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Research Article

Dissecting the biochemical and hormonal changes of thidiazuron on defoliation of cotton CO17 (*Gossypium hirsutum*) to enhance mechanical harvest efficiency

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Abstract
Using chemical defoliants to remove cotton leaves represents a groundbreaking shift in cotton cultivation. The mechanization of cotton harvest is increasing, but a substantial amount of foliage that remains on the plant even at maturity is the major barrier for mechanical harvest. Properly completing mechanical and manual harvests requires artificial leaf detachment through defoliants. Still there is no proper defoliant concentration, application times and mechanism of action available. Therefore, the present study aimed to find an effective defoliant and application time to enhance mechanical harvest efficiency, along with a clear description of the mechanism of actions in cotton CO17 (*Gossypium hirsutum*). The field experiment was conducted during the year 2019-20 and used five concentrations of Thidiazuron defoliant (100, 150, 200, 250 and 300ppm) and Ethephon@0.5% (T_2) in cotton variety CO17 to study the physiological, biochemical and hormonal responses at 120, 127 and 134 days after sowing. As a result, the concentrations of plant growth hormones, indole-3 acetic acid (4.9 fold), zeatin (32.7%) and gibberellic acid (7 fold) reduced. In contrast, abscisic acid (48.6%), jasmonic acid (34.9%), salicylic acid (2.15 fold) increased in the T_7- Thidiazuron + Diuron (300 ppm) treatment followed by T_5-Thidiazuron + Diuron (200 ppm). Additionally, the antioxidant enzymes ascorbate peroxidase, peroxidase, catalase, superoxide dismutase, cellulase in leaves, petiole and bolls were decreased due to defoliant T_5- Thidiazuron + Diuron (200 ppm) followed by T_7-Thidiazuron + Diuron (300 ppm), indicating that the hormone concentration, antioxidative and hydrolytic enzymes are ruled out and forces the defoliation process.

Keywords: Antioxidants, Cotton, Calcium, Chemical defoliants, Leaf abscission, Magnesium, Plant hormones

INTRODUCTION

Chemical defoliants induce leaf senescence, abscission and premature leaf drop in plants. Cotton is mechanically picked in many affluent nations and thidiazuron is utilized as a chemical defoliant. Defoliants such as Thidiazuron and Ethephon are classed as harvest assistance chemicals since they are commonly

used to ease the progress of mechanical harvesting of crops (Wang *et al.,* 2023). Cotton foliage should be removed before harvesting to improve cotton fibre quality and reduce debris during machine picking (*Zhang et al., 2023*). However, the efficacy of this sort of defoliant might alter depending on environmental conditions and the mechanism of thidiazuron works with diuron remains unknown. The mechanism of action of defoliants, concentration and correct application time are necessary for proper cotton harvest management (Nisler *et al.,* 2016).

The defoliants encourage endogenous ethylene synthesis, inhibiting the auxin transports and eventually induces early leaf abscission (Xu *et al.,* 2019). The changes in the hormone concentration may induce hydrolytic enzyme synthesis, which dissolves the cell membrane and nutrient concentration of petioles (Du *et al.,* 2014). Antioxidant enzymes that are produced when a plant is under stress might diminish the impact of this cell membrane damage. But, defoliants could decrease antioxidant enzyme activity and form an abscission layer in the leaf abscission zone (Jin et al., 2020). The present study aimed to know the effect of chemical defoliants on biochemical and hormonal parameters during leaf abscission of cotton CO17 (*Gossypium hirsutum*).

MATERIALS AND METHODS

During defoliation by various defoliants, the physiological, biochemical and hormonal state was determined using a cotton variety called "CO 17" (*G. hirsutum*) with a special nature of medium duration (130-140 days) and erect and compact plant traits. The experiment was conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore (111N; 771E; 426.7m MSL) from 2019 to 2020.

Design of experiment and data collection

Using the split plot statistical design, the plots were arranged for the main plot as a time of defoliants application and the subplot as treatments with three replications. The Difference between means was analyzed using the two-way Least significant difference (LSD) test at 5% probability level.

Treatments and time of applications

Six defoliants with one control viz., T_1 Control, T_2 Ethephon (0.5 %), T_3 Thidiazuron + Diuron (100 ppm), T_4 Thidiazuron + Diuron (150 ppm)), T_5 Thidiazuron + Diuron (200 ppm), T_6 Thidiazuron + Diuron (250 ppm), T_7 Thidiazuron + Diuron (300) were considered as sub plot, and three times of application viz., spray at 120 DAS, spray at 127 DAS), spray at 134 DAS) were considered as the main plot. From which, randomly ten plants were selected on each replication after four days of defoliant spray to study the various biochemical (Catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, cellulase, calcium and magnesium and hormonal (auxin, gibberellic acid, zeatin, jasmonic acid, salicylic acid) features.

Plant growth hormones (LC-MS method)

The phytohormones were quantified in the best three treatments and control. Phytohormones viz., Indole acetic acid (IAA), Abscissic acid (ABA), Gibberellic acid (GA) and Zeatin (ZT), Jasmonic acid (JA), Salicylic acid (SA), were quantified using Liquid Chromatography Mass Spectrometry (LC-MS) by the procedure suggested by Muller *et al.* (2011). About 0.5g of leaf sample was mixed with 1.5 ml of methanol and water mixture (8:2) and the suspension was vortexed for 30 minutes. Then, the mixture was centrifuged at 15000 X g for 5 minutes at 4°c and the supernatant was transferred to a sterile microfuge tube and concentrated it using vacuum concentrator (Eppendorf vacufuge, USA). The dried extracts were dissolved in 100 micro liters of HPLC grade methanol and sonicated for 10 minutes. After sonication, the mixture was centrifuged at 15000 x g for 10 minutes at 40 c. The supernatant was filtered using 0.2 µm membrane filter and quantified using LC-MS (SHIMADZU -8040, China). The standard stock solutions of JA, SA, IAA, ABA, GA AND ZT (Sigma Aldrich) were prepared using methanol.

Estimation of biochemical parameters

0.1 g of fresh leaf tissue was homogenised with 1.5 mL of phosphate buffer (50 mM, pH 6.5) before being centrifuged at 12,000 rpm for 15 minutes at 4 °C to measure the antioxidant enzymes Ascorbate peroxidase (APX), Peroxidase (POX), Catalase (CAT) and Superoxide dismutase (SOD), as well as Cellulase. The ascorbic acid-dependent reduction of H_2O_2 at 290 nm was assessed using the ascorbate peroxidase (APX) activity assay method reported by (Asada, 2006). The APX activity was calculated using the extinction coefficient of 2.8 mM cm^{-1} and the enzyme activity was reported as m min^{-1} mg $protein^{-1}$. POX activity was determined by following the procedures of Hammerschmidt *et al.* (1982).The reaction mixture of 0.01 M Phosphate buffer and 2 ml of 0.25% guaiacol was added with 0.1M Hydrogen Peroxide. Increase in absorbance was noted at 420 nm. The activity was expressed as n mole min^{-1} mg $protein^{-1}$. Catalase activity was estimated by the protocols of Rubio *et al.* (2002) by the addition of 0.5g leaf sample with 2.5 ml of 100mM potassium phosphate buffer and 0.5 ml of 2.5mM H_2O_2 reaction mixture. The activity was measured by dehydration of H_2O_2 using the spectrophotometer at 240nm for 1minutes and expressed as µmol g^{-1} of sample. Superoxide dismutase activity was determined by nitroblue tetrazolium (NBT) method by measuring the photo-reduction of

NBT at 560 nm and expressed as units min^{-1} g^{-1} of fresh leaf tissue (Beyer and Fridovich, 1987). One unit of SOD activity is equivalent to amount required inhibiting photo reduction of NBT by 50 %.

Estimation of cell wall degrading enzyme

Extraction buffer with a composition of 100 mM Tris-HCl, 0.5% PVPP, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 10 mM DTT, 0.15 mM PMSF, 0.3% (w: v) X-100 Triton, and 300 ppm sodium azide, was used to prepare the extract from leaf, petiole, and boll samples that were taken from the field. The extract was diluted 1:9 (v: v) in water and centrifuged at 10,000 g for 20 minutes before being filtered through filter paper for an hour. The viscosity method was used to assess this extract's cellulase (CEL) activity (Veloz-Garcia *et al.,* 2010). A unit of enzyme was defined as specific activity (U mg $protein^{-1}$), which is the reciprocal of the number of hours required to achieve a 50% viscosity reduction for the enzyme $X10^3$.

Estimation of calcium and magnesium nutrient content in leaf

Like phytohormones, nutrients were also quantified in the best three treatments with control. Calcium (Ca) and magnesium (Mg) get complexed by EDTA in the order of Ca, followed by Mg. Ca is estimated first by using a murexide indicator in the presence of sodium hydroxide at pH 12. Then, Ca+Mg were estimated using Eriochrome Black-T in the presence of ammonium chloride and ammonium hydroxide buffer solution at pH 10. Values were expressed as per cent (Wilkinson, 1956; Koehler *et al.,* 1996).

RESULTS AND DISCUSSION

The term defoliation describes the process of spontaneous separation of organs from the mother plant. In general, it is considered that the balance in the plant hormones determines the abscission to occur in the leaves. However, chemical defoliants act as an agent that induces the abscission layer formation and promotes leaf shedding.

Response of various plant hormones against chemical defoliants

The present findings indicated that the use of defoliants had a negative impact on indole-3 acetic acid (IAA) concentration. Thidiazuron+Diuron@ 200 ppm (T_5) treatment showed a 4.9 fold reduction in IAA concentration, while Ethephon@0.5% (T_2) treatment showed a 4.5 fold reduction. The level of zeatin was likewise decreased by about 32.7% in the Ethephon@0.5% (T_2) and Thidiazuron+Diuron@ 200 ppm (T_5) treatments, respectively. Thidiazuron+Diuron@ 300 ppm (T_7) treatment resulted in a seven-fold decrease in Gibberellic acid (GA_3) followed by 2.2 fold reduction in Thidiazuron+Diuron@ 200 ppm (T_5) treatment. In contrast, Ethephon (0.5%) had the highest amount of Abscissic acid (ABA) (48.6%) followed by the Thidiazuron+Diuron@ 300 ppm (T_7) and Thidiazuron+Diuron@ 200 ppm (T_5) treatments. Salicylic acid concentration was also higher in the Thidiazuron+Diuron@ 200 ppm (T_5) treatment and Thidiazuron+Diuron@ 300 ppm (T_7) treatment at 134 DAS increased 34.9% of jasmonic acid concentration (Table 1a and 1b).

Plant hormones play a major role in cotton defoliation, and endogenous concentrations of ethylene, ABA, and auxin are involved in leaf abscission in various plant species. Due to chemical defoliants, the endogenous concentration of ABA was increased at the stem side and the IAA was decreased at the leaf side (Israeli *et al.,* 2019). Auxin is an important plant growth promoter and reduction of this hormone is the first signal in leaf abscission. This reduction may induce ethylene synthesis, leading to leaf senescence and abscission (Lim *et al.,* 2007). However, Shen *et al.* (2013) found that higher accumulation of IAA in leaf petiole portion can trigger the leaf abscission process during the boll-opening stage of cotton. Cytokinins are called antisenescence hormones that delay the leaf senescence and abscission process. In the present study, the level of kinetin was reduced due to the application of the defoliant thidiazuron+diuron, and the reduction of this hormone may induce defoliation. Cytokine also promotes nutrient mobilization into treated areas which may take the creation of source-sink relations (Laila *et al.,* 2020). There is no direct relationship between GA and leaf defoliation, but it acts as an antagonist to ABA. GA may play a role as ROS scavengers to delay leaf defoliation by producing antioxidant enzymes . GA and Cytokinins have a synergistic regulatory role in cotton leaf senescence. In addition to ethylene, ABA is also involved in the absorption process (Yu *et al.,* 2009).

Salicylic acid (SA) influences different physiological and biochemical activities in plants and is key in regulating their growth and yield. It may also directly induce protection from the cell membrane damage by reduced electrical leakage, H_2O_2 in leaves and dehydration losses by triggering the antioxidant defense system and protein accumulation. Evidence suggests that cotton leaf abscission is regulated by ROS signaling induced by thidiazuron defoliant in cotton. These defoliants inhibit the cytokinin oxidase/dehydrogenase, which is involved in the oxidative breakdown of cytokinin and increases endogenous cytokinin concentration in cotton plants (Hayat *et al.,* 2010). Methyl jasmonate or jasmonic acid (JA) is also considered an enhancer of leaf abscission in different plants. This hormone is involved in the formation of a secondary abscission zone in the petiole. JA combined with IAA can also induced

Table 1a. Influence of defoliants and time of application on plant growth hormones content in the leaf of the cotton CO17

Treatments	Plant Growth Hormones											
	Indole 3 acetic acid (IAA) (µg g^{-1} of sample)				Zeatin (µg g^{-1} of sample)				Gibberellic acid (GA) (µg g^{-1} of sample)			
	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean
T_1 - Control	34.34	39.38	42.60	38.77	14.8	29.0	23.1	22.3	3.8	2.6	2.9	1.6
T_2 -Ethephon (0.5 %)	9.42	2.72	9.08	7.07	15.5	16.7	18.1	16.8	3.4	0.4	1.2	1.6
T_5- Thidiazuron + Diuron (200 ppm)	6.17	6.42	7.01	6.53	11.7	21.9	23.1	18.9	0.3	0.7	0.5	0.5
T_7 - Thidiazuron + Diuron (300 ppm)	11.77	12.21	10.28	11.42	15.3	30.3	26.0	23.9	0.2	0.3	0.3	0.2
Sed	0.07	0.23	0.35	0.40	0.17	0.27	0.44	0.46	0.009	0.021	0.032	0.035
CD (0.05)	0.21**	0.48 **	0.75**	0.84**	0.46**	0.56**	0.96**	0.98**	0.024**	0.044**	0.0694**	0.075**

** Denotes significance at the 0.01 level of probability

Table 1b. Influence of defoliants and time of application on plant growth hormones content in the leaf of the cotton CO17

Treatments	Plant Growth Hormones (4 days after defoliants spray)											
	Abscissic acid (ABA) (µgg^{-1} of sample)				Salicylic acid (SA) (µg $g^{-1}$of sample)				Jasmonic acid (JA) (ng $g^{-1}$of sample)			
	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean
T_1 – Control	49.01	71.33	61.57	63.97	0.35	0.75	0.67	0.59	17.1	26.8	32.4	32.1
T_2 -Ethephon (0.5 %)	59.46	75.38	65.34	95.06	0.77	0.92	1.13	0.94	21.9	30.8	28.3	27.0
T_5- Thidiazuron + Diuron (200 ppm)	58.85	80.00	69.21	69.35	1.04	1.18	3.37	1.86	27.8	35.8	37.3	33.6
T_7 – Thidiazuron + Diuron (300 ppm)	61.46	117.09	80.35	86.30	0.58	1.27	0.81	0.89	26.5	54.9	41.6	41.0
Sed	0.61	0.87	1.44	1.51	0.02	0.01	0.02	0.02	0.34	0.35	0.63	0.61
CD (0.05)	1.69**	1.84**	3.21**	3.18**	0.05**	0.02**	0.06**	0.04**	0.94**	0.74**	1.44**	1.28**

** Denotes significance at the 0.01 level of probability

the leaf secondary zone formation in cotton plants (Saniewski *et al.,* 2016). In the present study, the exogenous application of Thidiazuron + Diuron and other defoliants induced IAA and JA concentration that may be the reason for higher defoliation.

Response of various biochemical characters against chemical defoliants

Ascorbate peroxidase (APX), Peroxidase (POX), Catalase (CAT), and Superoxide dismutase (SOD) (Tables 2 and 3) showed significant differences in treatments and interactions of defoliants and time of application. Thidiazuron+Diuron@ 200 ppm (T_5) defoliants showed lower Ascorbate peroxidase activity with 4.9 m min^{-1} mg $protein^{-1}$ values in 134 DAS. When Ethephon@0.5% (T2) was sprayed at 134 DAS, the Superoxide Dismutase (SOD) activity increased to 145.6%, and Thidiazuron + Diuron (200 ppm) was applied at 127 DAS, it increased to 142.3%. In comparison to the control, Thidiazuron + Diuron (200 ppm) and Ethephon@0.5% (T2) sprays at 120 DAS decreased peroxidase by 151.5 and 140 percent, respectively. Catalase activity was drastically reduced when Thidiazuron + Diuron (200 ppm) was applied at 134 and 120 DAS (44.2 and 59.2 unit min^{-1} g-1, respectively).

Reactive oxygen species, viz., singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radical, are synthesized concerning the plant stress (Xie *et al.,* 2019). However, the excessive ROS degrades the cellular contents such as proteins, lipids, and nucleic acid, which significantly results in leaf abscission. In the present study, the estimated antioxidant enzymes were lower compared to the control, indicating that the ROS plays a major role in leaf defoliation with respect to the chemical defoliants imposed. The hydrogen peroxide and superoxide radicals are important endogenous regulators for cotton defoliation. Higher accumulation of ROS in the leaf abscission zone site has been implicated in leaf abscission. Lipids, proteins, and nucleic acids can all be harmed by these excess ROS. Similar results were observed in cotton plants by applying thidiazuron (0.1%), which caused continuous hydrogen peroxide (H_2O_2) generation and decreased antioxidant concentration level, resulting in leaf abscission. These findings demonstrated that defoliants could induce an abiotic stress that can drive cotton leaf abscission (Jin et al., 2020). ROS have a key role in how plants control the abscission of their leaves. High levels of ROS generation at the abscission zone activate cell wall hydrolytic enzymes, which then cause cell walls to be digested and cell membranes to degrade, which in turn causes leaves to abscise *(*Yoon *et al., 2020).*

In present study of leaf cellulase content, Thidiazuron + Diuron (200 ppm) (T_5) caused the highest percentage of the increase (62.4%), followed by thiadiazuron + diuron (300 ppm) (T_7) by 39.3 %. However, in the peti-

Table 2. Influence of defoliants and time of application on ascorbate peroxidase and peroxidase enzymes activity of the cotton CO17

Treatments	Antioxidant enzymes activity (4 day after defoliants spray)							
	Ascorbate Peroxidase (µm min^{-1} mg $protein^{-1}$)				Peroxidase (nmole min^{-1} mg $protein^{-1}$)			
	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean
T_1- Control	14.8	13.3	11.2	18.5	271.4	198.1	182.6	217.4
T_2- Ethephon (0.5 %)	6.4	4.1	4.6	12.5	113.1	110.9	187.2	137.1
T_3- Thidiazuron + Diuron (100 ppm)	4.9	5.8	6.5	16.7	109.8	112.4	167.3	129.8
T_4- Thidiazuron + Diuron (150 ppm)	5.4	6.8	8.9	17.5	205	195.7	158.1	186.3
T_5- Thidiazuron + Diuron (200 ppm)	6.4	5.2	4.9	11.6	107.9	105.1	128.7	113.9
T_6- Thidiazuron + Diuron (250 ppm)	8.9	6.4	6.5	16.4	186.8	117.2	146.4	150.1
T_7- Thidiazuron + Diuron (300 ppm)	5.9	6.6	5.5	13.5	145.1	147.7	112.9	135.2
Sed	0.068	0.061	0.119	0.106	1.011	1.971	3.318	3.414
CD (0.05)	0.189**	0.125**	0.271**	0.289**	2.806**	3.998**	6.962**	6.925**

** Denotes significance at the 0.01 level of probability

Table 3. Influence of defoliants and time of application on catalase and superoxide dismutase enzymes activity of cotton CO17

Treatments	Antioxidant enzymes activity (4 day after defoliants spray)							
	Catalase activity (units min $^{-1}$ g^{-1})				Superoxide dismutase (unit min $^{-1}$ g^{-1})			
	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean
T_1- Control	154.7	162.4	181.1	166.1	19.5	17.2	16.7	17.8
T_2- Ethephon (0.5 %)	87.4	79.7	83.4	83.5	10.1	16.3	6.8	11.1
T_3- Thidiazuron + Diuron (100 ppm)	103.2	100.4	80.3	94.6	8.5	7.9	11.9	9.4
T_4- Thidiazuron + Diuron (150 ppm)	97.9	91.6	63.3	84.3	11.0	10.8	9.8	10.5
T_5- Thidiazuron + Diuron (200 ppm)	59.2	56.5	44.2	53.3	8.3	7.1	10.2	8.5
T_6- Thidiazuron + Diuron (250 ppm)	101	102.5	94.6	99.4	8.5	8.9	8.2	8.5
T_7- Thidiazuron + Diuron (300 ppm)	77.1	71.0	67.9	72.0	11.1	9.8	7.1	9.3
Sed	0.52	1.23	2.04	2.14	0.011	0.134	0.243	0.232
CD (0.05)	1.44**	2.50**	4.24**	4.33**	0.311**	0.272**	0.531**	0.472**

** Denotes significance at the 0.01 level of probability

ole section, Thidiazuron + Diuron (300 ppm) (T_7) treatment was shown to have the highest concentration of cellulase (95.1%), followed by Thidiazuron + Diuron (200 ppm) (T_5) treatment (68.6%). However, Thidiazuron + Diuron (200 ppm) treatment (T_5) had greater cellulase content in the bolls (28.2%), while all other treatments had lower levels of cellulase production (Table 4). The increased cellulase activity could be attributed to the effects of the defoliant on the plant's biochemical and hormonal processes.The cell wall hydrolytic enzymes are triggered with the help of Indole -3-acetic acid and Abscisic acid-dependent manner. In other hand continuous productions of H_2O_2 activates the cellulase enzymes and consequently induces abscission. Because ROS cleave plant cell wall polysaccharides and loosen the cell wall, the enhanced H_2O_2 levels in the late period may be associated with the cell wall degradation process. Such results were also found by Li *et al.* (2019) and explained that it may be due to the membrane integrity under stress with excessive ROS production, including H_2O_2 and O_2 .

Response of plant nutrients against chemical defoliants

In the present study, the decrease per cent of calcium content in leaves was observed in thiadiazuron + diuron (300 ppm) (T_7) defoliant spray at 134 DAS (3.12 %). In terms of magnesium, the decreased value was noted in Ethephon@0.5% (T_2) defoliant spray at 134 DAS (0.82 %) (Table 5). Plant nutrients are important for plant

Table 4. Influence of defoliants and time of application on cellulase content (units g^{-1}) of the cotton

Treatments	Cellulase content (units g^{-1})											
	Leaves				Petioles				Bolls			
	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean
T_1- Control	11.2	11.5	12.5	11.7	24.5	24.9	24.5	24.6	10.9	11.2	11.0	11.0
T_2- Ethephon (0.5 %)	11.7	15.5	13.3	13.5	27.9	30.2	33.5	30.5	9.1	10.3	10.7	10.0
T_3- Thidiazuron + Diuron (100 ppm)	13.5	18.1	16.5	16.0	29.5	34.7	36.3	33.5	10.9	11.1	11.2	11.1
T_4- Thidiazuron + Diuron (150 ppm)	15.3	13.5	19.5	16.1	30.5	34.0	39.5	34.7	8.3	10.1	11.6	10.0
T_5- Thidiazuron + Diuron (200 ppm)	17.4	16.5	23.2	19.0	37.2	39.6	41.3	39.4	13.7	14.2	14.5	14.1
T_6- Thidiazuron + Diuron (250 ppm)	14.6	16.6	11.5	14.2	24.5	37.3	31.4	31.1	11.0	11.0	10.8	10.9
T_7- Thidiazuron + Diuron (300 ppm)	17.0	14.8	17.3	16.3	39.3	45.5	47.8	44.2	12.9	13.5	14.4	13.6
Sed	0.082	0.190	0.315	0.329	0.360	0.426	0.773	0.739	0.079	0.116	0.201	0.200
CD (0.05)	0.228**	0.385**	0.655**	0.666**	0.999**	0.865**	1.692**	1.498**	0.219**	0.234**	0.432**	0.406**

** Denotes significance at the 0.01 level of probability

Table 5. Influence of defoliants and time of application on calcium and magnesium content in the leaf of the cotton

Treatments	Calcium (%)				Magnesium (%)			
	120 DAS	127 DAS	134 DAS	Mean	120 DAS	127 DAS	134 DAS	Mean
T_1 - Control	4.08	3.28	4.16	3.84	1.44	1.10	1.01	1.18
T_2 -Ethephon (0.5 %)	4.24	3.92	4.40	4.19	0.77	0.58	0.82	0.72
T_5- Thidiazuron + Diuron (200 ppm)	3.92	3.04	3.12	3.36	1.68	1.49	1.73	1.63
T_7 - Thidiazuron + Diuron (300 ppm)	4.56	3.52	3.68	3.92	0.91	1.78	1.73	1.47
Sed	0.0439	0.0382	0.0722	0.0662	0.0108	0.0161	0.0264	0.0279
CD (0.05)	0.1218**	0.0803**	0.1697**	0.1391**	0.0301**	0.0338**	0.0585**	0.0585**

** Denotes significance at the 0.01 level of probability

structure development and proper functions. Calcium's primary function in plant growth is to provide structural support and major composition in cell walls. It also serves as a secondary messenger when plants are physically or biochemically stressed and play a key role in regulating plant senescence. Hasanuzzaman *et al.* (2020) reported that a decreased level of Ca^{2+} concentration in leaves can increase lipid peroxidation and accumulation of ROS. Magnesium is the major nutrient, particularly in cotton plants. It is also present in the central portion of chlorophyll amid four nitrogen atoms. It activates many enzymes required in plant processes and stabilizes the nucleic acids (Kroh *et al.,* 2020).

Conclusion

The chemical defoliants T_7- Thidiazuron + Diuron (300 ppm) followed by T_5- Thidiazuron + Diuron (200 ppm) caused changes in the concentrations of several plant hormones like Indole 3 acetic acid, zeatin, gibberellic acid and salicylic acid. The indole-3 acetic acid (4.9 fold), zeatin (32.7%) and gibberellic acid (7 fold) were reduced. In contrast, abscisic acid (48.6%), jasmonic acid (34.9%), salicylic acid (2.15 fold) were increased. These changes in hormone levels likely triggered signaling pathways and physiological responses leading to leaf abscission. Moreover, the defoliants T_5- Thidiazuron + Diuron (200 ppm) affected the activity of antioxidant enzymes, decreasing ascorbate peroxidase, catalase, and superoxide dismutase levels. These antioxidant enzymes are essential for neutralizing reactive oxygen species and protecting plant cells from oxidative damage. The decreased activity of these enzymes may have facilitated the breakdown of cellular components during the leaf abscission process. In addition, the defoliants increased the activity of the cell wall-degrading enzyme cellulase. This enzyme breaks down the cell wall, a critical step in shedding leaves during abscission. Together, these combined modifications in hormone profiles, antioxidative enzyme activity, and cell wall degradative enzyme activity worked synergistically to induce the leaf abscission process in the cotton CO17.

Conflict of interest

The authors declare that they have no conflict of interest.

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