Allelopathic Effects of Redroot Pigweed's on Photosynthesis Performance, Photochemistry, and Photosynthetic Genes Expression of Cucumber &Wheat plant

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Abstract

In this study the cucumber and wheat plants were used to clarify the mechanism of redroot pigweed's allelopathic effects on photosynthesis. In order to reach that goal, plants were cultivated hydroponically, treated by redroot pigweed's leachate and finally chlorophyll fluorescence and photosynthetic gas exchange parameters, photosynthetic pigments content and the expression of photosynthetic genes (PsbA and PsbS) and allelochemical interaction with proteins of studied genes were analyzed. After exposure to the allelopathic stress, significant differences in the majority of photosynthetic characteristic were observed in the studied species. Redroot pigweed allelopathy led to alteration in photosynthesis performance, photochemistry, and photosynthetic genes expression of cucumber and wheat plants, ultimately its results were observed in morphological traits of plants. Molecular docking study strongly confirmed the possibility of direct binding and action of allelopathic compounds with allelopathic action's target proteins. Overall, this study showed that compared to cucumber, the wheat plant is able to withstand the damaging effects of amaranth allelopathy on photosynthesis in order to resist and survive.

1 INTRODUCTION

Plants are usually exposed to various biotic and abiotic stresses under the both natural and agricultural conditions (Inderjit and Einhellig, 1993; Maqbool *et al.,* 2013). Allelopathy is sub-discipline of chemical ecology and is one of the most important biotic stresses affecting plant growth and developments (Einhellig 1995; Achigan-Dako*et al.,* 2014). This stress is a multidimensional stress and its effects on plants have been observed at molecular, biochemical, physiological, morphological, and even ecological levels (Inderjit and Einhellig, 1993; Kohli *et al.,* 2001; Gniazdowska and Bogatek, 2005).

The decline in leaf chlorophyll content is an early general response of crops to allelopathic stress, which is probably the result of imbalance in cell's homeostasis (Borella*et al.,* 2014; Dehghani*et al.,* 2014; Singh and Sunaina, 2014). In addition, carotenoids' content alters in response to the allelopathy as well (Kohli*et al.,* 2001; Ahrabi *et al.,* 2011; Dehghani *et al.,*2014). Furthermore, the plants' anthocyanins and even flavonoids pigment contents increase in response to the allelopathic stress. Anthocyanins and flavonoids are the well-known low molecular weight antioxidant compounds whose concentrations increase under many stress conditions (Ahrabi *et al.,* 2011).

Effects of allelopathy on photosynthetic pigments ultimately lead to influence on the photosynthesis as one of the most important metabolic pathways in the plants. The direct impacts of allelopathy on plant photosynthesis are mainly inhibition and/or damage to the proteins involved in photosynthesis apparatus, increasing the decomposition of photosynthetic pigments, and change in expression of photosynthetic genes. Moreover,

alteration in chloroplasts' structure leads to reduction of photosynthetic pigment contents, decline in energy and electron transfer due to reducing of ATP synthesis activity, and decreasing in stomatal conductance and transpiration rate which can indirectly influence plant photosynthesis (Meazza *et al.,* 2002; Yu *et al.,* 2003; Wu *et al.,* 2004; Yu *et al.,* 2006; Bakhshayeshan-Agdam *et al.,* 2020). Influencing the function of PSII is the main effect of allelopathic stress on photosynthesis (Wink and Latzbruning 1995; Wasternack and Hause 2013; Sunmonu and Van Staden 2014; Achigan-Dako *et al.,* 2014). Accordingly, the D1 subunit of this photosystem is highly susceptible to stress, which are directly or indirectly destructed by increasing free radicals' concentration in the chloroplasts (Gonzalez et al., 1997; Shao *et al.,* 2009; Uddin*et al.,* 2012). Interfering of allelopathic compounds with the hormonal signal transduction pathways (such as ABA) can also involve in allelopathic effects on plants' photosynthesis (Inderjit and Einhellig, 1993; Weir *et al.,* 2004).

The responsible compounds in allelopathy phenomenon are called allelochemicals (Kohli *et al.,* 2001; Singh *et al.,* 2001; Bakhshayeshan-Agdam *et al.,* 2020). In plants, these compounds are non-nutritive and species- or tissue- specific substances and are mainly produced as secondary metabolites. In addition, decomposition of organic materials by microbes can lead to releasing of some allelochemicals. Other organisms such as fungi and algae can produce some allelopathic compounds (Inderjit and Nilsen, 2003). Allelochemicals belonging to different groups of plant compounds and various classifications for these compounds were proposed by researchers; but none of suggested classifications are inclusive (Inderjit and Einhellig, 1993; Chou, 1999; Shao-Lin *et al.,* 2004; Weir *et al.,* 2004; Leslie, 2005). Generally, phenolic compounds, alkaloids, non-protein amino acids, terpenoids, saponins, and benzoxazinones are most important allelochemicals in plants (Kohli*et al.,* 2001; Khan *et al.,*2010; Razavi, 2011; Soltys*et al.,* 2013).

Redroot pigweed (*Amaranthus retroflexus* L.) is one of the most invasive weeds worldwide with well-known allelopathic effects. This plant has a high invasion power and many crops are susceptible and vulnerable to its invasion (Bhowmik and Doll, 1982; Chou, 1999; Ahrabi*et al.,* 2011; Shahrokhi *et al.,* 2012). Moreover, redroot pigweed is in the list of resistant plants to herbicides. Extracts of this plant have diverse allelopathic effects on receiver plants such as growth inhibition of various plants species (Costea *et al* ., 2004; Mlakar *et al.,* 2012; Shahrokhi *et al.,* 2012; Bakhshayeshan-Agdam *et al.,* 2015).

Although there have been many studies on allelopathy, especially redroot pigweed's allelopathy, none of these studies have specifically focused on the effects of allelopathy on photosynthesis. Hence, current research has aimed to investigate redroot pigweed's allelopathic effects on photosynthesis performance, photochemistry, and photosynthetic gene expression including PsbA and PsbS of cucumber and wheat plants which are identified as sensitive and resistant species to allelopathic effects of redroot pigweed, respectively (Bakhshayeshan-Agdam *et al.,* 2015). Finally, in this study, it was attempted to investigate the direct interaction of amaranth allelopathic compounds detected in the shoots of studied plants (Table S1), by molecular docking.

2 MATERIALS AND METHODS

2.1 Plant sample and leachate preparation

Redroot pigweed fresh materials were collected from crop fields of Khosroshahr (East Azerbaijan, Iran) and thoroughly powdered after oven drying under lab conditions. The plant species was identified by Agricultural and Natural Resources Research Centre of Iran (ANRC, Tabriz, East Azerbaijan, Iran).

For leachate preparation, 0.25 gr of powdered material were suspended in 100 ml of sterile double distilled water and mixed for 24 hours using a horizontal rotary shaker (120 rpm). Then, the suspension was filtered using two layers of sterile cheesecloth and filtrate considered as leachate 0.25 % (Shahrokhi *et al.,* 2012; Mlakar *et al.,*2012; Bakhshayeshan-Agdam *et al.,* 2015).

2.2 Plant culture and treatment

Seeds of cucumber (*Cucumis sativus* L. "Basmenj") and wheat (*Triticum aestivum* L. "Pishgam") were supplied by Agricultural and Natural Resources Research Center of Iran and stored at 4 °C until cultivation. Firstly, plant seeds were sterilized using sodium-hypochlorite solution (1% v/v) for 5 minutes, cultivated

in sterile perlite and moisturized by sterile distilled water for 7 days. Thereafter, uniform seedlings were transferred to Hoagland growth medium and grown for 7 days. Uniform 14 days old seedlings were treated by redroot pigweed leachate. Hoagland solution was used as the only solution for control plants. When allelopathic symptoms (such as chlorosis, decline in plant's growth and development, and so on- almost after three days) were observed, bio-assays were conducted.

2.3 Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were determined using a portable fluorometer (OSF1, ADC Bioscientific Ltd.,UK) after adapting the leaves (youngest fully expanded leaf in all treatments) to dark for 10 minutes. Initial (F_0), maximum (F_m), and variable ($F_v = F_m$-F_0) fluorescence values were measured by the device. Other parameters were calculated using the following formulas (Rühle *et al.,* 2014).

Maximal quantum yield of PSI = F_v/F_m

Φ_{II} (effective quantum yield of PSII)=F'_v/F'_m

NPQ= 1 - $[(F'_m - F'_0)/(F_m - F_0)]$= 1 - (F'_v/F_v)

ETR= Φ_{II} (PAR × 0.84 × 0.5)

q_0= $(F_0 - F'_0)/F_0$= 1 - (F'_0/F_0)

q_P= $(F'_m - F_s)/(F'_m - F'_0)$

q_A= $(F_m - F'_0)/F_m$= 1 - (F'_0/F_m)

Rfd= $(F_m - F_s)/F_s$= (F_m/F_s) - 1

SV_0= $(F_0 - F'_0)/F'_0$= (F_0/F'_0) - 1

2.4 Photosynthetic gas exchange parameters

Photosynthetic gas exchange parameters were measured using a portable leaf chamber analyzer (ADC, LCA4, UK) on youngest fully expanded leaf in all treatments. During the measurements, the leaf chamber temperature was 25±1 °C, CO_2 concentration varied between 350-400 ppm and photosynthetic photon flux density (PPFD) was 300 μmol m^{-2} s^{-1}. Water use efficiency (WUE) was also calculated by using net photosynthesis (A) and transpiration (E) values using following formula (Jiang *et al.,* 2006).

WUE= A/E

2.5 Photosynthetic pigments content

For the extraction of pigments, fresh leaf samples were homogenized with acetone (>99%) at 4 °C and filtered by Whatman No. 42 filter paper. Finally, the absorbance of extracts was recorded at 662, 645, 495, 480, and 470 nm and pigments contents were calculated using following formulas as μg g^{-1}FW (Dere *et al.,*1998; Buldaa *et al.,* 2008).

Chlorophyll a= C_a= $11.75A_{662}$ - $2.350A_{645}$

Chlorophyll b= C_b= $18.61A_{645}$ - $3.960A_{662}$

Carotenoids= C_{x+c}= $1000A_{470}$ + $2.270C_a$ - $81.4C_b/227$

β-Caroten= $C_{\beta\text{-}\delta\rho}$= $17.16A_{495}$ - $3.96A_{480}$

Lutein= C_{Lut}= $11.51A_{480}$ - $20.61A_{495}$

2.6 Molecular studies

According to the results of redroot pigweed's allelopathic effects on chlorophyll fluorescence, photosynthetic gas exchange parameters and photosynthetic pigments; PsbA gene encoding D_1 protein in the central part

of Photosystem II and PsbS gene encoding CP_{22} protein in the Photosystem II antenna were selected for molecular studies.

2.7 Primers

Specific primers were designed using wheat and cucumber gene sequences for PsbA (Figure S1) and Arabidopsis for PsbS (Figure S2) stored at the NCBI gene bank (https://www.ncbi.nlm.nih.gov). Primer design was performed using Primer 3 software, and Beacon Designer; and Snape Gene software were used to examine the parameters associated with the primers (Thornton and Basu, 2011). 18S rRNA housekeeping gene was also used as internal control (Fu *et al.,* 2014). Designed primers were synthesized by Metabion Company (Germany) with high purity and did not need purifying (Table 1).

2.8 RNA extraction

In order to acquire total RNA extraction, shoot samples were powdered in the liquid nitrogen (100 mg) using 1000 µl Trizol reagent (Sigma-Aldrich). Then, 200 µl of chloroform was added into the solution and after 15 minutes of incubation at room temperature, the samples were centrifuged at 12,000 g for 10 minutes at 4 °C. The RNA was precipitated in the aqueous phase using propanol and saturated salt solution (0.4 M sodium citrate and 1.2 M sodium chloride) and thereafter washed with 70% ethanol. The resulting samples were carefully air-dried and finally air-dried RNAs were dissolved using DEPC (CinnaGen) water and stored at -80 °C until cDNA synthesis. After extracting, the RNA quantity was determined by detection of its absorption ratio at 280 and 260 nm (the acceptable absorption ratio for these two wavelengths is 1.5 to 2.1) using picodrop (Table S2). RNA quality was investigated by its loading in agarose gel (Razeghi and Leister, 2013) (Figure 1A).

TABLE 1

2.8 RT-PCR

Reaction samples for RT-PCR analysis included iQTM SYBR Green Supermix, specific primer pairs for PsbA and PsbS genes (see Table 1), and cDNA template. A control reaction without a cDNA template (NTC) was also considered to investigate the possibility of contamination and dimer formation by primer pairs. 18S rRNA was also used as the internal control gene and the PCR temperature program for each primer pair was adjusted according to Table 1. cDNA amplification was monitored using SYBR Green fluorescence readings at 530 nm at the end of each expansion period (Razeghi and Leister, 2013).

2.9 Molecular docking analysis

Molecular docking simulations were performed by Autodock 4.2 (Rashtbari et al., 2017; Yekta*et al.,* 2017). All water molecules were removed by Auto Dock Tools and polar hydrogen atoms, KOLLMAN atoms charge were added to PsbA and PsbS, and docking calculations were performed using Lamarckian genetic algorithm (LGA). The Grid Box was placed at center of PsbA (X center: -8.337, Y center: -50.07, Z center: 38.514) and PsbS (X center: -13.053, Y center: -5.89, Z center: 23.526). The grid map for spacing the midpoints of XXÅ for PsbA and PsbS were 0.508 and 0.375 angstroms, respectively. The numbers of points in the XYZ dimension were 126*126*126 angstroms, respectively. After docking analysis, the lowest binding energy conformation was selected for each target. The interactions of the PsbA and PsbS protein complexes with thymol, carvacrol, beta-elemene, trans-caryophyllene, germacrene-D, spathulenol, and 5alpha-pregnane compounds were also analyzed using UCSFChimera and Discovery Studio 4.1 Client.

2.10 Experimental design and statistical analysis

The experiment was conducted as completely randomized design (CRD). Plants were cultivated in pots containing sterile perlite and each treatment was conducted by three replications. T-test was used for analysis of variance by SPSS software (ver. 16) and reading was considered significant when p [?] 0.01. Figures were prepared using Microsoft excel 2007 software.

3 RESULTS

3.1 Chlorophyll fluorescence parameters

Redroot pigweed's leachate affected chlorophyll fluorescence parameters of cucumber and wheat species differently (Table 2). Leachate treatment significantly increased the F_0, F_m, F_v, NPQ, q_0, q_A, Rfd, and SV_0 values in cucumber (p [?]0.01). The same treatment in wheat increased F_0, NPQ, and SV_0 and decreased ETR and F_v/F_m parameters significantly (p[?]0.01).

TABLE 2

3.2 Photosynthetic gas exchange parameters

In cucumber plant, an increase in stomatal conductance and a decrease in stomatal resistance and water use efficiency were detected which were statistically significant (p *[?]* 0.01). In wheat plant, a significant decrease was observed in net assimilation rate, transpiration rate and stomatal conductance, while treatment with amaranth leachate led to significant increase (p *[?]* 0.01) in stomatal resistance and water use efficiency in comparison to the control (Table 3).

TABLE 3

3.3 Photosynthetic pigments content

Treatment with redroot pigweed leachate significantly affected photosynthetic pigment concentrations of cucumber and wheat plants (Table 4). In cucumber plant, chlorophyll b, total chlorophyll, total carotenoid, and β-caroten increased and chlorophyll a to b ratio and lutein content decreased, which was statistically significant (p *[?]* 0.01). In wheat plant, chlorophyll a was significantly decreased by amaranth leachate, which resulted in a significant decrease in total chlorophyll and chlorophyll a to b ratio (p *[?]* 0.01). Total carotenoid in wheat was not affected utterly by applied concentrations of leachate, however, β-caroten content was significantly decreased (p *[?]* 0.01).

TABLE 4

3.4 PsbA and PsbS photosynthetic gene expression

Expression analysis of PsbA and PsbS genes showed that treatment of wheat and cucumber plants by amaranth leachate caused a change in the expression of these genes, which also differed between studied species (Figure 1C). Leachate treatment significantly decreased PsbA gene expression in cucumber, but this gene expression was significantly stimulated in wheat plants (p *[?]* 0.01). PsbS gene expression in both cucumber and wheat plants reduced by application of amaranth leachate, but it was only statistically significant in the cucumber plant (p *[?]* 0.01). This difference was also seen in the gel images of RT-PCR products of the studied genes (Figure 1B).

FIGURE 1

3.5 Molecular docking studies

Molecular docking studies were carried out by AutoDock 4.2 in order to predict the preferred binding state as well as the binding site of the thymol, carvacrol, beta-elemene, trans-caryophyllene, germacrene-D, spathulenol, and 5alpha-pregnane compounds (as well-known allelochemicals of amaranth) with PsbA and PsbS proteins. These compounds are highly bound to these proteins. According to the results (Table 5), the 5alpha-pregnane compound with more negative binding energy as well as its lower inhibition constants compared to other compounds indicates a more stable and better binding to PsbA and PsbS encoded proteins. After the 5alpha-pregnane, the trans-caryophyllene, germacrene-D, spathulenol, beta-elmenene, thymol, and carvacrol compounds were placed in next rank, respectively. The site effect and amino acids involved in the binding of these compounds are shown in Figure 2 to 8.

TABLE 5

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

FIGURE 8

4 DISCUSSION

4.1 Chlorophyll fluorescence parameters

An increase in the minimum fluorescence of chlorophyll (F_0) in both cucumber and wheat plants was observed in this study. This effect could be an indication of the reaction centers degradation in the photosystem II, alteration in structure and content of photosynthetic pigments under stress conditions. Decline in quinone A (Q_A) capacity and its lower oxidation rate due to the slow electron flow along the electron transport chain and eventually the inactivation of photosystem II can be another reason. When quinone molecules form, the first electron acceptors in photosystem II, are in the oxidized state, the system has the lowest fluorescence. As the quinone reduces, the reaction centers in photosystem II are closed and no more electrons transfer to photosystem I, which gradually leads to an increase in fluorescence. In this enhanced fluorescence mode, the photosystem II centers are in the state of maximum chlorophyll fluorescence (F_m). Increasing F_0 under stress conditions will allow the system to reach Fm faster and reduce its efficiency for quinone reduction and electron transfer (Soheili Movahhed *et al.,* 2017). In this study, maximum chlorophyll fluorescence in cucumber increased but in wheat remained constant. Moreover, increasing F_m improved electron transfer state in cucumber. It seems that changes in beta-carotene concentration in cucumber and wheat plants were involved in this observation. Chlorophyll variable fluorescence (F_v) is an indicator to show complete quinone reduction and its increase in cucumber plant confirms the improvement of its photosynthetic status. Reduction of maximum quantum efficiency of photosystem II (F_v/F_m) in wheat plant reveals disruption of electron transfer in photosynthetic electron transfer chain of this plant. The oxidized quinone B (Q_B) accumulation under such conditions indicates no electron transfer from the reduced Q_A to the Q_B. Its reason is not clear well with the available data in the literature and the present investigation, but it seems that the decreasing CO_2assimilation due to the stomata closure in wheat plant leads to non-consumption of products derived from photosynthetic electron transfer chains ($NADPH/H^+$ and ATP) and the increasing of the reduced feredoxin. As a result of the accumulation of reduced ferredoxin, free radicals production is stimulated and leads to destruction of thylakoid membrane proteins, which are involved in the photosynthetic electron transport chain. This event reduces the rate of electron transfer, increases the chlorophyll fluorescence, and reduces the function of photosystem II and ultimately destroys the D1 subunit of this photosystem. Excessive opening of cucumber stomata (although is not beneficial for plant in the long period of time because of higher water transpiration) causes hypersensitivity to the redroot pigweed's allelochemicals. However, stomata opening leading to higher utilization of $NADPH/H^+$ and ATP in the chloroplasts for CO_2 fixation, declines the amount of reduced ferredoxin, controls the level of chlorophyll fluorescence, and finally prevents or reduces the production of free radicals. Increasing non-photochemical quenching of chlorophyll (NPQ and SV_0) in both plants after treatment by amaranth leachate indicates that the system is under challenge and both plants try to decline the reduced ferredoxin content and consequently the production of free radicals by heat dissipation. According to the obtained results of this study, the rate of non-cyclic electron transfer (ETR) decreased in wheat plant. Therefore, it seems that in this plant the production of reduced ferredoxin and free radicals is enhanced due to closing of the stomata. Then, by increasing the amount of free radicals in the photosynthetic space, D_1 subunit of photosystem II is destroyed. As a result, electron transfers to the ferredoxin and its reduction is prevented. Due to the inability to utilize this strategy, the cucumber plant, offers excessive opening of stomata and continues carbon fixation to decline the reduced ferredoxin.

4.2 Photosynthetic gas exchange parameters

Plants that were treated by amaranth leachate showed significant differences in photosynthetic gas exchange parameters compared to control. In wheat plant, a sharp decrease in net assimilation rate, transpiration rate, and stomatal conductance was observed. It seems that both stomatal and non-stomatal factors have been involved in decreasing photosynthesis rate in wheat plant. On the other hand, although decreasing stomatal conductance and increasing stomatal resistance as stomatal factors have been involved in photosynthesis decline in this plant, these are not all the reasons however. Chlorophyll fluorescence studies as well as the results of photosynthetic pigments contents indicate the involvement of non-stomatal factors in reducing photosynthesis. In the treated cucumber plants, the parameters of net assimilation rate, transpiration rate, and stomatal conductance increased. The interpretations provided for the wheat plant also justify the behavior of the cucumber plant in relation to the photosynthetic gas exchange parameters. Photosynthetic gas exchange parameters in wheat plant were more affected than cucumber, but by studying the water use efficiency and stomatal resistance, contrary evidence was witnessed. Significant decrease in water use efficiency and stomatal resistance was observed in cucumber plants treated by redroot pigweed leachate, whereas photosynthesis and transpiration rate of this plant was not increased significantly. In wheat plant, despite decrease in photosynthesis and transpiration rate due to decrease in stomatal opening, increase in water use efficiency and stomatal resistance were observed. When increasing photosynthesis, cucumber plant was exposed to very extreme water loss situation, while wheat increased water use efficiency by increasing stomatal resistance and decreasing water loss.

4.3 Photosynthetic pigments content

Treating with redroot pigweed leachate led to leaf yellowing (chlorosis) in majority of plants in this study. The cause of this chlorosis in wheat plant is the decrease in chlorophyll content of leaves, a phenomenon that has been noticed in the literature (Borella *et al.,* 2014, Dehghani *et al.,* 2014, Singh and Sunaina, 2014). Probably one of the reasons for the decrease in photosystem II efficiency and photosynthesis fall down in wheat is the decrease in leaf chlorophyll content. In cucumber species, the cause of leaf yellowing was different from that in wheat. In the other hand, under allelopathic stress condition, cucumber plants showed a significant increase in carotenoid pigment content, which could be interpreted as a resistance mechanism in this plant. There is no doubt that one of the detrimental effects of amaranth allelopathy is the induction of oxidative stress in plants (Bakhshayeshan-Agdam *et al.,* 2019). The xanthophyll cycle is one of the well-known mechanisms involved in resistance to oxidative stress (Salehi-lisar and Bakhshayeshan-agdam, 2016) and an increase in the concentration of components of this cycle can serve to the benefit for plants under stress. After chlorophyll a, chlorophyll b is one of the most important photosynthetic pigments. Besides, the ratio of chlorophyll a to b is also determinant in plants and studies show a decrease in this parameter in both plants. In addition to total carotenoid content, beta-carotene and lutein concentrations as two carotenoid pigments that play a key role in photosystem II antennae were also investigated. Beta-carotene is a pigment that supplies the energy needed to chlorophyll stimulation, reducing its content and function in photosystem II antenna causes reducing of chlorophyll excitation and decreasing of photosystem II function in electron transport (Telfer, 2002), -an event that was observed in wheat plant. Lutein was known as the most important xanthophyll in plants which forms 60% of total xanthophyll and 40% of total leaf carotenoid. This pigment acts as a non-photochemical quenching of chlorophyll in reaction centers and decline in its content is compensated by enhancing other carotenoids involved in photosystem II antenna such as beta-carotene (Dall'Osto et al., 2006), this occurrence was observed in cucumber plant.

4.4 Molecular studies

The results of gene expression analysis showed that allelochemical compounds altered the expression of PsbA and PsbS genes directly or indirectly. Interestingly, these changes were dependent on plant species; so it seems that the strategy and ability of each plant for tolerating allelopathic stress determines the fate of changes in gene expression. Increasing PsbA gene expression in wheat plant (which encodes D1 subunit of photosystem II due to increasing chlorophyll fluorescence) declines the use of reduced coenzymes, reduction of stomatal opening, and subsequently reduction of CO_2input and carbon fixation and ultimately reduction of photosystem II efficiency and increasing risk of D1 subunit degradation in this photosystem are quite obvious.

In fact, by continuously replacing degraded D1 protein, wheat plant retains the production of $NADPH/H^+$ and ATP to fixate respiratory carbon and avoid water loss, whereas the decline in cucumber's PsbA gene expression caused cucumber not to be able to continuously replace degraded D1 protein. According to the increasing chlorophyll fluorescence in this plant and extreme risk of D1 subunit degradation, cucumber plant increased the stomatal opening and photosynthesis rate to reduce the excess electron in the photosynthetic electron transport chain. The results of PsbS gene expression confirm the interpretations provided for both plants. The expression of this gene was decreased in both plants, but it was statically significant (p *[?]* 0.01) only in cucumber plant (99.93%). Decline in PsbS gene expression led to the destruction of the optical antenna components. Destruction of antenna structure, reducing the energy supply for excitation of reaction centers, especially in photosystem II, led to better control of excess electron entry into the photosynthetic electron transfer chain. The results of molecular docking showed that the proteins encoded by PsbA and PsbS can interact directly with thymol, carvacrol, beta-elemene, trans-caryophyllene, germacrene-D, spathulenol, and 5alpha-pregnane compounds. These compounds are well-known allelochemicals in plants. Therefore, it can be concluded that allelochemicals not only indirectly (such as induction of oxidative stress) but also directly are able to decrease protein's function by binding to their structure. All of the above mentioned compounds have the ability to bind to proteins encoded by the PsbA and PsbS genes, and among them the 5alpha-pregnane found in wheat shoot has the best structure for this binding.

5 CONCLUSION

Redroot pigweed allelopathy leads to alteration in photosynthetic gas exchange parameters of studied plants and the suppression of photosynthetic electron transfer chain. In addition, increasing electron leakage due to decline in reduced coenzymes utilization and increasing free radicals in the photosynthetic space has been observed. The partial return of emitted electrons to the photosystem II reaction centers along with the cyclic electron transfer chain results in the continuation of ATP production without $NADPH/H^+$ production. Free radicals' presence in the photosynthetic space as well as the constant return of electrons to photosystem II reaction centers make the structure of this photosystem vulnerable, causing subunits to be destroyed, especially D1 subunits. In addition to the indirect effect, some allelochemicals present in the amaranth leachate, are also able to directly bind to D1 subunits and destroy its structure. Finally, redroot pigweed allelopathy can affect the expression of photosynthetic genes in the treated plants and the quantity and quality of these effects are dependent on studied plant species.

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TABLE 1 The sequences of the designed primers, the resulting protein name and its molecular weight (KDa), the length of the fragment amplified by the primers (bp) and the PCR program for the main genes and the internal control

Subunit	Gene	MW	Sequence	Fragment length
Main genes	**Main genes**	**Main genes**	**Main genes**	**Main genes**
PsbA	D_1	39	F: AATAGGGAACCGCCGAATAC R: GTATGCGTCCTTGGATTGCT	220
PsbS	CP_{22}	22	F: CTCAGCCCAAAGTTCACCAT R: CCACCAGACGTTCCAAAGAT	175
Internal control	**Internal control**	**Internal control**	**Internal control**	**Internal control**
RID2	18S rRNA	32	F: GAGAAACGGCTACCACATCCA	252

Subunit	Gene	MW	Sequence	Fragment length
			R: CCCAACCCAAGGTCCAACTAC	

TABLE 2 The effects of redroot pigweed leachate on chlorophyll fluorescence parameters of cucumber and wheat plants. The data represent the mean of three replications ± SD and different letters in each parameter for each species indicate significantly different values at$p$ [?]0.01

Parameters	Cucumber	Cucumber	Wheat	Wheat
	-Leachate	+Leachate	-Leachate	+Leachate
$\mathbf{F_0}$	146.00±4.00^b	162.66±2.89^a	151.33±3.21^b	199.66±3.20^a
$\mathbf{F'_0}$	140.30±3.41^a	147.39±0.43^a	139.08±3.35^a	165.19±13.01^a
$\mathbf{F_m}$	771.00±29.82^b	897.00±13.23^a	538.33±11.37^a	436.66±37.88^a
$\mathbf{F'_m}$	577.00±28.21^a	575.33±26.54^a	538.33±11.37^a	436.66±47.88^a
$\mathbf{F_v}$	616.00±19.31^b	741.00±21.79^a	582.00±37.61^a	615.00±13.53^a
$\mathbf{F'_v}$	436.69±31.17^a	427.94±26.62^a	399.25±8.15^a	371.47±19.33^a
$\mathbf{F_v/F_m}$	0.79±0.02^a	0.82±0.01^a	0.81±0.04^a	0.73±0.03^b
$\mathbf{F'_v/F'_m}$	0.76±0.01^a	0.74±0.01^a	0.74±0.01^a	0.61±0.09^a
NPQ	0.30±0.04^b	0.42±0.04^a	0.30±0.09^b	0.55±0.13^a
ETR	127.05±2.84^a	124.90±2.03^a	124.60±0.26^a	103.16±15.37^b
$\mathbf{q_0}$	0.04±0.009^b	0.09±0.01^a	0.08±0.004^a	0.10±0.09^a
$\mathbf{q_P}$	0.95±0.01^a	0.96±0.01^a	0.95±0.03^a	0.99±0.03^a
$\mathbf{q_A}$	0.81±0.002^b	0.84±0.01^a	0.81±0.02^a	0.80±0.02^a
Rfd	3.76±0.25 ^{b}	4.51±0.26^a	3.62±0.69^a	3.86±0.22^a
$\mathbf{SV_0}$	0.04±0.01^b	0.1±0.01^a	0.09±0.005 ^{b}	0.2±0.09^a

F'_0, F_0: Minimal fluorescence from dark- and light-adapted leaf, respectively (PSII centers open).; F'_m, F_m: Maximal fluorescence from dark- and light-adapted leaf, respectively (PSII centers closed).; F'_v, F_v: Variable fluorescence from dark- and light-adapted leaves, respectively (QA reduction).; F_v/F_m: Maximum quantum yield of PSII photochemistry.; F'_v/F'_m: PSII maximum efficiency, provides an estimate of the maximum efficiency of PSII photochemistry at a given PPFD, which is the PSII operating efficiency if all the PSII centers were open (QA oxidized).; NPQ: Non-photochemical quenching.; ETR: Electron transport rate.; q_0: Relative changes of minimum fluorescence of chlorophyll a.; q_P: Photochemical quenching coefficient.; q_A: Absolute quenching.; Rfd: Ratio of chlorophyll fluorescence decrease to steady-state or vitality index.; SV_0: Non-photochemical quenching of minimum fluorescence of chlorophyll a

TABLE 3 The effects of redroot pigweed leachate on photosynthetic gas exchange parameters of cucumber and wheat plants. The data represent the mean of three replications ± SD and different letters in each parameter for each species indicate significantly different values at p [?]0.01

Parameters	Cucumber	Cucumber	Wheat	Wheat
	-Leachate	+Leachate	-Leachate	+Leachate
A ($\mu\mu o\lambda\ \mu^{-2}\ \varsigma^{-1}$)	2.37±0.15^b	2.89±0.10^a	2.17±0.41^a	1.39±0.25 ^{b}
E ($\mu\mu o\lambda\mu^{-2}\ \varsigma^{-1}$)	0.84±0.21^b	1.10±0.01^a	1.60±0.23^a	0.36±0.12^b
g_s (mol m^{-2} s^{-1})	2.10±0.74^b	2.93±0.56^a	0.54±0.05^a	0.09±0.02^b
r_s (mol m^{-2} s^{-1})	2.62±0.66^a	0.64±0.5 ^{b}	2.40±0.79^b	14.88±3.84^a
$\Omega\Upsilon$E ($\mu\mu o\lambda\ \mu o\lambda^{-1}$)	3.13±0.71^a	2.62±0.11^b	2.01±0.49^b	4.33±0.59^a

A: Net assimilation rate.; E: Transpiration rate.; g_s: Stomatal conductance.; r_s: Stomatal resistance.; WUE: Water Use Efficiency

TABLE 4 The effects of redroot pigweed leachate on photosynthetic pigments content of cucumber and wheat plants. The data represent the mean of three replications ±S D and different letters in each parameter for each species indicate significantly different values at p [?]0.01

Parameters	**Cucumber**	**Cucumber**	**Wheat**	**Wheat**
	-Leachate	+Leachate	-Leachate	+Leachate
ϑλα (μγγ^{-1} ΦΩ)	43.98±3.57 [a]	44.77±1.66[a]	140.57±16.08[a]	105.33±10.46[b]
ϑλβ (μγγ^{-1} ΦΩ)	44.69±2.25[b]	69.77±2.60[a]	20.86±3.60[a]	24.52±2.45[a]
ϑλα+β (μγ γ^{-1} ΦΩ)	88.67±3.94[b]	114.54±4.27[a]	161.43±15.58[a]	129.85±10.63[b]
Chl$_{a/b}$	1.13±0.15[a]	0.64±0.01[b]	7.16±1.10 [a]	4.33±0.65[b]
ἀρ (μγ γ^{-1} ΦΩ)	24.31±5.36[b]	37.08±1.63[a]	35.64±2.68[a]	35.11±2.86[a]
β-ςαροτεν (μγ γ^{-1} ΦΩ)	22.42±4.39[b]	29.72±4.89[a]	18.51±1.54[a]	14.81±1.02[b]
Λυτειν (μγγ^{-1} ΦΩ)	14.09±3.78[a]	8.16±2.96[b]	34.91±2.76[a]	32.01±2.35[a]

Chl$_{a(b)}$: Chlorophyll a(b).; Chl$_{a+b}$: Total Chlorophyll.; Car: Carotenoide

TABLE 5 Binding energy (ΔG) and inhibition constants (KI) of the interaction between studied allelochemicals as ligands in molecular docking and PsbA and PsbS protein complexes

Parameters	**PsbA**	**PsbA**	**PsbS**	**PsbS**
	ΔG (Kcal mol^{-1})	KI (μM)	ΔG (Kcal mol^{-1})	KI (μM)
Thymol	-4.35	647.78	-4.01	1.15
Carvacrol	-4.25	760.45	-4.17	880.93
Beta-elemene	-4.82	291.42	-4.71	352.31
Trans-caryophyllene	-5.79	57.03	-5.36	118.61
Germacrene-D	-5.71	65.43	-5.80	55.88
Spathulenol	-5.31	127.33	-4.74	322.15
5Alpha-pregnane	-7.31	4.42	-7.04	6.95

FIGURE 1 Gel images of extracted RNAs from the leaves of cucumber and wheat plants **(A)** . RT-PCR products for PsbA and PsbS genes of cucumber and wheat plants **(B)** , C: Cucumber, W: Wheat, -L: -Leachate, +L: +Leachate, NTC: No Template Control. The effects of redroot pigweed leachate on the expression of PsbA and PsbS genes of cucumber and wheat plants **(C)** , Results of relative expression and gel images of RT-PCR products match in both plant species. The data represent the mean of three replications ±SD and different letters in each parameter for each species indicate significantly different values at p [?]0.01

FIGURE 2 Best docked conformations for Thymol-PsbA system (A) and Thymol-PsbS system (B) complexes

FIGURE 3 Best docked conformations for Carvacrol-PsbA system (A) and Carvacrol-PsbS system (B) complexes

FIGURE 4 Best docked conformations for Beta-elemene-PsbA system (A) and Beta-elemene-PsbS system (B) complexes

FIGURE 5 Best docked conformations for Trans-caryophyllene-PsbA system (A) and Trans-caryophyllene-PsbS system (B) complexes

FIGURE 6 Best docked conformations for Germacrene-D-PsbA system (A) and Germacrene-D-PsbS system (B) complexes

FIGURE 7 Best docked conformations for Spathulenol-D-PsbA system (A) and Spathulenol-D-PsbS system (B) complexes

FIGURE 8 Best docked conformations for 5Alpha-pregnane-D-PsbA system (A) and 5Alpha-pregnane-D-PsbS system (B) complexes

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