

ADDENDUM 2

Study on soil microflora of *B.t.* (MLS9124) and non-*B.t.* cotton soils

Study No.14:

Title : Effect of *B.t.* cotton (MLS9124) on the soil microflora
Organization : Metahelix Life Sciences Private Limited, Bangalore
Status : Kharif 2006 - Completed

Objective:

The objective of this study was to assess the effect of the *B.t.* cotton plants expressing the *cryIC* gene developed by Metahelix Life Sciences, on the soil microflora.

Introduction:

Soil microflora also play a crucial role in the soil properties. The soil microflora largely depends on the type of soil, temperature, moisture, plant growth, nutrients, pH, and many other factors which may vary between locations but also within a single plot and over very small distances (OECD, 2007)

Methodology:**Soil Sample Collection**

Soil samples were collected by digging out soil around the *B.t.* and the Non *B.t.* plant rhizosphere area (up to 20 cm from the plant) to a dimension of 15 cm height X 7 cm diameter. Three such samples were collected for each field and pooled and mixed together into a single. Similar sampling was taken from the non-rhizosphere zone (25 to 40 cm away from the plant). Samples were collected from both *B.t.* and Non *B.t.* fields from two locations namely Attur in Tamil Nadu and Guntur in Andhra Pradesh, where the trials were conducted.

The soil samples were analyzed at the GKVK campus of University of Agricultural Sciences at Bangalore, Karnataka.

UNIVERSITY OF AGRICULTURAL SCIENCE, GKVK, BANGALORE

No. Ag. Micro./PBFS / 81/ 2007-08

Popularization of Biofertilizer Scheme
Dept. of Agril. Microbiology
GKVK, Bangalore - 560 065
Dated: 23/ 01 / 2008

To

The Director of Research
University of Agricultural Sciences
GKVK, Bangalore - 560065

Sir,

Sub: Analysis report of soil samples sent by Metahelix Life Sciences Pvt. Ltd - reg

Ref: — No. DR/T-1(95)/2007-08. Dt. 8/5/2007

With reference to the above subject, I am here with submitting the results of Bt and Non Bt. soil samples of cotton fields of Attur (T.N.) and Guntur (A.P.) drawn and sent by **Meta-helix Life Sciences Pvt. Ltd, Plot No. 3, KIADB, 4th Phase, Bommasandra, Bangalore - 560099.**

I request you to kindly communicate the analytical results enclosed here with to the concerned.

This is for your kind information and for further needful.

Yours faithfully,


(K.R. SREERAMULU)

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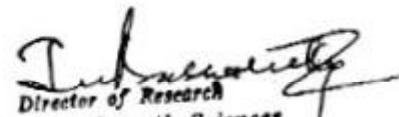
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DR/T-1(95)/2007-08 dt 23-1-08

Forwarded for consideration and needful

To

Dr. M. J. Vasudeva Rao

MLs Meta helix life sciences Pvt Ltd
Bangalore-99


Director of Research
University of Agril. Sciences
GKVK Campus, Bangalore-560 065

REPORT ON MICROBIOLOGICAL STUDIES OF Bt. AND NON Bt. SOILS OF COTTON

INTRODUCTION

Bacillus thuringiensis (Bt) is a gram positive, aerobic, endospore forming bacteria has a great potential as a bio control agent in controlling many of the insects belonging to *Lepidoptera*, *Coleoptera*, *Diptera* and nematodes. In this study an attempt was made to analyze the rhizosphere micro flora of cotton grown in two locations of India viz., Attur (Tamil Nadu), Guntur (Andhra Pradesh). The soil samples were collected by Meta-helix Life Sciences Private Ltd., Bangalore and the soil samples sent by them are analyzed for microbial population.

MATERIAL AND METHODS

The microbial populations were estimated by using serial dilution plate count technique. The population of soil bacteria was estimated on soil extract agar as per the procedure out lined by Bunt and Rovira (1955), fungi on Martin Rose Bengal streptomycin sulphate agar (Martin,1950), actinomycetes on Kusters agar (Kuster and Williams,1964) and yeast population on yeast extract agar (Windle Taylor,1958). Similarly the beneficial microbial population in soil viz., *Rhizobium* population on Yeast Extract Mannitol Agar, *Azotobacter* population on Ashby's medium as per the procedure of Norris (1959), *Azospirillum* population on Dobereiner's medium (Dobereiner *et al.*,1976) and Phosphate Solubilizing Bacteria on Pikovskaya's medium (modified by Sundara Rao and Sinha, 1963). The population of organic matter degrading bacteria *cellulomonas* was estimated by using Hans medium.

RESULTS

The population of general microflora of Attur and Guntur soils has been showed in Table 1, 2 and the population of beneficial microflora of Attur and Guntur soils has been shown in Table 3 and 4 respectively.

Table 1: General Microbial population of Bt. and non Bt. soils of Attur.

Sl. No.	Bt./ Non Bt.	Rhizosphere/ Non rhizosphere	ATTUR SOIL SAMPLES		
			Microbial population (cfu g ⁻¹ soil)		
			Bacteria (No.x10 ⁶)	Fungi (No.x10 ³)	Actinomycetes (No.x10 ⁴)
1	Non Bt.	001 R	15.50	9.00	5.25
2	Non Bt.	001 NR	7.00	6.25	4.75
3	Bt.	002 R	10.75	3.00	1.00
4	Bt.	002 NR	04.25	5.50	2.00

- Note:**
1. Soil samples have not been collected by the University of Agril. Sciences, GKVK, Bangalore, soil samples were collected only by Meta helix Pvt. Ltd. and has been sent for microbial analysis at this laboratory.
 2. The information contained in this report is based only for this batch of the sample submitted for analysis and is valid only for 30 days from the date of issue and not university is responsible for any alterations in the data.
 3. The Results of the analysis is not valid for any legal and commercial purposes

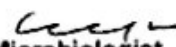

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Table 2: General Microbial population of Bt. and non Bt. soils of Guntur.

Sl. No.	Bt./ Non Bt.	Rhizosphere/ Non rhizosphere	GUNTUR SOIL SAMPLES		
			Microbial population (cfu g ⁻¹ soil)		
			Bacteria (No.x10 ⁶)	Fungi (No.x10 ⁶)	Actinomycetes (No.x10 ⁴)
1	Non Bt.	001 R	18.25	8.75	4.00
2	Non Bt.	001 NR	10.75	6.00	3.25
3	Bt.	002 R	8.50	3.75	1.00
4	Bt.	002 NR	6.00	5.25	2.50

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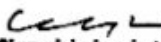

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Table 3: Beneficial microbial population of Bt. and non Bt. soils of Attur

SL No.	Bt./ Non Bt.	Rhizosphere/ Non rhizosphere	ATTUR SOIL SAMPLES					
			Microbial population (cfu g ⁻¹ soil)					
			<i>Rhizobium</i> (No.x10 ⁶)	<i>Azotobacter</i> (No.x10 ⁶)	<i>Azospirillum</i> (No.x10 ⁶)	PSB (No.x10 ⁶)	PSF (No.x10 ³)	<i>Cellulomonas</i> (No.x10 ⁴)
1	Non Bt.	005 R	8.25	7.50	7.75	3.50	0.00	2.00
2	Non Bt.	005 NR	4.75	5.25	4.25	2.00	2.75	1.25
3	Bt.	006 R	8.00	6.75	2.50	3.00	0.00	0.00
4	Bt.	006 NR	2.25	4.00	2.00	1.25	1.00	1.75

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Table 4: Beneficial microbial population of Bt. and non Bt. soils of Guntur

Sl. No.	Bt./ Non Bt.	Rhizosphere/ Non rhizosphere	GUNTUR SOIL SAMPLES					
			Microbial population (cfu g ⁻¹ soil)					
			<i>Rhizobium</i> (No.x10 ⁶)	<i>Azotobacter</i> (No.x10 ⁶)	<i>Azospirillum</i> (No.x10 ⁶)	PSB (No.x10 ⁶)	PSF (No.x10 ³)	<i>Cellulomonas</i> (No.x10 ⁴)
1	Non Bt.	005 R	8.75	10.25	6.50	5.75	4.25	3.00
2	Non Bt.	005 NR	6.50	5.50	4.25	3.50	2.00	1.00
3	Bt.	006 R	2.50	5.00	5.75	2.25	0.00	0.00
4	Bt.	006 NR	2.25	4.00	2.50	2.00	1.00	1.00

- Note:** 1. Soil samples have not been collected by the University of Agril. Sciences, GKVK, Bangalore, soil samples were collected only by Meta helix Pvt. Ltd. and has been sent for microbial analysis at this laboratory.
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Results and Conclusions :

Measurement of microbial activity is normally through the presence of culturable microbes in the soil. Overall the soils exposed to *B.t.* did not show significant variation in the population of microflora (Visser et al., 1994; Devare *et al.* 2004; Motavalli *et al.* 2004). Microbial population of *B.t.* and non *B.t.* cotton expressing Cry1Ac was found to be comparable (Manjunath, 2005; APCoAB. 2006)

The effect of transgenic plants on soil populations of non-target bacteria and fungi, could be either transient or do not have any effect at all. Dunfield & Germida (2003) concluded that the changes in the microbial community structure associated with genetically modified plants were temporary and did not persist into the next field season.

In the present study, the soil microbial population was comparable between the soils surrounding *B.t.* cotton, MLS9124 event expressing Cry1C protein and the non *B.t.* counterpart. This observation indicates that MLS *B.t.* cotton, event MLS9124, does not have adverse effect on soil microflora.

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