Alhaithloul HAS and Soliman MH (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 2, Article number 12709
DOI:10.15835/nbha50212709
Research Article

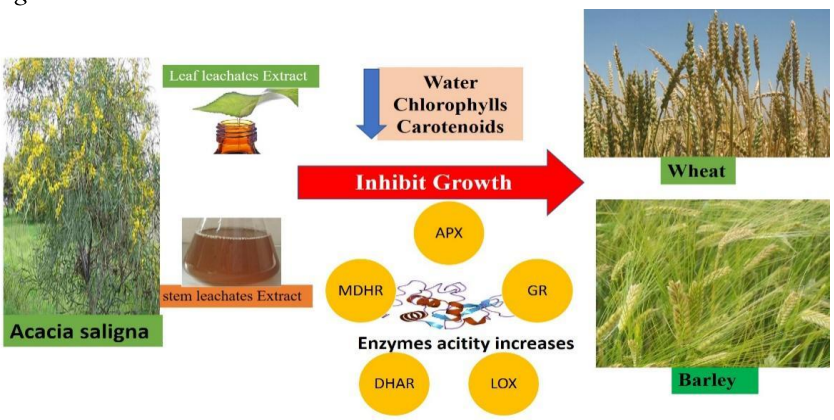
Responses of wheat and barley to *Acacia saligna* leaf and stem extracts: influence on growth and ascorbate-glutathione cycle

Haifa Abdulaziz Sakit ALHAITHLOUL[1], Mona H. SOLIMAN[2,3]*

[1]*Jouf University, College of Science, Biology Department, Sakaka 2014, Kingdom of Saudi Arabia; haifasakit@ju.edu.sa*
[2]*Cairo University, Faculty of Science, Botany and Microbiology Department, Giza 12613, Egypt; hmona@sci.cu.edu.eg (*corresponding author)*
[3]*Taibah University, Faculty of Science, Biology Department, Al-Sharm, Yanbu El-Bahr, Yanbu 46429, Kingdom of Saudi Arabia; mhhussein@taibahu.edu.sa*

Abstract

The present study aimed to study the effect of dry leaf and stem leachates of *Acacia saligna* on wheat's growth and enzyme functioning (*Triticum aestivum*) and barley (*Hordeum vulgare*). Leaf leachates (LL) and stem leachates (SL) of *A. saligna* were applied through root and nutrient solution in different concentrations i.e., 5, 10, and 15%. Treatment of LL and SL declined the growth in terms of height and dry weight in both tested plants in concentration-dependent manner with the maximal decline due to 15% LL. In addition, content of relative water, total chlorophylls, and carotenoids decreased in both wheat as well as barley. The activity of ascorbate peroxidase, monodehydro ascorbate reductase, dehydroascorbate reductase, and glutathione reductase increased considerably due to the treatment of LL and SL. The indigenous tolerance mechanisms in wheat and barley seedlings were further strengthened in wheat and barley by increased accumulation of glycine betaine, glutathione, and ascorbate in response to LL and SL treatment. Additionally, the activity of lipoxygenase and protease were increased significantly due to LL and SL treatment with a maximal increase at higher concentrations. From the present study it can be concluded that extracts of leaf and stem of *A. saligna* inhibit the growth of wheat and barley significantly with a concomitant increase in the functioning of the ascorbate-glutathione (AsA-GSH) cycle. Further, both crop species showed comparable responses to *A. saligna* leachates.



Graphical abstract

Received: 24 Mar 2022. Received in revised form: 13 Apr 2022. Accepted: 15 Apr 2022. Published online: 23 May 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Keywords: *Acacia saligna*; allelopathy; ascorbate-glutathione cycle; barley; lipoxygenase; wheat

Introduction

Several factors regulate plant growth and development. Plants release different kinds of chemicals into their surroundings, resulting in alterations in the growth of other plant species growing in their vicinity (Cruz-Ortega *et al*., 2007; Tomar and Agarwal, 2013; Kamran *et al.*, 2021). Among the plant chemicals, secondary metabolites are considered as key contributors to such effects. Such interferences in the growth of plants by these metabolites are called as allelopathy (Maqbool *et al*., 2013). Allelopathy is one of the key factors determining the growth of plants in the natural environment. Allelochemicals are released into the soil by decomposition of plant parts, root exudation, and volatilization (Inderjit and Duke, 2003; Cruz-Ortega *et al*., 2007). Allelochemicals released can impart beneficial or inhibitory effects on the growth and development of other plant species depending on their concentration and nature (Tomar *et al*., 2015; Chopra *et al*., 2017; Mir *et al*., 2021; Huang *et al*., 2021). Inhibitory effects of allelochemicals include reduced germination, reduction in photosynthetic pigments, and enzyme functioning due to the oxidative effects (Ma *et al*., 2020; Mir *et al*., 2021). It has shown that allelopathic effects of plant exudates or leachates are due to the presence of significant quantities of secondary metabolites (Kumbhar and Patel, 2016; Tomar *et al*., 2015; Li *et al*., 2021). Several research studies have confirmed the induction of oxidative stress due to the treatment of plant extracts (Huang *et al*., 2020; Zhang *et al*., 2020). Plant interactions resulting due to allelochemicals have been suggested to trigger oxidative damage (Bogatek and Gniazdowska, 2007).

Oxidative stress results due to the excess production of reactive oxygen species (ROS) in different cellular organelles thereby hampering the functioning of plant species (Ahanger *et al*., 2017; Zaid and Wani, 2019; Hasanuzzaman *et al*., 2019, 2020). Araniti *et al.* (2018) has demonstrated a significant increase in apoptotic bodies and necrotic cells in *Arabidopsis* due to the treatment of allelochemical, rosmarinic acid. Hydrogen peroxide, superoxide, and hydroxyl are the key ROS molecules that can affect deleteriously plant growth and development when present in higher concentrations (Ahanger *et al*., 2017). To counter the damaging effects of the allelopathic stress-induced ROS toxicity plants upregulate the antioxidant system and accumulate protective molecules (Tomar *et al*., 2015; Ma *et al*., 2020; Huang *et al*., 2020). Among the antioxidant molecules, the components of the ascorbate-glutathione cycle have a vital role in the maintenance of optimal ROS levels and the redox status (Anjum *et al*., 2010; Hasanuzzaman *et al*., 2019, 2020). In addition, the increased accumulation of compatible osmolytes is also improved to counter the oxidative damage to sensitive assimilatory pathways and the associated components (Tomar and Agarwal, 2013; Chen *et al*., 2015; Sadiq *et al*., 2020).

Wheat and barley are two important crop plants within the Poaceae family that are cultivated for food and contribute significantly to world food security. Various environmental factors influence their growth, development, and productivity. While as *Acacia saligna* is invasive plant species with the potential efficiency to invade agricultural land, thereby can affect the growth of crops significantly. Invasive exotic plant species can influence other crop plants' growth and distribution patterns (Tomar *et al*., 2014). With this view in the present study, it was hypothesized that the extracts of *A. saligna* can influence the growth and chlorophyll synthesis of wheat and can modulate the functioning of the ascorbate-glutathione cycle and oxidative stress parameters.

Materials and Methods

Preparation of leaf and stem extracts of Acacia saligna

Leaf and stem parts of *A. saligna* were harvested and washed with distilled water. After drying the tissues at room temperature, samples were powdered finely. After that 5, 10, and 15 gm of powdered leaf and stem of *A. saligna* were added to 100 mL distilled water. After thorough shaking were left for 48 hours at room temperature. Thereafter, the extracts were passed through muslin cloth and subsequently filtered through Whatman No. 1 filter paper. After centrifuging the extract at 3000 pm, the final volume was made to 100 mL and was stored at 4 °C until further use.

Experimental treatments

Wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) seeds were surface sterilized with sodium hypochlorite (5%) for 5 minutes and washed thoroughly by distilled water. Sterilized seeds were sown in earthen pots (25 cm diameter) filled with soil, sand, and compost in the ratio of 4:1:1. At the time of sowing, pots were wetted with 200 mL Hoagland solution. After germination number of seedlings per pot was thinned to 10 per pot and irrigated with 200 mL Hoagland solution every alternate day. Ten days after seedling growth, pots were divided into different groups and treated with different leaf and stem leachates concentrations. The details of treatments used were as: (a) Control (Hoagland), (b) 5% leaf leachate (LL), 10% LL, 15 LL, 5% stem leachate (SL), 10% SL and 15% SL. For leachates preparation, the components of Hoagland solution were dissolved in leachate solutions. After twenty days of the leachate treatment plant, tissue was harvested and analysed for different parameters described below.

Estimation of photosynthetic pigments, gas exchange parameters, and chlorophyll fluorescence

100 mg fresh leaf tissue was macerated in acetone using pestle and mortar. After centrifuging the homogenate at 3000 rpm for 20 minutes the absorbance of the supernatant was read at 480, 645, and 663 nm (Arnon, 1949). For measurement of photosynthetic efficiency, intercellular CO_2 concentration, and stomatal conductance, an infrared gas analyzer (CID-340, Photosynthesis System, Bioscience, Washington, USA) was used and measurements were recorded on a fully expanded leaf. Chlorophyll fluorescence parameters including maximum photochemical efficiency, photochemical quenching, and non-photochemical quenching were measured using Chlorophyll Fluorometer (PAM 2500; Walz, Germany) and leaves were dark-adapted for 30 min.

Estimation of RWC and glycine betaine

The relative water content (RWC) of the leaf was measured according to Smart and Bingham (1974). Briefly, twenty leaf discs were punched from the fresh leaves of control and treated seedlings and their fresh weight (FW) was recorded immediately. Thereafter, the same leaf discs were immersed in Petri dishes filled with distilled water, and after one-hour, turgid weight (TW) was taken. Same discs were oven-dried at 80 °C for 24 hrs, and dry weight (DW) was recorded. The calculation was done using the following formula:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

For the estimation of glycine betaine (GB) dry powdered wheat and barley leaves were extracted in distilled water. After extraction 2N H_2SO_4 was added to the samples, a cold KI-I2 reagent was subsequently added. After that the mixture was centrifuged at 10,000rpm for 15 minutes and the supernatant was aspirated, and the periodide crystals were dissolved in 1, 2-dichloroethane. The optical density was recorded at 365 nm and for calculation, the standard curve of GB was used (Grieve and Grattan, 1983).

The activity of protease and lipoxygenase

Protease activity was determined according to the method of Green and Neurath (1954) as described by Mir *et al.* (2021). Fresh 100 mg tissue was extracted in 50 mM phosphate buffer (pH 7.4) supplemented with 1% PVP. After centrifuging at 5000 rpm, the supernatant was reacted with casein at 40 °C for one hour. After that chilled 20% TCA was added and the resultant was incubated for 3 hours at 4 °C. After centrifuging the samples for 15 minutes at 5000 rpm supernatant was reacted with Folin-Ciocalteu's reagent in an alkaline medium was added and the optical density was recorded at 660 nm. The activity was expressed as an amount of tyrosine released per mg protein. The activity of lipoxygenase was measured according to the method of Doderer *et al.* (1992). After extraction of tissue linoleic acid was used as a substrate to determine the activity of lipoxygenase in the supernatant and change in absorbance was monitored at 234 nm. An extinction coefficient of 25 mM^{-1} cm^{-1} was used for calculation.

Assay of activities antioxidant enzymes

Antioxidant enzymes were extracted by homogenizing fresh 1 gm leaf tissue in chilled phosphate buffer (50 mM; pH 7.0) supplemented with 1% polyvinyl pyrrolidine, 0.1 mM PMSF, and 1 mM EDTA using a prechilled pestle and mortar. Thereafter the extract was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was collected and used as an enzyme source for enzyme activity as described below:

For assaying the activity of ascorbate peroxidase (APX, EC 1.11.1.11) method of Nakano and Asada (1981) was used in an assay mixture of 1 mL containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, and enzyme extract. The reaction was initiated by the addition of hydrogen peroxide and a change in absorbance at 290 nm was recorded for 3 minutes. For calculation, an extinction coefficient of 2.8 mM^{-1} cm^{-1} was used.

For the activity of glutathione reductase (GR; EC 1.6.4.2), the assay mixture contained sodium phosphate buffer (100 mM; pH 7.8), nicotinamide adenine dinucleotide phosphate (0.1 mM; NADPH), oxidized glutathione (0.5 mM; GSSG), and enzyme extract. Change in optical density was recorded at 340 nm for 2 min, and an extinction coefficient of 6.2 mM^{-1} cm^{-1} was used for calculation (Foyer and Halliwell, 1976).

The activity of DHAR (EC: 1.8.5.1) was assayed in an assay mixture containing 50 mM potassium phosphate buffer (pH 7.0), 2.5 mM GSH, 100 μM DHA, and enzyme extract. Change in absorbance was monitored at 265 nm for 2 min and a calculation extinction coefficient of 14 mM^{-1} cm^{-1} was used (Nakano and Asada, 1981).

The activity of MDHAR (EC: 1.6.5.4) was measured according to the method of Hossain *et al.* (1984). Assay mixture (1 mL) containing Tris–HCl buffer (50 mM; pH 7.5), NADPH (0.2 mM), AsA (2.5 mM), 0.5 U of ascorbate oxidase and enzyme extract. Change in absorbance was recorded at 340 nm for 2 min and an extinction coefficient of 6.2 mM^{-1} cm^{-1} was used for calculation.

Estimation of ascorbate and reduced glutathione

The content of ascorbate (AsA) was estimated by following the method described by Mukherjee and Choudhuri (1983). Fresh plant material was homogenized in 6% TCA using pestle and mortar. After centrifuging the extract at 5000 rpm for 10 min, the supernatant was reacted with dinitrophenylhydrazine (2%) and thiourea (10%). After boiling the tubes in a water bath for 15 min samples were cooled and 80% H_2SO_4 was added. Absorbance was taken at 530 nm and calculation was done using a standard curve of ascorbate. For estimation of reduced glutathione (GSH) content, fresh leaf tissue was homogenized in phosphate buffer (pH 8.0) using pestle and mortar. The extract was centrifuged at 3000rpm for 15 minutes and 0.5 mL supernatant was mixed with 0.5 mL of 5, 5-dithiobis-2-nitrobenzoic acid. After 10 minutes the absorbance was recorded at 412 nm and calculation was done using a standard curve of GSH (Ellman, 1959).

Assay of glyoxylase I and glyoxylase II activity and the content of methylglyoxal

For assaying the activity of glyoxylase I method described by (Hasanuzzaman *et al.*, 2011) was followed. The assay mixture containing 0.1 M phosphate buffer, GSH (100 mM), 16 mM $MgSO_4$ and reaction were started by 35 mM methylglyoxal and change in absorbance at 240 nm was taken for 2 min. The method as described by (Nahar *et al.*, 2017) was followed for assaying Glyoxylase II activity. The assay mixture contained Tris-buffer (pH 7.2) containing DTNB, S-D-lactoylglutathione, enzyme, and formation of GSH was monitored at 412 nm. Content of methylglyoxal was estimated according to the method of (Wild *et al.*, 2012) and absorbance was read at 288 nm.

Statistical analysis

The data presented is the mean (±SE) of three replicates. ANOVA was performed and the least significant difference was calculated at LSD $P<0.05$.

Results

Results showing the effect of LL and SL of *A. saligna* on the plant height and dry biomass are shown in Table 1. Relative to control plants treated with LL and SL exhibited declined height and dry weight with the maximal decline due to the higher concentrations, however, LL imparted much decline as compared to SL. At 15% leachate concentrations, the percent decline in plant height was 47.11% and 29.80% for wheat and 32.97% and 18.69%for barley due to LL and SL respectively. The decline in plant dry weight was 41.00% and 41.51% in wheat and barley respectively due to 15% LL treatment however decline due to 15% SL was only 29.23% and 24.09% respectively (Table 1).

Table 1. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the height (cm) and plant dry weight (gram per plant) in wheat and barley

Treatment	Plant height		Plant dry weight	
	Wheat	Barley	Wheat	Barley
Control	34.66±2.08	30.33±2.08	1.293±0.105	1.190±0.095
5% LL	31.61±1.51	29.33±1.52	1.141±0.057	1.011±0.028
10% LL	25.66±1.52	24.66±2.08	0.8983±0.064	0.8690±0.033
15% LL	18.33±2.5	20.33±1.52	0.7628±0.033	0.6976±0.058
5% SL	33.33±2.08	29.66±2.08	1.267±0.058	1.108±0.096
10% SL	29.33±1.52	27.33±1.15	1.048±0.095	0.9542±0.046
15% SL	24.33±1.52	24.66±2.08	0.9150±0.017	0.9033±0.021

Values are mean (±SE) of three replicates.

Content of total chlorophylls and carotenoids also showed a steep decline with increasing concentrations of leachates of leaf and stem (Table 2). The decline was less at lower concentrations and maximum at higher concentrations. In wheat total chlorophylls declined by 9.17%, 23.30% and 45.79% due to 5%, 10% and 15% LL, and by 4.18%, 16.62% and 35.18% due to 5%, 10% and 15% SL treatment over control. In barley observed decline in total chlorophylls was 2.79%, 23.50% and 41.54% due to 5%, 10% and 15% LL, and by 8.5%, 19.45% and 37.08% due to 5%, 10% and 15% SL treatment over control. A similar trend was observed in carotenoids with the maximal decline of 47.81% (wheat) and 57.36% (barely) at 15% LL treatment and 21.81% (wheat) and 46.77% (barely) at 15% SL (Table 2).

Table 2. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the total chlorophylls (mg /g FW), carotenoids (mg /g FW) and relative water content (RWC, percent) in wheat and barley

Treatment	Total Chlorophylls		Carotenoids		RWC	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control	2.103±0.195	2.400±0.264	0.5051±0.0698	0.8000±0.034	89.23±3.95	90.76±3.16
5% LL	1.910±0.104	2.333±0.152	0.4727±0.0311	0.6870±0.133	83.70±3.17	82.51±2.66
10% LL	1.613±0.109	1.836±0.158	0.3434±0.0311	0.4622±0.043	72.66±3.96	75.20±3.91
15% LL	1.14±0.234	1.403±0.115	0.2636±0.0304	0.3411±0.011	63.73±3.80	67.53±2.76
5% SL	2.015±0.103	2.196±0.176	0.5021±0.0154	0.7150±0.019	84.06±3.68	87.06±3.49
10% SL	1.753±0.121	1.933±0.230	0.4322±0.0112	0.6289±0.062	76.83±5.40	79.34±4.03
15% SL	1.363±0.076	1.51±0.079	0.3949±0.0259	0.4258±0.023	67.40±2.91	73.63±4.08

Values are mean (±SE) of three replicates.

Relative to control, treatment of LL and SL resulted in a significant decline in the stomatal and non-stomatal characters of photosynthesis in both barley and wheat (Figures 1 and 2). The maximal decline was observed due to a higher concentration of leachates. Percent decline in photosynthesis, stomatal conductance and intercellular CO_2 was 34.14, 24.15, and 21.01% (due to 15% LL) and 26.01, 12.72 and 12.55% (due to 15% SL) in barley and 37.55, 17.15 and 24.46% (due to 15% LL) and 23.39, 11.33 and 10.07% (due to 15% SL) in wheat over control (Figure 1A-C). The decline in fluorescence parameters like PSII (Fv/Fm), qP, and ETR was obvious in both barley and wheat however NPQ was observed to register an increase. In barley Fv/Fm, qP and ETR decreased maximally by 14.91, 24.67, and 31.96% due to 15% LL over control while as in wheat the decline was 15.84, 26.66, and 37.06% respectively. NPQ increased due to both LL and SL with a maximum increase of 34.24% (barley) and 38.93% (wheat) due to LL. (Figure 2A-D).

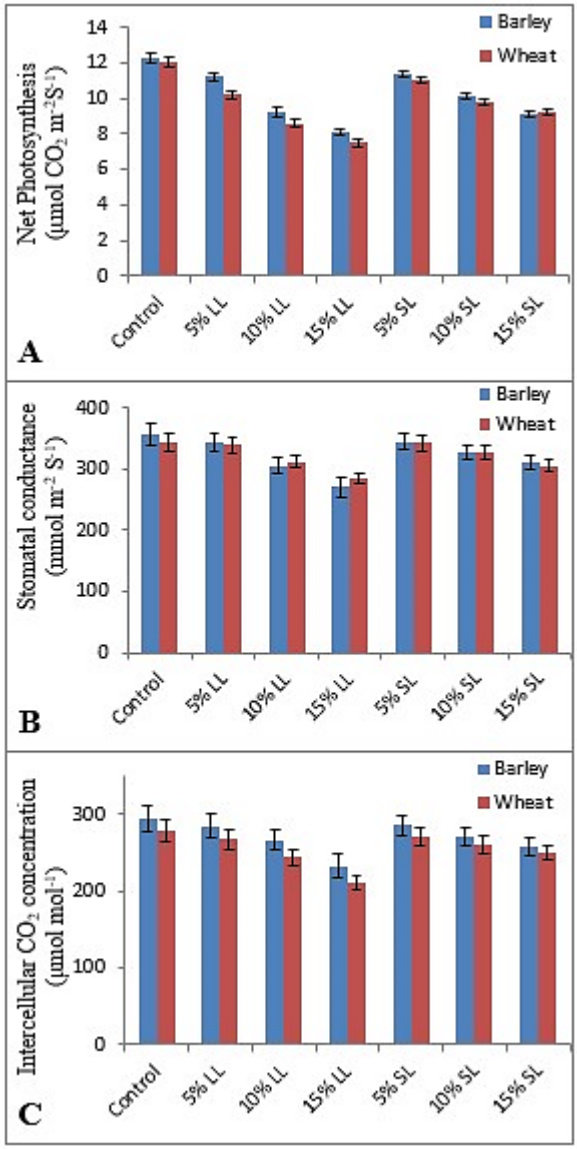


Figure 1. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the (A) net photosynthesis, (B) stomatal conductance and (C) intercellular CO2 concentration in wheat and barley

Values are mean (±SE) of three replicates.

Relative water content (RWC) declined with the treatment of leaf and stem leachates with maximal decline registered due to higher concentrations of leachates of both leaf and stem. Leaf leachates triggered more decline in RWC of both plants (Table 2). Relative to control, RWC declined by 28.57% and 24.46% due to 15% LL and 15% SL in wheat while a decline of 34.39% and 18.87% was observed in barley due to 15% LL and 15% SL respectively (Table 2).

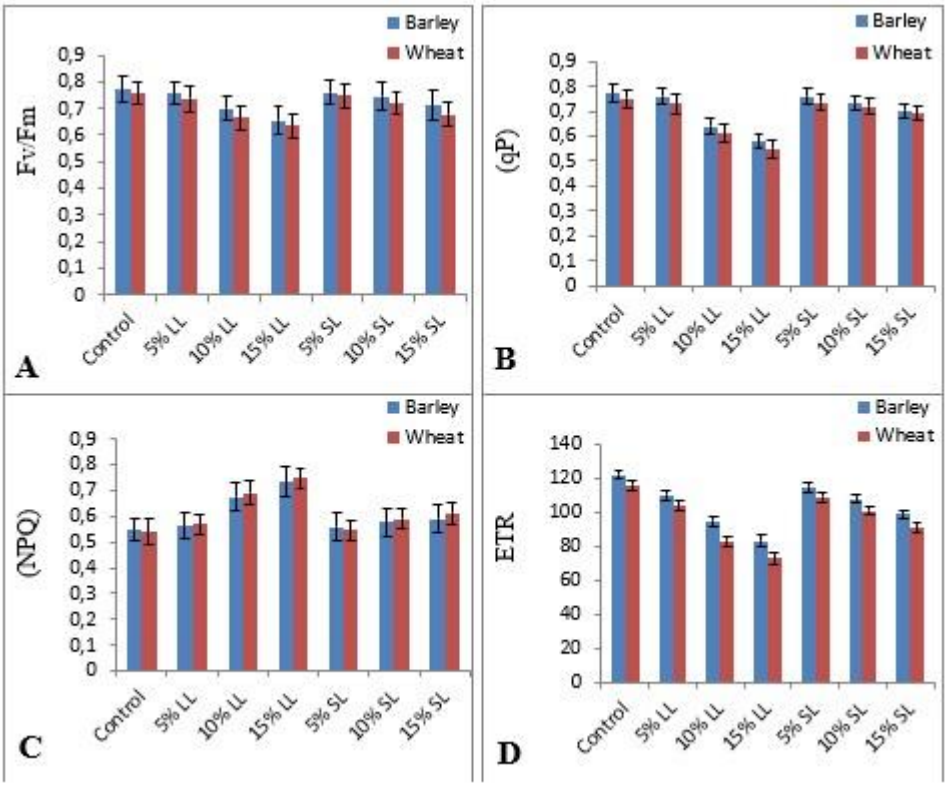


Figure 2. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on (A) PSII activity, (B) photochemical quenching, (C) non photochemical quenching and (D) electron transport rate in wheat and barley
Values are mean (±SE) of three replicates.

Treatment of leaf and stem leachates increased the activity of lipoxygenase and protease in both wheat and barley plants (Figure 3A and B). Wheat seedlings showed relatively higher activities of lipoxygenase and protease as compared to barley. The activity of lipoxygenase increased maximally by 178.08% and 140.79% in wheat and barley due to 15% LL treatment and showed 124.90% (wheat) and 106.43% (barley) increase due to 15% SL treatment over control (Figure 3A and B). Similarly, protease exhibited a maximal increase of 82.20% and 79.23% in wheat and barley at 15% LL treatment (Figure 3A and B).

Seedlings treated with leachates of leaf and stem of *A. saligna* registered an increase in the content of glycine betaine in a concentration-dependent manner with maximal content observed in seedlings treated with 15% of leachates. Relative to control seedlings, glycine betaine increased maximally by 83.69% and 49.60% due to 15% concentrations of LL and SL respectively in wheat, while as in barley percent increase was 54.21% and 22.76% (Figure 4).

The treatment of leaf and stem leachates of *A. saligna* resulted in increased functioning of the ascorbate-glutathione cycle (Figure 5 and 6). In wheat, the activity of APX, MDHAR, DHAR, and GR increased maximally by 56.77, 65.20, 78.46%, and 60.81% due to 15% LL and by 28.51, 37.17, 61.20%, and 43.24% due to 15% SL over control. However, in barley increase in APX, MDHAR, DHAR, and GR was 58.21, 68.32, 70.31%, and 38.14% in seedlings treated with 15% LL and 27.14, 31.39, 45.07%, and 18.23% with 15% SL (Figure 5). Contents of GSH and AsA also exhibited an increase with leachate treatment. Relative to control, GSH maximally increased at a higher concentration of leachates with a percent increase of 32.05% and 13.91% in wheat and 41.70% and 14.44% in barley due to 15% LL and 15% SL respectively (Figure 6). However, the content of AsA increased maximally by 23.45% and 11.64 % due to 10% LL and SL in wheat, while barley exhibited a maximal increase of 29.81% and 18.26% due to 15% LL and SL (Figure 6).

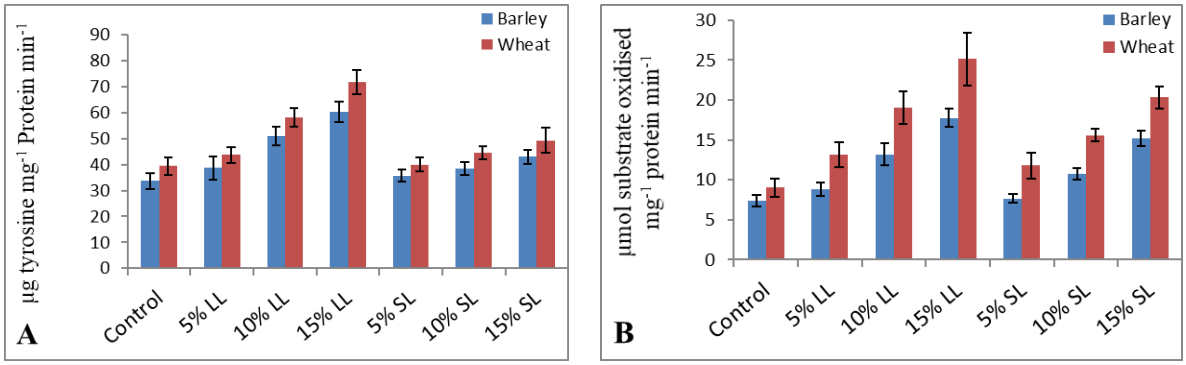


Figure 3. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the activity of (A) protease and (B) lipoxygenase in wheat and barley
Values are mean (±SE) of three replicates.

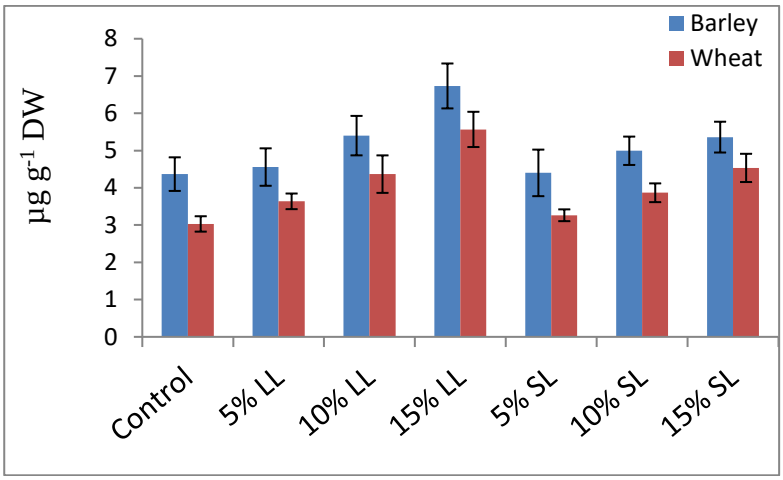


Figure 4. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the content of glycine betaine in wheat and barley
Values are mean (±SE) of three replicates

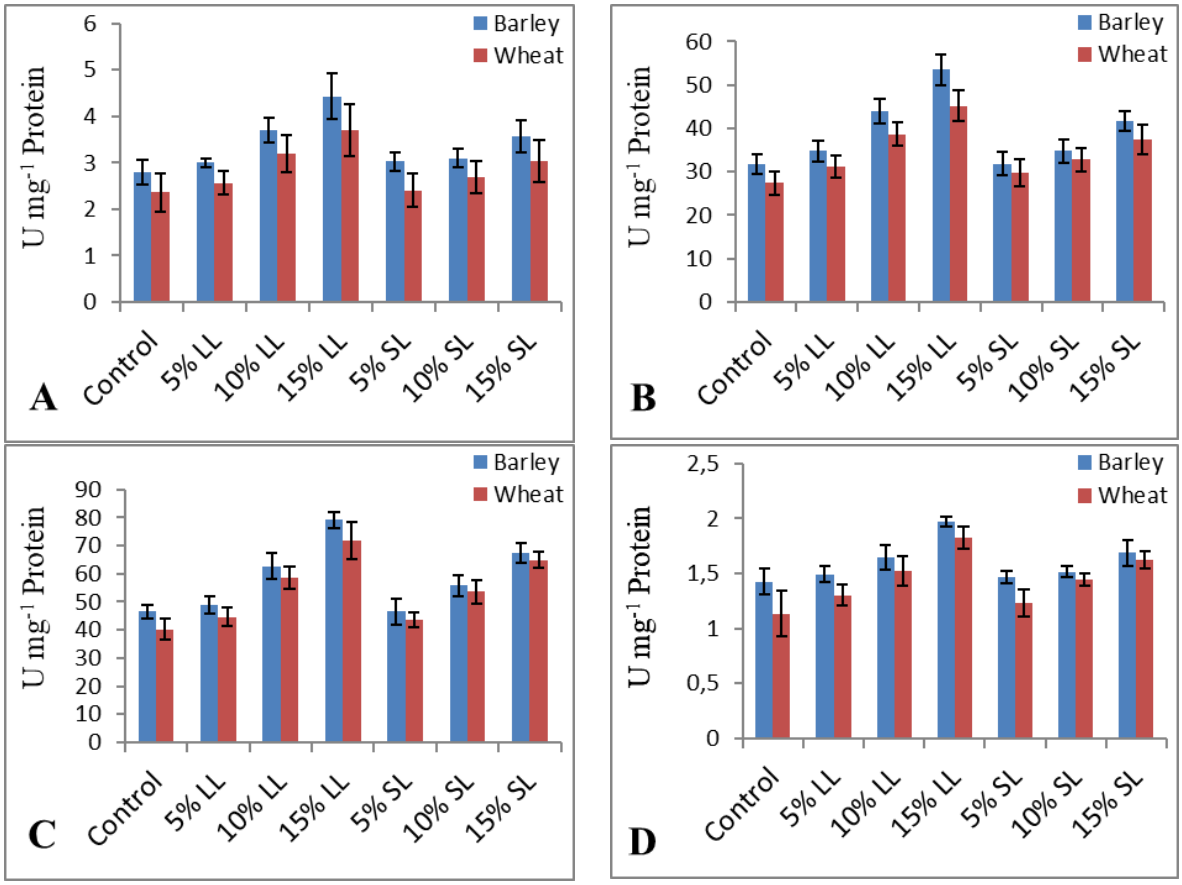


Figure 5: Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the activity of (A) ascorbate peroxidase, (B) monodehydroascorbate reductase, (C) dehydroascorbate reductase and (D) glutathione reductase in wheat and barley
Values are mean (±SE) of three replicates.

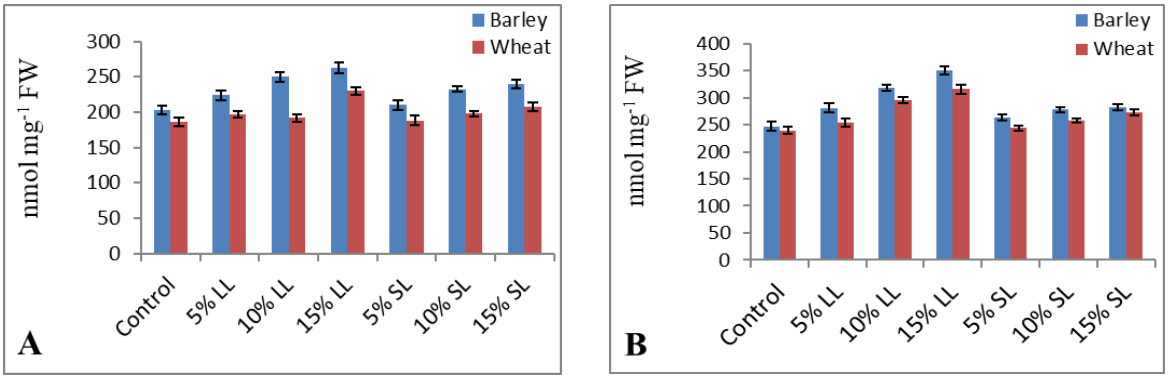


Figure 6. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the content of (A) ascorbic acid and (B) reduced glutathione in wheat and barley
Values are mean (±SE) of three replicates.

Effect of LL and SL of *A. saligna* on the activity of glyoxylase I and the glyoxylase II is shown in figure 7A and B. Relative to control, activity increased significantly due to LL and SL and attained a maximal increase at 15% concentration of leachates. Relative to control the activity of glyoxylase I and the glyoxylase II increased maximally by 46.41% and 53.43% in barley and by 31.99% and 53.19% in wheat due to 15% LL. Content of methylglyoxal increased maximally by 15% LL and SL. In 15% LL treated barley and wheat methylglyoxal increased by 84.50% and 74.80% respectively (Figure 7C).

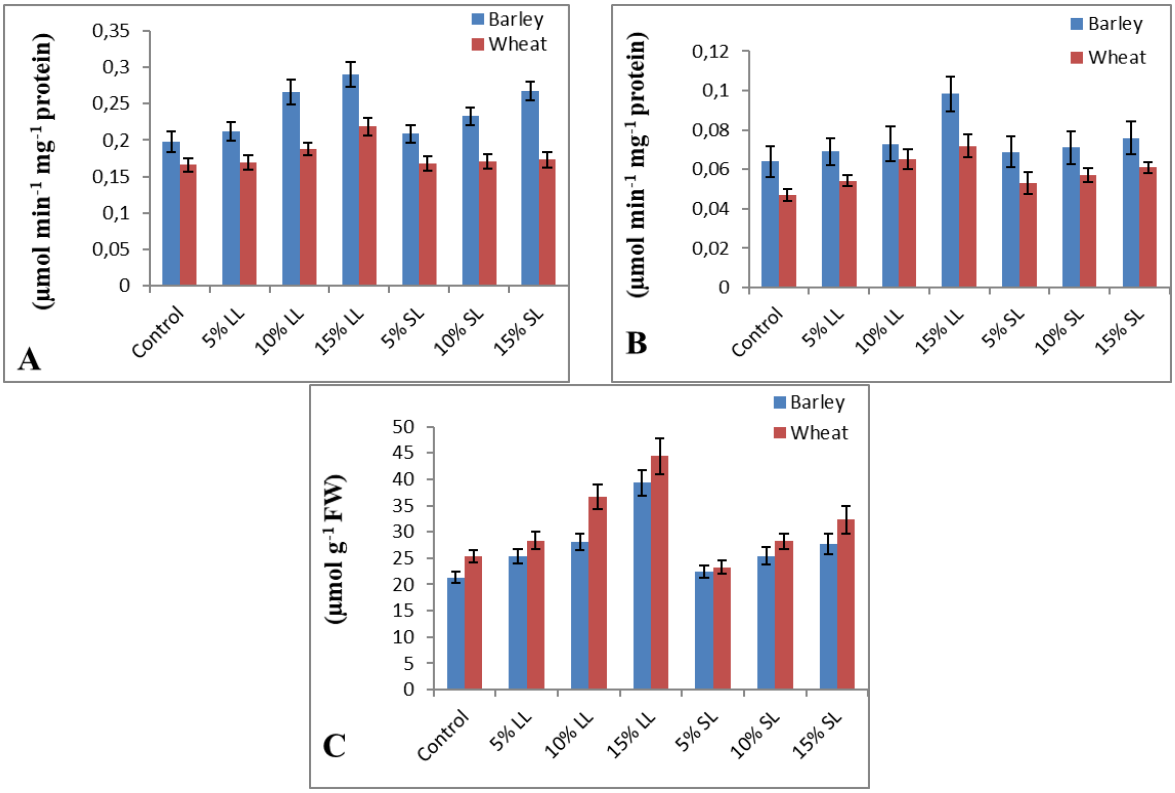


Figure 7. Effect of different concentrations of dry leaf leachate (LL) and stem leachate (SL) of *A. saligna* on the activity of (A) glyoxylase I, (B) glyoxylase II and (C) methylglyoxal content in wheat and barley Values are mean (±SE) of three replicates.

Discussion

Allelopathy stress is of serious threat to global food security. *A. saligna* shows invasive habits and can cause a considerable decline in the growth of existing flora (El-Gawad and El-Amier, 2015). Most of the studies have attributed the allelopathic potential of plant species to the presence of vital secondary metabolites that can inhibit metabolism either through hindering biochemical or physiological functioning (Scavo *et al.*, 2019; Mir *et al.*, 2018, 2021). Allelochemicals can affect the endogenous hormone levels by influencing their metabolism (Yang *et al.*, 2005) therefore can impart damaging effects on plant growth. Moreover, the damage to plant growth is due to allelochemical mediated modulation of the soil characteristics (Scavo *et al.*, 2019). The present study also observed that extracts of *A. saligna* declined the height and dry mass accumulation of wheat and barley plants significantly in a concentrations-dependent manner. Reduced growth and biomass in *A. saligna* treated wheat and barley can be attributed to: (a) reduction in cell division, (b) impaired hormonal homeostasis, and (c) restricted water uptake leading to oxidative damage. Earlier several studies have reported a decline in cell division due to the treatment of allelochemicals, mostly secondary metabolites (Hallak *et al.*, 1999; Cai and Mu, 2012). Reduced growth due to *A. saligna* extracts can be due to secondary metabolites. Treatment of *Aglaia odorata* extracts inhibited the growth of *Allium cepa* by hampering the mitosis (Teerarak *et al.*, 2012). In soybean, treatment of *Datura stramonium* extracts significantly reduced root growth due to declined cell division leading to a reduction in root elongation (Cai and Mu, 2012). In wheat, Tomar and Agarwal (2013) have also demonstrated reduced growth in terms of plant height and biomass accumulation due to treatment of *Jatropha* sp. leachates. Similarly, Javaid *et al.* (2006) has reported a significant decline in shoot biomass of *Parthenium hysterophorus* due to the treatment of allelopathic crop extracts.

Reduced growth and biomass production in *A. saligna* extract-treated wheat and barley plants were accompanied with a significant decline in the total chlorophylls and carotenoids. Similar to our results the decline in the synthesis of chlorophyll and carotenoids due to treatment of plant extracts have been reported by others as well (Tyagi and Agarwal, 2011; Shah *et al*., 2017; Tomar *et al*., 2015). Plant extracts reduce the uptake of mineral elements like nitrogen that form a key component of chlorophyll (Tomar and Agarwal, 2013). Extracts from *Datura stramonium* reduce the chlorophyll content of *Cenchrus ciliaris* and *Neonotonia wightii* resulting in declined growth (Elisante *et al*., 2013). Allelochemicals induce oxidative stress and can trigger damage to chlorophyll pigments by increasing ROS formation. Under stress conditions, the activity of chlorophyll synthesis enzymes is down-regulated while those involved in degradation are upregulated (Turan and Tripathi, 2012). In the present study, the treatment of *A. saligna* extracts may have triggered the accumulation of ROS and the degradation of pigments with a concomitant decline in the biosynthetic components. Besides this, the declined chlorophyll synthesis in *A. saligna* treated seedlings were observed to exhibit a considerable decline in stomatal and non-stomatal parameters of photosynthesis. Reduced photosynthesis, intercellular CO_2, stomatal conductance, PSII activity, photochemical quenching, and electron transport rate in leachate treated plants were evident more in LL treatments. Reduced entry of CO_2 results due to reduction in a number of stomata and closure due to the effects of stresses on water relation (Lawson, 2009). Allelochemicals adversely affect the chlorophyll fluorescence by declining PSII functioning and photochemical quenching (Hussain and Reigosa, 2011). Reduced electron transport in plants due to pressure exerted by invasive plants results due to the presence of allelochemicals (Zhang *et al*., 2016). Besides this, the decline in acceptors and donors of photons and electrons in the photosynthetic units has been considered a reason for declined photochemical efficiency (Zhang *et al*., 2016). Reduced photosynthesis, stomatal conductance, intercellular CO_2 and PSII functioning due to allelopathic stress severely affects carbohydrate metabolism and growth (Ding *et al*., 2019).

Further, it was observed that treatment of dry leachates resulted in an increase in the activity of lipoxygenase and protease, with a much obvious increase in seedlings treated with leaf leachates. Increased lipoxygenase under stressful conditions contributes to greater lipid peroxidation, affecting the structural and functional stability of membranes (Nahar *et al*., 2016). Under stressed condition activity of lipoxygenase (Hasanuzzaman *et al*., 2012; Ahanger *et al*., 2019) and protease (Ahanger and Agarwal, 2017; Ahanger *et al*., 2019) is increased significantly. Reports discussing the impact of allelopathic stress on lipoxygenase and protease are not available. Proteases have an essential role in the degradation and are crucial for cellular protein homeostasis and recycling as well as cleavage of signaling proteins (Stael *et al*., 2019). Lipoxygenases bring hydroperoxidation of polyunsaturated fatty acids leading to the generation of phytooxylipins (Viswanath *et al*., 2020). The products of the lipoxygenase pathway have essential functions as growth regulators, odours, and signaling molecules that have significant importance in germination, fruit ripening, and senescence (Rosahl, 1996; Viswanath *et al*., 2020). Dry leachate treatment resulted in up-regulation of lipoxygenase activity in wheat and barley seedlings, which may have contributed to the regulation of the mentioned processes. However, further studies are required to unravel the exact role under allelopathy stress. In plants, proteases have regulated growth, programmed cell death, abscission, fruit ripening and nitrogen homeostasis (Liu *et al*., 2018; Martinez *et al*., 2019), and nutrient mobilization under environmental stresses (Diaz-Mendoza *et al*., 2014; Gomez-Sanchez *et al*., 2019).

The activity of the antioxidant enzymes and the content of non-enzymatic antioxidants increased in both barley as well as wheat seedlings due to the treatment of leaf and stem extracts. Enzymes including APX, DHAR, MDHAR, and GR are the key enzymatic components of the AsA-GSH cycle in plants. Their up-regulation significantly represses the damaging effects of excess ROS (Hasanuzzaman *et al*., 2020). Earlier studies have also reported increased activity of antioxidant enzymes in plants treated with plant extracts (Lara-Nuñez *et al*., 2006; Tomar *et al*., 2015; Ghimire *et al*., 2020). Several allelochemicals have been reported to

induce the more significant accumulation of toxic ROS (Zhang *et al.*, 2007; Huang *et al.*, 2020). Upregulated functioning of the AsA-GSH cycle mediates efficient neutralization of ROS like hydrogen peroxide from the sensitive organelles like chloroplast and mitochondria thereby protecting the electron transport (Hasanuzzaman et al. 2020). Treatment of *Casuarina equisetifolia* leachates has been reported to increase the activity of APX, GR, and CAT in *Hernandia nymphaeifolia* (Zhang *et al.*, 2017). In the present study both tested crop plants maintained increased activity of AsA-GSH enzymes which could have assisted in preventing damage to photosynthetic electron transport. Besides this, the increased AsA-GSH functioning also contributes to redox homeostasis by maintaining ascorbate and glutathione concentrations (Ahanger *et al.*, 2017; Nahar *et al.*, 2017; Soliman *et al.*, 2020a, b). Ascorbate and glutathione can directly scavenge ROS and also act as substrates for enzymatic components, thereby further strengthening the mechanisms for quick elimination of excess ROS (Foyer and Noctor, 2011). In addition, their role in improving protection against oxidative effects has been reported (Naz *et al.*, 2016; Hasanuzzaman *et al.*, 2018). Besides this, the content of methylglyoxal and the functioning of glyoxylase cycle enzymes were upregulated in plants treated with leachates of *A. saligna*. Enzymes of the glyoxylase cycle are specific in eliminating the excess accumulated methylglyoxal to alleviate its damaging effects on the cellular structures and their functioning (Alhaithloul *et al.*, 2019; Ahmad *et al.*, 2020; Ahmad *et al.*, 2021). Research reports discussing the allelopathic impact on the methylglyoxal content and the functioning of glyoxylase cycle enzymes are not available. Methylglyoxal is toxic to cells and can affect germination, photosynthesis, and root growth. Excess generation of methylglyoxal modulates the plant responses to adverse conditions by regulating stomatal movements, ROS production, calcium ion concentration in the cytoplasm, activating the inward rectifying potassium channels, and the expression of stress-responsive genes (Hoque *et al.*, 2016). Decline growth in wheat and barley due to the allelopathic effects of *A. saligna* leaf and stem extracts can be attributed to excessive production of ROS and methylglyoxal production resulting in photoinhibition. However, further studies can be worthwhile.

Accumulation of osmolytes in response to allelopathic stress has been reported (Wang *et al.*, 2009; Tomar and Agarwal, 2013; Omezzine *et al.*, 2014). Allelochemicals can impart osmotic and ionic stress thereby restricting the water uptake and influencing the normal plant functioning and performance (Tomar and Agarwal, 2013; Araniti *et al.*, 2017). In the present study, the content of glycine betaine increased due to LL and SL treatment depicting the potential of wheat and barley to counter the damage. Accumulation of osmolytes prevents excess ROS accumulation and helps to maintain the tissue water content, thereby protecting major cellular processes (Ashraf and Foolad, 2007; Masood *et al.*, 2016; Ali *et al.*, 2020). Glycine betaine influences key plant functioning, including enzyme activity, nutrient uptake, and photosynthesis (Ali *et al.*, 2020).

Conclusions

Treatment of LL and SL from *A. saligna* inhibited the growth of wheat and barley by triggering damage to key macromolecules evident as increased protease and lipoxygenase activity in them. Declined RWC, chlorophyll, and carotenoids also justify the damaging effects of LL and SL. The activity of AsA-GSH cycle enzymes and the content of GSH, AsA, and glycine betaine increased significantly to counter the damage. Further studies are required to unravel the exact mechanisms.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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