Study No.16:

Title : Estimation of Cry1C in the soils of *B.t.* cotton (MLS9124) fields
Organization : Metahelix Life Sciences Private Limited, Bangalore
Status : Kharif 2006 - Completed

Objective:

The objective of this study was to detect and estimate the Cry1C protein at different depths of soils in the rhizosphere and non rhizosphere areas, from the fields of transgenic *B.t.* cotton containing *cry1C* gene and Non *B.t.* cotton.

Introduction:

Bacillus thuringiensis is a common soil dwelling gram positive bacterium. However there are concerns about the safety of the *B.t.* protein residues coming out of the genetically modified plants into the soil environment. There are several reports that *B.t.* protein does not persist in soil and is usually quickly degraded (Palm ***et al***. 1996; Sims and holden, 1996; Saxena ***et al***. 2004; Shen ***et al***. 2006). Soil environment has been shown to play a major role in the degradation of *B.t.* proteins (Shen ***et al***. 2006 and Sun ***et al***. 2007)

Methodology:

Soil samples collected were from two locations, namely Attur in Tamil Nadu and Guntur in Andhra Pradesh immediately after the crop period were the field trials were conducted for *B.t.* cotton. Each soil sample was collected in triplicates from the rhizosphere (up to 20 cm from the plant) and non rhizosphere area (25 cm away from the plant up to 40 cm) at depths of 25, 50, 75 and 100 cm. The triplicates from similar depths were pooled and mixed well and 0.5 g of each pooled sample was used for the Cry1C protein estimation.

The QuantiPlate kit for Cry1C detection from Envirologix® was used to quantify the residues of Cry1C in the soils from *B.t.* cotton fields. A set of Cry1C protein standards at three different concentrations namely 1, 5 and 10 ppb was used in the assay. To ensure that extraction was proper, soil sample from Non *B.t.* field was spiked with *B.t.* cotton seed powder and used as a control.

Briefly, 0.5 g of soil sample was weighed into 2.0 ml microtubes and 1 ml of extraction buffer was added. Samples were tho roughly mixed using a vortex machine and the soil was sedimented by centrifugation at 10000 rpm for 5 min. 100 µl of each sample was loaded into the ELISA plates from the kit and incubated for 15 minutes at room temperature.

To each well 100 µl of enzyme conjugate was added and incubated at room temperature for 60 min. The plates were then washed and 100 µl of substrate was added and incubated for 30 minutes at room temperature. Then 100 µl of stop solution was added and the absorbance was read at an optical density (OD) of 450 nm in Tecan Sunrise^{TM} plate reader.

Results and Conclusions:

The soil samples from two locations from both *B.t.* and Non *B.t.* fields were tested for the Cry1C protein content. All the soil samples from both the rhizosphere and the non rhizosphere zones at different depths showed very low OD value similar to that of the negative control sample (Tables 1 and 2). The range of detection using this kit was as low as 1 ppb (parts per billion) to 10 ppb. The high OD value of soil sample spiked with *B.t.* cotton seed powder indicates that extraction was comparable across all samples (Table 3).

Table 1: Estimation of Cry1C in soils at different depths from *B.t.* and Non *B.t.* fields at Attur

Location	Rhizosphere				Non Rhizosphere			
Attur, TN	25 cm	50 cm	75 cm	100 cm	25 cm	50 cm	75 cm	100 cm
5174 N *B.t.* field sample 1	0.095 (<LOD)	0.097 (<LOD)	0.103 (<LOD)	0.103 (<LOD)	0.095 (<LOD)	0.093 (<LOD)	0.093 (<LOD)	0.084 (<LOD)
sample 2	0.083 (<LOD)	0.083 (<LOD)	0.098 (<LOD)	0.091 (<LOD)	0.087 (<LOD)	0.077 (<LOD)	0.08 (<LOD)	0.075 (<LOD)
sample 3	0.081 (<LOD)	0.085 (<LOD)	0.093 (<LOD)	0.076 (<LOD)	0.073 (<LOD)	0.08 (<LOD)	0.076 (<LOD)	0.067 (<LOD)
5174 *B.t.* field sample 1	0.09 (<LOD)	0.09 (<LOD)	0.096 (<LOD)	0.098 (<LOD)	0.081 (<LOD)	0.086 (<LOD)	0.08 (<LOD)	0.08 (<LOD)
sample 2	0.106 (<LOD)	0.096 (<LOD)	0.096 (<LOD)	0.091 (<LOD)	0.083 (<LOD)	0.075 (<LOD)	0.079 (<LOD)	0.071 (<LOD)
sample 3	0.08 (<LOD)	0.074 (<LOD)	0.083 (<LOD)	0.077 (<LOD)	0.082 (<LOD)	0.074 (<LOD)	0.066 (<LOD)	0.067 (<LOD)

<LOD: Less than Limit of detection

Table 2: Estimation of Cry1C in soils at different depths from *B.t.* and Non *B.t.* fields at Guntur

Location	Rhizosphere				Non Rhizosphere			
Guntur, AP	25 cm	50 cm	75 cm	100 cm	25 cm	50 cm	75 cm	100 cm
5124 NBt field Sample 1	0.078 (<LOD)	0.071 (<LOD)	0.071 (<LOD)	0.075 (<LOD)	0.069 (<LOD)	0.07 (<LOD)	0.07 (<LOD)	0.07 (<LOD)
Sample 2	0.07 (<LOD)	0.082 (<LOD)	0.083 (<LOD)	0.075 (<LOD)	NT*	NT	NT	NT
5124 *B.t.* field Sample 1	0.079 (<LOD)	0.083 (<LOD)	0.083 (<LOD)	0.076 (<LOD)	0.076 (<LOD)	0.075 (<LOD)	0.083 (<LOD)	0.075 (<LOD)
Sample 2	0.076 (<LOD)	0.075 (<LOD)	0.07 (<LOD)	0.068 (<LOD)	NT	NT	NT	NT

*NT: Not Tested; <LOD: Less than Limit of Detection

Table 3: OD values of standards and controls used in the ELISA based estimation of Cry1C protein in Soil samples

Controls	OD_{450}
Negative Control	0.072
1 ppb Standard	0.302
5 ppb standard	1.174
10 ppb standard	1.748
Spiked sample**	2.512 (>LOQ)

**Soil from Non *B.t.* field spiked with *B.t.* cotton seed powder
>LOQ: greater than Limit of Quantification

The low OD values for the soil samples from Non *B.t.* fields show that Cry1C protein is not present in the soil. Similar low values in the soil samples from *B.t.* fields suggest that Cry1C protein, if present, is below the limit of detection. We can conclude that the Cry1C protein will not form residues in soil and hence be considered not to pose any concern to the soil environment.

References:

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