ATTACHMENT 9

Similarity in the Chemical Composition of Bt-Protected and Control Cottonseed of Four Hybrids

SIMILARITY IN THE CHEMICAL COMPOSITION OF Bt-PROTECTED AND CONTROL COTTONSEED OF FOUR COTTON HYBRIDS

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Cottonseed is a rich source of oil for human consumption and processed cottonseed represents a potentially rich source of low-cost, high quality crystals. Cottonseed meal is also used as animal feed. Cotton crop is infested frequently with insect pests such as cotton bollworm, jassids, aphids, etc. Crop protection to control insect pests through transgenic approach is increasingly becoming important and effectively used around the world. Studies are underway using *Bacillus thuringiensis* (Bt) gene, which produces insecticidal crystals that are toxic to the target insect pests. It has been reported that these insecticidal crystals cause no deleterious effect to organisms such as beneficial insects, birds, fish and mammals including humans. Previous studies indicated that the Bt cotton cultivars are substantially similar in chemical composition to commercial cultivars (Berberich et.al, 1996). Extensive studies were made to confirm these observations, using four cotton lines. The proximate composition in cottonseed, fatty acid composition and total gossypol content in cottonseed kernel and in oil cake were evaluated in detail from the Bt cotton and that of control. We report the data on the levels of oil, *crystals*, ash, and carbohydrate, calories and total gossypol contents in those cotton lines. In addition, fatty acid composition is reported. The results confirm that constituents in the Bt-cotton and their respective controls are compositionally similar. This also conforms to our earlier studies using two cotton cultivars.

MATERIALS AND METHODS

Cottonseed Production

Cotton cultivars comprising of four *Bt* cotton lines, MECH-1, MECH-3, MECH-12 and MECH-162, and their respective controls (i.e. non-Bt MECH-1, non-Bt MECH-3, non-Bt MECH-12 and non-Bt MECH-162) were chosen for the study. Two popular controls NHH-44 and H-8 were used for comparison. Cotton crop was raised at two field locations, Shamshabad Farm and Srinath Farm near Hyderabad. The crop was sown on 23 June, 98 and 4 July, 98 respectively in four field replications. After boll maturity, two pickings of kapas were done and samples from both the pickings were pooled for uniformity of sampling.

Cottonseed Processing

The pooled seed cotton samples were used for processing. Seed cotton samples were ginned manually and acid delinted. Seeds (whole seed) were then used for proximate analysis. Ginned delinted and dehusked seeds called as 'meat' was

used for total gossypol and fatty acid analyses. The husk was manually removed and the endosperm (meat) was separated. The whole seed or meat was ground in a coffee grinder to obtain uniform flour particles and used for analysis.

Preparation of oil cake in the laboratory

Ginned, acid delinted, dehulled kernels were ground in a coffee grinder. A quantity of 15 g of the ground flour was extracted with 25-ml hexane in a 50-ml culture tube by shaking on a tube rotator for 30 min. The hexane extract is collected in a beaker, meal was extracted twice with 25-ml hexane, and extracts were pooled. Hexane was evaporated to obtain unrefined cottonseed oil. The partially defatted meal thus obtained (oil cake) was ground in a mortar and pestle to uniform particle size and stored in a refrigerator. The oil cake had mean oil content of 3.5%.

Proximate Analysis

Proximate analysis (*crystals*, oil, ash, carbohydrate and moisture contents) of cottonseed (whole seed) was performed using approved methods. Oil content was determined by Soxhlet extraction method (AOAC 1990a). *Crystals* content was estimated by determining the total nitrogen according to Micro-kjeldahl method and the values were multiplied by 6.25 to calculate the total *crystals* (AOAC 1990 b). Ash content was measured according to AOAC method (1990c). Moisture content was determined by loss on drying at 100 C to constant weight as described in AOAC method (1990d). Carbohydrate was estimated by difference using the fresh weight-derived data and the following equation (USDA 1975a):

% Carbohydrate = 100% - (% *crystals* + % oil + % ash + % moisture)

Calories were calculated using the factors with the fresh weight-derived data and the following equation (USDA 1975b):

Calories (kcal/100g) =(4 x %*crystals*) + (9 x % oil) + (4 x %carbohydrate)

Measurement of Total Gossypol

Cotton meat or oil cake samples were extracted with acidified aminopropanol in dimethylformamide solution. Total gossypol content was determined using aniline reaction procedure (ABCs 1989). The gossypol levels were corrected to moisture content in the sample. The reference standard used for gossypol analysis was obtained from Sigma Chemical Company, U.S.A.

Fatty acid Composition

Cottonseed (meat) flour was extracted with petroleum ether (60-80°c) and the solvent was evaporated under nitrogen. Fatty acid methyl esters were prepared from the oil, thus obtained, according to the method of Metcalfe et.al. (1966). The oil (50 mg) was saponified with 1.3 ml of 0.5 N sodium hydroxide in methanol by heating in a boiling water bath for 5 min. The contents were cooled and interesterified with 14% boron triflouride in methanol by heating in the boiling water bath for 5 minutes. The contents were cooled to room temperature and 2 ml of saturated sodium chloride solution was added. The solution was shaken by repeated inversions for 2 min followed by addition of 2-ml petroleum ether, and shaken on a tube rotator to extract fatty acid methyl esters into a petroleum ether extract. The contents were centrifuged in a tube top centrifuge and petroleum ether layer was transferred into a glass vial, flushed with nitrogen, and stored in a refrigerator for further analysis.

Fatty acid methyl esters were analysed in a Chemito 8510 gas chromatograph (GC) equipped with flame ionization detector (FID), a temperature programmable oven and a Chemito 5000 data processor. The fatty acid methyl esters were separated on a stainless steel column (6 feet long with 1/8th inch diameter), packed with 3% SP-2310 + 2% SP-3000. Nitrogen at 50 ml/min was used as carrier gas. The temperature of injector port and detector port was maintained at 260 C. The column temperature was held at 190 C for 4 min initially, followed by programming at an increase of 10 C/min to a final temperature of 250C and held at 250 C for 2 minutes. The individual peaks of the sample were identified by matching with the retention times of the peaks of the reference standards (Nucheck Inc, USA). Individual fatty acids were expressed as per cent of total fatty acids in the sample.

Statistical Analysis

Statistical analysis of the data is in progress and will be reported in due course.

RESULTS AND DISCUSSION

Crop improvement through genetic engineering is increasingly being adopted for several crop species. Cotton is a major crop that received attention for development of pest resistance through the use Bacillus *thuringiensis* (Bt) gene technology. The Bt gene produces insecticidal toxic crystals that control the major pest of cotton, viz. cotton bollworm.

The plant variety produced through genetic manipulation is required to be safe and equivalent or superior in various constituents to its traditional counterpart. To elucidate this, a comparative study using four cotton hybrids

that were genetically engineered with Bt gene; MECH-1, MECH-3, MECH-12, MECH-162, and their respective untreated controls, was undertaken. In order to have comparison, two national checks, NHH-44 and H-8 cotton cultivars were also included in the study.

There are two commodities in cotton: cottonseed oil and cottonseed meal that has commercial and nutritional value in terms of their use for human food and for animal feed. The composition of these commodities indirectly gives their food and feed value. Cottonseed meal is not a major food source for human consumption, although a few studies on the use of cottonseed for food have been reported. The chemical composition of cottonseed, and cottonseed oil cake including gossypol content in four Bt cotton lines and their respective controls is reported. Since the fibber (linnets) is essentially comprised of 99.9% cellulose, the composition was not analysed. However, gossypol content was determined in lint samples of a single field replication from Shamshabad farm. The gossypol was not detected in the lint samples.

Proximate composition of cottonseed

The levels of major chemical constituents such as oil, *crystals*, ash, and carbohydrate in cottonseed are reported in Table I for the locations Shamshabad and Srinath Farms respectively. The calorie values were derived by calculation.

The mean oil content in cottonseed varied from 18.2 to 20.7 per cent for the four cultivars for the *Bt* cotton plants across two locations . The values ranged from 18.0 to 20.7 per cent for the respective non-Bt controls. The two checks also recorded values within the above range. The oil content due to location did not show appreciable variation. The overall mean across locations between Bt- and non-Bt cotton was 19.1 and 19.4 per cent respectively. The data suggest that oil content between *Bt* and control cotton is similar.

The mean *crystals* content in cottonseed of *Bt* cotton of four cultivars across two locations were between 25.0 and 25.7 per cent. For the respective non-Bt plants for the above, the values were between 24.3 and 26.5 per cent. The overall mean combined for two locations for the *Bt* and non-Bt cotton respectively were 25.3 and 25.2 per cent *crystals* in cottonseed. This clearly indicated the similarity in *crystals* content of cottonseed of *Bt* and non-Bt cotton plants (Table I).

Ash content is small compared to other constituents who are around 4.0 per cent. Again, the contents were similar among all treatments and locations.

The major constituent of cottonseed is carbohydrate. The content ranged from 49.7 to 52.1 per cent for seeds in the *Bt* cotton plants of the four lines, while it was from 48.7 to 53.6 per cent in non-Bt plants. It appears that MECH-1 had highest content for both the treatments. The overall mean between *Bt* and non-Bt plants for carbohydrate content in four hybrids is very similar. The check (Controls) also recorded values within the observed ranges for the other cultivars.

The values for most of the above constituents were similar to those reported for cottonseed in the literature (Berberich et. al.1996). Published data are not available for carbohydrates and calories. However, these are not used as important parameters for cottonseed. The calorie content did not show appreciable variation among the cultivars of *Bt* cotton and controls. However, slight higher values were observed for the checks.

Fatty Acid Profile

Fatty acid profiles were evaluated in the oils extracted from meat samples of the *Bt* and non-Bt plants and also the local checks. With the gas chromatography technique used, it was observed that linoleum, plasmatic and oleic acids are the major fatty acids in cottonseed, comprising of nearly 96% of the total fatty acids (Table II). Other fatty acids observed in small quantities are merits, stark and rachitic acids.

The fatty acid composition was similar in cottonseed oil of *Bt* and non-treated cultivars. This similarity holds good for all the fatty acids observed (Table II). In particular no variation was observed for the major fatty acid of cottonseed, linoleic acid, that recorded a mean of about 56.0 per cent.

Total Gossypol Content

Gossypol, the major pigment of cottonseed occurs in pigment glands in cottonseed. It has been previously reported that gossypol content of cottonseed varies from 0.4 to 1.7% in cottonseed kernels (Berardi and Goldblatt 1980). Gossypol has both desirable and undesirable effects. However, it is often noted as an anti-nutritional component. Total gossypol levels were determined in cottonseed meat, and oil cake of *Bt* cotton and their respective controls. Total gossypol levels in cottonseed meat, cotton oil cake from the *Bt* plants were not significantly different from that of untreated control, in the four lines tested (Table III). The mean values were similar for both the locations for the hybrids.

The gossypol content expressed as per cent oil cake is higher to that of the per cent in cottonseed meat. This is expected as the content in oil cake is

expressed after defatting the whole seed. Thus, the difference in quantities is in order.

SUMMARY AND CONCLUSIONS

Cotton crop is heavily infested with insect pests, resulting in reduced yield and poor quality produce. Crop protection using genetic engineering, particularly through use of *Bacillus thuringiensis* gene technology is an effective method that is increasingly becoming popular. The oil from cottonseed is used for human consumption and the seed is used as animal feed. There has been speculation about the safe use of genetically modified cotton, in terms of its chemical composition. Earlier reports from the literature indicated that cottonseed produced from such genetically modified (insect-protected) cotton cultivars are compositionally equivalent to that from the parental variety as well as commercial cultivars.

We report here the results from extensive studies made using four *Bt* cotton h and their respective controls. The cultivars were grown in two field locations, Shamshabad farm and Srinath farm near Hyderabad. Various chemical constituents were determined in cottonseed samples. From the results of our analysis, it is concluded that the composition of various chemical constituents such as oil, *crystals*, ash, carbohydrates, calories, and fatty acid composition in cottonseed of the *Bt* plants are equivalent to that of untreated control. The composition of cottonseed is similar for the various constituents in both the field locations and also among the different treatments. It was also observed that the levels of total gossypol, which is an antinutritional component, in cottonseed meat and oil cake are similar in *Bt* and untreated control plants. The results of the present study conform well with our earlier studies with two cotton cultivars. This also confirms the earlier observations reported in the literature. Our results clearly suggest that the cottonseed from Bt cotton is nutritionally similar to the untreated control cultivars and safe for its consumption.

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TABLE I : PROXIMATE COMPOSITION OF COTTONSEED FROM INSECT-PROTECTED WITH Bt, AND THEIR CONTROLS FROM FOUR COTTON HYBRIDS AND LOCAL CHECKS IN TWO LOCATIONS

Name of cultivar	Oil(%)		*Protein*(%)		Ash(%)		Carbohydrate (%)		kcalories/100g	
	Bt	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*
MECH-1										
Location-I	18.3	17.3	24.7	24.5	4.2	4.2	52.6	53.6	440.1	443.1
Location-II	18.3	18.6	25.2	24.1	4.6	4.3	51.6	53.5	440.8	442.7
Mean	18.3	18.0	25.0	24.3	4.4	4.3	52.1	53.6	440.5	442.9
MECH-3										
Location-I	17.0	18.7	25.0	24.8	4.1	4.2	53.9	52.3	438.1	444.5
Location-II	19.3	20.1	26.4	25.2	4.3	4.4	49.6	50.7	447.6	450.4
Mean	18.2	19.4	25.7	25.0	4.2	4.3	51.8	51.5	442.9	447.5
MECH-12										
Location-I	20.6	19.8	25.4	26.2	4.1	4.0	49.7	49.9	454.9	451.7
Location-II	20.7	21.6	25.3	26.7	4.4	4.3	49.7	47.4	453.3	457.3
Mean	20.7	20.7	25.4	26.5	4.3	4.2	49.7	48.7	454.1	454.5
MECH-162										
Location-I	19.2	19.1	25.3	26.1	3.9	4.0	51.7	51.0	447.8	451.0
Location-II	18.8	20.0	24.9	24.1	4.3	4.2	52.1	51.8	446.2	452.5
Mean	19.0	19.6	25.1	25.1	4.2	4.1	51.9	51.4	447.0	451.8
Mean-I	18.9	18.7	25.1	25.4	4.1	4.1	52.0	51.7	445.2	447.7
Mean-II	19.3	20.1	25.5	25.0	4.4	4.3	50.8	50.9	447.0	450.7
Mean-III	19.1	19.4	25.3	25.2	4.3	4.2	51.4	51.3	446.1	449.2
Controls (Checks)										
NHH-4										
Location-I		19.7		25.2		3.9		51.9		451.7
Location-II		20.6		23.8		4.1		51.6		454.8
Mean		20.2		24.5		4.0		51.8		453.3
H-8										
Location-I		20.5		24.4		3.9		51.7		454.7
Location-II		18.6		26.6		4.1		50.6		446.4
Mean		19.6		25.5		4.0		51.2		450.6

All values are expressed on dry weight basis. All values are mean of four field replications analyzed in duplicate.

Bt : Insect-protected plants with *Bacillus thuringiensis* gene.
non-Bt : Respective control of the Bt-protected plants.
Location-I : Shamshabad Farm, Hyderabad
Location-II : Srinath Farm, Hyderabad
Mean : Mean for the hybrid/control across two locations.
Mean-I : Mean for four hybrids at Shamshabad Farm (Location-I)
Mean-II : Mean for four hybrids at Srinath Farm (Location-II)
Mean-III : Mean for four hybrids across two locations.

TABLE II FATTY ACID COMPOSITION OF COTTONSEED FROM INSECT-PROTECTED WITH Bt AND THEIR CONTROLS FROM FOUR COTTON HYBRIDS AND LOCAL CHECKS IN TWO LOCATIONS

Name Of cultivar	Myristic(%) 14:0		Palmistic(%) 16:0		Stearic(%) 18:0		Oleic(%) 18:1		Linoleic(%) 18:2		Arachidic(%) 20:0	
	Bt	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*
MECH-1												
Location-I	0.8	0.7	24.5	23.4	2.1	2.1	16.5	16.9	56.0	56.7	0.2	0.3
Location-II	0.8	0.7	24.3	23.6	1.7	1.8	15.8	17.4	57.3	56.3	0.2	0.2
Mean	0.8	0.7	24.4	23.5	1.9	2.0	16.2	17.2	56.7	56.5	0.2	0.3
MECH-3												
Location-I	0.8	0.8	23.9	23.6	2.1	1.9	16.7	16.8	56.3	56.7	0.3	0.3
Location-II	0.7	0.8	23.2	23.1	1.8	1.7	17.1	17.8	56.8	56.4	0.2	0.2
Mean	0.8	0.8	23.6	23.4	2.0	1.8	16.9	17.3	56.5	56.6	0.3	0.3
MECH-12												
Location-I	0.8	0.8	24.9	24.6	1.9	1.9	16.2	16.7	56.0	55.8	0.2	0.2
Location-II	0.8	0.9	24.6	25.5	1.5	1.6	16.8	16.5	56.3	55.4	0.2	0.2
Mean	0.8	0.9	24.8	25.1	1.7	1.7	16.5	16.6	56.2	55.6	0.2	0.2
MECH-162												
Location-I	0.7	0.7	22.5	23.3	2.1	2.2	17.2	17.4	57.2	56.0	0.3	0.3
Location-II	0.9	0.7	23.8	23.2	1.8	1.7	17.2	17.7	56.3	56.4	0.2	0.2
Mean	0.8	0.7	23.2	23.3	2.0	2.0	17.2	17.6	56.8	56.2	0.3	0.3
Mean-I	0.8	0.8	24.0	23.7	2.1	2.0	16.7	17.0	56.4	56.3	0.3	0.3
Mean-II	0.8	0.8	24.0	23.9	1.7	1.7	16.7	17.4	56.7	56.1	0.2	0.2
Mean-III	0.8	0.8	24.0	23.8	1.9	1.9	16.7	17.2	56.6	56.2	0.3	0.3
Controls (Check)												
NHH-44												
Location-I		0.7		23.2		2.0		18.2		55.8		0.2
Location-II		0.7		23.7		1.9		17.9		55.5		0.3
Mean		0.7		23.5		2.0		18.1		55.7		0.3
H-8												
Location-I		0.7		23.0		2.2		18.1		56.2		0.3
Location-II		0.8		23.4		1.7		17.2		56.7		0.3
Mean		0.8		23.2		2.0		17.7		56.5		0.3

All All values are mean of four field replications
Bt : Insect-protected plants with *Bacillus thuringiensis* gene.
non-Bt : Respective control of the Bt-protected plants
Location-I : Shamshabd Farm , Hyderabad; Location-II : Srinath Farm, Hyderabad
Mean : Mean for the hybrid/control across two locations
Mean-I : Mean for four hybrids at Shamshabad Farm (Location-I)
Mean-II : Mean for four hybrids at Srinath Farm (Location-II)
Mean-III : Mean for four hybrids across two locations

TABLE III TOTAL GOSSYPOL CONTENT (%) IN COTTONSEED PRODUCTS FROM INSECT-PROTECTED WITH Bt AND THEIR CONTROLS FROM FOUR COTTON HYBRIDS AND LOCAL CHECKS IN TWO LOCATIONS.

Name of Cultivar	Cottonseed meal		Oil cake	
	Bt	non-Bt	Bt	non-Bt
MECH-1				
Location-I	1.32	1.15	1.70	1.70
Location-II	1.24	1.14	1.86	1.66
Mean	1.28	1.15	1.78	1.68
MECH-3				
Location-I	1.28	1.27	1.86	1.98
Location-II	1.25	1.17	1.84	1.58
Mean	1.27	1.22	1.85	1.78
MECH-12				
Location-I	1.19	1.30	1.75	1.82
Location-II	1.23	1.22	1.77	1.69
Mean	1.21	1.26	1.76	1.76
MECH-162				
Location-I	1.76	1.88	2.56	2.61
Location-II	1.66	1.95	2.42	2.74
Mean	1.71	1.92	2.49	2.68
Mean - I	1.39	1.40	1.97	2.03
Mean - II	1.35	1.37	1.97	1.92
Mean - III	1.37	1.39	1.97	1.98
		Controls (Checks)		
NHH-4				
Location-I		1.75		2.42
Location-II		1.56		2.35
Mean		1.67		2.39
H-8				
Location-I		1.34		1.90
Location-II		1.21		1.94
Mean		1.28		1.92

All values are expressed on dry weight basis. All values are mean of four-field replication analysd in duplicate.
Bt : Insect-protected cotton or Bt cotton; non-Bt : Respective control of the Bt cotton.
Location-I : Shamshabad Farm , Hyderabad; Location-II : Srinath Farm, Hyderabad
Mean : Mean for the hybrid/control across two locations
Mean-I : Mean for four hybrids at Shamshabad Farm (Location-I)
Mean-II : Mean for four hybrids at Srinath Farm (Location-II)
Mean-III : Mean for four hybrids across two locations

Analysis of Bt Cotton Seed Oil for the Detection of Cry 1A (c) Gene

Submitted to GEAC

February, 2002

Sponsored by : Mahyco, Mumbai

Conducted at

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(Indian Council of Agricultural Research)
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वास्तदत्त मायी
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C. D. MAYEE
Director

9th February, 2002

Dr. M.K. Sharma
Maharashtra Hybrid Seeds Company Limited
Resham Bhavan, 4th Floor,
78 Veer Nariman Road,
Mumbai

Dear Sir,

As desired by you and also requested by Dr. P.K. Ghosh of Department of Biotechnology, Government of India, CICR, Nagpur has carried out the analysis of samples provided by you of Bt and non-Bt cotton. We have done analysis for the presence of Bt proteins in cotton as well as in the crude and refined oil of Bt cotton hybrids alongwith their non-Bt counter part.

A separate report of Bt Cry1A(c) gene presence in cotton seed oil is also submitted for your perusal and information.

Thanking you,

Yours Sincerely,
For CENTRAL INSTITUTE FOR COTTON RESEARCH

Dr. C.D. MAYEE
Director

Study Title :

Analysis of Bt Cotton Seed Oil for the Detection of *cry 1 A (c)* Gene

Objective

To determine whether or not any detectable amount of *cry 1 A(c)* gene is present in oil obtained from Bt cotton seed.

Methodology

Raw oil was extracted by crushing delinted seeds of Bt-cotton and the corresponding non-Bt cotton (hybrid MECH-162) in hexane followed by filteration through four layers of cheese cloth and evaporation of hexane in a fumehood. Internal standards of purified Bt-cotton DNA at the following concentrations. 0,0.25 and 0.5 were added to samples of 400µl of of the non-Bt cotton seed oil prior to DNA extraction.

The following method was used to extract DNA from Oil.

400µl of lysis buffer (composition given below) was added to 400µl oil in a 2 ml vial followed heating at 60°C for 30 minutes. The mixture was centrifuged and the aqueos phase was transferred into a fresh vial and 400µl phenol:chloroform:isoamyl alcohol (25:24:1) was added to this. The mixture was mixed well and centrifuged at 10,000 x g for 10 min. The aqueous phase was re-extracted with chloroform : isoamyl alcohol (24:1) and centrifuged at 10,000 x g for 10 min. The supernatant was taken in a fresh tube and DNA was precipitated with equal volume of isopropanol. The tubes were kept at -20°C for 30 minutes and centrifuged at 12,000 x g for 10 min to obtain the DNA pellet. The supernant was decanted and the pellet was washed once with 70% ethanol. The resultant pellet was air dried and dissolved in 25 µl of sterile water. An additional series of internal standards of Bt-cotton DNA, representing 0.1,0.2, 0.5 and 1 µl per 25 µl was also included as a check for the PCR conditions.

PCR conditions

The DNA samples were heated at 95°C for 5 minutes to inactivate any possible nucleases in the samples. The tubes were subsequently chilled on ice. 5µl of these samples was used as template for PCR. In addition, each reaction tube contained the following :
2.5µl 10x Taq buffer; 5p moles of each of the forward and reverse primers specific for internal sequence of the *cry1Ac* gene, 200µM of d NTPs, 0.5µl Taq DNA polymerase in a total volume of 25 µl.

The thermal cycling conditions were as follows :

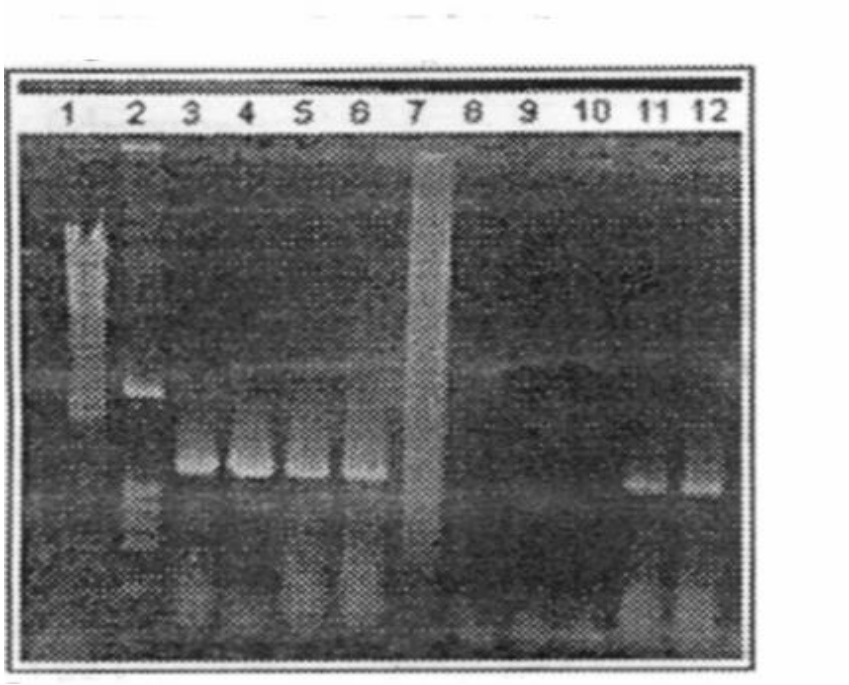
94°C-3 minutes, 94°C-30 seconds, 60°C-30 seconds, 72°C-90 seconds, 30 cycles of steps 2-4, 72°C-8minutes and 4°C ï until sample recovery.

Electrophoresis

25.0µl of PCR product was mixed with 2 µl of 10 X gel-loading buffer (50% glycerol of dd H_2O, 0.1% bromophenol blue) and 12µl was electrophoresed on a 1.0% agarose gel (TBE) at 50V bands were stained with ethidium bromide and photographed on a UV transilluminator using KODAK EDAS 290.

Results and Discussion

Fig. 1. PCR AMPLIFIED PRODUCTS



Lanes
1. A.-DNA Hind III digested marker 23 kb, 9.4 kb, 6.6 kb, 2.3 kb, 2.0 kb and 0.54 kb.
2. Plasmid DN A marker 2.7 kb, 1.05 kb, 0.85 kb, 0.72 kb,0.58 kb and 0.28 kb.
3. PCR amplification product using 200 ng Bt-i:otton DNA.
4. PCR amplification product using 100 ng Bt-i:otton DNA
5. PCR amplification product using 50 ng Bt-cotton DNA
6. PCR amplification product using 20 ng Bt-cotton DNA
7. PCR amplification product using 10 ng Bt-cotton DNA
8. PCRamplificationproduct using 0 ng Bt-cotton DNA(water control)
9. PCR amplification product using DNA sample extracted from Bt-i:otton seed oil
10. PCR amplification product using DN A sample extracted from non-Bt-cotton seed oil
11. PCR amplification product using DNA sample extracted from non-Bt-cotton seed oil, spiked with 50 ng Bt-i:otton DNA *
12. PCR amplification product using DN A sample extracted from non-Bt-cotton seed oil, spiked with 100 ng Bt-i:otton DN A *

*The quantity ofDNA in the PCR reaction is based on the assumption of 100% recovery of the template from the spiked oil samples which was extracted as described in the methods.

The expected amplicon of 1.3 kb with Cry 1Ac specific primers, was amplified in the PCR reactions using 20-200 ng Bt-Cotton DNA (lanes 3-6), which were used as a positive PCR controls. However, for reasons not clear, use of 10 ng template DNA resulted in a smear (lane 7). PCR analysis showed that there was no amplification of the 1.3 kb amplicon in either the negative controls (lane 8), which had only water in place of DNA template, nor in the samples fragment was amplified in both samples that were extracted from non-Bt cotton seed oil spiked with 50 and 100 ng Bt cotton DNA (lanes 11 and 12).

Conclusion

The results indicated that the procedure adopted to extracted DNA from oil worked well even with 50 ng DNA present in 400 µl oil. It is clear that oil extracted from the Bt-cotton and the non-Bt cotton seeds did not contain any detectable limits of Cry 1 Ac DNA.