Enumeration of Bio-inoculants

Before imposing above treatments, soil samples were collected for the enumeration of *T. viride* and *Pseudomonas fluorescens*. Soil samples at the depth of 15cm were collected and the soil samples were

processed. One gram of soil samples from each replicated treatment was used for serial dilution. The required replicated platings were done on Trichoderma viride Selective Medium (TSM) for the enumeration of *T.viride* under room temperature in 7 days of incubation period. The mean Colony Forming Unit (CFU) has been recorded and presented in table-1. Replicated platings were done on King's B medium for the enumeration of Pseudomonas fluorescens under room temperature in 5 days of incubation period. The mean Colony forming Unit (CFU) has been recorded and presented in table 1. Soil samples were collected for the post experimental enumeration of *T. viride* and Pseudomonas fluorescens population same as that of pre experimental enumeration of bio-agents and results were recorded and presented in the following table 1. Before imposing AMF in the treatments, root bits were collected from each replicated treatments and stained for the assessment of mycorrhizal colonization.

RESULTS AND DISCUSSION

In the present investigation, selection of Basal stem rot affected coconut gardens for conducting field experiment for the management of basal stem rot disease at farmer's holdings were done during 2012-2013. Field trial for the management of basal stem rot of coconut in farmer's holding at Thennangudi village, Peravurani and in CRS, Veppankulam for the year 2012-13 was done.

The initial and final disease index and initial and final nut yield was recorded before and after imposing the above treatments at the respective fields and the data are presented in the following tables 2-3. The coconut-based cropping system with coconut as main crop and pepper, banana and pineapple as component crops showed an abundance of *Gigaspora* species, *Gigaspora* decipens and G.gigantea in coconut rhizosphere. In India, a number of fungi belonging to four genera viz., *Glomus, Gigaspora, Sclerocystis and Acaulospora* have been found to form mycorrhizal association with coconut (Thomas *et. al.*, 1991).

On the contrary, in both the field trial, there was no colonization of mycorrhiza in the roots. The mean final fungal population (T-viride) ranged from 0.437 to 3.75 x 10³ CFU / gm dry soil (Thennagudi) and 0.625 to 3.437 x 10^3 CFU/gm dry soil (CRS, Veppankulam table 4-5. All the three species of Trichoderma namely T.viride, T.harzianum and T.hamatum multiplied rapidly in neem cake as well as wheat grains and moderate growth was observed in farm yard manure. In the field trials, conducted at Veppankulam for the management of BSR with the antagonistic fungus T.harzianum either applied alone or in combination with neem cake, significant disease suppression was observed in neem cake+T.harzianum applied coconut trees and the population of fungi, bacteria, actinomycetes and Trichoderma increased significantly (Bhaskaran et. al., 1984). The mean Trichoderma viride population was increased in the treatment T1=Trichoderma viride Talc based formulation – 200gm/palm/year + 5 kg neemcake/ year, after application of respective bio-agent along with 5 kg of neemcake and other treatments are at par.

The mean final bacterial population ranged from 0.562 to 2.062 x 10^5 CFU/gm dry soil (Thennagudi) and 0.687 to 1.25 x 10^5 CFU/gm dry soil (CRS, Veppankulam).

 T_1 (Super *Pseudomonas* alone) and T_3 (Super *Pseudomonas* + Arbuscular Mycorrhizal Fungi (AMF)) were the best treatments with respect to the bacterial

S. No	Treatments		r Forming Unit g dry soil	% increase or decrease over the contro.	
		Pre treatment	Post treatment		
1.	Super Pseudomonas alone	1.187	2.419	128.5	
	-	(1.30)	(1.71)		
2.	AMF (Arbuscular Mycorrhizal Fungi) alone	0.937	0.812	- 23.8	
		(1.20)	(1.15)		
3.	S.Pseudomonas +AMF	1.187	2.641	147.6	
		(1.30)	(1.77)		
4.	Control	1.0	1.035		
		(1.22)	(1.24)	-	
	SEd	0.163	0.29	-	
	CD = (0.05)	0.356	0.565	-	

Table 1
Pre and Post Experimental Enumeration of Bacterial Population on King's B Medium Under Pot Culture

* Mean of four replications, (+) - Increase, (-) - Decrease

Treatments	Thennangudi field trial during 2012-2013						
	Mean Disease Index			Mean Nut Yield			
	Pre- Experimental	Post- Experimental	%Increase / Decrease over the initial	,	Post- Experimental	%Increase / Decrease over the initial	
T1 = <i>Trichoderma viride</i> Talc based formulation – 200gm/palm/year + 5 kg neemcake/year	23.75	18.11	5.64	103.25	115.25	12	
T2 = <i>Pseudomonas fluorescens</i> Talc based formulation – 200 gm/palm/year + 5 kg neemcake/year	50.04	43.49	6.55	96.25	118	21.75	
T3 =3 <i>Bacillus subtilis</i> (B.S. mixture) Talc based formulation- 300 gm/palm/year + 5 kg neemcake/year	31.76	21.57	10.19	96.00	124	28	
T4 = <i>Super Pseudomonas</i> Talc based formulation- 200 gm/palm/year + 5 kg neemcake/year	29.71	18.87	10.84	93.25	126	32.75	
T5 = Arbuscular Mycorrhizal Fungi (AMF) (0.5kg) – 500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture	39.10	36.00	3.10	99.50	109.25	9.75	
T6 = Soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/ Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval;	36.98	25.50	11.48	99.50	129	29.5	
T7 = Root feeding with Tridemorph (2ml in 100ml of water) at quarterly interval	45.60	39.61	5.99	90.75	101.5	10.75	
T8= Root feeding with Hexaconazole (2ml in 100ml of water) at quarterly interval	68.13	65.67	2.46	98.75	109.75	11	
T9 = Control	11.68	36.97	-25.29	100.75	81.75	19	
CD @5%	36.63	29.65 NS		11.84 NS	6.96		

 Table 2

 Effect of Different Treatments against Basal Stem Rot Disease of Coconut Disease Index and Nut Yield at

 Thennangudi during 2012-2013

(+) - Increase in value (-) - Decrease in value

population growth table-6-7. The mean fungal and bacterial population was increased after application of respective bio-agents along with neemcake.

In the field trial, the final disease index ranged from 18.11 to 65.67 (Thennagudi) and 10.17 to 56.78 (CRS, Veppankulam). There is no difference among the treatments over the control in terms of diseas index in both the farmer's holdings.

The mean final nut yield ranged from 81.75 to 129 (Thennagudi) and 84.25 to 127 (CRS, Veppankulam). There is a significant difference among the following treatments T6= Soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval,T4= *Super Pseudomonas* Talc based formulation- 200 gm/palm/year + 5 kg neemcake/ year and T=3 *Bacillus subtilis* (B.S. mixture) Talc based formulation- 300 gm/palm/year + 5 kg neemcake/ year over the control in terms of nut yield in Thennagudi farmer's holding. Treatments T4 was found to be best with respect to the nut yield in CRS, Veppankulam trial T6,T3 and T2 are ranked next.

Veppankulm during 2012-2013							
Treatments			Veppankulm field	l trial during 2	012-2013		
	Disease Index			Nut Yield			
	Pre- Experimental	Post- Experimental	%Increase / Decrease over the initial	Pre- Experi- mental	Post- Experimental	% Increase/ Decrease over the initial	
T1 = <i>Trichoderma viride</i> Talc based formulation – 200gm/palm/year + 5 kg neemcake/year	32.33	28.60	3.73	107.50	110	2.5	
T2 = <i>Pseudomonas fluorescens</i> Talc based formulation – 200 gm/palm/year + 5 kg neemcake/year	21.71	16.96	4.75	108.25	117.75	9.5	
T3 =3 <i>Bacillus subtilis</i> (B.S. mixture) Talc based formulation- 300 gm/palm/year + 5 kg neemcake/year	26.63	16.61	10.02	113	123.25	10.25	
T4 = Super Pseudomonas Talc based formulation- 200 gm/palm/year + 5 kg neemcake/year	46.52	36.24	10.28	105.5	127	10.25	
T5 = Arbuscular Mycorrhizal Fungi (AMF) (0.5kg) – 500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture	24.40	20.79	3.61	103.5	110.5	7.0	
 T6 = Soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval; 	54.42	44.41	10.01	106.25	126.5	20.25	
T7= Root feeding with Tridemorph (2ml in 100ml of water) at quarterly interval	37.08	31.20	5.88	100	102.5	2.5	
T8= Root feeding with Hexaconazole (2ml in 100ml of water) at quarterly interva	61.63	56.78	4.85	100.50	107.75	7.25	
T9 = Control	32.78	10.17	22.61	106.25	84.25	-22	
CD @5%	45.679 NS	36.99 NS		16.62	12		

 Table 3

 Effect of Different Treatments against Basal Stem Rot Disease of Coconut Disease Index and Nut Yield at

 Veppankulm during 2012-2013

(+) - Increase in value (-) - Decrease in value



Application of bio-inoculant *-Trichoderma viride* along with 5kg of neem cake at Thennagudi village,Thanjavur district



Soil drenching with 1% of Bordeux Mixture at CRS,Veppankulam,Thanjavur district

Treatments	*Mean colony Form gram of dry		
	Pre Experimental Trichoderma population	Post Experimental Trichoderma population	%Increase / Decrease over the initial
T1 = <i>Trichoderma viride</i> Talc based formulation - 200gm/palm/year + 5 kg neemcake/year	1.312 (1.35)	3.750 (2.06)	-2.438
T2 = <i>Pseudomonas fluorescens</i> Talc based formulation - 200 gm/palm/year + 5 kg neemcake/year	0.812 (1.15)	2.625 (1.77)	-1.813
T3 =3 <i>Bacillus subtilis</i> (B.S. mixture) Talc based formulation- 300 gm/palm/year + 5 kg neemcake/year	0.625 (1.06)	2.187 (1.64)	-1.5625
T4 = <i>Super Pseudomonas</i> Talc based formulation - 200 gm/palm/year + 5 kg neemcake/year	1.187 (1.30)	2 (1.58)	-0.813
T5 = Arbuscular Mycorrhizal Fungi (AMF) (0.5kg) – 500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture	0.875 (1.17)	1.312 (1.35)	-0.4375
T6 = Soil drenching with 40 Litres of 1% Bordeaux mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval;	1.375 (1.37)	1.062 (1.25)	0.3125
T7= Root feeding with Tridemorph (2ml in 100ml of water) at quarterly interval	0.562 (1.03)	0.437 (0.97)	0.1245
T8= Root feeding with Hexaconazole (2ml in 100ml of water) at quarterly interva	0.687 (1.09)	0.812 (1.15)	-0.1255
T9 = Control	0.937 (1.20)	0.687 (1.09)	0.2495
CD = (0.05):	0.801NS	0.7437	

Table 4	
Enumeration of <i>T-viride</i> on <i>Trichoderma</i> selective medium (TSM) at Thennakudi field trial during 2012 - 13	3

(+) - Increase in value (-) - Decrease in value

Table 5

Enumeration of *T-viride* on *Trichoderma* Selective Medium (TSM) at CRS, Veppankulam Field Trial during 2012-13

Treatments	*Mean colony For one gram of d		
	Pre Experimental Trichoderma population	Post Experimental Trichoderma population	%Increase / Decrease over the initial
T1 = <i>Trichoderma viride</i> Talc based formulation –	1.187	5.1	0.3902
200gm/palm/year + 5 kg neemcake/year	(1.30)	(2.37)	
T2 = <i>Pseudomonas fluorescens</i> Talc based formulation	1.062	3.725	0.2965
– 200 gm/palm/year + 5 kg neemcake/year	(1.25)	(2.06)	
T3 =3 Bacillus subtilis (B.S. mixture) Talc based	1.062	2.175	0.1715
formulation- 300 gm/palm/year + 5 kg neemcake/year	(1.25)	(1.64)	
T4 = <i>Super Pseudomonas</i> Talc based formulation	1.500	1.925	0.25
– 200 gm/palm/year + 5 kg neemcake/year	(1.41)	(1.56)	
T5 = Arbuscular Mycorrhizal Fungi (AMF) (0.5kg)	1.312	1.825	0.2965
-500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture	(1.35)	(1.52)	
T6 = Soil drenching with 40 Litres of 1% Bordeaux	1.25	1.775	0.375
mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval;	(1.32)	(1.51)	
T7= Root feeding with Tridemorph (2ml in 100ml of	0.625	1.325	0.34375
water) at quarterly interval	(1.06)	(1.35)	
T8= Root feeding with Hexaconazole (2ml in 100ml	0.687	1.175	0.32775
of water) at quarterly interva	(1.09)	(1.29)	
T9 = Control	0.812	1.75	0.359
	(1.15)	(1.50)	
CD = (0.05):	0.737NŚ	1.442	

(+) - Increase in value (-) - Decrease in value

Management of Basal S	tem Rot Disease in Coconut through	Bio-incoculants and Chemicals

Treatments	*Mean colony For one gram of a		
	Pre Experimental bacterial population	Post Experimental bacterial population	%Increase / Decrease over the initial
T1 = <i>Trichoderma viride</i> Talc based formulation	1.187	1.312	-0.125
 200gm/palm/year + 5 kg neemcake/year 	(1.30)	(1.35)	
T2 = <i>Pseudomonas fluorescens</i> Talc based formulation	0.625	1.75	-1.125
– 200 gm/palm/year + 5 kg neemcake/year	(1.06)	(1.50)	
T3 =3 Bacillus subtilis (B.S. mixture) Talc based	0.875	1.875	-1
formulation- 300 gm/palm/year + 5 kg neemcake/year	(1.17)	(1.54)	
T4 = <i>Super Pseudomonas</i> Talc based formulation	1.125	2.062	-0.937
– 200 gm/palm/year + 5 kg neemcake/year	(1.27)	(1.60)	
T5 = Arbuscular Mycorrhizal Fungi (AMF) (0.5kg)	0.875	1	-0.125
– 500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture	(1.17)	(1.22)	
T6 = Soil drenching with 40 Litres of 1% Bordeaux	0.75	0.562	0.188
mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval;	(1.12)	(1.03)	
T7 = Root feeding with Tridemorph (2ml in 100ml of	0.625	0.812	-0.187
water) at quarterly interval	(1.06)	(1.15)	
T8= Root feeding with Hexaconazole (2ml in 100ml	0.75	0.562	0.188
of water) at quarterly interva	(1.12)	(1.03)	
T9 = Control	1.125	1.125	0
	(1.27)	(1.27)	
CD = (0.05):	0.578 NS	0.6972	

	Table 6		
Enumeration of Bacterial Po	pulation on King's B medium at	Thennangudi Field Trial duri	ng 2012 – 13

(+) - Increase in value (-) - Decrease in value

Table 7
Enumeration Bacterial Population on King's B at CRS, Veppankulam Field Trial during 2012-13

Treatments		rming Unit(x10 ³)/ dry soil sample		
	Pre Experimental bacterial population	Post Experimental bacterial population	% Increase / Decrease over the initial	
T1 = <i>Trichoderma viride</i> Talc based formulation	0.75	1.52	0.25	
– 200gm/palm/year + 5 kg neemcake/year	(1.12)	(1.42)		
T2 = <i>Pseudomonas fluorescens</i> Talc based formulation	0.937	5	0.20	
– 200 gm/palm/year + 5 kg neemcake/year	(1.20)	(2.35)		
T3 =3 Bacillus subtilis (B.S. mixture) Talc based	0.87	5.32	0.28	
formulation- 300 gm/palm/year + 5 kg neemcake/year	(1.17)	(2.41)		
T4 = <i>Super Pseudomonas</i> Talc based formulation	0.06	4.95	-0.57	
– 200 gm/palm/year + 5 kg neemcake/year	(0.75)	(2.33)		
T5 = Arbuscular Mycorrhizal Fungi (AMF) (0.5kg)	1.00	1.70	0.18	
– 500 gm/palm/year +10 kg Vermicompost Vermiculite based Mass culture	(1.22)	(1.48)		
T6 = Soil drenching with 40 Litres of 1% Bordeaux	1.25	2.00	0.31	
mixture -once in 3 months/Year + Root feeding with Tridemorph (2ml in 100ml of water)- at quarterly interval;	(1.32)	(1.58)		
T7 = Root feeding with Tridemorph (2ml in 100ml of	0.68	2.00	0.32	
water) at quarterly interval	(1.09)	(1.58)		
T8 = Root feeding with Hexaconazole (2ml in 100ml	0.75	1.42	0.25	
of water) at quarterly interva	(1.12)	(1.39)		
T9 = Control	0.937	2.12	0.26	
	(1.20)	(1.62)		
CD = (0.05):	0.542 NS	1.5845		

(+) - Increase in value (-) - Decrease in value

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