SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH

(A Unit of Shriram Scientific and Industrial Research Foundation)

19, University Road, Delhi-110007
Telephone: 91-011-27667267,27667983,27667860
Fax: 91-011-27667676, 27667207 E-mail: sridhi@vsnl.com
Visit us at http://www.shriraminstitute.org

ISO-9001 Certified Institute

TEST CERTIFICATE

000096422

Issued to :
NATH BIO-GENES (INDIA) LTD
309, MEGHDOOT
96, NEHRU PLACE
NEW DELHI - 110019

Kind Attn: MR M S KHAN , CHIEF MANAGER

J.O.No. 301-101-0442
Reg.No. 1135262
Date 07-02-2005
GC-01 (REV-03)
Your Ref.No. -
Date 07.01.2005

Sample Particulars :
One sample described as Non BT Cotton seeds was received.

TEST RESULTS

(As on received basis)

S.No	Tests	Observed Value	Protocol
1.	Protein (N*5.30) % by mass	19.9	IS:7219-1973
2.	Oil Content % by mass	20.2	SP-18
3.	Total Carbohydrate as Invert sugar % by mass	22.8	SP-18
4.	Ash % by mass	4.0	SP-18
5.	Total Gossypol % by mass	0.93	SP-18

****

DOR: 12.01.2005
DOC: 07.02.2005

AUTHORISED SIGNATORY
Dy.Director/AD-Chief
Sr.Scientist/Scientist

NOTE 1. The result listed refer only to the tested sample(s) and applicable parameter(s). Endorsement of products is neither allowed nor implied.
2. Total liability of our institute is limited to the invoiced amount.
3. Samples will be destroyed one month from the date of issue of test certificate unless otherwise specified.
4. This report is not to be reproduced wholly or in part and cannot be used as an evidence in the Court of law and should not be used in any advertising media without our special permission in writing.
5. In case any reconfirmation of contents of this test certificate is required, please contact our office.

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TEST CERTIFICATE

00009842

Issued to :
NATH BIO-GENES (INDIA) LTD
309, MEGHDOOT
94, NEHRU PLACE
NEW DELHI - 110019

Kind Attn: MR M S KHAN , CHIEF MANAGER

Sample Particulars :
One sample described as BT Cotton seeds was received.

J.O.No. 501-101-0441
Reg.No. 1135262
Date 07-02-2005
GC-01 (REV-05)
Your Ref.No. -
Date 07.01.2005

TEST RESULTS

(As on received basis)

S.No	Tests	Observed Value	Protocol
1.	Protein (N*5.30) % by mass	20.0	IS:7219-1973
2.	Oil Content % by mass	20.7	SP-18
3.	Total Carbohydrate as Invert sugar % by mass	21.3	SP-18
4.	Ash % by mass	3.9	SP-18
5.	Total Gossypol % by mass	0.87	SP-18

****

DOR: 12.01.2005
DOC: 07.02.2005

AUTHORISED SIGNATORY
By: Director/AD-Chief
Sr.Scientist/Scientist

NOTE
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Presence of GFM Cry1 A Gene and Protein in Bt-Cotton Seed Oil

Pure Cotton Seed Oil tested at Shriram Institute for Industrial Research at Delhi was found devoid of Bt-Protein as well as Bt-Gene.

In the case of Bt-corn where root exudates of Bt protein are known to occur all through the duration of crop, a very extensive and intensive study was conducted recently by the European Union (Griffiths et al. 2005). Field trials were established and monitored at three European sites (Denmark, Eastern France and South-West France). The concluding statement of this French-British-Danmish multi-Institutional project was: "The effect of the Bt maize was small and within the normal variation expected in these agricultural systems".

5. *Base Line Susceptibility of Helicoverpa armigera*

Procedure:

Laboratory cultures were established by collecting about 300 late instar larvae of *Helicoverpa armigera* from the cotton field in the following locations in the cotton belt of North, South and Central India. Abohar (Punhjab), Guntur (A.P), Rajkot and Vadodara (Gujarat), Isarwadi, Jalgaon and Yeotmal (M.S.).

The larvae were reared on a semi synthetic diet, which contains chickpea as its main component. Larvae were inspected regularly to ensure that they remained pathogen-free. The colony was maintained in a culture room with a mean temperature of 28ºC, 60% RH and with a photoperiod of 14:10 (L:D). About 200 moth representing each location were obtained. Each mating cage contained about 50 moths and eggs were collected daily. These eggs were surface sterilized in 0.05% sodium hypochlorite solution and incubated for hatching. The F1 generation neonate larvae were used for bio-assays. The Bt protein was assayed by diet-incorporation method. Seven different concentrations ranging from 0.02 to 8 µg/ml of diet were used. Newly hatched, active larvae were transferred onto the solidified diet in the 24-cell insect-rearing tray with a fine hair brush (1 larvae / well). After larval transfer, insect-rearing trays were covered with semi-permeable wrap and lead was closed. Each treatment was replicated three time and at least 20 larvae formed one experimental unit. Mortality of larvae was scored every 24 hrs. for seven days. The larvae were marked dead when they did not move when prodded. The surviving larvae were severely inhibited and were weighted on the final day of experiment. Each bio-assay was repeated two times. In each experiment,

mortality data at different concentrations were used to calculate lethal concentration (LC_{50} and LD_{50}), their respective 95% fiducial limits and slope.

The results of probit regression analysis of dose response mortality data for the bioassays of 'GFM Cry1A' in neonate *H. armigera* are shown in the Figure below. The LC50 Values for neonates ranged from 0.14 to 0.68 µg of GFM Cry1A/ml of diet.

The population from Yeotmal had the lowest LC_{50} Values, whereas, population from Rajkot had the highest LC_{50} Values. The LC_{50} Values ranged from 1.23 to 4.38 µg of GFM Cry1A / ml of diet.

Dose Mortality Response of *H. armigera* 'Ecotypes' to *GFM* Cry1A

Location	State	LC50	95% Fiducial limit		LC90	95% Fiducial limit		Slope ± SE
			Lower	Upper		Lower	Upper	
Abohar	Punjab	0.16	0.09	0.27	1.52	0.6	3.84	1.48 ± 0.21
Aurangabad	Maharastra	0.26	0.16	0.43	2.05	0.84	5.01	1.48 ± 0.23
Yeotmal	Maharastra	0.14	0.08	0.23	1.23	0.51	2.95	1.30 ± 0.22
Jalgaon	Maharastra	0.36	0.22	0.67	3.4	1.2	9.62	1.26 ± 0.23
Jalna	Maharastra	0.49	0.31	0.77	2.6	1.11	6.07	1.76 ± 0.32
Vadodara	Gujarat	0.55	0.35	0.87	2.73	1.21	6.16	1.84 ± 0.33
Rajkot	Gujarat	0.68	0.41	1.14	4.38	1.63	11.76	1.59 ± 0.30
Guntur	Andhra Pradesh	0.63	0.39	1.01	3.51	1.46	8.41	1.71 ± 0.32

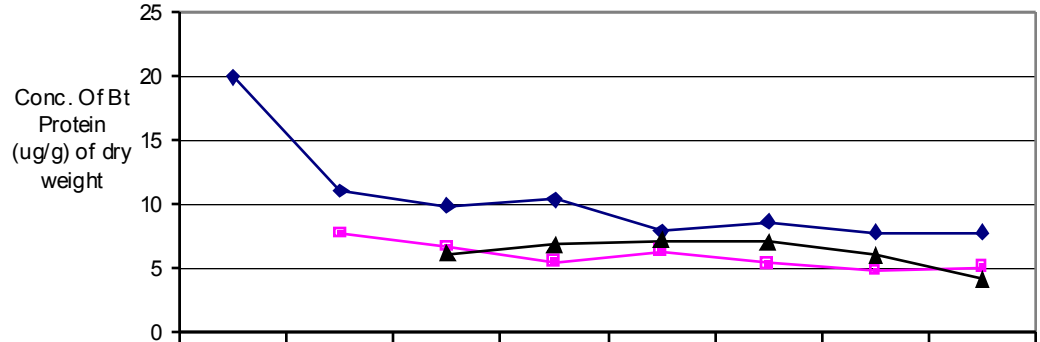
GFM Cry1A protein was found to be toxic to all geographic populations tested

The LC50 values for neonates ranged from 0.14 to 0.68 ug of GFM Cry1A/ml of diet. The population from Yeotmal had the lowest LC50 values, whereas, population from Rajkot had the highest LC50 values. The LC90 values ranged from 1.23 to 4.38 ug of GFM Cry1A/ml of diet.

4. Bt-Protein Expression Profile

Monoclonal antibody ELISA plates (M/s DesiGen Diagnostics) were used to screen as well as for quantification of the Bt protein. These kits was tested in our laboratory repeatedly, for consistency of results. Such kits have been used to generate a database in respect of Bt-protein Expression Profile at different stages of crop age, of different plant parts (terminal leaf, square and boll rind). The expression profile of different Bt hybrids especially those that have shown promising performance is presented in figures 1 to 3.

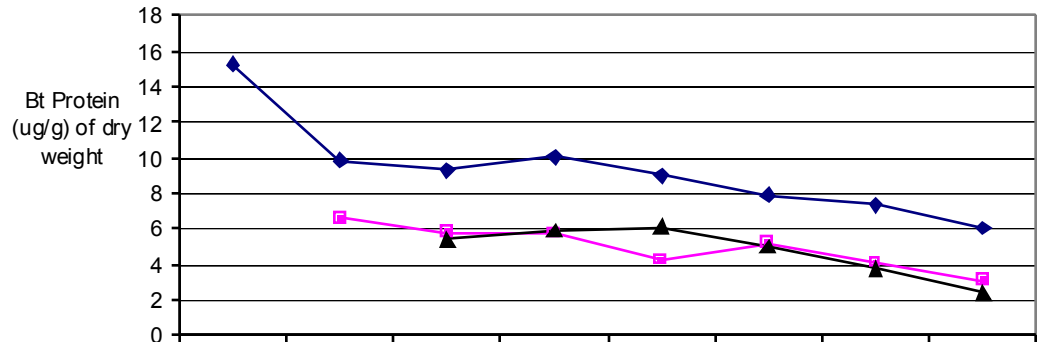
Fig.:1 Mean Bt Protein concentration In Dfferent Plant Parts of NCEH-6 Bt from Punjab State



	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	12 DAS	135 DAS
Term Leaf	19.99	11.00	9.81	10.30	7.80	8.54	7.76	7.71
Square		7.68	6.61	5.44	6.28	5.26	4.74	5.03
Boll Rind			6.03	6.78	7.08	6.99	5.90	4.01

DAS : Days after sowing

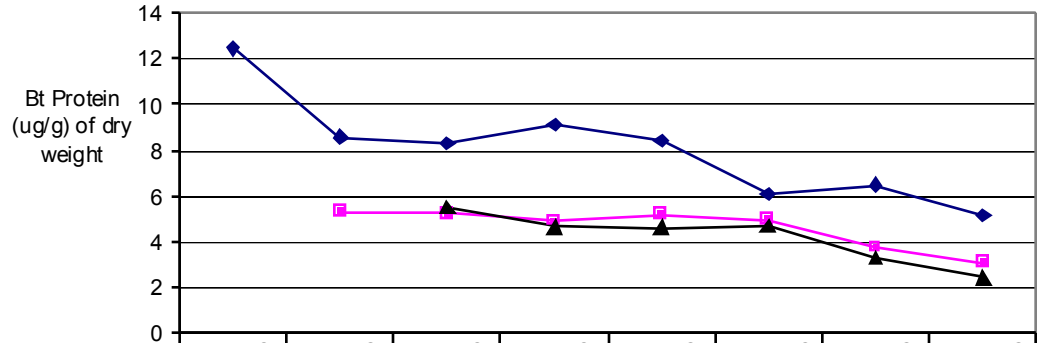
Mean Bt Protein concentration In Dfferent Plant Parts of NCEH-6 Bt from Haryana State



	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	12 DAS	135 DAS
Term Leaf	15.24	9.84	9.30	10.08	9.01	7.90	7.35	6.07
Square		6.60	5.82	5.76	4.26	5.22	4.12	3.12
Boll Rind			5.42	5.91	6.09	5.00	3.80	2.41

DAS : Days after sowing

Mean Bt Protein concentration In Dfferent Plant Parts of NCEH-6 Bt from Rajasthan State



	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	12 DAS	135 DAS
Term Leaf	12.48	8.57	8.3	9.11	8.41	6.07	6.44	5.12
Square		5.30	5.20	4.90	5.16	4.93	3.72	3.06
Boll Rind			5.48	4.62	4.6	4.67	3.26	2.41

DAS : Days after sowing