ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 13, Issue, 12, pp.19775-19779, December, 2021

DOI: https://doi.org/10.24941/ijcr.42503.12.2021

RESEARCH ARTICLE

ALLELOPATHIC IMPACT OF BT AND NON-BT COTTON ON GRAM (*CICER ARIETINUM*) SEEDLINGS

Sudhir Kumar*, Neerja Singh and Manjula K Saxena

Department of Botany, University of Rajasthan, Jaipur

ARTICLE INFO

Article History:
Received 15th September, 2021
Received in revised form
29th October, 2021
Accepted 10th November, 2021
Published online 29th December, 2021

Keywords
Allelopathy,
Bt Cotton, Toxic,
Leachate and Germination.

Corresponding author:
Sudhir Kumar

ABSTRACT

An experiment was set up to determine the allelopathic impact of Bt cotton and Non Bt cotton on the growth ofgram seedlings in Petri plates. The aqueous leachate of dry powder of Bt and NBt cotton leaves of different concentrations (w/v) was examined for their allelopathic effect. The experiment was further repeated in plastic disposable pots to confirm the results. The observations revealed that Bt cotton leachate inhibited the germination, and growth of seedlings of gram significantly at P<0.01. This is the first report on toxic effect of Bt and NBt cotton on growth of crop seedlings.

Citation: Sudhir Kumar, Neerja Singh and Manjula K Saxena. *"*Allelopathic impact of bt and non-bt cotton on gram (*Cicer Arietinum*) seedlings*", 2021. International Journal of Current Research, 13, (11), 19775-19779.*

INTRODUCTION

The Cotton crop attracts a number of insects in the field. In India 166 species of insect are reported from the field of cotton (Dhawan, 2019; Dhawan, 2011). The bollworm infection caused heavy loss of yield of Cotton in the past (Ismail, 2019; Vonzun, 2019). Therefore, a man-made insecticide Bt Cotton has been adopted in 2002 in India (Dhillon and Sharma, 2012). The Bt toxin after the death of Bt Cotton plant reaches to the soil via root exudes or deteriorating residue (Motavalli *et al*., 2004). There has not been much study on the effect of Cry endotoxin on ecosystem, soil, nutrient dynamics, crops, and other insects and animals (Bruinsma *et al*., 2003;Yasin, *et al*., 2016).Therefore, the allelopathic impact of Bt and NBt was investigated on gram using seed germination and seedling growth bioassay first and in pot experiment thereafter to confirm the results.

MATERIAL AND METHODS

Preparation of aqueous leachate of NBt and Bt cotton leaves: Leaves of Bt and NBt cotton were used to prepare aqueous (aq.) leachate for experiments. The leachate of three concentrations viz 1%, 3% and 5% (dry weight/volume) were prepared by dissolving 1, 3 and 5g plant material dried leaf powder in 100mL distilled water respectively and kept at room temperature for 24 h. This solution was filtered through muslin cloth and filtrate was used for experiment as leachate.

Seed germination and seedling growth bioassay in Petri Plate-Experiment 1: Growth bioassay was set up on gram (*Cicer arietinum*) seeds in sterilized Petri dishes under laboratory conditions. Petri dishes (20) were lined with two layered dry sterilized filter paper disc. Seed (10) were distributed at equal distance in on filter paper in each Petri dishes and 5 ml of aq. leachate of different concn of Bt and NBt cotton varieties and distilled water in control were poured to each Petri dish initially and 3 ml each after 2,4,6 and 8 days after sowing (DAS). Five replicates of aq. leachate of different concn of Bt and NBt cotton leaves viz 1%, 3% and 5% (dry weight/volume) were set up to conduct gram seed germination and seedling growth bioassay for testing phytotoxicity of Bt and Conventional NBt cotton. The Petri dishes were placed in dark in BOD incubator at 20 to 22°C. The seedlings were harvested 7 DAS. Various parameters viz. % germination, root and shoot length of seedling, dry weights were measured.

Allelopathic impact of soil of Bt and NBt fields on gram in Pots-Experiment II: The Bt and NBt field soil (500g) was placed on sieve separately and washed by pouring distilled

water over it two three times and collected in conical flasks and termed washing 1 (Bt) and washing 2 (NBt) for further experiment. The allelopathic impact of these washing 1 and 2 were investigated using seed germination and seedling bioassay on three crops.

Allelopathic impact of Bt and NBt on gram seedlings in pots-Experiment III: The pot experimentwase also performed to confirm the results obtained with Petri plates. Small disposable pots (8×6) were filled with 60 g dry garden soil.They were moistened with water. Top soil around 5 cm layer of each pot was removed in separate container and dry powder(60 g) of Bt and NBtleaves were mixed to make it 5%w/w and refilled in disposable pots with 5 replicates of each set. Ten seeds of gram were placed at equidistance. Growth parameters viz., germination, shoot length, root length, fresh weight and dry weight were observed after the harvest of experiment 10 DAS.

Antibacterial Bioassay-Experiment IV: The bioactivity of Bt and NBt extracts of cotton leaves was further tested by antimicrobial bioassay. Pure cultures of 2 bacteria, *Escherichia coli* (Gram-ve) and *Staphylococcus aureus* (Gram+ve) were obtained frommicrobiology laboratory, SMS Medical College, Jaipur and grown in Nutrient agar (NA) medium (prepared by autoclaving 8% Nutrient agar of Difeco-Laboratories, Detroit, USA) in distilled water at 15 lbs. psi for 25-30 min and incubating at 37°C for 48 h.The bioassay was performed following wells diffusion method. Petri plates were evaluated for bioactivity test by calculating the diameter of inhibition zone (in mm) using ruler. All sets were replicated three times. Average values were computed. The allelopathic potential were measured quantitatively on the basis of the inhibition zone, and activity index.

RESULTS

Effect of Bt and NBt cotton on gram seedlings in Petri plates-Experiment I

Data Analyses The allelopathic impact of Bt and NBt cotton plants on the growth of gram is shown in Table 1. Germination % was decreased to 60±6of C at 3 and 5 % concn of aqueous leachate of Bt cotton significant at $p<0.01$. However, NBt leaves had no impact on germination of gram seeds, but it greatly retarded at all concn of Bt cotton leaves.The stem and root length also declined significantly ($p<0.01$) in Bt cotton leaves however, little impact was seen in NBt cotton leaves. The maximum inhibiting effect was noted in dry weight.

Allelopathic impact of soil of Bt and NBt fields in Petri plates on gram seedlings Experiment II: The allelopathic potential of soil of Bt and NBt cotton fields on gram seedlings is shown in Table 2. The soil of Bt cotton field exhibited toxicity to the growth of gram seedlings. The germination % was observed 88±4, 70±3 and 84±4 in control, Bt and NBt field soil. **Data Analyses** Bt field soil greatly reduced the length of stem of gram seedlings from 16.28±1cm in control to 8.72±0.80 in Bt field soil significant at $p<0.01$. Similarly, root length and dry weight also declined in Bt field soil (11.83±0.55cm and 0.98±0.04g in Bt soil and 17.01±0.43cm and 1.41±0.08g in control respectively.

Allelopathic impact of Bt and NBt leaves on gram seedlings in pots - Experiment III: The results of allelopathic impact of Bt and NBt cotton on the growth of gram plants in pots is shown in Table 3. Bt leaves reduced the germination % of gram seeds from 86±4 in C to 76±5% in Bt at 5 % concn. However, NBt improved the same to 88±4 % in comparison to C.

Data Analyses Bt leaves lessened the shoot length and root length significantly at $p<0.01$(11.47±0.81 and 9.94±0.27 cm) than 15.59±0.28 and 12.54±0.33cm in control at 5% after 14 days. However, there is little enhancement in the length of gram plants in the presence of NBt leaves. Likewise, there was almost no difference in their dry weight in both sets. Bt leaves had greater inhibitory effect on gram plants than NBt in pot experiment in comparison to control.

Antibacterial Bioassay-Experiment IV: The allelopathic potential of Bt and NBt cotton plants was also investigated using antibacterial bioassay. The results of well diffusion method are summarized in Table 4. The impact was seen on two bacterial strains *Escherichia coli* and *Staphylococcus aureus*. Ciprofloxacin was used as standard for comparison. The aqueous leachate of 3 % of Bt and NBt leaves was used for antibacterial bioassay. Bt was found toxic to the growth of *E. coli.*

Data Analyses: It produced 61.76±3.33 % zone of inhibition (ZOI) significant ($p<0.01$) in comparison to standard whereas Bt made 91.60±4.67% ZOI. Bt and NBt leachate was found highly toxic to the growth of pus forming bacteria *S. aureus* significant at $p<0.01$ and produced 72.12±3.23 and 53.87±3.02 % ZOI in comparison to standard. The activity Index of both bacteria were measured as 0.61 and 0.91 in *E. coli* and 0.53 and 0.72 in *S. aureus*. The higher index in Bt cotton confirms higher allelopathic potential in comparison to NBt.

DISCUSSION

The production of cotton gradually but slowly increased in 1970-71 from 5000-000 Bales, 000ha to 15000/- bales/ in 96-97. After a short fall in 2002-2003, it again enhanced to more than 25000 in 2008-2009 (Suresh *et al*., 2015). Cotton production decreased in 2002 but increased thereafter because Bt cotton, a man-made insecticide has been adopted by the farmers in India. Yield increased from 180kg/ha in 1980 to 200 kg/ha in next 20 years but doubled (424 kg/ha) in 2009-10. Nevertheless, the same tremendously enhanced thereafter and reached 785 kg/ha in 2013-14 (Cotton Advisory Board, 2014).
In 2007-08 Bt cotton yield was 1376 kg/ha in 310 plots where NBt cotton 1112 kg/ha in 80 plots (Lalitha et al., 2009). Approximately 0.25 g ha Cry endotoxin has been documented in the field of Bt Crop (*Blackwood* and *Buyer, 2004*). However, there are reports that Cry 1 Ac did not affect ecological and environmental conditions of the field neither metabolic nor biochemical (Velmurugan and Sahu, 2013;Yasin, *et al*., 2016; Sherene *et al*., 2019; Mandal *et al*., 2019). The Bt cotton has been adopted in India in 2002. Yield increased from 180kg/ha in 1980 to 200 kg/ha in next 20 years but doubled (424 kg/ha) in 2009-10. It has been a dispute from last few years that transgenic plants have potential to alter the plant residue features and rhizosphere because they directly come in contact with soil. Soil chemistry is greatly affected by the presence of Cry gene. It significantly decreased 12-27% bacterial population and enzymes activities present in soil viz., acid phosphatases, phytase, nitrogenase and dehydrogenase in Bt cotton compared with non-Bt cotton fields.

Table 1. Effect of different concn of leachate of NBt and Bt cotton leaves on the growth of gram (*Cicer arietinum*) seedlings

		NBt			Bt		
Parameters	Control	1%	3%	5%	1%	3%	5%
Germination %	90±6	87±3	90±6	87±3	73±9	60±6*	67±9*
Stem length, cm	16.23±1.18	15.53±0.76	15.89±0.68	15.49±0.66	8.83±0.66**	8.76±1.18**	7.68±0.47**
Root length, cm	15.65±0.46	15.47±0.41	17.84±0.61*	16.31±0.77	12.56±0.60*	11.87±0.55**	10±0.25**
Fresh weight, g	7.51±0.46	7.20±0.77	7.87±0.45	8.08±0.30	5.61±0.25*	5.11±0.23**	5.08±0.27*
Dry weight, g	1.86±0.07	1.57±0.14	1.29±0.06	1.31±0.17	0.98±0.06**	0.84±0.03**	0.70±0.12**

Mean±SE. *=p< 0.05, **=p<0.01. All columns of growth parameters are significantly compared Bt with NBt as standard using Tukey test after ANOVA

Table 2. Allelopathic impact of soil of Bt and NBt cotton fields on the growth of gram (*Cicer arietinum*) seedlings in Petri plates

Parameters	Control	NBt	Bt
Germination, %	88±4	84±4	70±3**
Shoot length, cm	16.28±1	15.75±1.11	8.72±0.80**
Root length, cm	17.01±0.43	16.24±0.40	11.83±0.55**
Total fresh weight, g	7.82±0.12	7.63±0.23	5.69±0.49**
Total dry weight, g	1.41±0.08	1.32±0.03	0.98±0.04**

Table 3. Allelopathic impact of Bt and NBt on gram (*Cicer arietinum*) seedlings in pot experiment

Parameters	Control	NBt	Bt
Germination, %	86±4	88±4	76±5
Shoot length, cm	15.59±0.28	16.31±0.58	11.47±0.81**
Root length, cm	12.54±0.33	13.12±0.27	9.94±0.27**
Total fresh weight, g	8.43±0.36	8.24±0.39	5.92±0.52**
Total dry weight, g	1.61±0.09	1.05±0.08	1.65±0.07**

Table 4. Effect of methanolic leaf extract of NBt and Bt cotton on bacteria

Methanolic extract of leaves	Zone of Inhibition (ZOI), mm					
	Bacteria					
	Escherichia coli			*Staphylococcus aureus*		
	Standard	ZOI	AI	Standard	ZOI	AI
NBt cotton	18.23±0.28	11.26±0.08**	0.61	19.73±0.33	10.63±0.08**	0.53
Bt cotton	18.23±0.28	16.70±0.2**	0.91	19.73±0.33	14.23±0.03**	0.72

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard, Mean ± SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software

Figure 1. Allelopathic impact of aqueous leachate of Bt and NBt cotton leaves on gram seedlings

Figure 2. Allelopathic impact of soil of Bt and NBt cotton fields on gram seedlings

Figure 3. Allelopathic impact of aq. leachate of NBt and Bt cotton on gram in pots

Antibacterial bioassay

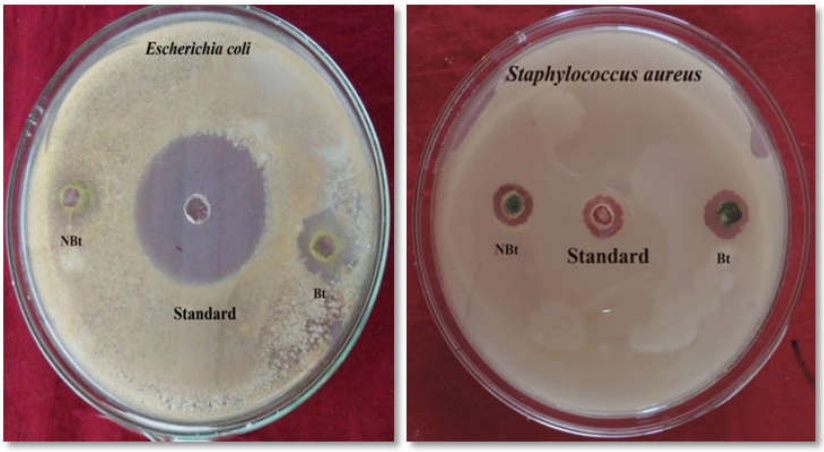

Figure 4. Effect of methanolic leaf extract of NBt and Bt cotton

However, no effect was observed in fungal and nitrifiers counts, esterase and alkaline phosphatase activities (Tarafdar *et al*., 2012). The Cry proteins enter in rhizosphere after death of Bt cotton plant. Its persistence nature has been documented (Muchaonyerwa *et al*., 2004; Karuri *et al*., 2013). It can survive there up to 140 days (Palm *et al*., 1996). Autotoxicity of NBt cotton plant has been reported that inhibited its own seedlings in decomposing residue of Bt cotton (Li and Zhang, 2016). Additionally, decreased soil fertility and nutrients have been described in long term cotton cultivation due to infestation with *Fusarium*sp.and *Verticillium*sp. (Neri *et al.,* 2005).However, exudates of Bt cotton may alter the microorganisms in the field (Singh *et al*., 2013). The present findings indicated the inhibitory effect of two bacteria in antibacterial bioassay. Bt cotton did not alter the soil chemistry (Sarkar *et al*., 2009). Insecticidal proteins have been reported to bound on clays and humic acids (Stotzky, 2004). There has not been any study on allelopathy of cotton. Recently allelopathic potential of Bt and NBt cotton leaves have been reported using duckweed bioassay (Kumar *et al*., 2020). The present findings concluded that both NBt and Bt leaves had negative effect on gram seed germination and seedling growth. Nevertheless, its effect was severe with Bt leaves. The rhizospheric soil of Bt cotton plant also produced toxic effect on growth of gram seedlings. However, no significant effect was seen in case of NBt rhizospheric soil except dry weight. The allelopathic potential of Bt cotton leaves was also confirmed by antibacterial bioassay. The Bt cotton leaves strongly inhibited the growth of bacteria,*E. coli* and *S. aureus*in comparison to NBt. The present findings highlighted that allelopathic potential of Bt leaves was apparent on growth crop seedlings and also confirm that the soil is also influenced.

ACKNOWLEDGEMENT

One of US (Neerja Singh) is gratefully acknowledged UGC, New Delhi for providing JRF NET fellowship. We are also thankful to the Department of Botany, University of Rajasthan, Jaipur for providing facilities in the department for this research.

REFERENCES

Dhawan, A.K.2019, Integrated Pest Management in Cotton. In:*Integrated Pest management in the Tropics*, New India Publishing Agency, New Delhi.pp 499-575.

Dhawan, AK V. Kumar,K.Singh, and S.Saini, 2011. Pest Management Strategies in Cotton Soc., *Sustainable Cotton Production*, Ludhiana: 127.

Blackwood CB and Buyer JS(2004). Soil microbial communities associated with Bt and non-Bt corn in three soils. Journal of Environmental Quality,33: 832-836.

Bruinsma M, Kowalchuk GA and Van Veen JA (2003). Effects of genetically modified plants on microbial

communities and processes in soil. Biology and Fertility of Soils,37: 329-337.

Dhillon, M.K. and Sharma, H.C., 2012. Paradigm shifts in research on host plant resistance to insect pests. *Indian Journal of Plant Protection*, *40*(1), .1-11.

Ismail SM (2009). Influence of some insecticide sequences on the injurious insect- pests of cotton plants. Bull Natl Res Cent, 43: 149.

Karuri H, Amata R, Amugune N and Waturu C, (2013). Effect of Bt cotton expressing Cry1Ac and Cry2Ab2 protein on soil nematode community assemblages in Mwea, Kenya. Journal of Animal &Plant Sciences,19(1): 2864-2879

Khush G,(2001). Green revolution: the way forward. Nature Reviews Genetics,2: 815-822.

Kuruganti K,(2009). Bt Cotton and the Myth of Enhanced Yields. Economic and Political Weekly, 44(22):29-33

Li Y and Zhang Q, (2016). Effects of naturally and microbially decomposed cotton stalks on cotton seedling growth. Archives of Agronomy and Soil Science, 62(9): 1264-1270.

Mandal A, Thakur JK, Sahu A, Manna MC, Rao AS, Sarkar B and Patra A,(2018). Effects of Bt-cotton on biological properties of Vertisols in central India. Archives of Agronomy and Soil Science, 65 (5):670-685.

Motavalli PP, Kremer RJ, Fang M and Means NE, (2004). Impact of genetically modified crops and their management on soil microbially-mediated plant nutrient transformations. Journal of Environmental Quality,33: 816-824.

Muchaonyerwa P, Waladde S, Nyamugafata P, Mpepereki S and Ristori Go,(2004). Persistence and impact, on microorganisms, of *Bacillus thuringiensis* proteins in some Zimbabwean soils. Plant Soil,266: 41-46.

N. Lalitha, B. Ramaswami and PK. Viswanathan.2009, India's experience with Bt cotton: case studies from Gujarat and Maharashtra. In: R. Tripp (ed.) *Biotechnology and Agricultural Development: Transgenic Cotton, Rural Institutions and Resource-Poor Farmers*, Routledge, New York, 135–167.

Neri D, Sugiyama N, Inujima A, (2005). Effects of organic residues on strawberry root growth. International Journal of Fruit Science,5:127–137.

Palm, C.J., Seidler, R.J., Schaller, D.L. and Donegan, K.K., 1996. Persistence in soil of transgenic plant produced Bacillus thuringlensis var. kurstaki δ-endotoxin. *Canadian journal of Microbiology*, *42*(12), 1258-1262.

Sarkar B, Patra AK, Purakayastha TJ and Megharaj M, (2009). Assessment of biological and biochemical indicators in soil under transgenic *Bt* and non-*Bt* cotton crop in a sub-tropical environment. Environmental Monitoring and Assessment 156: 595–604.

Sherene T, Kavimani R and Kumar B,(2019). Studies on the impact of growing Transgenic cotton on Soil Health in major Bt cotton growing areas of Tamil Nadu, India. Biological Forum – An International Journal,11(1): 18-23.

Shewry PR and Hey SJ,(2016). Do we need to worry about eating wheat? Nutrition bulletin, 41(1):6–13.

Shewry PR and Hey SJ,(2015). The contribution of wheat to human diet and health. Food and energy security,4(3): 178–202.

Singh RJ, Ahlawat IPS and Kumar K,(2013). Productivity and profitability of the transgenic cotton-wheat production system through peanut intercropping and FYM addition. Experimental Agriculture, 49: 321–335.

Stotzky G,(2004). Persistence and biological activity in the soil of the insecticidal proteins from Bacillus thuringiensis, especially from transgenic plants. Plant and Soil,266: 77-89.

Suresh A, Ramasundaram P, Samuel J and Wankhade S,(2015), Cotton Cultivation in India Since the Green Revolution: Technology, Policy, and Performance. Review of Agrarian Studies,4(2), http://ras.org.in/cotton cultivation in India since the green revolution.

Tarafdar JC, Rathore I and Shiva V,(2012). Effect of Bt-Transgenic Cotton on Soil Biological Health. Applied Biological Research,14(1): 1-9.

Velmourougane Kand Sahu A,(2013). Impact of transgenic cottons expressing Cry*1Ac* on soil biological attributes. Plant Soil Environment,59:108–114.

Vonzun S, Messmer MM, Boller T, Shrivas Y, Patil SS and Riar A,(2019). Extent of Bollworm and Sucking Pest Damage on Modern and Traditional Cotton Species and Potential for Breeding in Organic Cotton. Sustainability,11: 6353.

Wright AL, Hons FM, Lemon RG, McFarland ML, Nichols RL, (2007). Stratification of nutrients in soil for different tillage regimes and cotton rotations. Soil and Tillage Research,96:19–27.

Yasin S, Asghar HN, Ahmad F, Zahir ZA, Waraich EA, (2016). Impact of *Bt*-cotton on soil microbiological and biochemical attributes, Plant Production Science, 19(4): 458-467.

****