Scholars Journal of Agriculture and Veterinary Sciences **e-ISSN 2348–1854**
Sch J Agric Vet Sci 2015; 2(2A):102-107 **p-ISSN 2348–8883**
©Scholars Academic and Scientific Publishers (SAS Publishers)
(An International Publisher for Academic and Scientific Resources) DOI: 10.36347/sjavs.2015.v02i02.005

Evaluation of two Chinese Bt cotton genotypes against the African bollworm, *Helicoverpa armigera* (Hubner) damage under Sudan rainfed conditions

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Abstract: The chemical insecticides have been the main method of pest control that is costly (30-40% of the total cost), polluting to the environment and damaging to the quality of life. The experiments were carried-out in Gedarif State at two site viz., North, South Gedarif and Damazin (Blue Nile State) during 2011/12 and 2012/13 seasons. Bollworms are the main pest inflecting major loss to the yield and constitute about 50% of the total insect control cost. The objective of this research was to evaluate two Chinese Bt cotton genotypes against the African bollworm, *Helicoverpa armigera* (Hubner) damage under rainfed conditions. Two Bt-cotton genotypes; a Seeni 1 (Chinese 1) and Seeni 2 (Chinese 2) cotton introduced by China-Aid Agricultural Technology Demonstrations Center for evaluation and release for commercial production in Sudan. The two genotypes carry Cry1A gene from *Bacillus thuringiensis* (Bt) which is specific toxin against Lepidoptera larvae to protect cotton crop against bollworms. Results obtained showed that the damage by the bollworms was significantly lower or negligible on the two Bt-genotypes compared to the local checks across the three sites and overall % bolls damage was 1% on the Bt genotypes CN-C01 and CN-C02 compared to 82 and 89 % on the two local checks Abdin and Hamid. The number of bolls per plant at harvest was significantly higher on the two Bt-genotypes (18 to 20) than the two local checks Abdin and Hamid. The seed cotton yield (SCY) was significantly higher in Damazin followed by South Gedarif and North Gedarif. The overall SCY of the Bt-genotypes CN-C01 and CN-C02 was 762 and 720 kg/ha, respectively, compared to 362 and 347 kg/ha for the two checks Abdin and Hamid, respectively. The GOT of the Bt-genotypes was significantly higher ranging between 42-44%, while that of the local checks ranged between 32-35%. The lint yield of the Bt-genotypes was 312 and 301 kg/ha while that of the local checks was 118 and 121, respectively. The lint yield of CN-C01 and CN-C02 were increase 160 and 151% over the average of the two checks.
Keywords: Bt cotton, CN-C01, CN-C02, *Bacillus thuringiensis*, bollworms, *Helicoverpa armigera* Lint, damage, Cry1A gene.

INTRODUCTION

Cotton cultivation began in Sudan in the Nuba mountains in the 1920s of the last century in Equatoria in 1948, and in the South of the Blue Nile in 1952, and in South Gedarif (Samsum)in 1964 [2]. Cotton is one of the main cash crops in Sudan and has contributed significantly to the economy of the country in the past. It is grown under irrigation and rain-fed [1]. Yield have been fluctuating between a minimum of 779 to a maximum of 1748 kg/ha in the irrigated sector compared to between 94 and 952 kg/ha in the rain-fed sector. Insect pests are the major constrains contributing to high cost of production, low cotton yield and less income from cotton production. The chemical insecticides have been the main method of pest control that is costly (30-40% of the total cost), polluting to the environment and damaging to the quality of life. Bollworms are the main pest inflecting major loss to the yield and constitute about 50% of the total insect control cost [1]. Bt cotton is developed by genetic engineering through transfer of Bt genes from a soil born Bacteria called *Bacillus thuringiensis* (Bt). These genes enable the plants to produce a protein that is toxic to caterpillar pests such as the cotton bollworms [11]. In 1996, the first Bt cotton varieties were introduced commercially in the United States, Mexico and Australia [6]. Chinese Academy of Agricultural Sciences (CAAS) has developed and patented local Bt gene (Cry1A) and insert it into local varieties [7-8]. Bt-cotton varieties have been marketed since 1997 in China to help control attacks of some cotton pests, viz., *Helicoverpa armigera*. It is estimated that Bt-cotton is currently grown on about 70% of the total Chinese cotton-growing area. Most studies have explained this broad distribution by the specific advantages of Bt-cotton particularly the reduction in pesticide use [1]. Two Chinese Bt genotypes were introduced by the China-aid Agricultural Technology Demonstration Center (CATDC), Elfaw. One of these two genotypes was released in 2012 for commercial use in the Sudan for both rainfed and irrigated sector.

MATERIALS AND METHODS

The experiments were carried-out in Gedarif State at two site viz., North, South Gedarif and Damazin during 2011/12 and 2012/13 seasons. The experiments were arranged in randomized complete block design (RCBD), with four replications. The two Bt-genotypes and the two local checks (Abdin and Hamid) were evaluated at two sites under rain-fed conditions. Plot size was 7 X 10 m. Spacing was 0.8 m between rows and 0.45 m between holes three plants were left after thinning. Sowing date was between 20, and 12 July for 2011 and 2012, respectively.

Two Bt-cotton genotypes; a Seeni 1 (Chinese 1 open- pollinated) and Seeni 2 (Chinese 2 -Hybrid) cotton introduced by CATDC for evaluation and release for commercial production in Sudan. The two genotypes carry Cry1A gene from *Bacillus thuringiensis* (Bt) which is specific toxin against Lepidoptera larvae to protect cotton crop against bollworms. The two genotypes were approved for commercial production by Chinese National Authority in 2004 and 2008, respectively. The two genotypes were widely adopted in Shandong province and currently under production in China. Two local cotton varieties; Abdin and Hamid were included in the evaluation as local checks.

Field experiments for two seasons across two rainfed sites (North and South Gedarif). Cultural practices followed the recommendation for cotton in Sudan. Weeding was performed manually and ½ N urea was applied once. No chemical was applied for insect control. Data were recorded for insect damage, genotypes agronomic traits, seed cotton yield, yield components and fiber quality parameters.

Data were collected on the following parameters:

1. Number and percent damaged fruiting bodies early in the season from September to October at varying intervals.
2. Number and percent of damaged bolls/ plant and number of larvae/ plant in 5-10 randomly selected plants/ plot at 1st boll setting and 1st boll opening stage.
3. Seed cotton yield (SCY).
4. Yield components and fiber characteristics

Main agronomic and morphological characteristics such as flower and boll opening dates, plant height, number of monopodial and sympodial branches and hairiness.

Statistical analysis

Collected data was arranged and analyzed using Stat-view Statistical package (SAS, 1990) using ANOVA and correlation for test of.

RESULTS AND DISCUSSIONS

The rain fall during season 2011 at the three sites; North Gedarif (University Farm), South Gedarif (Doka) , and Damazin was 285 mm in 22days, 294 mm in 24 days and 576 mm in 32 days, respectively. The bollworms infestation was very high at Gedrarif and moderate to low at Damazin (Table 1 and 2). The damage by the bollworms was significantly lower or negligible on the two Bt-genotypes compared to the local checks across the three sites (Table2 and 3). Overall % bolls damage was 1% on the Bt genotypes CN-C01 and CN-C02 compared to 82 and 89 % on the two local checks Abdin and Hamid. The number of bolls per plant at harvest was significantly higher on the two Bt-genotypes (18 to 20) than the two local checks Abdin and Hamid (Table 2).

The SCY was significantly higher in Damazin followed by South Gedarif and North Gedarif. The overall SCY of the Bt-genotypes CN-C01 and CN-C02 was 762 and 720 kg/ha, respectively, compared to 362 and 347 kg/ha for the two checks Abdin and Hamid, respectively (Table 4 and 5). The GOT of the Bt-genotypes was significantly higher ranging between 42-44%, while that of the local checks ranged between 32-35%. The lint yield of the Bt-genotypes was 312 and 301 kg/ha while that of the local checks was 118 and 121, respectively [4]. The increase in lint yield of CN-C01 and CN-C02 was 160 and 151% over the average of the two checks.

Description and fiber characteristics

Seeni 1 :

1. Open pollinated variety (OPV)
2. Bt gene: GFM *Cry1A* China insect resistant cotton gene
3. Inserted victor: composed of 35S promoter, enhancer, Ω sequence, Kozak sequence, processing sequence, Poly(A) sequence (Fig. 3)
4. Plant: pagoda-shape, dark green, medium gland density, normal and medium size leaves, thin hairs.
5. Plant height: 71cm (rain-fed) -98 cm (irrigated)
6. Flower: pale creamy corolla
7. Boll-shape: round large
8. Symbodial braches: 8 (rain-fed)- 11 (irrigated)
9. Growth habit: semi-determinate
10. Days to first flower: 50 (rain-fed)
11. Days to 50% flowering: 54 days

12. Days to 50% boll opening: 95 (irrigated)-98 days (rain-fed)
13. Maturity: early maturing (137 days)
14. GOT: 41% (rain-fed)-43% (irrigated)
15. Fiber length: 27.6 mm
16. Fiber strength: 27.0 g/tex (HVI)
17. Fiber fineness: 4.5 mic

Table-1: Shedding number and % of the damage in shedding due to bollworms, of the Bt and non Bt- genotypes evaluated in season 2011 in confined open field trials under rain-fed conditions at Damazin, South Gedarif and North Gedarif

	Damazin		South Gedarif		North Gedarif		Combined	
Genotypes	Total shedding	% Damaged shedding	Total shedding	% Damaged shedding	Total shedding	% Damaged shedding	Total shedding	% Damaged shedding
CN-C01	10	0	25	9	22	3	57	4
CN-C02	3	0	22	2	23	2	48	1
Abdin	37	47	15	80	20	93	72	73
Hamid	35	45	16	86	20	86	70	72
P value		0.001		0.001		0.001		0.001
SE±		5.5		7.7		8.8		4.4
CV%		100		78		88		90

Table-2: Number of bolls/ plant, % of damaged bolls/ plant due to bollworms and number of bollworms (BW) larvae at boll formation and 1st boll opening stage, of the Bt and non- Bt genotypes evaluated in season 2011 under rain- fed conditions at Damazin, South Gedarif and North Gedarif

								South Gedarif		North Gedarif	
	No. bolls/ plant				% Damaged bolls/ plant			No. BW larvae at		No. BW larvae at	
Genotypes	Damazin	South Gedarif	North Gedarif	Combined	South Gedarif	North Gedarif	Combined	Boll formation	1st boll opening	Boll formation	1st boll opening
CN-C01	6	26	23	18	1	0	1	0	1	1	1
CN-C02	7	29	24	20	2	0	1	1	1	1	1
Abdin	5	3	3	4	87	77	82	12	19	9	13
Hamid	6	4	2	4	91	86	89	14	17	8	14
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SE±	0.2	3.1	2.8	1.5	8.2	8.2	5.7	0.4	0.4	0.3	0.3
CV%	15	82	84	90	84	99	90	64	65	52	56

Table-3: Seed cotton yield, GOT and their combined of the Bt and non- Bt genotypes evaluated under rain-fed conditions at Damazin, South Gedarif and North Gedarif in season 2011

	SCY (kg/ha)				GOT			
Genotypes	Damazin	South Gedarif	North Gedarif	Combined	Damazin	South Gedarif	North Gedarif	Combined
CN-C01	1150	626	509	762	42	40	40	41
CN-C02	1085	590	486	720	44	40	40	41
Abdin	931	96	60	362	32	33	34	33
Hamid	905	77	58	347	35	33	34	34
P value	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SE±	32.2	67.7	56.7	57.6	1.2	0.9	0.8	0.6
CV%	12	78	81	73	13	9	8	11

Table-4: Lint yield (kg/ha) and percent increase in lint yield of the Bt and non- Bt genotypes under rain-fed conditions at Damazin, South Gedarif and North Gedarif in season 2011

	Lint yield (kg/ha)							
Genotypes	Damazin	South Gedarif	North Gedarif	Combined	% over Abdin	% over Hamid	% over checks	% A over B
CN-C01	482	252	201	312	165	157	160	3
CN-C02	476	235	193	301	156	148	151	
Abdin	300	32	20	118				
Hamid	319	25	20	121				
P value	0.0001	0. 001	0.0001	0.0001				
SE±	23.6	27.9	22.9	23.5				
CV%	24	82	84	76				

% A over B: means % of increase of CN-C01 over CN-C02.

Table-5: Phonological characteristics, number of symbodial branches and plant height (cm) of the Bt and non-Bt genotypes evaluated under rain-fed conditions at Damazin, South Gedarif and North Gedarif in season 2011

Genotypes	Days to first flower[1)]	Days to first boll opening[2)]	No of sympodia[3)]	plant height (cm)
CN-C01	49	98	8	77
CN-C02	50	98	8	71
Abdin	49	97	8	85
Hamid	50	101	8	85
P value	0.8	0.0001	0.84	0.0001
SE±	0.25	0.49	0.12	1.6
CV %	0.02	0.02	0.06	0.14

1, 2 & 3: data from Damzin location only

DISCUSSION

The results obtained from the testing of the two Bt-genotypes CN-C01 and CN-C02 and the local checks Abdin and Hamid across the different irrigated and rain-fed environments revealed the consistence superiority of the Bt- genotypes over the local checks Abdin and Hamid. The two Bt-genotypes showed negligible damage due to bollworms in form of shedding or bolls damage while the local checks Abdin and Hamid suffered from significant damage in floral buds and bolls to the extend of to complete damage of bolls in sites with high bollworms infestation (Alfaw 2011/12). This was directly reflected in the higher seed cotton yield of the Bt- genotypes over the local checks. On the other hand the data indicated that the two Bt-genotypes had GOT significantly higher than the local checks. Thus the two Bt-genotypes CN-C01 and CN-C02 showed 142 and 129% increase in lint yield under irrigated conditions over the local checks, respectively. Under the rain–fed conditions the two Bt genotypes showed 160 and 151% increase in lint yield over the local checks, respectively.

Bollworms are major pest on cotton in Sudan. The old economic threshold (ETL) for pesticide spray is 10 eggs or small larvae/100 leaves and the new ETL is 30 eggs or 10 small larvae/100 leaves [2]. The number of insecticides sprays for bollworms ranges between 2 to 4 per season. As indicated in the bioassay and the evaluation trails there is no need for insecticides spray for protection of CN-C01 and CN-C02 against the bollworm. This contributes directly to reducing the production costs. The cultivation of the two Bt-genotypes in CN-C01 and CN-C02 will increase the lint yield by about 145% over the local varieties. This means that the cultivation of Bt cotton CN-C01 and CN-C02 will generate economic gain in terms of saving of cost of pesticides application and increase in yield. In addition, the use of the CN-C01 and CN-C02 will reduce the environmental pollution and hazards due to insecticide.

Biotech cotton was first planted in 1996, the first year of commercialization of biotech crops. Insect resistant cotton, featuring Bt-genes, and herbicide tolerant cotton were amongst the first products to be commercialized. Their impact has been substantial in all 13 countries where they have been commercialized, growing from less than one million hectares globally in 1996 to about 25 million hectares in 2011 [6]. Over 150 million hectares of biotech cotton have now been successfully planted in 13 countries since 1996. Seven million small farmers in China and another 7 million small farmers in India collectively planted a record 14.5 million hectares of Bt cotton. India celebrated a decade of successful cultivation of Bt cotton, which has achieved phenomenal success in transforming the cotton crop into the most productive and profitable crop in the country. The increase from 50,000 hectares of Bt cotton in 2002, (when Bt cotton was first commercialized in India) to 10.6 million hectares in 2011 represents an unprecedented 212-fold increase in

10 years. India enhanced farm income from Bt cotton by US$9.4 billion in the period 2002 to 2010 and US$2.5 billion in 2010 alone[3]. Thus, Bt cotton has transformed cotton production in India by increasing yield substantially, decreasing insecticide applications by about 50%, and through welfare benefits, contributed to the alleviation of poverty of 7 million small resource-poor farmers and their families in 2011 alone.

The data of the bollworm damage, yield and economic analysis of the Bt-genotypes CN-C01 and CN-C02 and the local checks presented in this paper is in consistence with the accumulated reports on Bt cotton across the globe [4, 10, 9, 11, 5 and 6]. The consistent expansion in the adoption of the Bt cotton to the extent that 82% of the global cotton is Bt and /or herbicide tolerant cotton is a solid testimony of the safety and benefit of these technologies. Therefore, the adoption and cultivation of the Bt- genotypes in the Sudan will lead to break through in cotton production and consequently improve farmer's welfare.

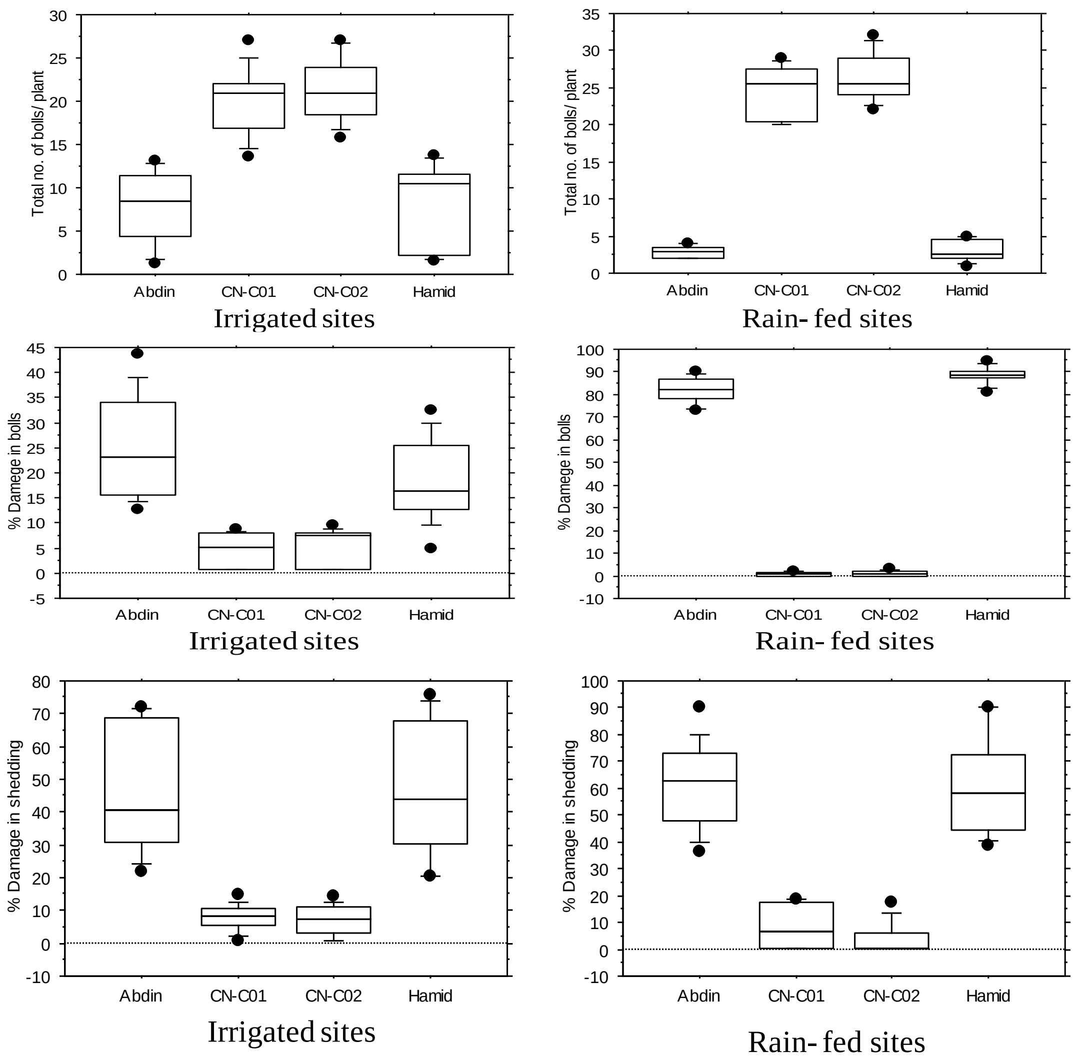

Fig-1: **Cell box of damage due to the bollworms In the Bt and non Bt genotypes evaluated under irrigation and rain fed conditions across 3 locations in each environment. The box indicates 25-75% of the scores. The line inside the box indicates the average.**

CONCLUSION

From the bollworms damage results it can obviously be concluded that *Cry1A* Bt- gene in the two genotypes CN-C01 and CN-C02 has sufficiently protected the cotton crop against the bollworms and bollworms damage has never exceeded 5% even in artificially inoculated cages. This confirms that Bt gene *Cry1A* is expressed in the environment adequately to kill both African and Egyptian bollworms in Sudan. Therefore, the CN-C01 and CN-C02 can be grown without the need for the intensive insecticides spraying for bollworms control. As a consequence the cost of production will be drastically reduced by up to 25%, and the environmental pollution, hazards to humans, animals and beneficial field organisms will also be reduced.

The results also indicated an increase of up to 95 % over the average of the two checks in seed cotton yield (SCY). The two Bt-genotypes have ginning out turn (GOT) above 40% which will add about 8- 9% in lint yield over the local checks. This, perhaps, the first time to propose for release of cotton varieties with GOT over 40 % in Sudan. Therefore, the adoption of CN-C01 and CN-C02 will generate benefits as a result of:

1. Crop protection against bollworms.
2. No insecticide application against the bollworms (about 25% reductions in cost of cotton production).
3. Less environmental pollution.
4. High seed cotton and lint yield.

Risk due to the gene in the environment and human health is not expected based on the preliminary analysis and experience gained in other countries adopting the technology for several years. The gene (*Cry1A*) went through stringent safety analysis in China and received approval from National Authority. It has been under production for 10 years in China.

Since the genotypes contain a single *Cry1A* gene, production strategy should be in place to prolong the efficacy of the gene against the bollworm and minimize the risk of development of early resistance against the Bt-gene [11- 12]. This can be through refuge plant cultivation or incorporation of another Bt-gene (stalked genes) to make double resistance genes and reduce the chance of resistance development for both of them at the same time.

Acknowledgements

Assistance from researchers; Sufian Suliaman, Nada Elsiddig, Hana Ibrahim at the BBRC, Contribution of Elashmawi S. Ali at the CATDC Alfaw and Mr. Haider A. M. Ahmed at the Ministery of Agriculture is highly acknowledged and appreciated. Comments and advice from Dr. Gazi Badawi, University of Khartoum, Faculty of Agriculture is acknowledge and appreciated. The secretary General of the Higher Council of Environment, Ministry of environment, Prof. Saadeldin I. Mohamed, the National Focal Point of the Convention of the Biological Diversity and the Bio-safety Protocol has been closely following and supporting the process ensuring the process in following the International Guide lines and procedures. This is highly acknowledged and noted with appreciation. This work was generously financially supported by the Federal Ministry of Agriculture and Irrigation, Sudan.

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