The Pharma Innovation Journal 2023; SP-12(10): 1787-1792

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(10): 1787-1792

www.thepharmajournal.com
Received: 23-07-2023
Accepted: 26-08-2023

Honnayya
Department of Agricultural Entomology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur, Karnataka, India

Sreenivas AG
Department of Agricultural Entomology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur, Karnataka, India

Kisan B
Department of Agricultural Biotechnology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur, Karnataka, India

Harischandra Nayak
Pesticide Residue and Food Quality Analysis, UAS, Raichur, Karnataka, India

Saroja N
Pesticide Residue and Food Quality Analysis, UAS, Raichur, Karnataka, India

Corresponding Author:
Honnayya
Department of Agricultural Entomology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur, Karnataka, India

Compensation of the loss in cry protein expression by additional application of ‘N’ fertilizers in elevated CO_2 and temperature on endotoxin production in *Bt* cotton

Honnayya, Sreenivas AG, Kisan B, Harischandra Nayak and Saroja N

Abstract

Elevated atmospheric CO_2 concentrations will cause plants to grow faster, lower nitrogen content per unit of plant tissue and generate higher carbon to nitrogen (C/N) ratios. We hypothesize that production of transgenic proteins were reduced, thus the loss in cry protein was mitigated by additional application of ‘N’ fertilizer. Based on the quantity of cry toxin produced in reference plot at 60 DAS was considered as a standard which later upon subsequent observations revealed that, in elevated CO_2, plants the cry toxin content in *e*CO_2 condition has increased over reference plot after the application of compensatory dose of N fertilizer. The *e*CO_2 treatments showed increased expression of Cry1Ac (2.88 and 2.10 µg/g) and Cry2Ab (53.48 and 43.77 µg/g) which was compensated by additional application of ‘N’ (6.14 gram of urea/plant) over the reference plot at 90 and 120 DAS respectively. The use of transgenic plants is becoming increasingly important and will continue to be so in the next decades. At the same time, atmospheric CO_2 increase will affect the effectiveness of this strategy. These observations have implications not only for agricultural use of transgenic plants, but also for the ecological consequences of transfer of *Bt* toxins to closely related wild plant genotypes.

Keywords: *Pectinophora gossypiella*, cotton, open top chambers, climate change, cry toxins

1. Introduction

Cotton, *Gossypium* spp. is one of the principle commercial fibre crops, which plays a prominent role in the national and international economy and is grown in tropical and subtropical regions of more than 111 countries. India is important cotton grower among all the cotton-producing countries globally and ranks first in area (129.57 lakh ha) with a production of 371 lakh bales and productivity of 487 kg lint ha^{-1}. Maharashtra, Gujarat, and Telangana are the major cotton growing states according for 69.60 per cent (87.59 lakh ha) in an area under cotton cultivation and 63.88 per cent (230 lakh bales) of cotton production in India. In Karnataka, it is cultivated over 5.50 lakh ha with a production of 18.00 lakh bales and productivity of 556 kg/ ha (Anon., 2021) [1].

Climate change greatly alters the relationship between plants and insects. Elevated CO_2 and high temperature are important environmental factors that affect the cotton development. Elevated CO_2 effect the plant-insect interaction by altering the chemical composition and physical properties of host plants (Bezemer and Jones, 1998) [2]. The impact of CO_2 on insect pests is found to be host mediated interaction. The increase temperature which changes the biochemical composition of plants and results in the rapid generation turnover of insects, which affect the survival, development, and reproduction of insect pest species due to changes in plants' nutrient quality under elevated CO_2 conditions (Hunter, 2001) [6].

Plants grown under elevated CO_2 conditions show higher photosynthetic rates that lead to higher carbon to nitrogen ratio resulting in lower nitrogen levels per unit of plant tissue that could affect the production of nitrogen-based proteins in transgenic plants. However, both direct and indirect effects of elevated CO_2 on herbivores should be studied, as such information will help to elucidate the interactions between host plants and phytophagus insects under future CO_2 enriched environment and to know the amount of nitrogen fertilizer required to partially compensate CO_2 effect and to produce *Bt* toxin protein in transgenic *Bt* cotton (Coviella *et al*., 2000) [3]. We hypothesized that elevated CO_2 leading to reduction in Nitrogen content plants grown in elevated CO_2 conditions and higher C: N ratio in the plants could affect the production of nitrogen-based proteins and also change in biochemical constituents in transgenic plants.

If this reduction is caused by lower N content, high N availability through fertilization should negate this effect. This would be significant because biological control of insect populations by means of transgenic plants is becoming an increasingly important pest management technique (Estruch *et al*., 1997) [4]. Although *Bacillus thuringiensis* proteins are the only transgenes commercially used for insect control, several other proteinaceous compounds such as proteinase inhibitors, amylase inhibitors, and lectins are being studied. Because transgenic plants producing nitrogen-based toxins are likely to be of widespread use well beyond the turn of the century, the expected increase in atmospheric CO_2 may impact the effectiveness of this strategy. Therefore, we used *Bt* transgenic plants as a model to assess the possible effects of future CO_2 atmospheric levels in the increasingly important use of transgenic plants producing proteins toxic to insects (Kumari *et al*., 2019) [9].

Most of the studies on the effects of elevated CO_2 focused on plants' biochemical composition, and very few studies were carried out on the effects of elevated CO_2 on insect host plant interactions. To understand these interactions found to be more important in predicting the impact of climate change on the expression and stability of host plant resistance to insect pests. Hence, to test whether the *Bt* protein content might affect the mitigation loss in cry protein in *Bt* cotton by additional application of 'N' fertilizer which will help to formulate effective management strategies to compensate the losses in cry protein content in *Bt* cotton plants.

2. Materials and Methods

Study on the influence of climate change variables on cry protein expression in *Bt* cotton hybrid was grown under different climate change regimes were carried out at the Centre for Agro climatic Studies, Main Agricultural Research Station, University of Agricultural Sciences, Raichur, 16.2044° N, 77.3341° E. Four circular OTC's (Open Top Chambers) were constructed by genesis group of technology, Mumbai with dimensions of five meters diameter and four meters height. In this different climate change treatments were set and the *Bt* cotton crop was sown.

These Open Top Chamber's were connected to Supervisory Control and Data Acquisition (SCADA) software system which could able to supply desired concentration of CO_2 and set temperature inside OTC's for 12 hours (morning 6 am to evening 6 pm). However, in the night hours due to the closure of the stomata the gaseous exchange is almost nil. Different climate change treatments simulated in different open top chambers are as follows,

Table 1: Details of climatic variables set in different OTCs are as follows

Treatments	Treatments details
T_1	Elevated CO_2 @ 550 ppm with 2 °C rise in temperature
T_2	Elevated CO_2 @ 550 ppm with normal temperature
T_3	Ambient CO_2 @ 410 ppm with 2 °C rise in temperature
T_4	Ambient CO_2 @ 410 ppm (Reference OTC)
T_5	Reference plot (open plot)

The *Gossypium hirsutum Bt* cotton hybrid *i.e*., Bindaas-7231 was raised individually in different open top chambers having different climate change treatments including reference plot (open field as a reference plot) in a 10 cement pots of (size; 42 x 32 sq. cm) containing a mixture of FYM, vermicompost, and soil in the ratio of 1:1:2. All agronomic practices for raising crop were followed as per the package of the University of Agricultural Sciences, Raichur (Table 1). Once the crop attained at 60 days the cry toxins (Cry1Ac and Cry2Ab) expressed was analyzed by ELISA method (Grothaus *et al*., 2006) [5] to quantify *Bt* toxin expression in leaves, flowers and bolls at 60 days of crop stage. Based on the quantity of cry toxin produced in reference plot/ open plot was considered as a standard check. By comparing this cry toxin produced in other treatments were calculated (Table 2) and accordingly the loss in cry toxin produced was compensated by the addition of urea. The details pertaining to it is presented below in tabular form.

Table 2: Concentration of cry toxin at 60 days of crop stage for compensatory dose of 'N'' fertilizer application

Treatments details	Cry toxin expression*			Compensatory dose of 'N' fertilizer in the form urea applied (g/plant)
	Cry1Ac (µg/gm)	Cry2Ab (µg/gm)	Mean cry toxins produced	
Elevated CO_2 @ 550 ppm with 2 °C rise temperature	2.42	48.26	25.34	6.14
Elevated CO_2 @ 550 ppm with normal temperature	2.53	50.34	26.43	5.15
Ambient CO_2 @ 410 ppm with 2 °C rise temperature	2.96	58.10	30.53	2.10
Ambient CO_2 @ 410 ppm (Reference OTC)	3.14	59.46	31.30	1.62
Reference plot (open plot)	3.35	64.99	34.17	--

* Average of 6 plants

2.1 Data analysis

Combined effects of CO_2 and temperature on cry toxin expression in *Bt* cotton were analyzed using a one-way analysis of variance. Treatment means were compared and separated using the least significant difference (LSD) at $p<0.01$. The data on growth parameters, physiological and biochemical parameters were analyzed using ANOVA. Statistical analysis was done by using SPSS software (version 16.0).

3. Results and Discussion

The lower N content per unit plant tissue caused by the elevated CO_2 resulted in lower cry toxin production by *Bt* cotton when nitrogen supply to the plants is a limiting factor. Results suggested that as currently designed, transgenic plants will require additional N fertilizer inputs to compensate the production of cry protein expression in eCO_2 conditions.

3.1 Compensate the loss in cry protein expression by additional application of 'N'' fertilizer

Promising *Bt* cotton hybrids *i.e*, Bindaas-1713 when subjected to climate change variables revealed that there was reduction in the endotoxin production by Cry1Ac and Cry2Ab under *e*CO_2 @ 550 ppm + 2 °C rise temperature compared to aCO_2 @ 410 ppm. So in order to mitigate/compensate the loss in cry protein by additionally urea was applied as source of 'N' fertilizer as explained in (Table 1). The results are obtained pertaining to this study is presented in the following headings.

3.2 Quantification of Cry1Ac and Cry2Ab toxin expression in Bindaas-7213 *Bt* cotton at 60, 90 and 120 days after sowing

Bt toxin expression was quantified by the ELISA method in leaves, flowers and bolls of Bindaas-7231 (*Gossypium hirsutum*) Bt cotton hybrid at 60 days after sowing under all the climate change treatments to know the effect of elevated CO_2 and temperature on the expression of cry proteins in different parts of *Bt* cotton hybrid. In all the treatments Cry1Ac and Cry2Ab, toxins were found more in leaves compared to flowers and bolls (Table 2 and Fig 1). The average amount of Cry1Ac and Cry2Ab toxins quantified were found to be highest in the reference plot (3.41 µg/g and 59.63 µg/g respectively), which was on par with aCO_2 @ 410 ppm treatment (3.35 µg/g and 58.20 µg/g) and aCO_2 @ 410 ppm + 2 °C rise temperature treatment (3.29 µg/g and 57.86 µg/g, respectively). On contrary, lowest amount of Cry1Ac (2.39 µg/g) and Cry2Ab (48.42 µg/g) was recorded in *e*CO_2 @ 550 ppm + 2 °C rise temperature treatment which was non-significant difference with *e*CO_2 @ 550 ppm treatment (Cry1Ac and Cry2Ab produced 2.48 µg/g and 48.55 µg/g of toxins, respectively) at 60 days after sowing (Table 2 and Fig 1). However, the percentage of cry toxins production was decreased in *e*CO_2 @ 550 ppm + 2 °C rise in temperature treatment Cry1Ac (29.91%) and Cry2Ab (18.80%) was reduced over reference plot at 60 days after sowing (Table 2 and Fig 1)

It is very clearly from the present study that endotoxin produced by Cry1Ac and Cry2Ab were affected greatly by climate change variables. This showed that climate change variables have great affected the plant growth and physiological component and also effect on cry toxin production, hence; additional 'N' fertilizer application was made to uplift the plant physiological components (N-based components). As a result of *Bt* cotton was grown in elevated CO_2 @ 550 ppm + 2 °C rise in temperature treatment which showed partially compensate the expression of cry toxins Cry1Ac (2.88 and 2.10 µg/g) and Cry2Ab (53.48 and 43.77 µg/g) which was compensated by applying (6.14 gram of urea/plant) (Table 1) as compared to reference plot Cry1Ac (3.64 and 2.84 µg/g) and Cry2Ab (63.47 and 58.86 µg/g) at 90 and 120 days after sowing, respectively (Table 3, 4 and Fig 1). However, the percentage of cry toxins production was reduced significantly in *e*CO_2 @ 550 ppm + 2°C rise temperature treatment Cry1Ac (20.88 and 26.06%) and Cry2Ab (15.11 and 25.64%) over a reference plot at 90 and 120 days of crop growth respectively (Table 3, 4 and Fig 1).

Present investigations are influence the effect of climate change variables on cry toxin production in *Bt* cotton hybrid when additional 'N' fertilizer was applied as a compensatory dose. It has partially compensated the endotoxin production in elevated CO_2 @ 550 ppm + 2 °C rise in temperature treatment over a reference plot.

These results are corroborated with the findings of Coviella *et al*. (2000) [3] who reported that transgenic plants grown in elevated CO_2 produced lower levels of *Bt* toxin than those plants grown in ambient CO_2 and high levels of nitrogen fertilization partially compensated for the *e*CO_2 effect and allowed the plants to produce *Bt* toxin levels closer to those of plants grown in ambient CO_2 conditions. Similarly, the present study is also corrugated with Shoulin Jiang *et al*. (2017) [13], who expected that N fertilization supply promote the expression of transgenic *Bt* toxin in transgenic *Bt* rice, particularly under elevated CO_2. The results are also in line with the findings of Patil (2007) [10], who revealed that increased cry protein concentration with the increase in 'N' fertilizer levels.

3.3 Yield of *Bt* cotton influenced by additional application of 'N' fertilizer to mitigate the loss in cry protein

In climate change treatments influenced the yield parameters of *Bt* cotton hence, after additional 'N' fertilizer application were made with enhanced yield of *Bt* cotton grown under *e*CO_2 @ 550 ppm + 2 °C rise in temperature treatment which was registered maximum yield (308.32 g/plant) at harvest of *Bt* cotton which was on par with *e*CO_2 @ 550 ppm treatment (296.84 g/plant) whereas, lowest yield was recorded in ambient CO_2 @ 410 ppm treatment (247.23 g/plant) at harvest of crop (Table 4).

These results of the investigation showed the trend of increased yield parameters and yield in the elevated climate change treatments due to increased biomass of the crop which was influenced by rubisco enzyme in *Bt* cotton. These results are supported by work of Kimball, (1983) [8] who noticed 30% increase in yield of *Bt* cotton when CO_2 levels were doubled. Similarly the studies made on tobacco by Xue *et al*. (2010) [15] Lucern by James *et al*. (2014) [7] and on cotton by Shreevani, (2015) [14] and Pooja, (2019) [12].

Present results are also corroborate with the findings of Patil *et al*. (2012) [11] who reported that the interaction effect were significant at 75 days, increase in fertilizer level targeted for higher yield recorded higher δ endotoxin content particularly in Bollgard-II.

Table 2: Influence of *e*CO_2 and temperature on cry toxin expression in different parts of Bindaas-7213 *Bt* cotton at 60 days after sowing

Treatments details	**Cry 1Ac (µg/g)**			**Average**	**% Reduction over control**	**Cry 2Ab (µg/g)**			**Average**	**% Reduction over control**
	Leaves	**Flowers**	**Bolls**			**Leaves**	**Flowers**	**Bolls**		
eCO_2 @ 550 ppm + 2 °C rise temperature	2.52±0.09^{b}	2.33±0.13^{b}	2.31±1.02^{b}	2.39±0.08^{b}	29.91	49.23±1.31^{b}	48.23±1.29^{b}	47.81±1.23^{b}	48.42±1.01^{b}	18.80
eCO_2 @ 550 ppm with normal temperature	2.65±0.12^{b}	2.36±0.10^{b}	2.43±0.23^{b}	2.48±0.06^{b}	27.27	48.82±1.94^{b}	48.58±1.12^{b}	48.24±1.06^{b}	48.55±1.17^{b}	18.58
aCO_2 @ 410 ppm + 2 °C rise temperature	3.41±0.15^{a}	3.28±1.02^{a}	3.17±0.09^{a}	3.29±0.18^{a}	3.52	58.29±1.91^{a}	57.92±1.06^{a}	57.36±2.07^{a}	57.86±1.05^{a}	2.97
aCO_2 @ 410 ppm (Reference OTC)	3.47±0.17^{a}	3.35±0.33^{a}	3.23±1.12^{a}	3.35±0.09^{a}	1.76	58.66±2.12^{a}	58.23±1.25^{a}	57.71±1.05^{a}	58.20±0.09^{a}	2.40
Reference plot (Open plot)	3.54±0.23^{a}	3.40±1.06^{a}	3.29±0.22^{a}	3.41±0.15^{a}	-	60.14±0.66^{a}	59.62±1.11^{a}	59.13±0.09^{a}	59.63±0.18^{a}	-
F.test	44.22**	42.82**	16.57**	34.53**	-	29.16**	34.21**	39.27**	34.21**	-
S. Em (±)	0.09	0.08	0.06	0.06	-	1.04	1.08	1.08	0.98	-
CD (P= 0.01)	0.27	0.24	0.18	0.18	-	3.12	3.24	3.24	2.94	-

**Significant @ 1%
Mean±SD are separated by least significant difference
Mean denoted by same letters in vertical column are not significantly different by DMRT

Table 3: Influence of *e*CO_2 and temperature on cry toxin expression in different parts of Bindaas-7213 *Bt* cotton at 90 days after sowing when 'N' fertilizer applied as compensatory dose

Treatments details	**Compensatory dose of 'N' fertilizer in the form urea applied (g/plant)**	**Cry 1Ac (µg/g)**			**Average**	**% Reduction over control**	**Cry 2Ab (µg/g)**			**Average**	**% Reduction over control**
		Leaves	**Flowers**	**Bolls**			**Leaves**	**Flowers**	**Bolls**		
eCO_2 @ 550 ppm + 2 °C rise temperature	6.14	3.00±1.09^{b}	2.90±1.06^{b}	2.73±0.09^{b}	2.88±0.13^{b}	20.88	54.23±1.07^{b}	53.92±1.90^{b}	53.48±1.36^{b}	53.88±1.44^{b}	15.11
eCO_2 @ 550 ppm with normal temperature	5.15	3.12±1.11^{b}	2.97±0.08^{b}	2.75±0.12^{b}	2.95±1.09^{b}	18.96	54.36±1.23^{b}	54.04±1.49^{b}	53.77±1.14^{b}	54.06±1.82^{b}	14.83
aCO_2 @ 410 ppm + 2 °C rise temperature	2.10	3.67±0.19^{a}	3.55±1.13^{a}	3.49±1.14^{a}	3.57±1.11^{a}	1.92	62.01±2.24^{a}	61.86±0.47^{a}	61.57±1.27^{a}	61.81±2.02^{a}	2.61
aCO_2 @ 410 ppm (Reference OTC)	1.62	3.75±1.01^{a}	3.57±1.19^{a}	3.49±1.17^{a}	3.60±0.21^{a}	1.10	62.40±1.77^{a}	62.25±1.03^{a}	61.93±0.19^{a}	62.19±1.29^{a}	2.02
Reference plot (Open plot)	-	3.76±0.14^{a}	3.62±0.21^{a}	3.54±0.16^{a}	3.64±1.04^{a}	-	63.67±0.19^{a}	63.45±1.35^{a}	63.28±1.42^{a}	63.47±1.37^{a}	-
F. test		32.62**	54.15**	67.23**	51.33**	-	30.52**	58.13**	27.65**	44.22**	-
S. Em (±)		0.09	0.05	0.05	0.06	-	0.94	0.82	0.75	1.08	-
CD (P= 0.01)		0.27	0.15	0.15	0.18	-	2.82	2.46	2.25	3.24	-

**Significant @ 1%
Mean±SD are separated by least significant difference
Mean denoted by same letters in vertical column are not significantly different by DMRT

Table 4: Influence of *e*CO_2 and temperature on cry toxin expression in different parts of Bindaas-7213 *Bt* cotton at 120 days after sowing when 'N' fertilizer applied as compensatory dose

Treatments details	Compensatory dose of 'N' fertilizer in the form urea applied (g/plant)	Cry 1Ac (µg/g)			Average	% Reduction over control	Cry 2Ab (µg/g)			Average	% Reduction over control	Seed cotton yield (g/plant)
		Leaves	Flowers	Bolls			Leaves	Flowers	Bolls			
eCO_2 @ 550 ppm + 2 °C rise temperature	6.14	2.26 ± 1.21^{b}	2.12 ± 0.19^{b}	1.92 ± 0.08^{b}	2.10 ± 0.18^{b}	26.06	44.21 ± 1.02^{b}	43.84 ± 1.14^{b}	43.25 ± 1.89^{b}	43.77 ± 1.13^{b}	25.64	308.32^{a}
eCO_2 @ 550 ppm with normal temperature	5.15	2.29 ± 0.04^{b}	2.14 ± 0.39^{b}	2.02 ± 0.15^{b}	2.15 ± 0.22^{b}	24.30	44.94 ± 1.69^{b}	44.28 ± 1.84^{b}	43.87 ± 1.07^{b}	44.36 ± 0.09^{b}	24.63	296.84^{a}
aCO_2 @ 410 ppm + 2 °C rise temperature	2.10	2.70 ± 1.03^{a}	2.61 ± 1.09^{a}	2.46 ± 1.12^{a}	2.59 ± 1.09^{a}	8.80	56.62 ± 2.04^{a}	56.31 ± 2.03^{a}	56.05 ± 0.15^{a}	56.33 ± 2.03^{a}	4.30	265.59^{b}
aCO_2 @ 410 ppm (Reference OTC)	1.62	2.83 ± 0.10^{a}	2.70 ± 0.07^{a}	2.59 ± 1.31^{a}	2.71 ± 1.12^{a}	4.58	57.97 ± 1.15^{a}	57.62 ± 1.13^{a}	57.23 ± 2.06^{a}	$57. 61\pm1.10^{a}$	2.12	247.23^{b}
Reference plot (Open plot)	-	2.96 ± 0.17^{a}	2.82 ± 1.01^{a}	2.73 ± 1.07^{a}	2.84 ± 0.13^{a}	-	59.03 ± 2.13^{a}	58.83 ± 1.17^{a}	58.74 ± 2.12^{a}	58.86 ± 2.05^{a}	-	254.71^{b}
F. test	-	32.57**	43.13**	87.25**	54.31**	-	48.59**	72.18**	51.09**	57.28**	-	38.59**
S.Em (±)	-	0.10	0.8	0.08	0.09	-	1.13	1.06	1.06	1.14	-	7.80
CD (P= 0.01)	-	0.30	0.24	0.24	0.27	-	3.39	3.18	3.18	3.42	-	23.50

**Significant @ 1%

Mean±SD are separated by least significant difference

Mean denoted by same letters in vertical column are not significantly different by DMRT

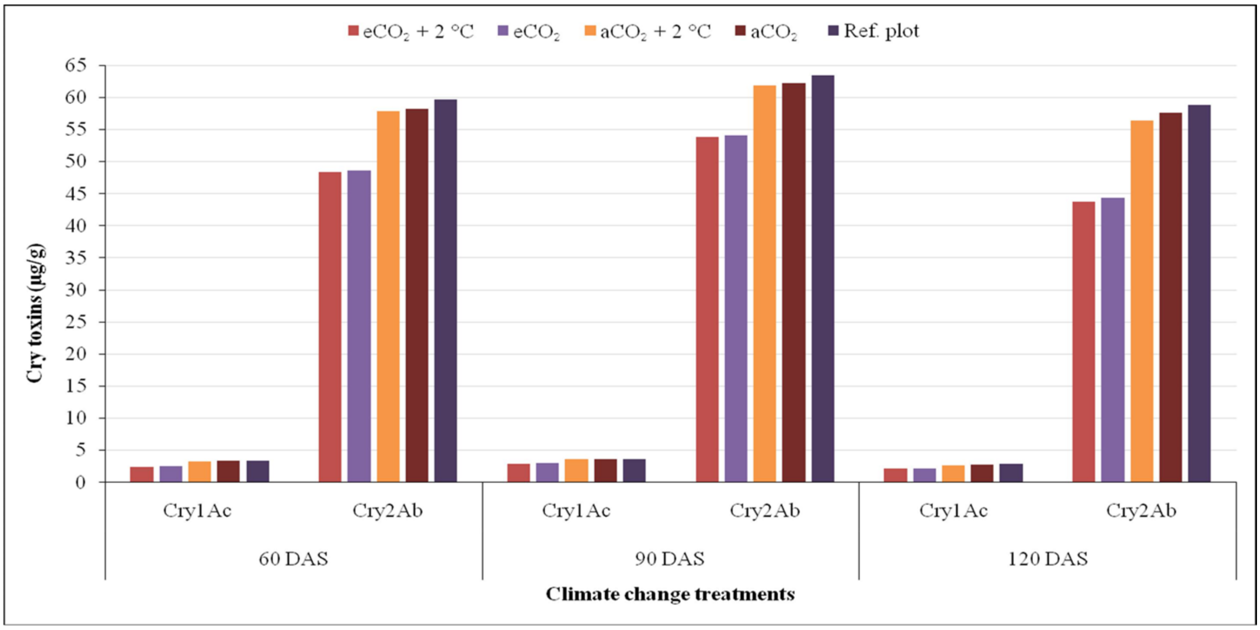

Fig 1: Effect of elevated CO_2 and temperature on cry toxin expression in Bindaas-7213 *Bt* cotton when additional N fertilizer applied as compensatory dose

4. Conclusion

The combined effect of elevated carbon dioxide and temperature can alter pest behaviour and weaken the expression of *Bt* toxin by changing the plant chemistry. Our results indicate that transgenic plants grown in elevated CO_2 produced lower levels of *Bt* toxin than those plants grown in ambient CO_2 or reference plot. High level of nitrogen fertilization was partially compensated for the CO_2 effect and allowed the plants to produce *Bt* toxin levels closer to those of plants grown in ambient CO_2.

Loss in cry protein was mitigated by additional application of N fertilizer at 60 DAS which later upon subsequent observations revealed that, in elevated CO_2, plants the cry toxin content in *e*CO_2 condition has increased over reference plot after the application of compensatory dose of N fertilizer. The *e*CO_2 treatments showed increased expression of Cry1Ac (2.88 and 2.10 µg/g) and Cry2Ab (53.48 and 43.77 µg/g) which was compensated by additional application of 'N' (6.14 gram of urea/plant) over the reference plot at 90 and 120 DAS respectively.

5. Acknowledgments

I am incredibly grateful to the Department of Agricultural Entomology, Department of Biotechnology, Centre for Climate Change and Pesticide Residue and Food Quality Analysis Laboratory, UAS, Raichur, for conducting my PhD research work without problem.

6. References

1. Anonymous. Area, production and productivity. The cotton corporation of India Ltd, 2021.
2. Bezemer TM, Jones TH. Plant insect herbivore interactions in elevated atmosphere CO_2, quantitative analysis and guild effects Oikos. 1998;82:212-222.
3. Coviella CE, Morgan DJW, Trumble JT. Interactions of elevated CO_2 and nitrogen fertilization: effects on production of *Bacillus thuringiesis* toxins in transgenic plants Environmental Entomology. 2000;29:781-787.
4. Estruch JJ, Carozzi NB, Desai N, Duck NB, Warren GW, Koziel MG. Transgenic plants: An emerging approach to pest control Nature Biotechnology. 1997;15:137-141.
5. Grothaus GD, Bandla M, Currier T, Giroux R, Jenkins GR, Lipp M. Immunoassay as an analytical tool in agricultural biotechnology Journal of AOAC International. 2006;89:913-928.
6. Hunter MD. Effect of atmospheric carbon dioxide on insect-plant interactions Agriculture and forest Entomology. 2001;3:153-159.
7. James MWR, Ben DM, Markus R, Andrrew NG, Scott NJ. Amino acid mediated impacts of elevated carbon dioxide and simulated root herbivory on aphid are neutralized by increased air temperature. Journal of Experimental Botany. 2014;10:1093-1113.
8. Kimball BA. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. Journal of Agronomy. 1983;75:779-788.
9. Kumari M, Verma SC, Bhardwaj SK. Effect of elevated CO_2 and temperature on crop growth and yield attributes of bell pepper (*Capsicum annuum* L.) Journal of Agricultural Meterology. 2019;21(1):1-6.
10. Patil BV, Bheemanna M, Hanchinal SG, Hosamani AC, Bansi AB. Status of pink bollworm, *Pectinophora gossypiella* (Saunders) on cotton at Raichur, Karnataka. Journal of cotton Research and Development. 2007;21:224-226.
11. Patil PAS, Chittapur BM, Udikeri SS. Leaf endotoxin content and performance of *Bt* cotton (*Gossypium hirsutum*) genotypes as influenced by fertility levels for target yields. Indian journal of Agricultural Sciences. 2012;82(7):645-649.
12. Pooja. Impact of climate change variables on the expression of cry toxins in *Bt* cotton and its impact on pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae). Ph.D. Thesis, 2019; Univ. Agric. Sci. Raichur (Karnataka, India).
13. Shoulin J, Yongging L, Yang D, Lei Q, Adnan BM, Teng L, *et al.* Impact of elevated CO_2 on exogenous *Bacillus thuringiensis* toxins and transgene expression in transgenic rice under different level of nitrogen. Scientific reports. 2017;7:147-158.
14. Shreevani GN. Effect of CO_2 and temperature on tri-trophic intraction of *Bt* cotton, aphid (*Aphis gossypii* Glover) and coccinellid (*Cheilomenes sexmaculata* Fab.) Ph.D. Thesis, 2015; Univ. Agric. Sci. Raichur (Karnataka, India).
15. Xue F, Lefu Y, Kang L, Feng G. Elevated CO_2 shift the focus of tobacco plant defences from cucumber mosaic virus to the green peach aphid. Plant. Cell & Environment. 2010;33:2056-2064.