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RESEARCH ARTICLE

ASSESSMENT OF ALLELOPATHY IN BT AND NON-BT COTTON USING DUCKWEED BIOASSAY

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ABSTRACT

The duckweed bioassay was performed in small disposable pots to examine the allelopathic potential of aqueous extracts of Bt cotton and Non-Bt cotton in laboratory. Fifteen pots were set up with five replicates, each filled with different concn of leachate i.e. 1, 3 and 5% w/v. The experiment was run for 21 days. Both Bt and Non-Bt extracts were highly toxic to the growth of duckweed. Aqueous leachates of Bt were proved more toxic than non-Bt and reduced the growth of *Spirodela polyrrhiza* (L.) Schleid. The number of fronds of duckweed declined more than 95% in Bt and 86% in non Bt whereas dry weight up to 75% and 62% in Bt and Non-Bt cotton respectively after 21 days. This is the pioneer study on allelopathic potential of cotton.

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INTRODUCTION

Allelopathy, coined by Molish (1935) is any process that involves secondary metabolites produced by plants and influences the growth of other organisms (Rice, 1992). The bioassays are the best technique to assess allelopathic potential of any leachate, extract orallelochemicals used in pharmacognosy, pharmaceuticals and toxicologic investigations(Leather and Einhellig, 1988).Bioassay can be performed using biological agent either plant or animals (Sutton and Portier, 1989; BioassaySotero, 2005). Seed germination and seedling growth bioassay is very common to examine toxicity of any extract. Wheat is commonly used in Petri plate methods however; lettuce growth bioassay has also been reported. Duckweed bioassay was first used to check the toxicity of terrestrial plant *Lantana camara* L. in aqueous medium and its allelochemicals (Jain *et al*, 1989). Further, it was studied thoroughly for its use in testing the toxicity of water quality thereafter successfully (Wang, 1990; Al-balawna *et al*, 2020).Furthermore, duckweed bioassay has been recommended especially when chemicals are in small quantity (Jain *et al*, 1989; Gopal and Goel, 1993). This is advantageous because it can be easily performed in a very small space. Besides, it is cheapest, easy to handle and count the number of fronds growing in small plastic disposable cups.

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As the growth of duckweed (*Lemna* sp. and *Spirodela polyrrhiza* (L.) Schleid) is very fast, bioassay completes within 15 or 21 days. *Gossypiumhirsutum* of family Malvaceae is native to warmer parts of tropical and subtropical areas of the old and new worlds. It is cultivated in Asia, Africa and South America for fibre, oilseed crop and animal protein (Choudhary, 2010). In many parts of India like Rajasthan, cotton is normally grown as dual crop system per year withwheat in winter. Allelopathy has not been reported in cotton so far. The present study was commenced to determine allelopathic potential of commercial cotton (Non-Bt) and Bt cotton using duckweed bioassay.

MATERIALS AND METHODS

In duckweed bioassay, *Spirodela polyrhiza* of family Lemnaceae was used as biological test material to check the allelopathic potential of aqueous leachate of Bt and NBt cotton. Numbers of fronds were counted and dry weight was measured at each harvest.

Preparation of aqueous leachate of Bt and NBt cotton: Leaves of Bt and conventional NBt cotton (Figure 1) were used to formulate their aqueous (aq.) leachate for experiment. 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 in wide mouth glass bottles covered with a lid respectively and kept at room temperature for 24 h.

This solution was filtered through double muslin cloth and filtrate was used for experiment as leachate.

Procedure of Duckweed bioassay: The leachates of different concentrations were used to check the phytotoxicity on the growth of duckweed. The experiments were setup in plastic disposable pots filled with leachates of different concentrations of 1,3 and 5% w/v of Bt and NBt leaves and tap water for control. Ten fresh plants of duckweed, each having two fronds with bud were placed in each pot. A total 20 with five replicates of each treatment were taken to set the experiment. The numbers of fronds in each plant were counted and dry weights of duckweeds were measured harvesting after 7, 14 and 21 days after being placed in pots. The data was tabulated and analysed statistically using Tukey test after ANOVA to compare treatments with controls.

RESULTS

Allelopathic potential of NBt and Bt cotton leaves determined for its toxicity using duckweed (*Spirodela polyrrhiza*) is shown in Table 1.

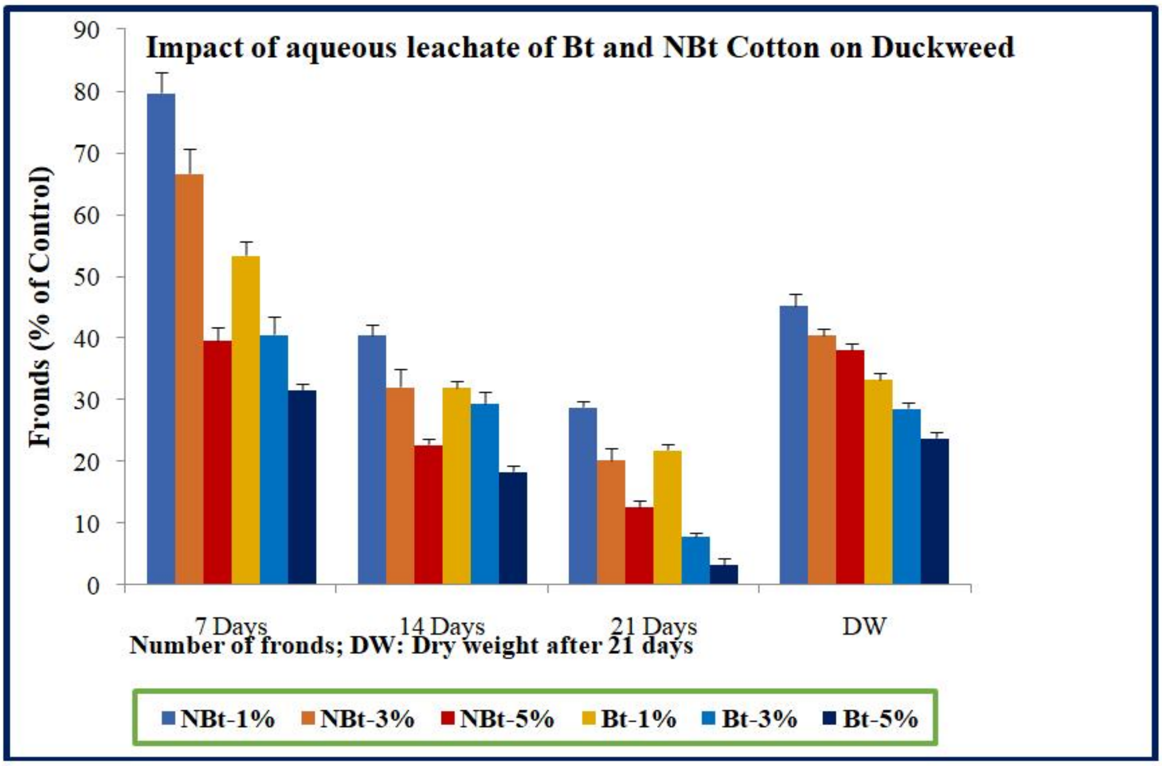
***Data Analyses*:** The number of fronds decreased from 34.50±0.65 (control) to 27.50±0.85(1%), 23.01±0.58 (3%) and 13.67±0.48 (5%) in NBt cotton leaves after 7 days. The leachate of Bt was more toxic to the growth of duckweed and decreased the number of fronds to 18.45±0.48, 13.98±0.48 and 10.89±0.82 at 1,3 and 5 % concentration of the leachate of Bt leaves after 7 days of experiment. The same pattern followed after 14 days. In third and final harvest after 21 days, the number of fronds decreased sharply significantly at p<0.01

Table 1 Allelopathic potential of NBt and Bt cotton using Duckweed Bioassay

Para-meters	Time interval	Control	Concentrations of aqueous leachate (w/v) of NBt and Bt Cotton			
			Sample	1%	3%	5%
Number of fronds	7 days	34.50±0.65	NBt	27.50±0.85**	23.01±0.58**	13.67±0.48*
		34.50±0.65	Bt	18.45±0.48**	13.98±0.48**	10.89±0.82**
	14 days	36.75±0.48	NBt	14.87±1.71**	11.78±0.87*	8.34±0.58**
		36.75±0.48	Bt	11.76±0.48**	10.78±0.71**	6.76±0.41**
	21 days	37.75±0.85	NBt	10.87±0.96**	7.56±0.65**	4.76±0.85*
		37.75±0.85	Bt	8.25±0.85**	2.97±0.63**	1.25±0.08**
Total dry weight (g)	Dry weight	0.42±0.02	NBt	0.19±0.01**	0.17±0.01*	0.16±0.01**
		0.42±0.02	NBt	0.19±0.01**	0.17±0.01*	0.16±0.01**
		0.42±0.02	Bt	0.14±0.01**	0.12±0.01*	0.10±0.00*

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

Figure 1. NBt (Left) and Bt (Right)seeds, cotton plants, Cotton bolls



Graph 1. Impact of aqueous leachate of Bt and NBt Cotton on *Spirodela polyrhhiza*

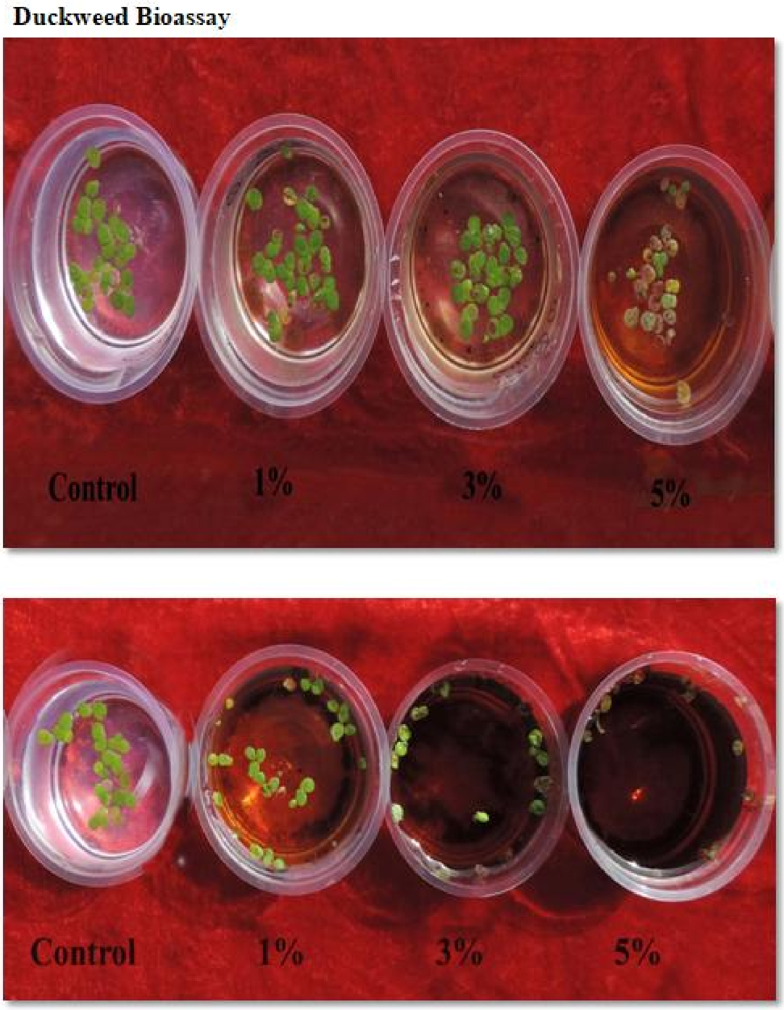


Figure 2. Growth of *Spirodela polyrhiza* (duckweed) in different concn of NBt (Top) & Bt cotton (Bottom).

level from 37.75±0.85 (control) to 7.56±0.65 (3%) and 4.76±0.85 (5%) in NBt leaves whereas 2.97±0.63 (3%) and 1.25±0.08 (5 %). The leaves of Bt cotton was more toxic than NBt leaves for the growth of duckweed *Spirodela polyrhiza* (Figure 2). The dry weight followed the same pattern. It was 0.42±0.02 g after 21 days in control and dropped tremendously to 0.19±0.01 at 1%, 0.17±0.01 at 3% and 0.16±0.01 at 5% (significant at $p<0.05$ level). The toxicity was more pronounced in Bt leaves and the dry weight decreased significantly at $p<0.01$ level to 0.14±0.01, 0.12±0.01 and 0.10±0.00 at 1,3 and 5 % respectively. Both Bt and NBt cotton exhibited toxicity and reduced the number of fronds and dry weight after 21 days of the experiment in comparison to control (Graph 1). The number of fronds decreased from 100 % in control to 21.85±1 % (at 1 % concn), 7.86±0.5% (at 3 %) and 3.31±0.3 % of C (at 5 % concn of aqueous leachates). The NBt values were 28.79±1, 20.02±2 and 12.60±1 respectively. Similarly, the dry weight tremendously reduced to 23.80±1 and 38.09±1 % of Control in Bt and NBt cotton respectively at 5 % concn after 21 days.

DISCUSSION

The allelochemicals are secondary metabolites that are produced as a result of defense mechanism in plants (Willis, 2007). The process of production of allelochemicals and their release outside the plant and in turn affecting other plant ‘s growth in positive or negative way is called allelopathy. This phenomenon strengthens the plant producing allelochemicals by providing defense mechanism. Auto-allelopathy has been reported in cotton.It has been shown that cotton inhibited the growth of its own seedlings. The extract of cotton (*Gossypium hirsutum*) at 150 ppm concn gradually reduced the number of leaves 93.91 %, dry weight 84% and accessary branches 75.7 % of control (Rezaee *et al*, 2014). Although, allelopathic effect was little in comparison to control. The cotton crop attracts maximum number of insects in the field (Dhawan, 2016). They were *Cotton bollworm (Helicoverpa armigera), Tobacco bollworm (Heliothisvirescens),* Pink Bollworm (*Pectinophora gossypiella*), Tobacco Thrip (*Frankliniella fusca*), *Cotton strainer bug (Dysdercus sidae), Leaf hopper (Amrasca biguttula)* and Cotton Aphid (*Aphis gossypii*). The cotton plants are known to synthesize phenolic compounds and flavonoids which help them to fight and keep insects away from cotton plants (Tan *et al*, 2013; Feng *et al*, 2013; Brunetti *et al*, 2013; Kranthi and Stone, 2020).

It is documented that allelochemicals are mostly phenolic compounds and flavonoids which are signaling molecules (Saxena *et al*, 2007; Saxena *et al*, 2012; Saxena, 2017).This study indicated strong allelopathic potential of Bt and Non-Bt cotton on *Spirodela polyrhiza.* Nevertheless, Bt cotton exhibited greater allelopathic potential than Non-Bt. In some area of India dual crop system predominates as cotton in summer and wheat in winter. The NBt as well as Bt cotton might cause toxic effect on the growth of its alternate crop which should be investigated.

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