

## Research Article

# Phytotoxic effect of plant extracts on physiology of cotton (*Gossypium hirsutum* L.) plants

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### Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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## HIGHLIGHTS

- Plant-derived compounds are alternatives of synthetic insecticides in sustainable agriculture.
- Different botanicals effects on physiology of cotton plants.
- Different botanicals influenced the photosynthesis of cotton crop.

## ABSTRACT

**Background:** Plant-derived compounds are alternatives of synthetic insecticides in sustainable agriculture.

**Objective:** This study investigated the phytotoxic effect of higher concentrations (2, 4, 8 and 16%) of four plants extracts (*Azadirachta indica*, *Mentha arvensis*, *D. stramonium* and *Citrus limonium*) on cotton plants. **Methods:** Each concentration was replicated four times to check the phytotoxic effect (CO<sub>2</sub>-in, CO<sub>2</sub>-out, H<sub>2</sub>O-in, H<sub>2</sub>O-out and photosynthesis absorption rate (PAR) in randomized complete block design. Data was recorded after 12, 24, 48 and 72 hours of spray with the help of Photosynthetic CL 340 meter.

**Results:** The results showed that CO<sub>2</sub>-in was more affected by the *D. stramonium* (131.65±0.38) at 8% concentration. The overall progress showed that *C. limonium* was more affected the CO<sub>2</sub>-in of cotton crop. CO<sub>2</sub>-out was less affected by the *C. limonium* (117.83±1.46) at 4% concentration than *M. arvensis* (116.99±1.25) at 8% concentration and *D. stramonium* (115.77±0.74) at 16% concentration, but was more affected by the *A. indica* (118.15±0.71) at 4%. H<sub>2</sub>O-in was more affected by the *C. limonium* (0.39±0.05) than *D. stramonium*, *A. indica* and *M. arvensis* at 16% concentration. H<sub>2</sub>O-out of cotton was least affected by the *D. stramonium* (7.63±0.01) at 2% and more affected by the *C. limonium* (1.56±0.15) at 16% concentration. PAR was more affected by the *A. indica* (931.47±8.39) at 4% concentration and least affected by the *M. arvensis* (1499.7±9.94) at 8% concentration.

**Conclusions:** Different dosages of various botanicals influenced the opening and closing of stomata and photosynthesis of cotton plants.

## 1 INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is known as world most important cash crop. It is utilized as a part of

various items like lint in textile; cottonseed is utilized as vegetable oil and feeds of animal. Cottonseed cake is a rich source of value protein (Sarwar et al., 2013). Cotton assumes a key part in monetary

improvement of both developed and developing nations. Cotton has been known as crude material for industrialization, riches and advancements of a nation, which give wage to various segments like well being, education and transportation.

Cotton is attacked by different insect pests in various stages, which cause lessening in the yield specifically or by implication. Roughly 160 species attack on the cotton at various stages like borers, sap-suckers and defoliators and cause around 60% yield misfortunes annually (Halbert and Manjunath, 2004). By feeding different insect pests reduce the quantity and decrease the quality by transmitting distinctive diseases (Manjunath, 2004). Numerous manufactured sprays are utilized to control insect pest and increase the fruit setting in the cotton crop (Dayan et al., 2009). Synthetic insecticides have undesirable impact on other non- target species, their residues remain in the food which is a reason of environmental pollution and ecosystem.

Synthetic insecticides must be replaced with those items which are friendly to environment. For a long time botanicals are being utilized option of manufactured insecticides for pests administration in light of the fact that these are more secure for condition and human wellbeing. Around 46 families of plants are utilized as botanicals which have insecticidal value (Isman and Machial, 2006)

Some farmers utilized high concentrations of insecticides to control the insect pests, yet there was no change in yield even diminishment. Development and yield of products lessened when OPI were utilized as a part of higher than suggested concentrations (Shehata and El-Khawas, 2003). It is observed that the broadly higher concentration of pesticide application caused negative effect on the physiology of the crop, generally on the photosynthesis of the crop. The development and yield of the crop additionally exasperates when the photosynthesis rate of product aggravate. The utilization of contact fungicide copper impact on the chloroplasts, photosynthesis and chlorophyll biosynthes (Petit et al., 2012).

Normally happening monoterpenes demonstrated phytotoxicity against maize plants, they impact on roots and leaves of maize crop. Carvone was most phytotoxic against maize than monoterpenes (Shehata and El-Khawas, 2003). Strawberry seedling is very influenced when treated with over 3% concentration of limonene (Ibrahim et al., 2001). The seedling of carrot and cabbage were exceptionally influenced when treated with the 9% concentration of

limonene (Ibrahim et al., 2004). Citrus photosynthetic was changed by the ramifications of pesticides (Jones et al., 1983). The present research was conducted to evaluate phytotoxic effects (CO<sub>2</sub>-in, CO<sub>2</sub>-out, H<sub>2</sub>O-in, H<sub>2</sub>O-out and photosynthesis absorption rate PAR) in cotton due to higher concentrations of different botanicals. In present research harmful effects of higher doses of botanicals were studied. The main objective was the evaluation of phytotoxic effects (CO<sub>2</sub>-in, CO<sub>2</sub>-out, H<sub>2</sub>O-in, H<sub>2</sub>O-out and photosynthesis absorption rate PAR) in cotton due to higher doses of different botanicals.

## 2 MATERIALS AND METHODS

The present study was carried out at College of Agriculture, University of Sargodha to check phytotoxic effect of high concentrations of four botanicals, Neem (*Azadirachta indica*), (*Mentha arvensis*), Datura (*Datura stramonium*), Lemonene (*Citrus limonium*), on cotton. The cotton variety MNH-886 was sowed with Chopa method on 30-May-2014. The row to row distance was 30 inches and plant to plant was 12 inches.

From the field of College of Agriculture, University of Sargodha fresh leaves of *D. stramonium* were collected. *A. indica*, *M. arvensis* and *C. limonium* leaves were collected from Bhakkar. The plant materials were washed with distilled water separately. For drying leaves were put at room temperature ( $\pm 25$  °C) for two weeks. It ensured that adequate air was streaming to evade damping. After shade drying leaves were granulated with electric processor for 45 seconds for making concentrated powder. At that point took leaves powder 40 grams put in conical flask and included ethyl liquor 320 mL in it. The mixture of powder and ethyl liquor was put on mechanical shaker for legitimate mixing for 72 hours. After blending the stock arrangement was put for 48 hours. The blend of stock solution was sifted with channel paper (What man channel paper No.1). Now the plant extract was prepared. A similar methodology was drilled for all plant leaves, respectively (Fiaz et al., 2012).

Plant Extract's Applications: Plant extracts were applied with hand worked sprayer. 80 plants were chosen and labeled. Each botanical concentration was replicated five times. Botanicals were utilized as foliar application with 2, 4, 8 and 16% concentrations on 80 chose plants. Three times splash was applied with interim of 20 days. Data was recorded 12, 24, 48 and 72 hours after the utilization of botanicals on

cotton. Data of CO<sub>2</sub>-in, CO<sub>2</sub>-out, H<sub>2</sub>O-in, H<sub>2</sub>O-out and Photosynthetic absorption rate (PAR) of cotton crop was recorded by Photosynthesis meter CI 340. M. Stat C 8.1 version was used for analysis of variance (ANOVA).

### 3 RESULTS AND DISCUSSION

#### 3.1 Phytotoxic impact of various concentrations of various botanicals against CO<sub>2</sub>-in on cotton crop

The results (Table 1) showed mean comparison of data regarding phytotoxic impact of various plant extracts against CO<sub>2</sub>-in of cotton crop. The results showed that 12 hours after spray CO<sub>2</sub>-in was more affected by *A. indica* (144.92±1.59) at 2% concentration with significant difference from *D. stramonium* (152.81±0.54), *M. arvensis* (159.84±0.69) and limonene (161.15±0.35). The 4% concentration of *D. stramonium* following 12 hours of spray was more viable when contrasted with different concentrates of botanicals. The result also showed that CO<sub>2</sub>-in of cotton crop was more influenced by *D. stramonium* (131.65±0.38) having significant difference and followed by *C. limonium* (134.58±0.47), *M. arvensis* (145.83±0.46) and *A. indica* (152.98±1.53) at 8% concentration. CO<sub>2</sub>-in

of cotton crop at the 16% concentration was less influenced of *A. indica* (158.53±0.30).

The results (Table 1) showed that 24 hours after spray the 2% concentration of *D. stramonium* (161.79±1.31) differ significantly and was less influenced the CO<sub>2</sub>-in of cotton crop when contrasted with all others treatments. The results indicated that 4% concentration of *C. limonium* (137.15±0.89) showed significant difference and highly affected the CO<sub>2</sub>-in of cotton. After 24 hours spray of 8% concentration of *M. arvensis* (145.70±0.46) minimum affected the CO<sub>2</sub>-in of cotton crop which was statistically at PAR with *A. indica* (143.85±0.39). The 16% concentrations of *A. indica* showed more toxicity (139.78±0.50) against CO<sub>2</sub>-in with non-significant difference from *M. arvensis* (142.81±0.57) while the remaining *D. stramonium* (150.15±2.00) and *C. limonium* (152.28±0.66) were less toxic.

The results (Table 1) also revealed that phytotoxic effect of *M. arvensis* and *D. stramonium* against CO<sub>2</sub>-in at 2% concentration after 48 hours did not differ significantly and followed by *A. indica* and limonene. The results also indicate that at 4% concentration *M. arvensis* (151.16±0.72) was least phytotoxic against CO<sub>2</sub>-in. Limonene 8% concentration following

**Table 1** - Phytotoxic effect of different plant extracts against CO<sub>2</sub>-in of cotton crop

Plant extracts	2% concentrations	4% concentrations	8% concentrations	16% concentrations
12 hours of spray				
<i>Azadirachta indica</i>	144.92±1.59 hi	150.61±0.89 ef	152.98±1.53 e	158.53±0.30 d
<i>Mentha arvensis</i>	159.84±0.69 cd	148.51±0.60 f	145.83±0.4 gh	135.62±0.42 j
<i>Datura stramonium</i>	152.81±0.54 e	144.14±0.1 hi	131.65±0.38 k	149.34±0.60 f
<i>Citrus limonium</i>	161.15±0.35 c	148.18±0.50 fg	134.58±0.47 j	142.96±0.3 i
Control	160.46±0.7282	168.71±0.4629	1771.66±0.50	173.76±0.22
24 hours of spray				
<i>Azadirachta indica</i>	154.12±0.24 de	47.84±0.41 hi	143.85±0.39 jk	139.78±0.5 lm
<i>Mentha arvensis</i>	156.61±0.34 cd	153.30±0.25 def	145.70±0.46 ij	142.81±0.5 jkl
<i>Datura stramonium</i>	161.79±1.31 b	140.49±0.80 ij	140.49±0.80 klm	150.15±2 fgh
<i>Citrus limonium</i>	148.89±2.10 ghi	141.7±1.50 m	141.7±1.50 kl	152.28±0.6 efg
Control	169.17±0.52	159.37±0.42	154.699±0.68	158.74±0.32
48 hours of spray				
<i>Azadirachta indica</i>	151.52±0.5 defh	147.58±1.79 ghi	152.18±1.4 cdefg	137.19±0.4 k
<i>Mentha arvensis</i>	147.3±0.43 ghi	151.16±0.7efgh	138.06±0.7 k	139.29±1.4 jk
<i>Datura stramonium</i>	147.26±0.80 hi	143.82±2.8 ij	148.64±1.7 fghi	150.8±2.1 efg
<i>Citrus limonium</i>	153.39±0.62 cdef	135.94±1.7 k	154.02±1.8 bcde	152.7±1.7 cdef
Control	158.68±1.08	158.61±1.08	156.10±1.15	152.70±0.9
72 hours of spray				
<i>Azadirachta indica</i>	152.5±0.53 cd	140.27±0.4 ij	139.89±0.3 ij	150.47±0.6 de
<i>Mentha arvensis</i>	148.30±0.17 ef	143.07±1.3 hi	139.04±0.8 j	146.58±1.9 fg
<i>Datura stramonium</i>	152.72±0.9 cd	145.37±1.9 fgh	140.12±0.8 ij	140.04±0.2 ij
<i>Citrus limonium</i>	137.32±1.3 j	143.27±1.5 ghi	157.74±0.5 b	142.66±0.9 hi
Control	155.97±0.6	156.61±0.4	161.28±0.52	155.06±0.6

Means ±SD were separated by LSD test.

48 hours was at fourth number with a specific end goal to influencing the CO<sub>2</sub>-in of cotton crop while and *M. arvensis* (138.06±0.72) was at 1<sup>st</sup>. The 16% concentration of *A. indica* and *M. arvensis* did not show significant difference from each other on the CO<sub>2</sub>-in of cotton crop.

The results (Table 1) also indicated that 2% concentration of *A. indica* (152.55±0.5) and *D. stramonium* (152.72±0.99) was statistically at PAR following 72 hours of spray and less phytotoxic against CO<sub>2</sub>-in of cotton crop. CO<sub>2</sub>-in of cotton crop was correspondingly influenced by the 4% concentration of *A. indica* (140.27±0.48), *M. arvensis* (143.07±1.31) and *C. limonium* (143.27±1.59) after 72 hours sprays while *D. stramonium* (145.37±1.98) was less toxic. The phytotoxicity of *M. arvensis*, *A. indica* and *D. stramonium* at 8% concentration after 72 hours was statistically at PAR with each other while limonene (157.74±0.5) was minimum lethal to CO<sub>2</sub>-in of cotton crop. The outcomes additionally delineate that CO<sub>2</sub>-in of cotton crop was more influenced by the 16% centralization of *D. stramonium* (140.04±0.24) following 72 hours of sprays.

### 3.2 Various botanicals phytotoxic impact at various concentrations against CO<sub>2</sub>-out of cotton crop

The results (Table 2) showed mean comparison of data regarding phytotoxic effect of different plant extracts against CO<sub>2</sub>-out of cotton crop. The results indicated that the 2% concentration of *A. indica* (136±0.75) after 12 hours differ significantly and less influenced the CO<sub>2</sub>-out of cotton crop as compared to *D. stramonium* (127.57±0.95), *C. limonium* (123.83±0.83) and *M. arvensis* (122.79±1.09) which were more phytotoxic. The finding at 4% concentration showed that *C. limonium* (122.42±1.30) was more phytotoxic to CO<sub>2</sub>-out of cotton crop. The results also indicated that the 8% concentration of *A. indica* (135.84±0.81) and *M. arvensis* (135±1.20) were less affected the CO<sub>2</sub>-out of cotton crop. The phytotoxic effect of 16% concentration of *M. arvensis* (123.14±1.54) and *C. limonium* (122.79±1.43) was statistically at PAR and more than the *A. indica* (130.17±1.17), but less than the *D. stramonium* (115.77±0.74).

The results (Table 2) also showed that the 2% concentration of *A. indica* (129.53±0.71) after 24 hours was more phytotoxic but statistically at PAR with *C. limonium* (132.35±0.99). All the botanicals did not differ significantly from each other at 4% concentration. The results demonstrated that the 8% concentrations of *A. indica* (133.3±0.77) and *M. arvensis* (133.52±

0.94) had correspondingly influenced the CO<sub>2</sub>-out of cotton crop. The results also revealed that the 16% concentration of *D. stramonium* (139.17±0.51) was more affected the CO<sub>2</sub>-out and was statistically at PAR with *M. arvensis* (141.88±0.82) 37 and *A. indica* (140.65±0.58).

The results (Table 2) also revealed that the 2% concentration of *C. limonium* (131.91±1.34) having significant difference from all the treatments and was less phytotoxic against CO<sub>2</sub>-out of cotton crop following 48 hours. The 4% concentration of *M. arvensis* (118.16±1.02) also differ significantly from all other treatments was more toxic against CO<sub>2</sub>-out. The results indicated that the 8% concentration of *M. arvensis* (126.15±0.7) and *D. stramonium* (127.09±0.44) did not differ significantly and were more phytotoxic against CO<sub>2</sub>-out of cotton crop. The 16% concentration of *C. limonium* (128.73±0.90) was more phytotoxic against CO<sub>2</sub>-out of cotton crop but statistically at PAR with *D. stramonium* (130.21±1.02) and *M. arvensis* (130.39±0.88).

The results (Table 2) also represented that after 72 hours at 2% concentration of *C. Limonium* (145.64±1.17), CO<sub>2</sub>-out word movement of cotton crop was less influenced which differ significantly from all other treatments. The 4% concentration of *D. stramonium* (122.95±1.14) was more phytotoxic against CO<sub>2</sub>-out of cotton crop having significant difference from all other treatments. The results revealed that 8% concentrations of *A. indica*, *M. arvensis*, *D. stramonium* and *C. limonium* have almost similar phytoxicity against CO<sub>2</sub>-out of cotton crop. These finding indicated that the 16% concentration of *D. stramonium* (129.64±2) following 72 hours of spray was more phytotoxic against CO<sub>2</sub>-out of cotton crop.

### 3.3 Phytotoxic impact of various concentrations of various botanicals against H<sub>2</sub>O-in of cotton crop

The results (Table 3) demonstrated that the concentration of 2% all treatments, 12 hours after spray, did not differ significantly from each other. The results revealed that cotton crop at 4% concentration of *A. indica* (4.63±0.11) and *D. stramonium* (4.73±0.10) were less phytotoxic and are statistically at par. The 8% concentration of *D. stramonium* (2.61±0.05) and *M. arvenis* (3.64±0.18) did not differ significantly from each other and were more phytotoxic than *A. indica* (3.74±0.16). The results also revealed that the 16% concentration of *A. indica* (2.42±0.03) was less phytotoxic and showed significant difference from all other treatments.

**Table 2** - Phytotoxic effect of different plant extracts against CO<sub>2</sub>-out of cotton crop

Plant extracts	2% concentrations	4% concentrations	8% concentrations	16% concentrations
12 hours of spray				
<i>Azadirachta indica</i>	136±0.7 f	143.83±0.8 e	135.84±0.8 f	130.175±1.1 g
<i>Mentha arvensis</i>	122.792±1 hi	137.45±0.7 f	135±1.2 f	123.14±1.5 hi
<i>Datura stramonium</i>	127.57±0.9 g	135.57±1.5 f	134.44±0.9 f	115.77±0.7 j
<i>Citrus limonium</i>	123.83±0.8 h	122.42±1.3 hi	119.90±1.7 i	122.79±1.4 hi
Control	157.32±0.8	150.06±0.7	167.47±0.8	173.06±0.9
24 hours of spray				
<i>Azadirachta indica</i>	129.53±0.7 hi	118.15±0.7 k	133.31±0.7 fg	140.65±0.5 d
<i>Mentha arvensis</i>	124.20±0.4 j	116.77±1.2 k	133.52±0.9 fg	141.88±0.8 de
<i>Datura stramonium</i>	127.81±0.9 i	119.95±1.0 k	126.74±1.2 ij	139.17±0.5 d
<i>Citrus limonium</i>	132.35±0.9 gh	117.83±1.4 k	135.92±1.0 ef	135.94±0.8 ef
Control	158.28±1.3	165.27±0.5	172.18±0.8	166.95±0.6
48 hours of spray				
<i>Azadirachta indica</i>	152.87±0.3 c	133.22±1.1 f	141.28±0.9 e	123.17±0.7 k
<i>Mentha arvensis</i>	123.08±0.1 k	118.16±1.0 i	126.15±0.7 ij	130.39±0.8 fgh
<i>Datura stramonium</i>	118.12±0.5 i	118.87±0.7 c	127.09±0.4 ij	130.2±1.02 gh
<i>Citrus limonium</i>	131.91±1.3 fg	125.01±0.2 jk	130.93±1.0 fgh	128.73±0.9 hi
Control	179.95±0.7	146.63±0.7	151.48±0.9	168.93±0.6
72 hours of spray				
<i>Azadirachta indica</i>	138.32±0.8 ef	127.81±0.9 j	133.72±1.2 gh	120.40±1.07 lm
<i>Mentha arvensis</i>	117.98±0.9 m	116.99±0.8 m	130.71±1.7 hij	131.99±1.1 ghi
<i>Datura stramonium</i>	135.12±1.3 fg	122.95±1.1 kl	128.22±1.2 ij	129.64± 2.0 hij
<i>Citrus limonium</i>	145.64±1.1 d	139.25±1.1 e	126.62±0.8 jk	132.53±1.5 gh
Control	171.94±0.7	165.64±0.5	161.40±0.9	172.99±0.7

Means ±SD were separated by LSD test.

**Table 3** - Phytotoxic effect of different plant extracts against H<sub>2</sub>O-in of cotton crop

Plant extracts	2% concentrations	4% concentrations	8% concentrations	16% concentrations
12 hours of spray				
<i>Azadirachta indica</i>	4.54±0.12 d	4.63±0.1 d	3.74±0.16 e	2.42± 0.03 f
<i>Mentha arvensis</i>	4.56±0.12 d	3.64±0.09 e	4.73±0.18 f	3.63±0.10 g
<i>Datura stramonium</i>	5.50±0.10 c	4.73±0.10 d	2.61±0.08 f	1.74±0.11 g
<i>Citrus limonium</i>	4.84±0.10 d	3.63±0.12 e	1.59±0.15 g	0.39±0.05 h
Control	7.29±0.02	8.49±0.07	7.39±0.01	8.59±0.11
24 hours of spray				
<i>Azadirachta indica</i>	5.37±0.09 d	4.63±0.14 e	3.43±0.13 f	2.77±0.09 g
<i>Mentha arvensis</i>	4.3± 0.05 e	3.45±0.13 f	3.74±0.04 f	1.56±0.17 h
<i>Datura stramonium</i>	4.50±0.03 e	2.45±0.16 g	1.39±0.12 h	1.53±0.12 h
<i>Citrus limonium</i>	4.35±0.09 e	2.50±0.07 g	3.46±0.09 f	2.48±0.14 g
Control	7.59±0.02	8.43±0.09	6.33±0.10	7.29±0.14
48 hours of spray				
<i>Azadirachta indica</i>	6.34±0.14 c	5.61±0.01 d	3.63±0.15 f	1.54±0.07 h
<i>Mentha arvensis</i>	4.34±0.13 e	3.72±0.09 f	3.46±0.10 f	1.59±0.10 h
<i>Datura stramonium</i>	3.56±0.04 f	2.36±0.15 g	1.45±0.20 h	1.46±0.13 h
<i>Citrus limonium</i>	4.49±0.10 e	4.36±0.08 e	3.50±0.12 f	3.57±0.20 f
Control	7.39±0.03	8.28±0.04	6.33±0.1	7.50±0.11
72 hours of spray				
<i>Azadirachta indica</i>	5.7±0.04 d	5.59±0.17 d	3.50±0.12 g	2.88±0.04 h
<i>Mentha arvensis</i>	4.57±0.04 e	3.59±0.02 fg	2.60±0.02 i	2.63±0.02 hi
<i>Datura stramonium</i>	3.60±0.02 fg	2.71±0.02 hi	2.64±0.05 hi	1.77±0.08 j
<i>Citrus limonium</i>	6.39±0.14 c	2.64±0.02 hi	5.74±0.01 d	3.77±0.09 f
Control	7.7±0.06	6.56±0.00	7.64±0.02	8.81±0.07

Means ±SD were separated by LSD test.

The results (Table 3) showed that the 2% concentration of *D. stramonium*, *M. arvensis* and *limonene* were statistically at PAR and more toxic against H<sub>2</sub>O-in of cotton crop than the *A. indica* (5.37±0.09). The outcomes demonstrated that *M. arvensis* (3.45±0.13) after 24 hours of spray at 4% concentration was more phytotoxic against H<sub>2</sub>O-in of cotton crop than the *A. indica* (4.63±0.14) but less toxic than the *limonene* (2.51±0.07) and *D. stramonium* (2.54±0.16). The 8% concentration of *D. stramonium* (1.39±0.12) differs significantly from all other treatments. The 16% concentration of *D. stramonium* (1.53±0.12) and *M. arvensis* (1.56±0.17) were statistically at PAR and the phytotoxicity of these two botanicals were more as compared to the *limonene* (2.48±0.14) and *A. indica* (2.77±0.09).

The results (Table 3) demonstrated that the *A. indica* (6.34±0.14) at 2% concentration following 48 hours of spray had significant difference and least toxicity against H<sub>2</sub>O-in of cotton crop while the other extracts were phytotoxic and more phytotoxic was *D. stramonium* (3.56±0.04). The results revealed that 4% concentration of *M. arvensis* differ significantly and had more phytotoxicity than all the other plant extracts. The results also showed that the *D. stramonium* at 8% concentration was more phytotoxic against H<sub>2</sub>O-in of cotton crop. While *limonene* (3.57±0.20) at 16% concentration phytotoxicity differ significantly and was less as compared to all the other plant extracts which were statistically at par.

The results (Table 3) also demonstrated that the *limonene* (6.39±0.14) at 2% concentration following 72 hours of spray had significant difference and was least toxic against H<sub>2</sub>O-in of cotton crop. The results revealed that *C. limonium* and *D. stramonium* had similar phytotoxicity at 4% concentration against H<sub>2</sub>O-in of cotton crop. The phytotoxicity of 8% concentration of different botanicals differ significantly and was in order as *limonene* > *A. indica* > *D. stramonium* > *M. arvensis* which were 5.74±0.01, 3.50±0.12, 2.64±0.05 and 2.60±0.02 respectively as compared to the average of control 7.64±0.02. The phytotoxicity at 16% concentration of *M. arvensis* (2.63±0.02) differs significantly and was more as compared to all other plant extracts.

### 3.4 Phytotoxic impact of various concentrations of various botanicals against H<sub>2</sub>O-out of cotton

The results (Table 4) demonstrated that the *limonene* at 2% concentration after 12 hours of spray had significant difference and was less phytotoxic

against H<sub>2</sub>O-out of cotton crop. The phytotoxic effects of *M. arvensis* (3.77±0.04) and *limonene* (3.66±0.00) at 4% concentration was statistically at PAR and were less than the *D. stramonium* (1.64±0.11) and *A. indica* (1.63±0.02). The 8% concentration of all botanicals did not differ significantly. Similarly 16% concentrations of all plant extracts were statistically at par.

The results (Table 4) showed that the phytotoxicity of *M. arvensis* (4.70±0.02) at 2% concentration after 24 hours of spray was statistically similar with those *C. limonium* (4.63±0.02). The 4% concentration of *M. arvensis* (1.70±0.02) differs significantly from rest of the three extracts which were statistically at par. The 8% concentration of *M. arvensis* (2.55±0.09) and *C. limonium* (2.53±0.02) did not show significant difference from each other. The 16% concentration of *A. indica* (1.66±0.04) did not differ significantly from *C. limonium* (1.56±0.09).

The results (Table 4) showed that the 2% concentration following 48 hours of spray of *M. arvensis* (4.64±0.05) significantly different in phytotoxicity against H<sub>2</sub>O-out of cotton crop from all other treatments. The 4% concentration of both *D. stramonium* and *C. limonium* demonstrated that they had at PAR effect on the H<sub>2</sub>O-out of cotton crop. The 8% concentration showed that the H<sub>2</sub>O-out of cotton crop was least affected by the *M. arvensis* which differ significantly from all treatments. *A. indica* (1.659±0) and *D. stramonium* (1.66±0.12) at 16% concentration had more affected H<sub>2</sub>O-out of cotton crop and did not have significant difference from each other.

The result (Table 4) revealed that at 2% concentration phytotoxicity of *C. limonium* (4.76±0) against H<sub>2</sub>O-out of cotton crop differ significantly and was more than all the other treatments. Similar results of *C. limonium* were also recorded at 4%, 8% and 16% concentrations.

### 3.5 Phytotoxic impact of various concentrations of various botanicals against PAR of cotton

The result (Table 5) showed that after 12 hours of spray, by 2% concentration the PAR of cotton crop was least affected of *M. arvensis* (1239.33±74.6) which differ significantly from all treatments while *M. arvensis* at 4% concentration was statistically at PAR with *A. indica*. The results revealed that phytotoxicity of *C. limonium* (1240.5±79.1) at 8% concentration against PAR differ significantly and was more than the *M. arvensis* (1499.7±42.8). The result also depicted

**Table 4** - Phytotoxic effect of different plant extracts against H<sub>2</sub>O-out of cotton crop

Plant extracts	2% Concentrations	4% Concentrations	8% Concentrations	16% Concentrations
12 hours of spray				
<i>Azadirachta indica</i>	3.66±0.01 d	1.63±0.02 f	2.64±0.02 e	1.64±0.02 f
<i>Mentha arvensis</i>	3.73±0.02 d	3.77±0.04 d	2.73±0.02 e	2.73±0.02 f
<i>Datura stramonium</i>	1.77±0.00 f	1.64±0.11 f	2.69±0.07 e	1.74±0.21 f
<i>Citrus limonium</i>	4.67±0.13 c	3.66±0.00 d	2.63±0.05 e	1.73±0.02 f
Control	7.78±0.00	6.63±0.05	6.48±0.18	7.60±0.17
24 hours of spray				
<i>Azadirachta indica</i>	5.62±0.03 c	4.73±0.02 d	3.74±0.02 e	1.6±0.04 g
<i>Mentha arvensis</i>	4.70±0.07 d	1.70±0.02 g	2.55±0.09 f	3.57±0.08 e
<i>Datura stramonium</i>	5.55±0.10 c	4.55±0.99 d	4.61±0.07 d	2.64±0.02 f
<i>Citrus limonium</i>	4.63±0.02 d	4.69±0.06 d	2.53±0.02 f	1.56±0.09 g
Control	7.67±0.07	7.36±0.04	7.25±0.07	7.33±0.07
48 hours of spray				
<i>Azadirachta indica</i>	3.60±0.06 e	2.60±0.03 g	2.56±0.04 g	1.65±0.00 h
<i>Mentha arvensis</i>	4.64±0.05 d	4.48±0.11 d	3.63±0.06 e	2.44±0.09 g
<i>Datura stramonium</i>	3.60±0.15 e	3.43±0.00 ef	2.43±0.05 g	1.66±0.12 h
<i>Citrus limonium</i>	5.52±0.07 c	3.38±0.07 ef	3.27±0.11 f	2.63±0.03 g
Control	7.44±0.18	7.32±0.03	7.31±0.07	6.44±0.05
72 hours of spray				
<i>Azadirachta indica</i>	6.72±0.02 c	5.76±0.05 d	4.70±0.07 e	4.70±0.02 e
<i>Mentha arvensis</i>	5.66±0.04 d	6.58±0.17 c	5.52±0.07 d	3.64±0.03 f
<i>Datura stramonium</i>	7.60±0.10 b	6.63±0.02 c	7.50±0.11 b	7.54±0.13 b
<i>Citrus limonium</i>	4.76±0.00 e	3.70±0.2 f	3.73±0.03 f	2.50±0.09 g
Control	8.63±0.03	7.63±0.11	8.70±0.02	7.66±0.08

Means ±SD were separated by LSD test.

**Table 5** - Phytotoxic effect of different plant extracts against PAR of cotton crop

Plant extracts	2% concentrations	4% concentrations	8% concentrations	16% concentrations
12 hours of spray				
<i>Azadirachta indica</i>	1053.41±17.09 i	931.47±36.10 k	1145.5±70.9 fg	1052.9±57.02 i
<i>Mentha arvensis</i>	1239.33±74.67 e	953.7±66.13 jk	1499.7±42.8 ab	1493.9±27.1 bc
<i>Datura stramonium</i>	989.2±98.66 j	1134.53±53.8 gh	1141.19±23.5 fg	1162.7±64.7 fg
<i>Citrus limonium</i>	1095.73±41.19 hi	1147.38±70.6 fg	1240.5±79.19 e	1267.3±28.4 e
Control	1186.12±3.10	1542.3±3.30	1453.41±74.03	1347.8±5.17
24 hours of spray				
<i>Azadirachta indica</i>	1330.9±8.4 de	1251.5±8.76 hi	1346.76±0.8 d	1263.74±15.2 ghi
<i>Mentha arvensis</i>	1313.5±5.7 def	1237.9±14.57 i	1311.39±20 def	1297.03±20.47 efg
<i>Datura stramonium</i>	1244.3±8.2 i	1287.2±19.4f gh	1169±1.08 j	1243.93±7.00 i
<i>Citrus limonium</i>	1246.1±9.37 i	1264.7±18.4 ghi	1425.65±7.2 bc	1227.48±9.18 i
Control	1402.8±1.15	1434.1±0.74	1391.45±1.17	1487.65±1.59
48 hours of spray				
<i>Azadirachta indica</i>	1380.44±0.66 b	1299.39±17.33 cd	1254.73±10.75 e	1227.45±5.41 e
<i>Mentha arvensis</i>	1243.85±12.50 e	1255.33±22.29 e	1015.82±6.65 g	1252.9±7.00 e
<i>Datura stramonium</i>	1265.2±12.92 de	1386.37±15.58 b	1358.29±17.12 b	1155.95±12.90 f
<i>Citrus limonium</i>	1311.1±8.68 c	1165.94±11.59 f	1169.14±8.68 f	1152.05±8.03 f
Control	1437.9±1.35	1470.3±1.59	1450.25±2.02	1437.43±3.42
72 hours of spray				
<i>Azadirachta indica</i>	1118.32±10 h	1065.05±12.15 i	1049.23±18.02 ij	1121.13±21.57 h
<i>Mentha arvensis</i>	1350.09±1.9 c	1342.1±7.56 c	1022.53±6.89 j	1277.67±5.57 ef
<i>Datura stramonium</i>	1266.25±5.04 f	1315.57±18.3 cd	1152.79±3.38 gh	1016.01±14.46 j
<i>Citrus limonium</i>	1160.27±16.07 g	1150.74±2.30 gh	1301.75±1.37 de	1177.18±3.91 g
Control	1459.76±1.78	1438.18±2.88	1439.93±1.15	1488.84±1.58

Means ±SD were separated by LSD test.

that the phytotoxicity of *A. indica* at 16% concentration was maximum ( $1052.93 \pm 57.02$ ) and differ significantly from all treatments.

The result (Table 5) revealed that after 24 hours of spray 2% concentrations of *C. limonium* ( $1246.11 \pm 40.3$ ) and *D. stramonium* ( $1244.38 \pm 35.4$ ) PAR affected maximum and were statistically at par. The results also demonstrated that *M. arvensis* ( $1237.93 \pm 62.7$ ) at 4% concentration was more PAR affected and had no significant difference from *A. indica* ( $1251.53 \pm 37.7$ ). The 8% concentration of *D. stramonium* ( $1169 \pm 4.6$ ) showed significant difference over all treatments and was more PAR affected. The 16% concentration of *D. stramonium* ( $1243.93 \pm 30.12$ ) and *C. limonium* ( $1227.48 \pm 39.5$ ) were statistically at PAR and differ significantly from all treatments.

The result (Table 5) revealed that after 48 hours of spray the PAR of cotton crop was less affected by the *A. indica* ( $138.44 \pm 2.8$ ) and differ significantly from all other treatments. The 4% concentration of limonene ( $1165.94 \pm 49.8$ ) was more phytotoxic and differs significantly from all treatments. Similar trend of limonene was also observed at 8% concentration. The PAR of cotton crop was more affected by the *D. stramonium* and limonene which did not show significant difference from each other.

The result (Table 5) showed that at 2% concentration, after 72 hours of spray, all the treatments differ significantly from each other. *A. indica* ( $1118.32 \pm 43.1$ ) more affected PAR of cotton crop at 2% concentration. Similar result was also recorded with 4% concentration of *A. indica*. The 8% concentration of *A. indica* ( $1049.23 \pm 77.5$ ) and *M. arvensis* ( $1022.53 \pm 29.6$ ) had more phytotoxicity against PAR of cotton crop and both were statistically at par. The PAR of cotton crop was more influenced by the *D. stramonium* ( $1016.01 \pm 62.2$ ) at 16% concentration.

The present finding showed that at 2% and 8% concentration, neem and mint affected the CO<sub>2</sub>-in of cotton crop. CO<sub>2</sub>-out was affected by mint and neem at 16% and 4% concentrations, respectively. Both neem and mint at 16% concentration affected the H<sub>2</sub>O-in of cotton crop. PAR of cotton crop was more affected by the neem at 4% concentration. The reason of such outcomes was that different dosages of different botanicals in various way influenced the opening and shutting of stomata and photosynthesis color of cotton crop. The dosages which cause higher

phytotoxicity of cotton crop, have more influence the stomata (influence the in word and out word development of CO<sub>2</sub> and H<sub>2</sub>O) and photosynthesis color (impact the PAR).

#### 4 CONCLUSIONS

The results of present research coincide with the finding of (Nijenstein and Ester, 1998), and (Ibrahim et al., 2004) who discovered that the concentrations of limonene 90 and 120 mL were more phytotoxic. The present investigation showed that the limonene 8% and 16% were more phytotoxic against CO<sub>2</sub>-in of cotton crop following 12 long periods of shower, at 4% affected the CO<sub>2</sub>-out, H<sub>2</sub>O-in was more influenced at 16% and H<sub>2</sub>O-out of cotton was more influenced at concentration of 16%.

#### 5 CONTRIBUTIONS

MNZ: designed and performed the experiments, analyzed the data, interpreted the results, and drafted the manuscript. NM, JI, and MFS: did the project administration, revised the manuscript and helped in data analysis. TI, HAB, MSB, and AU: helped in data collection, investigation and visualization.

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