

Bioefficacy of purified Cry1C protein on neonate larvae of *Spodoptera litura*

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Toxicity studies

As a preliminary validation, *Spodoptera frugiperda* (Sf9) cell lines were treated with the purified truncated Cry1C protein equivalent to that expressed in the Bt cotton event MLS9124 and an expected ballooning effect as a result of toxicity was observed. Subsequently, the purified protein was used in a bioefficacy test against the target pest, neonate larvae of *Spodoptera litura*.

Procedure:

8 µl of 2 different concentrations (3.2 ng/µl and 8 ng/µl) of the purified protein were painted on each surface of a non-transgenic cotton leaf disc of diameter 1 cm. The equivalent amounts of protein painted per cm² are 33 ng and 82 ng, on each surface respectively. Three different pesticide concentrations along with the 9124 event, non-transgenic cotton leaf and the dilution buffer were used as controls. One neonate larva was introduced on each leaf disc in eight (8) replicates and the mortality was observed at 24 hour intervals. The results are tabulated in table 1 below.

Results:

Table 1:

	Mortality							
	Day							
Sample	1	2	3	4	5	6	7	% mortality
Test concentration 1 3.2 ng/µl	2/8	3/8	3/8	3/8	5/8	5/8	5/8	62.5%
Test concentration 2 8 ng/µl	6/8	7/8	7/8	7/8	8/8	8/8	8/8	100%
Pesticide control 1	5/8	7/8	7/8	7/8	8/8	8/8	8/8	100%
Pesticide control 2	7/8	8/8	8/8	8/8	8/8	8/8	8/8	100%
Pesticide control 3	8/8	8/8	8/8	8/8	8/8	8/8	8/8	100%
MLS 9124	4/6	4/6	4/6	4/6	5/6	5/6	5/6	83.3%
Non transgenic cotton leaf	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0%

Key	
Pesticide control 1	Monocrotophos - 0.2% v/v
Pesticide control 2	Chloropyriphos - 0.2% v/v
Pesticide Control 3	Monocrotophos - 0.3% v/v

Conclusion:

The purified truncated Cry1C protein was efficacious against the neonate larvae of *Spodoptera litura* in a dose dependent manner. Although the extents of feeding in different replicates are not uniform, there is evidence that the efficacy of the purified Cry1C protein painted on a non-transgenic leaf surface is similar to that of the Bt cotton leaf disc carrying the event MLS9124.