



SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
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PROJECT No. : TOX-346F
STUDY : FEEDING STUDIES IN CATFISH
COMPOUND : Bt COTTONSEEDS
REPORT No. : 000046375
Date : 14.05.2007

FEEDING STUDIES IN CATFISH

WITH

Bt COTTONSEEDS

Report for:

METAHELIX LIFE SCIENCES PRIVATE LIMITED
PLOT NO.3, KIADB 4th PHASE, BOMMASANDRA,
BANGALORE-560 099, INDIA

Prepared by:

DEPARTMENT OF TOXICOLOGY
SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
(A Unit of Shriram Scientific & Industrial Research Foundation)

19, University Road, Delhi – 110 007
Tel. 27667267, 27667860, 27667432
Fax No. 91+11-27667676, 27667207
E. Mail : sridhi@vsnl.com



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SCIENTIFIC PERSONNEL INVOLVED IN THE STUDY

QUALITY ASSURANCE STATEMENT

Dr. RAJUL SAXENA, M.V.Sc.
 (Scientist Pathology)

This is to certify that the work described in the study report entitled 'Feeding Studies in Catfish' with 'Bt Cottonseeds (Sample II)' has been conducted with respect to the agreed study protocol.

SUPRIYA SEMWAL, M.Sc.
 (Project Trainee)

The report provides true and accurate record of results obtained.

D. NARAYANANAMY, M.Sc.
 (Project Trainee)

BY DIRECTOR

SCIENTIST PATHOLOGY

HEAD

TOXICOLOG

Rinu Bhat
**Sr. SCIENTIST
 QUALITY ASSURANCE**

Approved for Issue



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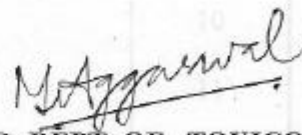
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SCIENTIFIC PERSONNEL INVOLVED IN THE STUDY

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STUDY DIRECTOR


SCIENTIST PATHOLOGY


HEAD, DEPT. OF TOXICOLOGY

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DEPUTY DIRECTOR
(MANAGEMENT)



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SUMMARY

In the assessment and evaluation of the toxic characteristics of a substance, determination of Feeding studies is initial step.

This study was hence, designed to conduct Feeding studies in catfish provided by M/s Metahelix Life Sciences Private Limited.

Three groups consisting of 50 fishes, individually, were designated for the study. The first group was kept as control that was dosed with fish feed only, the second group of animals was administered with the Non-Bt Cottonseeds, (Sample-I) in powdered form and supplemented with fish feed and the third group of animals was treated similarly with Bt Cottonseeds (sample II) in powdered form and supplemented with fish feed.

No toxic signs and symptoms /mortality was observed in any test group as well as the control group of animals.

Under the conditions of this study, the fish of Bt Cottonseeds (sample II) group did not induce any treatment related observable toxic effects, when compared to the corresponding group of "Non-Bt Cottonseeds (Sample-I) fish and the control group of fish.



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INTRODUCTION

This study was carried out to determine the survival and growth of catfish by feeding them on 'Bt Cottonseeds (Sample -II)' and 'Non - Bt Cottonseeds (Sample -I)' samples respectively.



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OBJECTIVE

To assess the growth and survival of catfish fed on a diet containing cottonseed meal derived from Bt Cottonseeds (Sample –II) as compared to that of the Non- Bt Cottonseeds (Sample –I) varieties for use as catfish feed. The processed and powdered cottonseed meal was incorporated into the catfish feed. The duration of the test was 28 days.

CHARACTERIZATION OF TEST AND CONTROL COTTONSEED:

The test and control cottonseeds will be characterized by the sponsor prior to their use in the study.



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TEST SUBSTANCE

The sponsor is responsible for the necessary characterization and evaluations of the test substance. The details of the test substance provided by the Sponsor are as follows:

PRODUCT NAME : NON-Bt COTTONSEEDS (SAMPLE I)
& Bt COTTONSEEDS (SAMPLE II)

SPONSOR : METAHELIX LIFE SCIENCES,
PRIVATE LIMITED

MATERIAL DESCRIPTION : YELLOWISH BROWN COLOURED
POWDER

PACKED IN : BROWN COLOURED PAPER
CARTONS

DATE OF COMMENCEMENT : 04-01-2007
OF STUDY

DATE OF COMPLETION : 31-01-2007
OF STUDY

(Note: For characterization details of test samples, see Annexure I provided by the sponsor).



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EXPERIMENTAL DESIGN

Duration : 28 days

Acclimatization : Test fish were acclimatized for at least 10 days in water of the quality to be used during the test period.

Name of the species : *Clarius batrachus* (Catfish)

Total No. of fish to be used : 150 fish

No. of fish / treatment : 50 fish (25 fish / aquarium)

Temperature : 16-22 °C (60-70 °C)

EXPERIMENTAL PROCEDURE

Identification

Each aquarium was identified by putting a label on it mentioning the name of the study, product name and aquarium number.



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FEED FORMULATION

Feed for the fish was formulated so that it contained crude protein, fats, supplements for vitamins and minerals, in addition to the cottonseed samples provided by the sponsor which was not exceeding 20 % of body weight.

Treatment Diets

Prior to offering the feed of above composition, it was analyzed for its composition. The feed offered was 2 % of body weight of the fish.

OBSERVATIONS

Observation Period : 28 days

Mortality and behavior of fish in the control group as well as the test groups were observed. Water temperature and dissolved oxygen were also monitored.

Feed Consumption :

Floating fish pellet meal was provided to the control group fish at the rate of 2% of body weight and it was noticed that whole feed provided to the fish was consumed.



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Likewise the test groups were also provided with the feed formulation at the dose rate of 2% body weight, which was consumed by the fish.

Body Weights :

Weight of fish initially i.e. on day 0, on day 14 and 28 were recorded and the mean weights calculated. The mean weights were considered for further study (Tables: 3-5).

Feed Conversion ratio :

Feed conversion ratio was calculated on the basis of average body weights of the fish (Table: 7).

Proximate Composition of Diets:

The analysis of composition of diets and fish fillets was done weekly (Table: 2).

Statistical Method:

All observed data are recorded and analyzed biostatistically.



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RESULTS:

Feed Consumption:

The formulated feed consumed by the fish of the test groups was similar to that of the control group.

Weight of fish:

The weight gain of fish of test groups was comparable to that of its control counterparts.

Toxic sign & symptoms:

No adverse effects including behavioral changes could be noticed in any of the test as well as the control group fish.

CONCLUSION:

Under the conditions of this study, the 28 days feeding studies on Catfish with 'Bt Cottonseeds (Sample -II) and Non - Bt Cottonseeds (Sample -I)', as a feed supplement, did not induce any observable toxic effects, when compared to its control counterparts.



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TABLE: 1

MEAN BODY WEIGHT OF FISH AT DIFFERENT INTERVALS

Groups	Day 0	Day 14th	Day 28th
Control	28.35±2.80	29.23±2.98	29.83±2.80
Non - Bt Cotton seeds (Sample -I)	28.33±2.80	29.21±3.29	29.78±2.64
Bt Cottonseeds (Sample -II)	28.46±3.12	29.46±2.85	29.95±2.32



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TABLE: 2

COMPOSITION OF THE FISH FEED ESTIMATED WEEKLY

Group	Parameters	Day 0	Day 7 th	Day 14 th	Day 21 st
Control	Protein(NX6.25) % by mass	14.4	16.2	15.4	10.4
	Moisture % by mass	40.6	41.0	41.6	39.7
	Crude Fat % by mass	4.3	3.8	3.3	5.4
	Ash % by mass	1.0	1.1	1.1	1.2
	Crude fibre % by mass	0.8	0.9	0.9	1.0
Non- Bt Cottonseeds (Sample-I)	Protein(NX6.25) % by mass	14.9	15.0	15.9	11.6
	Moisture % by mass	40.4	40.1	41.4	41.2
	Crude Fat % by mass	3.5	3.9	4.5	8.6
	Ash % by mass	0.9	1.0	0.8	1.4
	Crude fibre % by mass	1.9	2.0	2.0	2.3
Bt Cotton seeds (Sample-II)	Protein(NX6.25) % by mass	14.8	13.9	15.8	12.3
	Moisture % by mass	40.1	39.8	41.4	38.3
	Crude Fat % by mass	4.6	5.1	4.8	8.1
	Ash % by mass	1.2	1.1	1.4	1.4
	Crude fibre % by mass	2.0	2.1	2.3	2.2



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TABLE : 3 BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '0'

No. of Fish	Fish wt. on Day '0' Control group	Fish wt. on Day '0' Non- Bt Cottonseeds (Sample I)	Fish wt. on Day '0' Bt Cottonseeds (Sample II)
1	30.8	27.5	30
2	23.5	23.5	24.9
3	27.5	29.6	28.9
4	30.5	32.2	30.8
5	32.5	30.5	29.9
6	28.9	26.9	27.0
7	21.4	25.9	20.8
8	20.9	23.4	21.3
9	26.8	26.8	25.5
10	30.1	29.2	30.1
11	30.5	30.5	32.4
12	31.5	30.6	33.4
13	29.0	29.8	30.1
14	25.9	27.8	26.4
15	27.9	27.6	28.3
16	22.6	20.5	24.2
17	25.3	24.6	26.5



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TABLE : 3 (Contd) BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '0'

No. of Fish	Fish wt. on Day '0' Control group	Fish wt. on Day '0' Non-Bt Cottonseeds (Sample I)	Fish wt. on Day '0' Bt Cottonseeds (SampleII)
18	29.5	29.8	30.1
19	26.9	30.5	29.0
20	24.9	30.5	25.4
21	30.5	26.5	31.5
22	29.6	30.1	29.2
23	32.0	32.9	34.5
24	29.8	25.6	31.0
25	28.4	23.6	25.4
26	23.6	25.6	22.9
27	29.4	29.5	30.2
28	31.4	30.8	31.4
29	26.4	24.7	25.6
30	24.8	25.6	24.8
31	29.4	31.5	30.4
32	30.1	25.6	31.2
33	30.0	30.9	32.4
34	28.9	32.5	30.8

Contd -----



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TABLE : 3 (Contd) BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '0'

No. of Fish	Fish wt. on Day '0' Control group	Fish wt. on Day '0' Non-Bt Cottonseeds (Sample I)	Fish wt. on Day '0' Bt Cottonseeds (Sample II)
35	24.9	30.8	33.2
36	28.4	25.9	24.6
37	31.9	30.8	31.4
38	29.2	28.6	29.2
39	26.8	23.8	24.6
40	29.4	29.7	27.9
41	27.6	27.5	26.8
42	30.5	30.8	30.5
43	32.2	30.5	30.8
44	28.2	29.6	28.2
45	28.9	28.4	27.5
46	31.0	26.8	30.4
47	28.7	28.9	27
48	29.9	30.8	29
49	30.7	30.5	29.8
50	27.8	29.9	25.6
Mean±SD	28.35±2.80	28.33±2.80	28.46±3.12



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TABLE : 4 BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '14'

No. of Fish	Fish wt. on Day '14' Control group	Fish wt. on Day '14' Non-Bt Cottonseeds (SampleI)	Fish wt. on Day '14' Bt Cottonseeds (SampleII)
1	30.2	28.6	29.2
2	25.6	29.5	28.0
3	29.3	29.4	30.1
4	34.2	32.9	31.5
5	32.8	33.9	32.8
6	26.5	28.5	26.8
7	23.5	21.4	23.5
8	22.6	23.8	22.8
9	26.6	27.9	30.4
10	29.9	30.1	29.6
11	31.7	32.4	31.4
12	32.8	33.4	32.8
13	30.5	30.1	29.6
14	27.9	26.4	27.5
15	28.5	28.3	30.4
16	25.9	24.2	25.8
17	27.5	26.5	28.9

Contd-----



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TABLE : 4 (Contd) BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '14'

No. of Fish	Fish wt. on Day '14' Control group	Fish wt. on Day '14' Non-Bt Cottonseeds (SampleI)	Fish wt. on Day '14' Bt Cottonseeds (SampleII)
18	30.1	30.1	32.4
19	25.9	29.0	28.9
20	28.6	25.4	28.5
21	30.4	31.5	30.5
22	32.2	32.2	33.4
23	33.9	35.8	34.7
24	31.2	31.8	31.0
25	26.5	25.4	26.4
26	23.3	22.9	23.9
27	28.5	30.2	31.4
28	30.6	31.4	29.4
29	24.9	25.6	26.4
30	23.9	24.8	25.4
31	29.5	30.4	29.7
32	31.2	31.2	32.4
33	33.5	32.4	30.8
34	33.6	34.0	33.9

Contd-----



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TABLE : 4 (Contd) BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '28'

No. of Fish	Fish wt. on Day '14' Control group	Fish wt. on Day '14' Non-Bt Cottonseeds (Sample I)	Fish wt. on Day '14' Bt Cottonseeds (SampleII)
35	33.2	35.2	34.8
36	27.8	24.6	25.8
37	30.4	31.4	30.8
38	27.8	29.2	30.4
39	25.9	24.6	25.8
40	28.4	30.2	31.5
41	27.6	26.8	27.4
42	32.5	30.5	31.2
43	31.5	32.4	33.4
44	29.6	29.8	28.2
45	32.9	30.8	27.5
46	31.2	25.9	30.4
47	28.5	27.8	26.8
48	29.6	29.8	28.4
49	30.8	29.8	31.4
50	29.9	30.5	28.9
Mean ±SD	29.23±2.98	29.21±3.29	29.46±2.85



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TABLE : 5 BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '28'

No. of Fish	Fish wt. on Day '28' Control group	Fish wt. on Day '28' Non-Bt Cottonseeds (SampleI)	Fish wt. on Day '28' Bt Cottonseeds (SampleII)
1	32.2	31.0	30.5
2	26.5	28.6	29.5
3	29.5	31.5	30.2
4	34.8	30.8	33.4
5	33.6	28.9	29.6
6	29.4	32.5	31.5
7	25.6	26.8	26.8
8	24.5	24.4	25.9
9	26.5	25.5	28.9
10	31.4	31.8	30.5
11	31.4	32.0	33.2
12	34.5	34.5	35.6
13	31.5	30.1	33.2
14	27.8	27.3	28.4
15	29.5	28.4	29.1
16	27.6	25.6	26.4
17	27.6	27.3	28.2

Contd-----



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TABLE : 5 (Contd) BODY WEIGHTS OF CONTROL AND TEST FISH ON DAY '28'

No. of Fish	Fish wt. on Day '28' Control group	Fish wt. on Day '28' Non-Bt Cottonseeds (SampleI)	Fish wt. on Day '28' Bt Cottonseeds (SampleII)
18	31.5	30.3	31.2
19	32.5	32.4	30.5
20	26.5	26.2	24.5
21	31.5	32.8	31.5
22	32.2	29.2	29.6
23	34.9	34.8	34.5
24	32.1	32.2	33.1
25	28.6	26.1	27.8
26	25.6	23.4	25.6
27	30.2	29.4	30.1
28	31.9	31.5	32.5
29	24.9	28.5	26.4
30	29.6	30.1	26.5
31	31.5	28.7	29.4
32	29.4	30.8	29.0
33	32.9	31.9	32.1
34	33.2	33.5	32.0

Contd-----



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TABLE : 5 (Contd) BODY WEIGHTS OF FISH GIVEN DIFFERENT DOSES ON DAY '28'

No. of Fish	Fish wt. on Day '28' Control group	Fish wt. on Day '28' Non-Bt Cottonseeds (SampleI)	Fish wt. on Day '28' Bt Cottonseeds (SampleII)
35	31.5	33.5	30.2
36	29.5	27.9	28.5
37	30.9	32.8	30.2
38	33.6	30.4	31.5
39	24.6	25.1	29.5
40	29.6	27.9	28.6
41	26.8	27.3	28.2
42	25.6	31.2	32.2
43	30.2	30.8	31.4
44	29.4	30.8	31.5
45	29.6	30.2	29.0
46	30.8	29.2	30.2
47	26.8	31.4	30.2
48	28.6	30.1	29.4
49	32.6	31.2	30.2
50	28.5	30.4	29.4
Mean± SD	29.83±2.80	29.78±2.64	29.95±2.32



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Table No. 6

**AVERAGE INCREASE IN WEIGHT GAIN OF FISH AT
DIFFERENT GROUPS AT THE END OF EXPERIMENTATION**

S.No.	GROUP	AVERAGE INCREASE IN THE Wt. OF FISH
1.	Control	1.48
2.	Non-Bt Cottonseeds (Sample I)	1.45
3.	Bt Cottonseeds (Sample II)	1.49



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Table No. 7

FEED CONVERSION RATIO (FCR) OF DIFFERENT GROUPS AT THE END OF EXPERIMENTATION

S.No.	GROUP	FCR (in %)
1.	Control	3.98
2.	Non-Bt Cottonseeds (Sample I)	4.06
3.	Bt Cottonseeds (Sample II)	4.02

PROXIMATE FEED ANALYSIS

S. No.	TESTS	PROXIMATE FEED ANALYSIS AT THE START OF THE EXPERIMENT			PROXIMATE FEED ANALYSIS AT THE END OF THE EXPERIMENT		
		OBSERVED VALUE			OBSERVED VALUE		
		CONTROL	Bt COTTON	NON-Bt COTTON	CONTROL	Bt COTTON	NON-Bt COTTON
1.	MOISTURE, % BY MASS	39.7	41.2	38.3	43.4	34.7	38.4
2.	CRUDE FAT, % BY MASS	5.4	8.6	8.1	0.1	1.0	0.5
3.	CRUDE PROTEIN, (N x 6.25) % BY MASS	10.4	11.6	12.3	10.7	13.2	11.9
4.	CRUDE FIBRE, % BY MASS	1.0	2.3	2.2	0.2	0.9	0.9
5.	TOTAL ASH, % BY MASS	1.2	1.4	1.4	0.8	1.3	1.1



SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
Confidential

PROJECT No. : TOX-346F
STUDY : FEEDING STUDIES IN CATFISH
COMPOUND : Bt COTTONSEEDS
REPORT No. : 000046375
Date : 14.05.2007

Table No. 8

AVERAGE FEED CONSUMPTION DATA OF FISH

	Day 0-6		Day 7-13		Day 14-20		Day 21-28	
	Feed Given (gms)	Feed Consumed (Approx)	Feed Given (gms)	Feed Consumed (Approx)	Feed Given (gms)	Feed Consumed (Approx)	Feed Given (gms)	Feed Consumed (Approx)
Control	20	19.00	20	19.00	20	19.00	20	20.00
Non-Bt Cottonseeds (Sample I)	20	20.00	20	20.00	20	20.00	20	19.00
Bt Cottonseeds (Sample II)	20	20.00	20	19.00	20	20.00	20	20.00



SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH

(A unit of Shriram Scientific and Industrial Research Foundation)

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TEST CERTIFICATE

000046375

Issued to :
METAHELIX LIFE SCIENCES PVT. LTD.
PLOT NO. 3, KIADB 4TH PHASE,
BOMMASANDRA
BANGALORE - 560099KARNATAKA

J.O.No. TOX-346F
Reg.No. 4612570
Date 15-05-2007
GC-01 (REV-04)

Your Ref.No. --

Kind Attn: DR. M.J. VASUDEVA RAO , PRESIDENT

Sample Particulars:

One sample of "Bt Cottonseeds" was received for Feeding study in Catfish.

Date

TEST RESULTS

Material Description : Non-Bt Cottonseeds (Sample-I)- Yellowish brown coloured powder
Bt Cottonseeds (Sample-II)- Yellowish brown coloured powder.

Sponsor : Metahelix Life Sciences Private Limited
Plot no.3, KIADB 4th Phase, Bommasandra,
Bangalore-560 099, India.

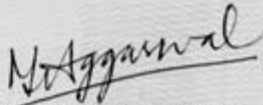
Result

Feeding study in Catfish

Under the conditions of this study, the 28 days feeding studies on Catfish with 'Bt Cottonseeds (Sample-II)' and Non-Bt Cottonseeds (Sample-I)', as a feed supplement, did not induce any observable toxic effects, when compared to its control counterparts.

(Annexure enclosed)

DOR : 06-11-2006
DOC : 14-05-2007


AUTHORISED SIGNATORY
(EMPLOYEE CODE: 6006)

19, University Road, Delhi - 110007.
E-Mail: qad@shriraminstitute.org Website: <http://www.shriraminstitute.org>

Ph: 91-11-27667267, 27667983, 27667860
Fax: 91-11-27667676, 27667207

PCR & ELISA CONFIRMATION OF BIOSAFETY COTTONSEED MATERIAL

Objective: Quality Control of the cottonseed material from cry1C-9124 based intrahirsutum hybrids to be used for the biosafety studies; despatched on 11th September 2006.

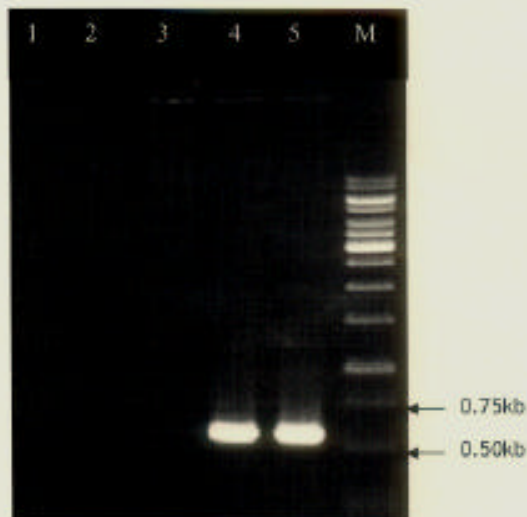
1. Confirmation of transgenic nature by PCR specific to the transgene
2. Confirmation of presence of Cry1C protein and its quantitation by ELISA

PCR confirmation

PCR was performed on Eppendorf Mastercycler Gradient machine with the following primers:

Upper: 5'-CCT CGC CAT TCT TCG TGA TTC C
 Lower: 5'-GGT TGG CCT CCC TTC CGT AGA TA

1. H₂O CONTROL
2. -VE CONTROL (LEAF)
3. NON TRANSGENIC SEED DNA
4. TRANSGENIC SEED DNA
5. +VE CONTROL



EXPECTATION- 0.58 KB

Results and conclusion

As expected amplification from cry1C was observed in case of transgenic and positive control proving the presence of the gene. Water and negative controls were clear indicating the absence of gene.

ELISA confirmation

Quantitative ELISA for Cry1C protein was performed using the Quantiplate kit for Cry1C (Envirologix, USA; Catalog No. AP 007) according to the manufacturer's protocol

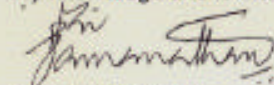
Sl no	Entry ID	A ₄₅₀	Cry1C concentration (µg/g on fresh wt)
1	Blank	0.09	NA
2	1 ppb standard	0.3	0.92
3	5 ppb standard	1.44	5.2
4	10 ppb standard	2.21	9.93
5	Nontransgenic	0.092	NA
6	Transgenic	2.9	13.08

Results

The absorbance value observed at 450nm for nontransgenic sample was nearly the same as blank and no colour development was observed in case of nontransgenic. Blue colour development was observed in case of transgenic samples indicating the presence of Cry1C protein.

Declaration

I hereby declare that the certificate of quality presented above is true to the best of my knowledge and is made on the basis of experiments carried out in our premises.



Val. Ramanathan

Head - Genomics