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Research Article

Ameliorative role of salicylic acid on morpho-anatomy and physiology of rapeseed (*Brassica napus* L.) under lead stress

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Abstract

Heavy metals, due to their pervasiveness is serious threat to crop productivity. Lead (Pb) is an incredibly toxic and unnecessary heavy metal that makes its entry into the plants via contaminated soil. Salicylic acid (SA), a plant-derived hormone has the ability to assist pants to strengthen their immune system against toxic metals like lead (Pb). This research intends to investigate the alleviating impact of salicylic acid on the morphology, anatomy and physiology of *B. napus* (rapeseed) plants during the toxicity of lead (Pb). The plants were treated with 3 levels of lead (lead nitrate) i.e. 1 mM, 2 mM, and 4 mM along with salicylic acid and without salicylic acid. Two levels of salicylic acid i.e. 0.5 mM and 1 mM were used in the form of foliar spray. The results indicated that Pb induced damage is alleviated by SA and the morphological traits were improved i.e root length (58%), shoot length (16%), root fresh weight (73%), shoot fresh weight (79%) and dry weight of root (66%) and shoot (74%), number of leaves (87%), leaf area (61%), and yield (78%) as compared to Pb stress. Similarly, the anatomical features i.e. epidermal thickness (46-63%), vascular tissue area (42-54%), cellular thickness (27-59%) of the plant also improved with SA treatment in comparison to Pb stress. The physiological parameter i.e chlorophyll pigments (chlorophyll a, b and total chlorophyll) were also increased by 2-4% with SA as compared to Pb toxicity. However, the higher level of SA (1 mM) proved less beneficial as compared to the lower level (0.5 mM) in mitigating the effects caused by stress. According to these outcomes, SA could serve as a helpful approach for enhancing the tolerance of *B. napus* to withstand stressed conditions, hence improving crop resilience in Pb-containing soils.

Keywords: anatomy; *Brassica*; heavy metals; lead; morphology; salicylic acid

Introduction

In the last few decades, soil pollution has gained international attention, with heavy metal pollution of agricultural soil being one of the most hotly debated issues (Rai *et al*., 2021). Heavy metals such as lead,

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chromium, nickel, cobalt, cadmium, zinc, and mercury, originating from activities like metal smelting, industrial waste, fertilizers, and sewage disposal, persist in the environment without degradation (Ejaz *et al*., 2023; Ghori *et al*., 2019). As these metals are non-biodegradable and unpredictably mobile or immobile (Ejaz *et al*., 2023), they destroy soil health and plant physiology, affecting soil pH and texture, and entering the food chain, pose severe risks to human health (Ghori *et al*., 2019). Under the toxicity of these metals, seed germination and seedling growth are hindered, and root development is affected, leading to reduced root growth and biomass (Ejaz *et al*., 2023). Heavy metals also stop plants from growing by interrupting the processes of cell metabolism and photosynthesis (Wang *et al*., 2020). Numerous plants have shown anatomical changes, including reduced root growth (Yadav *et al*., 2021) and transformed stem (Chaudhari *et al*., 2016) followed by leaf (Freitas-Silva *et al*., 2016) structure.

Lead (Pb) is one of the most dangerous heavy metal pollutants in the environment because it ranks second among pollutants due to its distribution and toxicity worldwide (Dalyan *et al*., 2020). Pb polluted the soil by reducing its pH and nutrients bioavailability and also disturbing its texture (Romero-Freire *et al*., 2015). Pb easily enters the plants from polluted soil (Rizwan *et al*., 2018). It is predominantly taken up by plant roots, and only a minimal amount is transported to the parts of plants above ground. The root system serves as the main site of Pb because roots absorb about 95% of Pb (Dalyan *et al*., 2018). Pb stress affects plant growth and development, reducing the plant's ability to absorb water and nutrients (Jayasri and Suthindhiran, 2016; Sidhu *et al*., 2016; Kumar *et al*., 2017). Metal (Pb) stress alters the relationship between plants and nutrients and disrupts the ratio of nutrients in plant tissues (Dalyan *et al*., 2020). It suppresses seed germination (Zhang *et al*., 2018) and early growth of seedlings (Seneviratne *et al*., 2017) by decreasing the function of hydrolytic enzymes such as α and β-amylase and also protease (Sidhu *et al*., 2017). In roots, Pb stress induces various anatomical modifications such as increased endodermis thickness and central cylinder (Zanganeh *et al*., 2021). Pb toxicity causes a remarkable reduction in plant growth and biomass, producing a decrease in root and shoot length and fresh and dry weights (Hattab *et al*., 2016; Venkatachalam *et al*., 2017). Lead (Pb) stress destroys photosynthetic pigments like chlorophyll or suppresses the formation of these pigments. As a result, total chlorophyll contents are reduced, which affects the photosynthesis process (Hadi and Aziz, 2015; Hou *et al*., 2018; Khan *et al*., 2018b). It suppresses the process of transpiration by reducing water content, decreasing leaf area, and disrupting stomata functioning (Alamri *et al*., 2018; Dalyan *et al*., 2020).

The youngest species of the Bassicaceae family, *Brassica napus* L. is commonly utilized as a potential oil crop and is known by a variety of names, such as oilseed rape, rapeseed, and colza. Canola was developed approximately 7,500 years before when *Brassica oleraceae* (cabbage) and *Brassica rapa* (turnip rape) were hybridized spontaneously (Raboanatahiry *et al*., 2021). On a global scale, *B. napus* production was calculated to be 73.6 million tons in 2020/2021 with a remarkable increase to 100.5 million tons in 2022/2023 (Borges *et al*., 2023). On the basis of the climate and genotype, *B. napus* takes 110-150 days to reach full growth and maturity. Mature seeds are round, 1.8-2.7 mm in diameter, and range in color from reddish-brown to a deep brown and sometimes black. Mature stems range in length from 120 to 150 cm (Raboanatahiry *et al*., 2021). Anatomically, its roots, stems, and leaves show typical structural features of Brassicaceae, including distinct vascular bundles and epidermal layers (Akbar and Begum, 2020). *B. napus* accumulates heavy metals like Ni, Cr, Pb, Cd, Zn, and Cu more in shoots than roots and heavy metal stress can severely impact its functioning (Ding *et al*., 2018). Several alterations in cellular and tissue structures occur during heavy metal stress which disrupts the anatomy of plants (de Freitas-Silva *et al*., 2016; Tupan and Azrianingsih, 2016). Strategies like adding amendments to soil can enhance *B. napus*'s capacity to mitigate heavy metal stress and improve growth (Khare *et al*., 2022).

Salicylic acid (SA), a plant hormone, was obtained from "Salix", the Latin word for the willow tree, for the very first time. It is a natural substance found in many plants and is also called ortho-hydroxybenzoic acid.

SA helps plants in many ways, like signaling, biotic and abiotic stress responses, plant defense, and thermogenesis (Wani *et al*., 2017). In 1899, the Bayer Company used SA to make aspirin (Arif and Aggarwal, 2018). When plants are exposed to harmful metals like cadmium (Cd), mercury (Hg), or lead (Pb), SA helps protect them. It improves respiration, and photosynthesis and modulates the antioxidant system in plants (Ghani *et al*., 2015; Zhang *et al*., 2015; Gondor *et al*., 2016; Hasanuzzaman *et al*., 2019). However, it's necessary to know that at elevated concentrations, salicylic acid can also perform the function of inhibitory regulator of plant growth (Sharma *et al*., 2020).

The aim of this study is to explore how salicylic acid can help rapeseed plants (*B. napus*) grow better and stay healthy in soil contaminated with lead. By examining how different levels of salicylic acid impact the growth, morphology, and anatomical responses of *B. napus* under lead stress, the research seeks to find practical ways to support rapeseed health and productivity in polluted environments. This study tends to improve farming practices by making rapeseed more resilient to lead contamination.

Materials and Methods

Experimental setup

The present research was performed in The Islamia University of Bahawalpur sub-campus Bahawalnagar to explore the impacts of salicylic acid (SA) in minimizing the lead stress on the morphology anatomy and physiology of *Brassica napus* var. AARI canola. The plant's seeds were obtained from the Ayub Agriculture Research Institute (AARI) in Faisalabad and planted in plastic pots (15 cm by 18 cm) in the field area of the university. The soil used for this experiment had sandy loam texture which was determined by feel analysis proposed by Thien (1979). The electrical conductivity of the soil was 0.20 (dSm^{-1}) which and pH was 7.8 which was determined by E.C. meter and pH meter respectively. In each pot, 5 seeds were sown at a depth of 2 cm containing 8 kg of soil and watered regularly. This potted experimental setup was established with a completely randomized design (CRD) method, consisting of one control group and 11 treatments. Each treatment contained 3 replications to ensure an accurate examination of the variables being studied. 3 solutions of lead [lead nitrate or $Pb(NO_3)_2$] with concentrations of 1 mM, 2 mM, and 4 mM were prepared by dissolving 0.331 g, 0.661 g, and 1.324 g of lead nitrate powder in 1 liter of distilled water, respectively. 2 concentrations of salicylic acid solutions, 0.5 mM and 1 mM were prepared by dissolving 0.07 g and 0.14 g of salicylic acid powder in 1 liter of distilled water. The treatments were: control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA (Figure 1). The germination of seeds started after 5 days of sowing. The treatments were given when the seedlings started to grow and reached an overall height of 5-7 cm after 14 days of their germination. 50 ml of lead nitrate solution was given to the plants with a foliar spray of salicylic acid every 7 days, and the process of this treatment application was meticulously repeated ten times during the 90 days experimental period. Throughout the entire duration of the experiment (90 days), the plants were irrigated as per requirement with normal water in order to maintain optimal growing conditions. After the completion of experimental period, the plants were harvested at the ripening of siliques from the pots and subjected to further analysis. 3 plants were selected from each pot for examination and their mean values were taken for statistical analysis.

Morphological attributes assessment

Various morphological attributes of *B. napus* have been assessed in this study. The plants' root, shoot, and silique lengths were measured with a measuring tape and recorded in centimeters (cm). The fresh weights of the root, shoot, and silique of plants were determined in grams (g) using a digital balance. For dry weight, the plants were oven dried at 50 °C until a constant weight was attained and then their dry weight was also determined in grams (g) using a digital balance. The number of leaves and siliques was counted manually. The leaf area (cm^2) of *B. napus* plants was assessed according to the formula given by Elings (2000):

Leaf area of the plant (cm) = length of leaf (cm) × maximum width of the leaf (cm) × 0.75

The Root-shoot ratio was obtained by applying a similar formula used by Sanders and Brown (1976):

Root-Shoot Ratio = Dry weight of the roots / Dry weight of the shoots

To obtain total yield, the seeds were taken out from the siliques of each plant, and their weight was determined in grams (g) using a digital balance.

Figure 1. *Brassica napus* L. plants under various treatments of lead (Pb) and salicylic acid (SA) showing variations in their morphological attributes

Control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Procedure for anatomical examination

For anatomical examination, the plants underwent thorough cleaning by washing them under tap water to eliminate all dirt and soil particles from their vegetative parts. The plant parts such as leaves, roots, and stems were cut into pieces approximately 2-3cm in length using scissors. These parts were then immersed in FAA (a mixture of formaldehyde, alcohol, and acetic acid) for a duration of 48 hours. Following the 48-hour period, the samples were transferred into 70% alcohol. A single staining dehydration method was employed for the preparation of slides, along with capturing their images at 4X, 10X, and 40X magnification of light microscope in the laboratory of The Department of Botany at The Islamia University of Bahawalpur sub-campus Bahawalnagar. Safranin and methylene blue were the stains utilized in this staining technique. For the observation of stomata and epidermal cells, the slides were prepared by carefully peeling the leaf epidermis using fine forceps and placing them on glass slides. A drop of glycerin is used as mounted medium for the peeled epidermis sections and the sections were covered with cover slips. These slides were observed at 10X and 40X magnifications of light microscope. The measurements of anatomical parameters were done by using Image J software.

Determination of chlorophyll content

The determination of contents of chlorophyll pigments i.e. chlorophyll-a, chlorophyll-b, and total chlorophyll was accomplished by performing the methodology described by Arnon (1949). 0.1 g of fresh leaf material was immersed in 5 ml of 80% acetone overnight. After that the optical density (O.D.) or absorbance of the extracted solution was assessed at wavelengths of 645, and 663 nm by running 80% acetone as blank solution.

Following formulas/equations were used for determining the contents of chlorophylls (chl a, chl b, total chl) and carotenoids.

$$\text{Chl. a (mg g}^{-1}) = [12.7(A_{663})\text{-}2.69(A_{645})] \times V/1000 \times W$$

$$\text{Chl. b (mg g}^{-1}) = [22.9(A_{645})\text{-}4.68(A_{663})] \times V/1000 \times W$$

$$\text{Total Chl. (mg g}^{-1}) = [20.2(A_{645}) + 8.02(A_{663})] \times V/1000 \times W$$

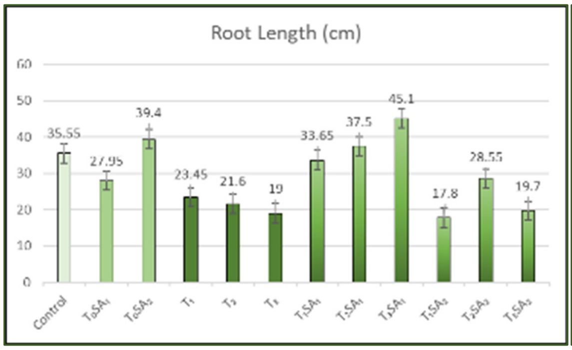
Statistical analysis

Statistix 8.1 software was used to analyze the data, and ANOVA (Analysis of variance) was run to assess the significance of the treatment effects. The least significant difference (LSD) test was used to compare the means of the treatments at a 0.05 probability level. Data was presented graphically using Microsoft Excel (2016).

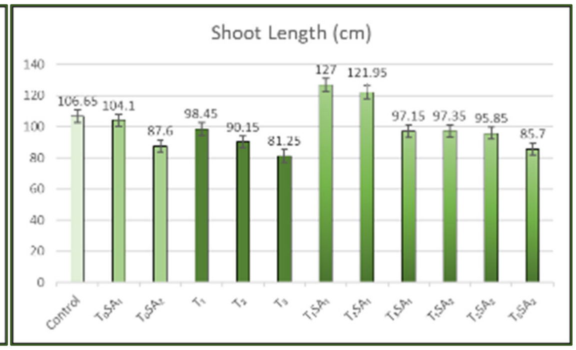
Results

Morphological results

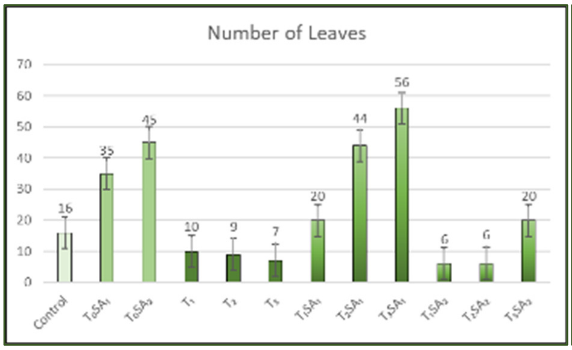
Different treatments with salicylic acid (SA) and lead nitrate $[Pb(NO_3)_2]$ affected the morphological parameters of *Brassica napus* L. (Figure 1). The results of the ANOVA (Table 1) highlighted that all the morphological attributes were significantly affected by the treatments, except the length of silique and root-shoot ratio. It has been seen that lead alone, especially T_3 [4 mM $Pb(NO_3)_2$] treatment, caused the least growth of the roots (19 cm) and shoots (81.25 cm). In contrast, when SA was combined with $Pb(NO_3)_2$, the lengths of the roots and shoots were significantly increased. T_3SA_1 (4 mM $Pb(NO_3)_2$ + 0.5 mM SA) produced the longest roots (45.1 cm), while T_2SA_1 (2 mM $Pb(NO_3)_2$ + 0.5 mM SA) produced the highest shoots (127 cm). The same trends were noticed in leaf development: combinations with higher SA levels (T_1SA_2, T_2SA_2) revealed inhibitory effects while maximum leaf count (56) was recorded in T_3SA_1. Elevated levels of $Pb(NO_3)_2$ or SA significantly decreased the leaf area. On the other hand, T_1SA_1 (1 mM $Pb(NO_3)_2$ + 0.5 mM SA) showed a notable increase (153.68 cm^2).


Root Length (cm)
35.55
27.95
39.4
23.45
21.6
19
33.65
37.5
45.1
17.8
28.55
19.7
Control

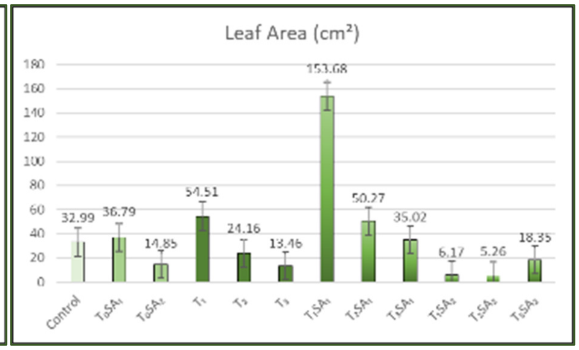
(A)


Shoot Length (cm)
106.65
104.1
87.6
98.45
90.15
81.25
127
121.95
97.15
97.35
95.85
85.7
Control

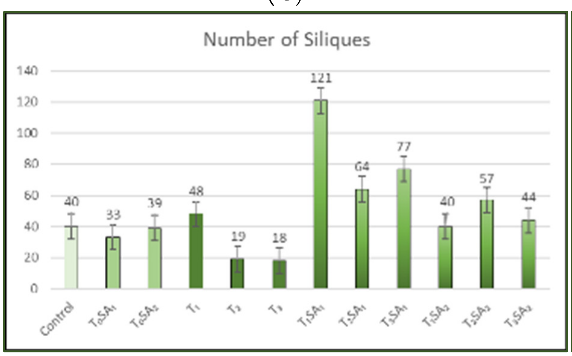
(B)


Number of Leaves
16
35
45
10
9
7
20
44
56
6
6
20
Control

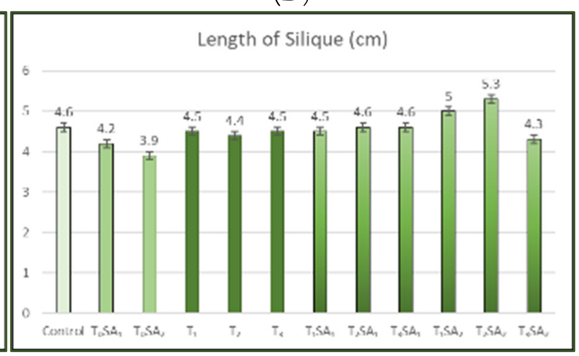
(C)


Leaf Area (cm²)
32.99
36.79
14.85
54.51
24.16
13.46
153.68
50.27
35.02
6.17
5.26
18.35
Control

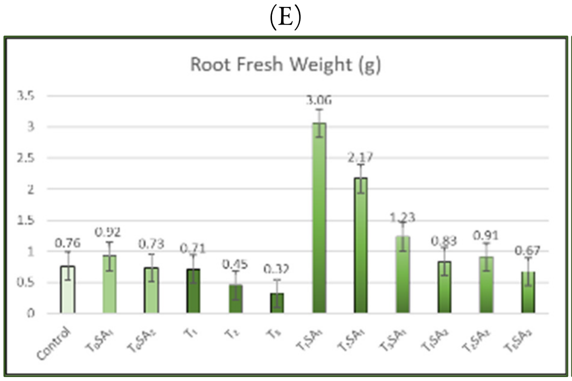
(D)


Number of Siliques
40
33
39
48
19
18
121
64
77
40
57
44
Control

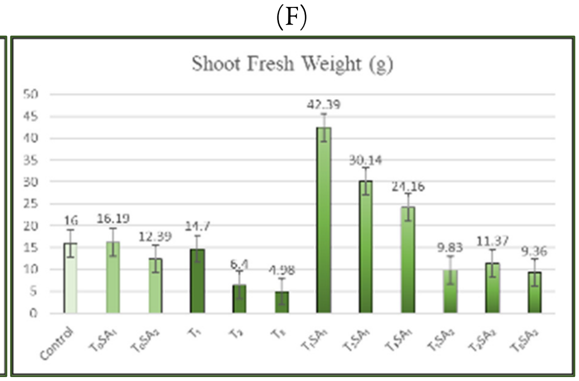
(E)


Length of Silique (cm)
4.6
4.2
3.9
4.5
4.4
4.5
4.5
4.6
4.6
5
5.3
4.3
Control

(F)


Root Fresh Weight (g)
0.76
0.92
0.73
0.71
0.45
0.32
3.06
2.17
1.23
0.83
0.91
0.67
Control

(G)


Shoot Fresh Weight (g)
16
16.19
12.39
14.7
6.4
4.98
42.39
30.14
24.16
9.83
11.37
9.36
Control

(H)

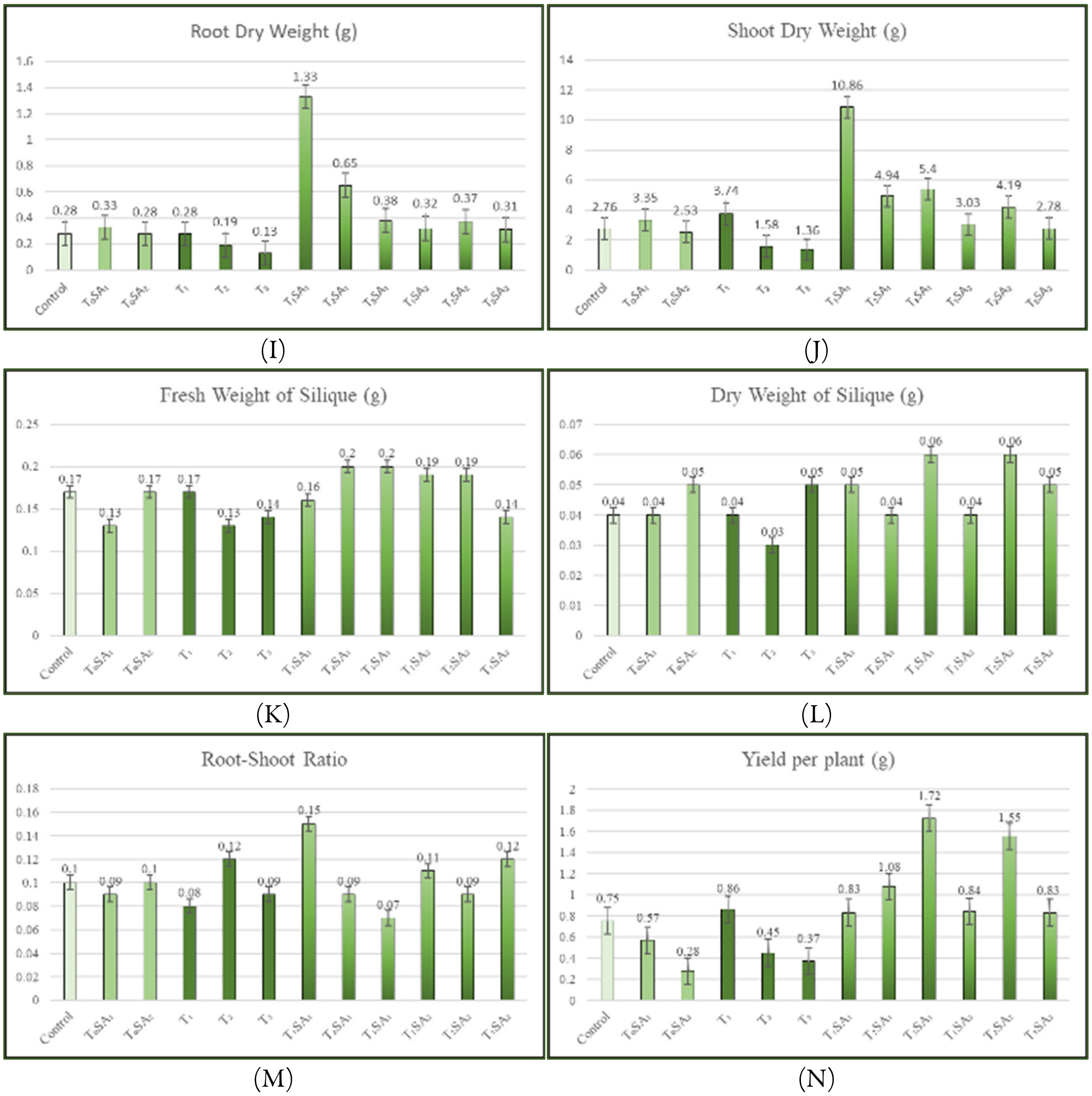


Figure 2. Morphological parameters, where (A) root length, (B) shoot length, (C) number of leaves, (D) leaf area, (E) number of siliques, (F) length of silique, (G) root fresh weight, (H) shoot fresh weight, (I) root dry weight, (J) shoot dry weight, (K) fresh weight of silique, (L) dry weight of silique, (M) root-shoot ratio and (N) yield per plant. The bar graphs showing the effect of different treatments on the morphological attributes of *Brassica napus* L. plants

The data is the mean with ± SE and error bars are denoting standard errors. Control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

In contrast to sharp declines under $Pb(NO_3)_2$ alone, the silique count peaked at 121 in T_1SA_1, indicating an enhanced function of SA. Elevated SA doses resulted in a modest increase in silique length, although this effect was statistically non-significant. The same pattern was seen in the fresh and dry weights of roots and shoots, with the lowest weight under T_3 and the maximum weight under T_1SA_1, highlighting SA's ability to reduce lead toxicity. Despite decreases in only SA- or $Pb(NO_3)_2$-treatments, combinations such as T_2SA_1 and T_3SA_1 also considerably increased the fresh and dry weights of siliques. T_1SA_1 displayed the highest allocation towards root growth, despite the fact that root-shoot ratios stayed relatively stable. T_3SA_1 had the highest yield per plant (1.72 g), demonstrating the synergistic effect of SA and $Pb(NO_3)_2$ in reducing stress toxicity and increasing plant productivity. By considering all the aspects, our findings suggest that salicylic acid significantly proved beneficial in reducing the growth inhibition caused by lead and restoring the morphological growth and production capacity of *Brassica napus* L., particularly when applied at lower dosages (0.5 mM).

Table 1. ANOVA for various morphological attributes of *Brassica napus* L. under the treatment of different concentrations of Lead nitrate and Salicylic acid and Lead nitrate + Salicylic acid

Morphological Parameters	Sum of Squares (SS)	Mean Square (MS)	F-value
Root Length (cm)	2708.44	246.222	3.44**
Shoot Length (cm)	6328.8	575.342	3.30**
No. of Leaves	10230.8	930.068	27.2***
Leaf Area (cm^2)	52894.1	4808.56	2.89*
No. of Siliques	25866.9	2351.54	3.42**
Length of Silique (cm)	4.19889	0.38172	2.11^{ns}
RFW (g)	31.6078	2.87343	4.67***
SFW (g)	5211.32	473.756	3.22**
RDW (g)	4.38839	0.39894	4.89***
SDW (g)	207.514	18.8649	3.40**
FWS (g)	0.02170	0.00197	3.13**
DWS (g)	0.00222	2.02E-04	2.91*
R-S ratio	0.01609	0.00146	2.00^{ns}
Yield per Plant (g)	6.30236	0.57394	7.11***

RFW = Root Fresh Weight, SFW = Shoot Fresh Weight, RDW = Root Dry Weight, SDW = Shoot Dry Weight, FWS = Fresh Weight of Silique, DWS = Dry Weight of Silique, R-S ratio = Root-Shoot ratio. ns = non-significant ($P > 0.05$); * = significant at $P < 0.05$; ** = highly significant at $P < 0.01$; *** = very highly significant at $P < 0.001$

Root anatomy

Measurement of anatomical parameters under various levels of lead nitrate [$Pb(NO_3)_2$] and salicylic acid (SA) treatments is a helpful tool to explore the toxicity of lead (Pb) in the roots of rapeseed plants. The thickness of cork (Figure 3) revealed significant effect of treatments by showing the values varied from 14.8 µm in T_3 [4 mM $Pb(NO_3)_2$] to 47.08 µm in T_1SA_1 [1 mM $Pb(NO_3)_2$ + 0.5 mM SA]. The area of root xylem (Figure 3) followed the trend similarly, with T_3 having the least area (67.2 µm^2) and T_1SA_1 having the most. The diameter and area of the xylem vessels (Figure 3) were considerably influenced; T_1SA_1 had the maximum values 55.77 µm and 27.11 µm^2, respectively) while T_1 and T_3 had the minimum. The area of root phloem (Figure 3) also varied, with T_0SA_2 (1 mM SA) having the largest area (25.96 µm^2) and T_2 [2 mM $Pb(NO_3)_2$] having the smallest area (5.3 µm^2). In the same way, T_2SA_1 [2 mM $Pb(NO_3)_2$ + 0.5 mM SA] had the largest vascular bundle area (166.43 µm^2), whereas T_3 had the lowest (141.27 µm^2) as shown in Figure 3. On the whole, the addition of salicylic acid (SA) diminished all the negative effects of lead (Pb) on these anatomical parameters particularly when applied at lower SA concentrations, hence improving the structure of root (Figure 4) as well as its function.

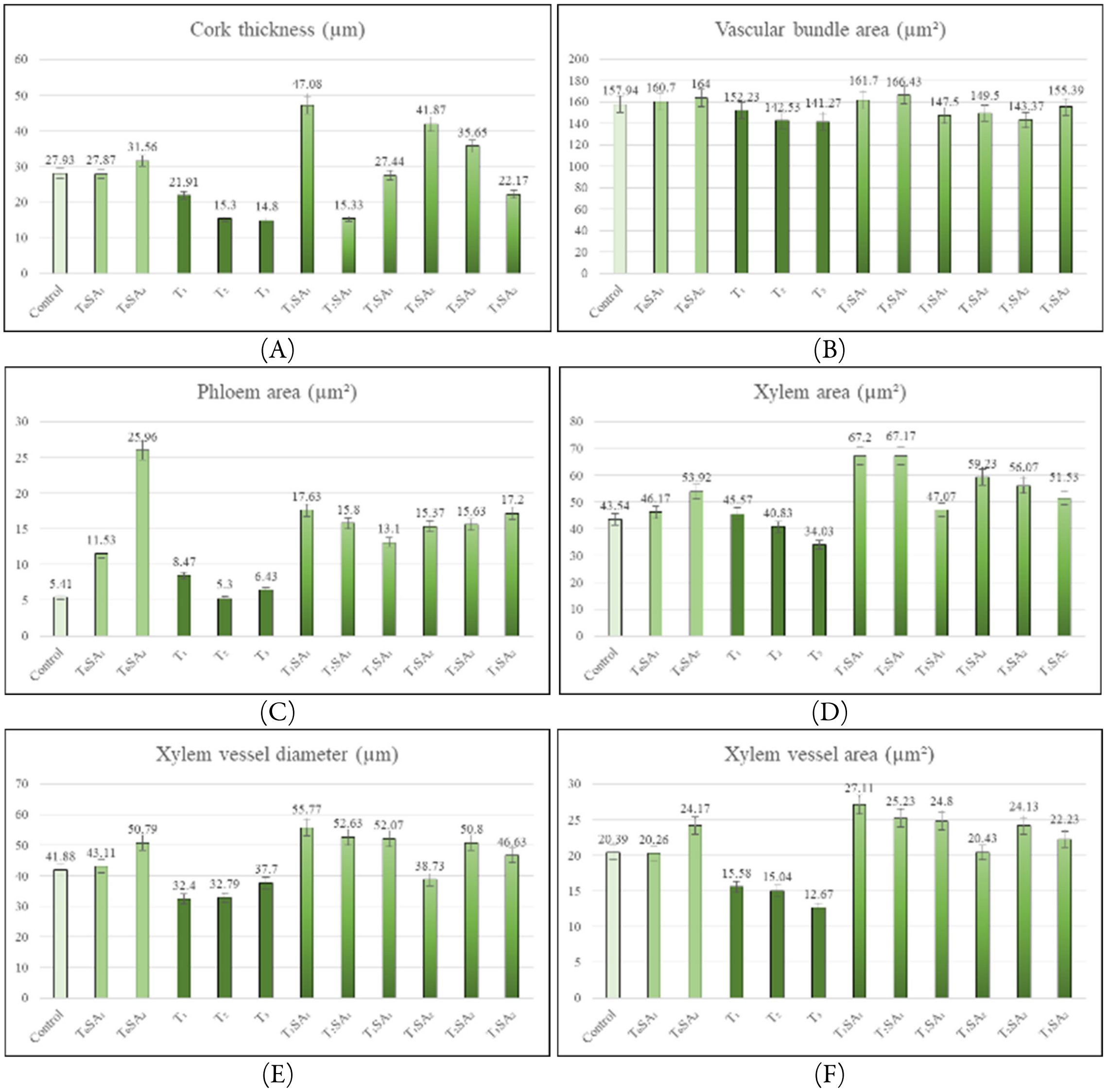
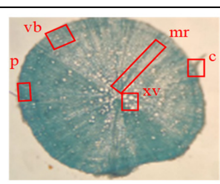
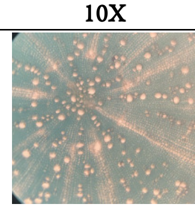
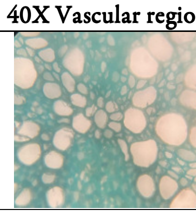
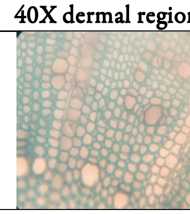
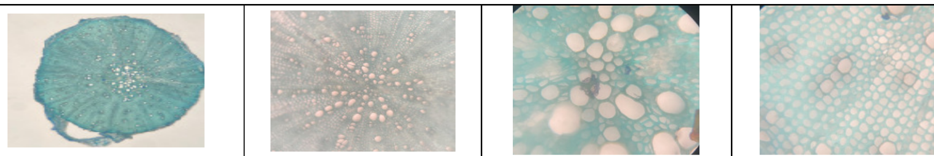
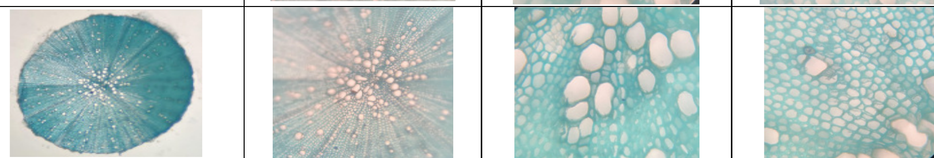
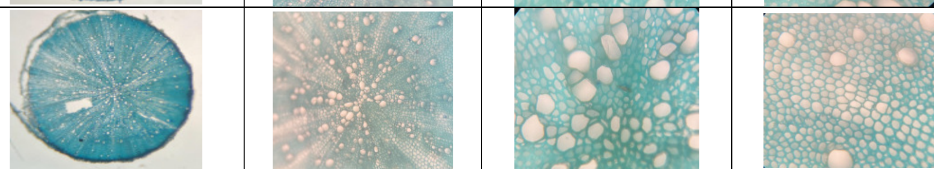
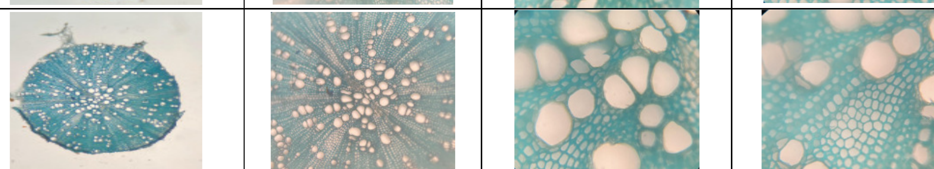
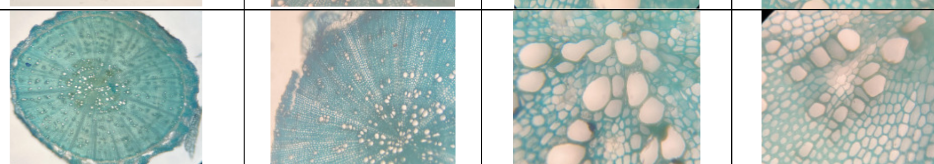
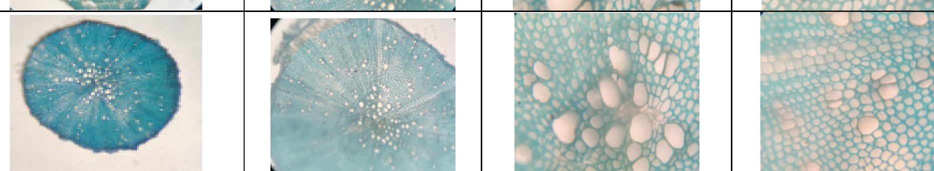
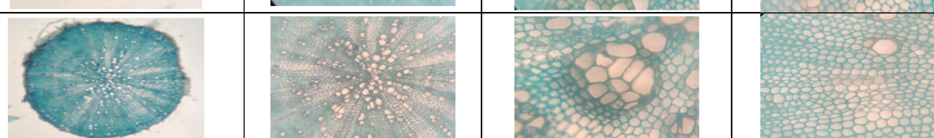
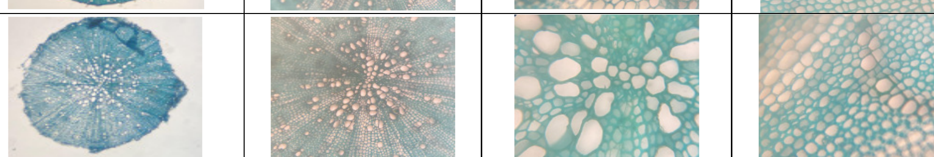
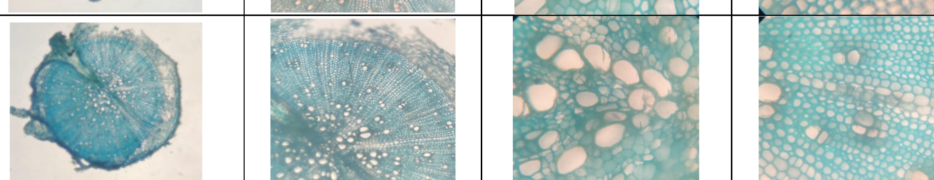
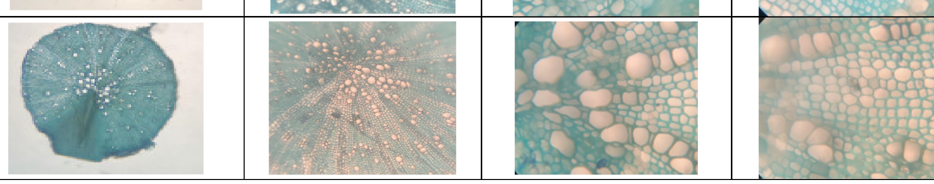


Figure 3. Root anatomical parameters, where (A) cork thickness, (B) vascular bundle area, (C) phloem area, (D) xylem area, (E) xylem vessel diameter and (F) xylem vessel area. The error bar graphs showing the effect of different treatments on the root anatomical parameters of *Brassica napus* L. plants

The data is the mean with ± SE and error bars are denoting standard errors. Control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Treat.	4X	10X	40X Vascular region	40X dermal region
Control				

T_oSA_1	
T_oSA_2	
T_1	
T_2	
T_3	
T_1SA_1	
T_2SA_1	
T_3SA_1	
T_1SA_2	
T_2SA_2	

T_3SA_2

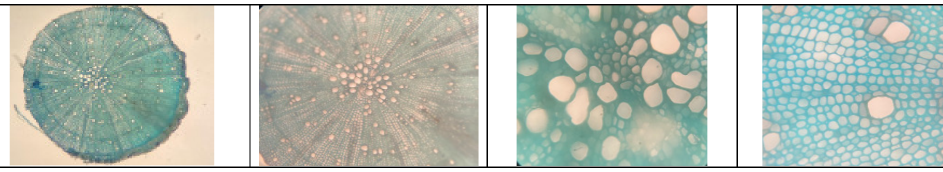


Figure 4. Comparison of root anatomy between different levels of lead nitrate, salicylic acid and lead nitrate + salicylic acid when applied at different time. Treat.: treatment, c: cork, vb: vascular bundles, p: phloem, xv: xylem vessels, mr: medullary rays.
Control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Stem anatomy

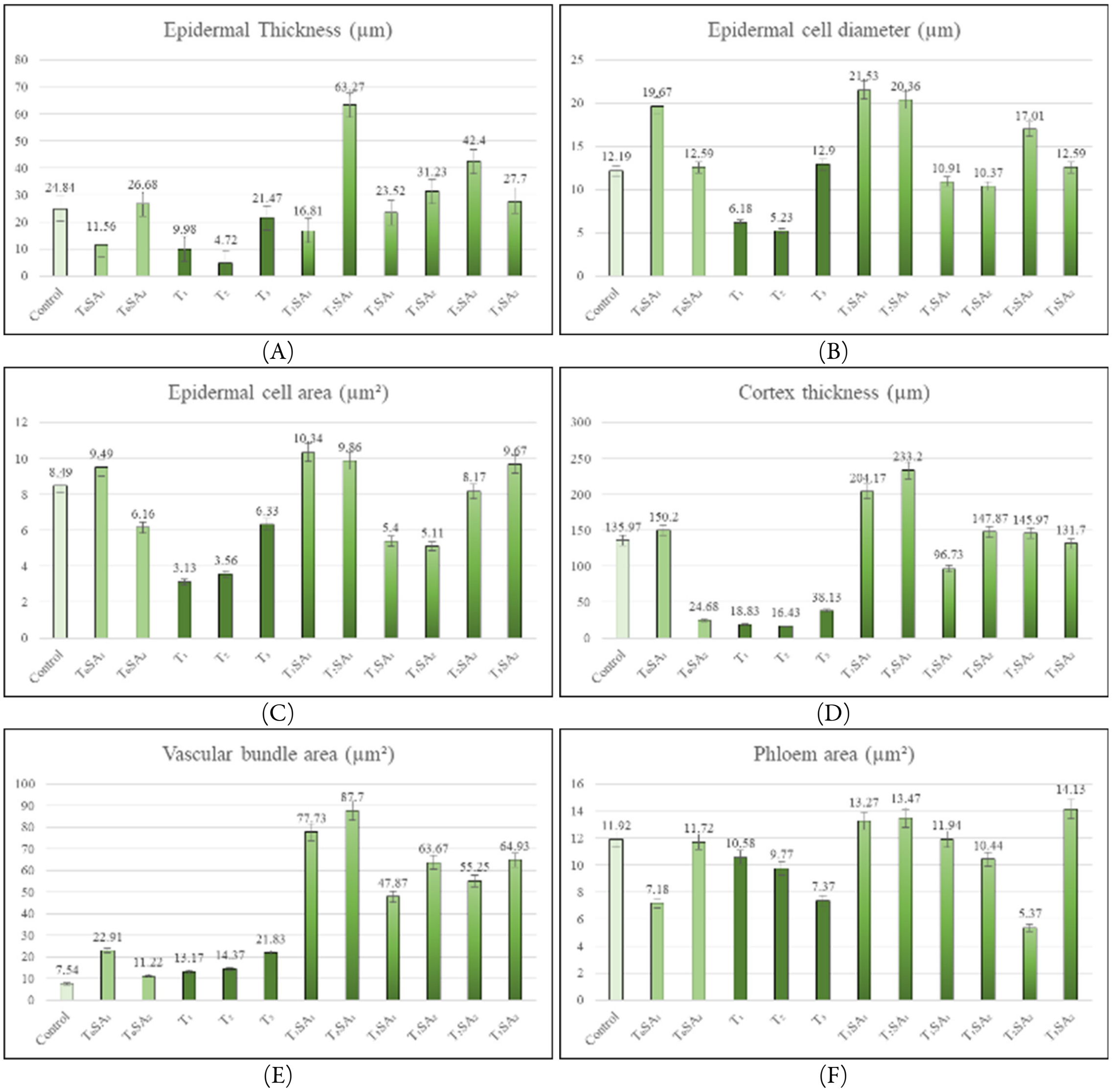
The treatments of $Pb(NO_3)_2$ and in combination with SA had a substantial effect on the stem's phloem and xylem areas, vascular bundle area, cortical thickness, xylem vessel area, and pith features in addition to the epidermal thickness, cell diameter, and area (Figure 5, Figure 6). The control group in all the parameters showed moderate values, while treatments produced significant differences. The thickness of epidermis, cortex, and area of vascular bundles (xylem and phloem) and pith were highest in T_2SA_1 (2 mM $Pb(NO_3)_2$ + 0.5 mM SA) and lowest in treatments containing $Pb(NO_3)_2$ alone such as T_1 [1 mM $Pb(NO_3)_2$], T_2 [2 mM $Pb(NO_3)_2$] and T_3 [4 mM $Pb(NO_3)_2$]. The ANOVA findings (Table 2) revealed high F-values and low p-values across parameters, indicating a robust treatment impact. The differences found were statistically significant.

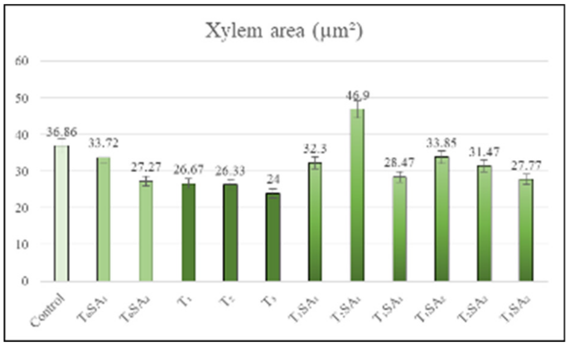
Table 2. ANOVA for various anatomical attributes of *Brassica napus* L. under the treatment of different concentrations of Lead nitrate and Salicylic acid and Lead nitrate + Salicylic acid

Anatomical Parameters	Sum of Squares (SS)	Mean Square (MS)	F-value
Root			
Cork Thickness (μm)	3571.93	324.721	27.9^{***}
Vascular Bundle Area (μm)	2871.74	261.068	11.1^{***}
Phloem Area (μm^2)	881.420	80.1290	33.8^{***}
Xylem Area (μm^2)	2737.65	248.877	13.5^{***}
Xylem Vessel Diameter (μm)	1464.40	133.127	2.67^{*}
Xylem Vessel Area (μm^2)	363.102	33.0093	3.51^{**}
Stem			
Epidermal Thickness (μm)	8141.06	740.096	37.8^{***}
Epidermal Cell Diameter (μm)	911.27	82.8425	7.72^{***}
Epidermal Cell Area (μm^2)	210.206	19.1097	7.26^{***}
Cortex Thickness (μm)	177523	16138.4	344^{***}
Vascular Bundle Area (μm^2)	27151.1	2468.28	328^{***}
Phloem Area (μm^2)	272.919	24.8108	9.91^{***}
Xylem Area (μm^2)	1287.98	117.089	8.18^{***}
Xylem Vessel Area (μm^2)	17504.5	1591.32	158^{***}
Diameter of Pith (μm)	320078	29098.0	577^{***}
Diameter of Pith Cells (μm)	6179.71	561.792	5.19^{***}
Area of Pith Cells (μm^2)	1948.29	177.117	1.93^{ns}
Leaf (Midrib)			
Upper Epidermis Thickness (μm)	678.356	61.6687	22.6^{***}
Lower Epidermis Thickness (μm)	408.746	37.1587	4.02^{**}
Vascular Bundle Area (μm^2)	1041.42	94.6741	15.7^{***}
Phloem Area (μm^2)	2361.73	214.703	9.82^{***}
Xylem Area (μm^2)	14125.7	1284.15	15.7^{***}

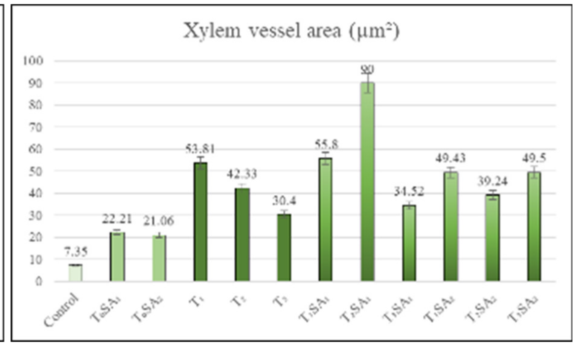
Xylem Vessel Diameter (µm)	52225.9	4747.81	12.6***
Collenchyma Cell Diameter (µm)	2088.77	189.888	5.18***
Leaf (Epidermis)			
Stomata Thickness (µm)	1526.36	138.760	12.4***
Epidermal Cell Thickness (µm)	665.33	60.4849	3.16**

ns = non-significant (P > 0.05); * = significant at P < 0.05; ** = highly significant at P < 0.01; *** = very highly significant at P < 0.001

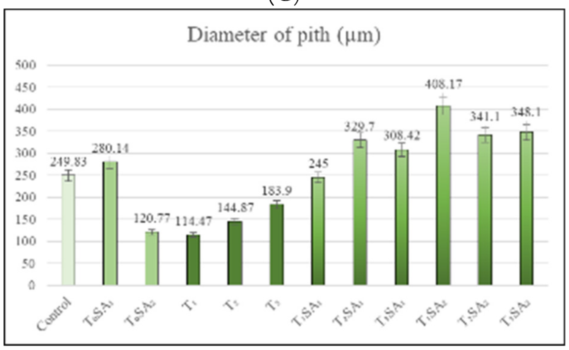




(G)



(H)



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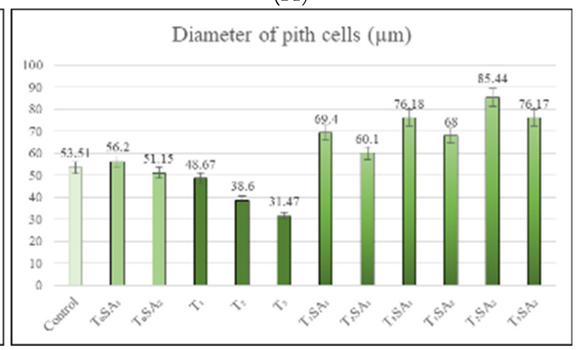

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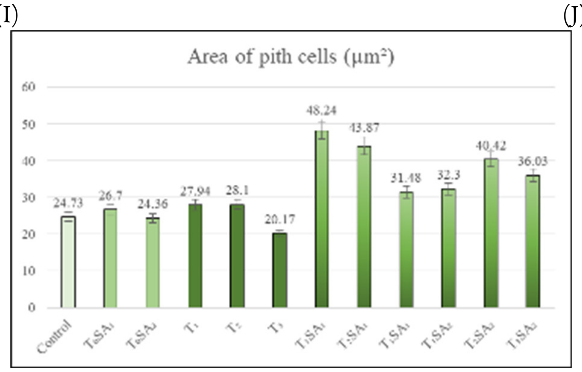

(K)

Figure 5. Stem anatomical parameters, where (A) epidermal thickness, (B) epidermal cell diameter, (C) epidermal cell area, (D) cortex thickness, (E) vascular bundle area, (F) phloem - area, (G) xylem area, (H) xylem vessel area, (I) diameter of pith, (J) diameter of pith cells (K) - area of pith cells. The bar graphs showing the effect of different treatments on the stem - anatomical parameters of *Brassica napus* L. plants The data is the mean with ± SE error barsare denoting standard errors. Control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

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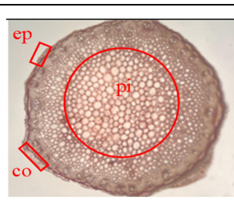
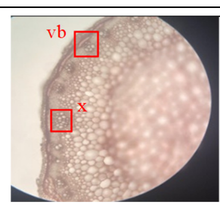
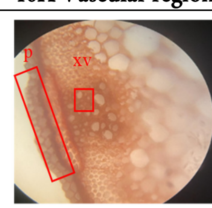
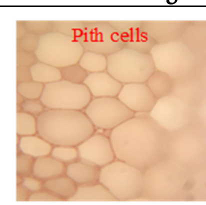
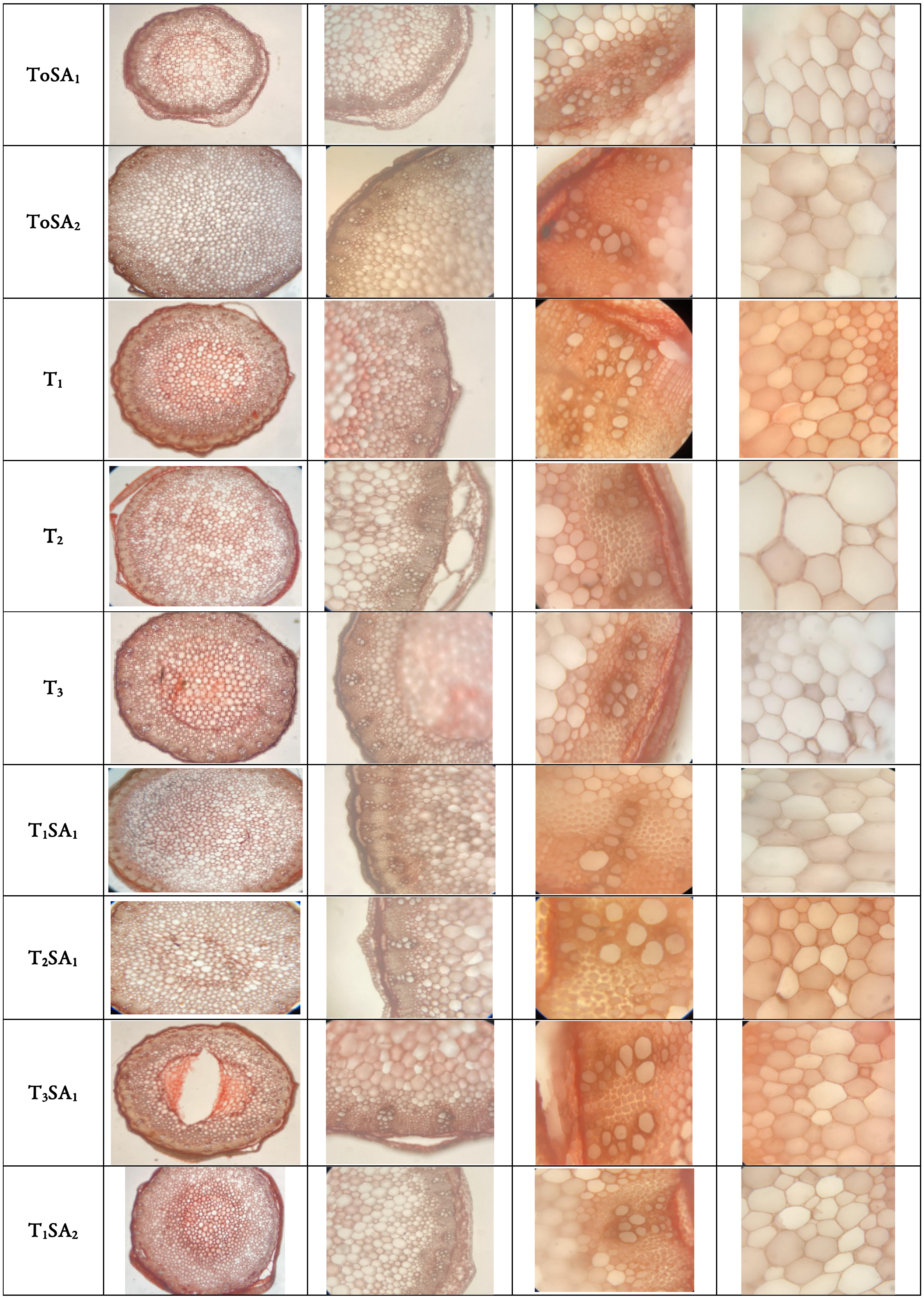
Control				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T_3
T_1SA_1
T_2SA_1
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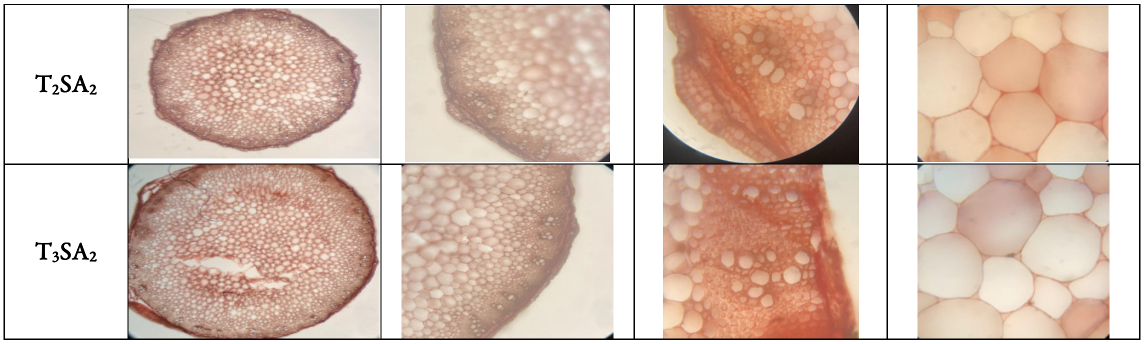
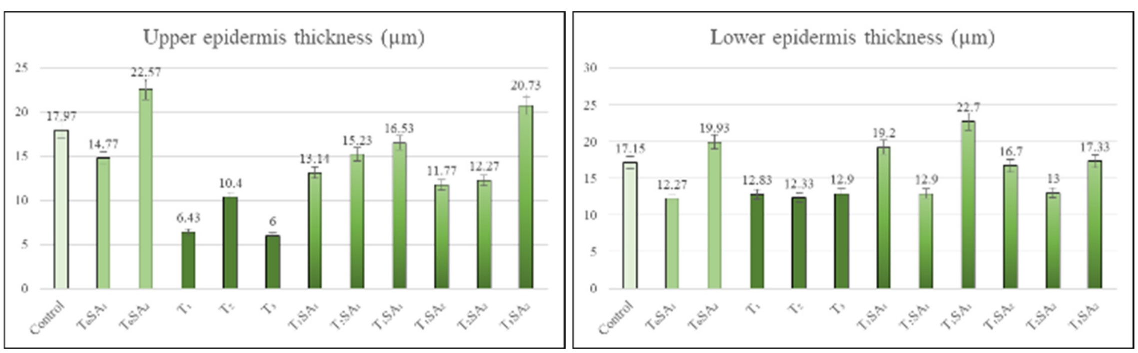


Figure 6. Comparison of stem anatomy between different levels of lead nitrate, salicylic acid and lead nitrate + salicylic acid when applied at different time. Treat.: treatment, ep: epidermis, co: cortex, vb: vascular bundles, p: phloem, x: xylem, xv: xylem vessels, pi: pith

Control = water only, T_0SA_1 = 0.5 mM SA, T_0SA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Leaf midrib anatomy

The anatomy of the leaf midrib (Figure 8) changed depending on the treatment with lead nitrate [$Pb(NO_3)_2$] and salicylic acid (SA). The upper epidermis thickness (Figure 7) was different in each case. T_0SA_2 (1 mM SA) had the thickest upper epidermal layer at 22.57 µm, while the thinnest was in treatment T_1 [1 mM $Pb(NO_3)_2$]. The same pattern was observed in the lower epidermis (Figure 7), with T_3SA_1 [4 mM $Pb(NO_3)_2$ + 0.5 mM SA] showing the thickest layer at 22.7 µm. The phloem area (Figure 7) was largest in the control group (42.53 μm^2), but it became smaller in other treatments, like T_1, which had only 9.8 μm^2. On the other hand, the vascular bundle area (Figure 7) was highest in treatment T_1SA_1 [1 mM $Pb(NO_3)_2$ + 0.5 mM SA] at 31.83 μm^2. The xylem area and xylem vessel diameter (Figure 7) were also altered. T_1SA_1 had the highest xylem vessel diameter (167.73 µm), and T_3SA_2 had the highest xylem area (73.47 μm^2). Collectively, the anatomical features of the leaf midrib improved when salicylic acid was combined with lead nitrate. This may have likely helped the plants to maintain their structure and improve nutrient transportation.



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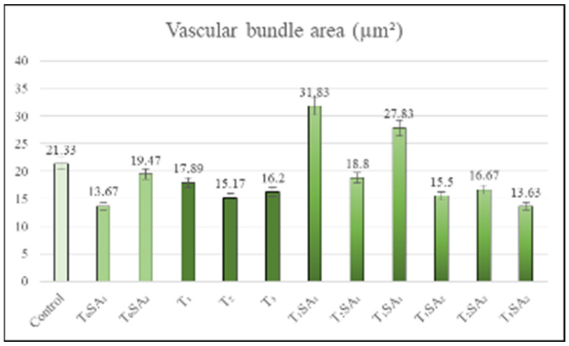
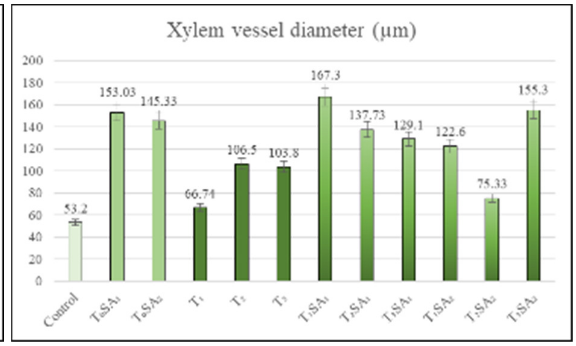


(C)



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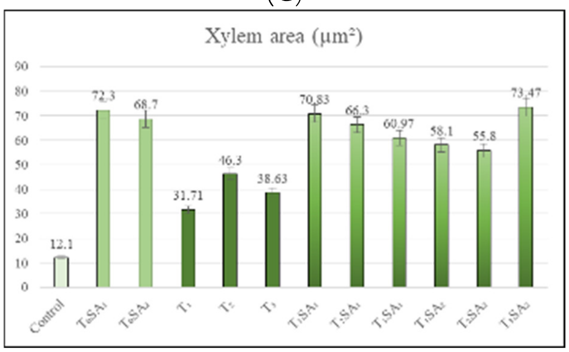

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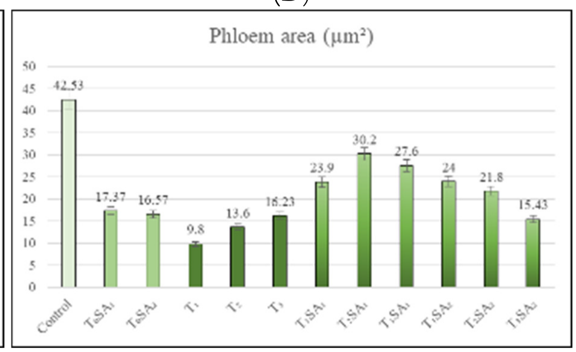

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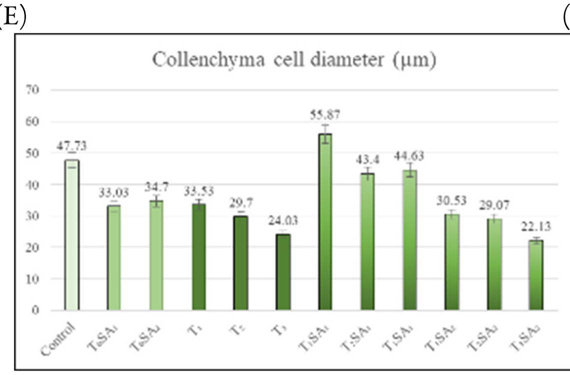

(G)

Figure 7. Leaf midrib anatomical parameters where (A) upper epidermis thickness, (B) lower epidermis thickness, (C) vascular bundle area, (D) xylem vessel diameter, (E) xylem area, (F) phloem area, (G) collenchyma cell diameter. The bar graphs showing the effect of different treatments on the leaf midrib anatomical parameters of *Brassica napus* L. plants

The data is the mean with ± SE error bars are denoting standard errors. Control = water only, $ToSA_1$ = 0.5 mM SA, $ToSA_2$ = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

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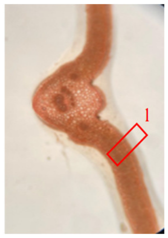
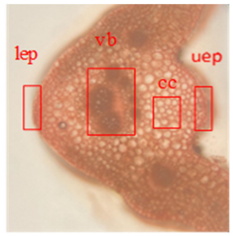
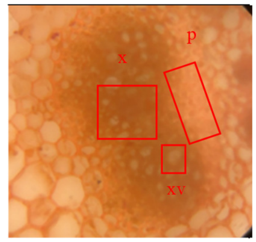
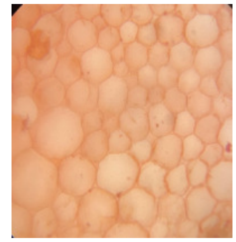
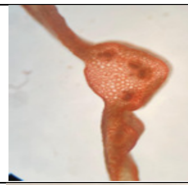
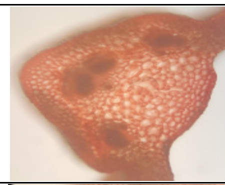
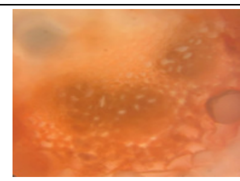
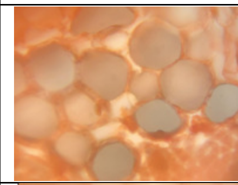
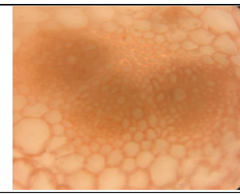
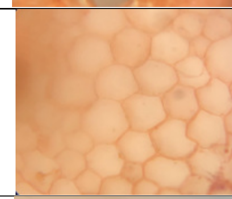
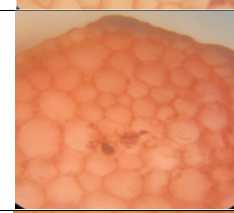
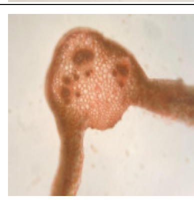
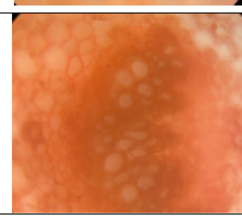
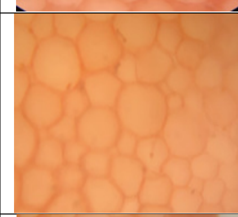
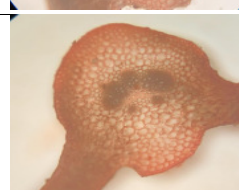
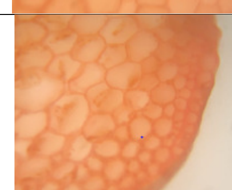
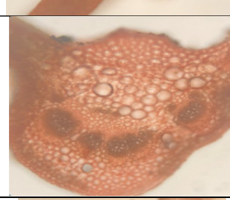
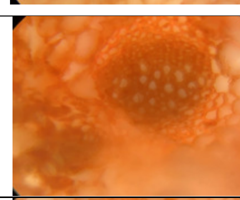
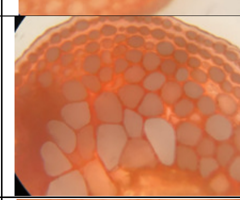
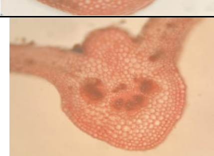
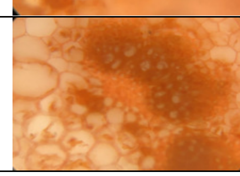
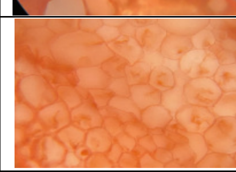
Control				
ToSA$_1$				
ToSA$_2$				
T$_1$				
T$_2$				
T$_3$				
T$_1$SA$_1$				
T$_2$SA$_1$				

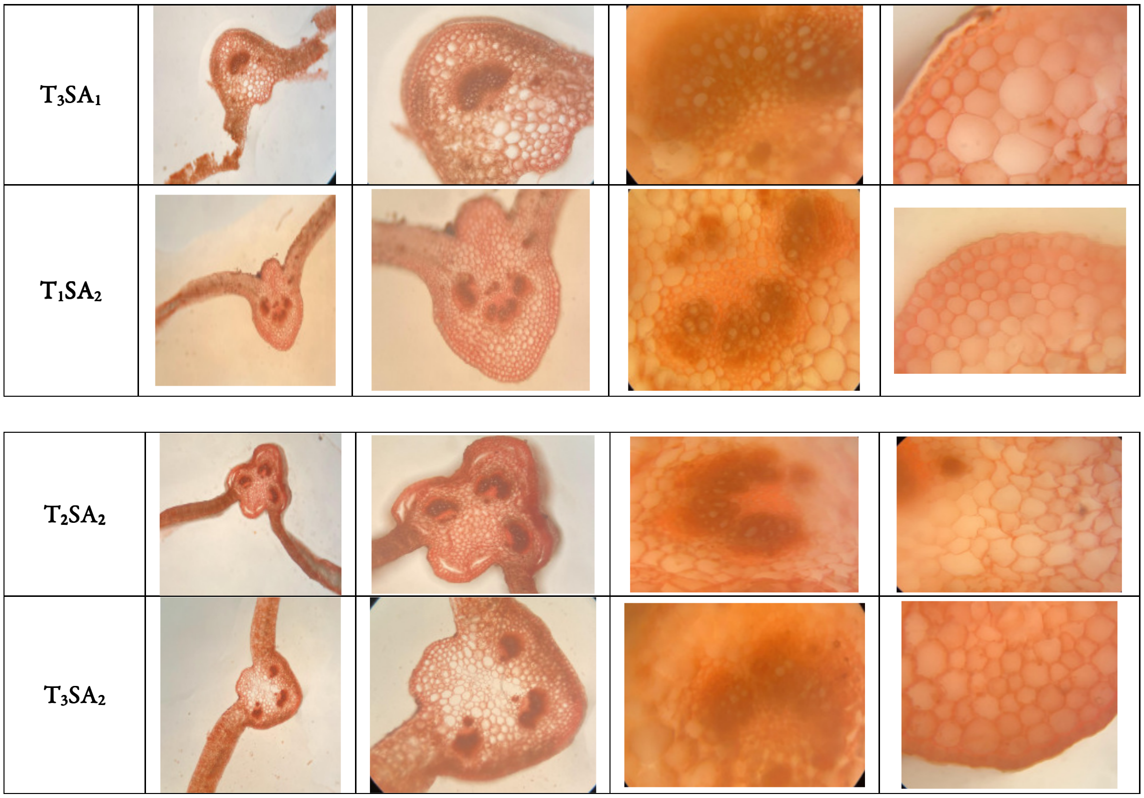
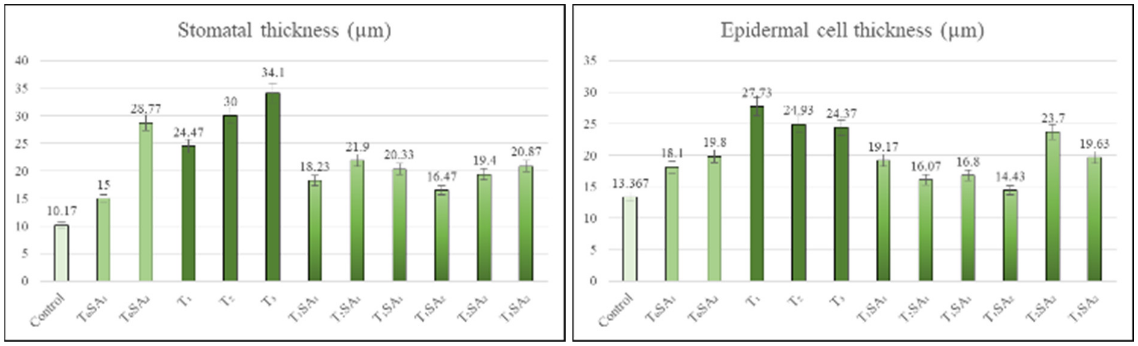


Figure 8. Comparison of leaf midrib anatomy between different levels of lead nitrate, salicylic acid and lead nitrate + salicylic acid when applied at different time. Treat.: treatment, l: lamina, uep: upper epidermis, lep: lower epidermis, vb: vascular bundles, x: xylem, p: phloem, xv: xylem vessels, cc: collenchyma cells Control = water only, T_oSA_1 = 0.5 mM SA, T_oSA_2 = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Leaf epidermis anatomy

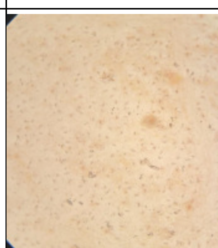
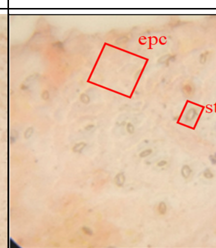
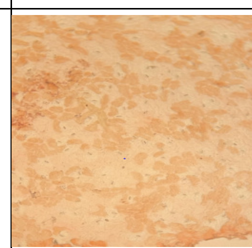
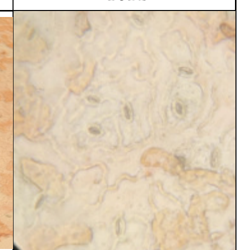
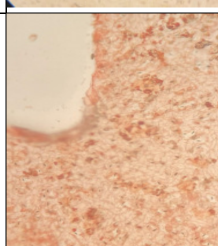
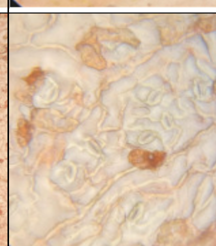
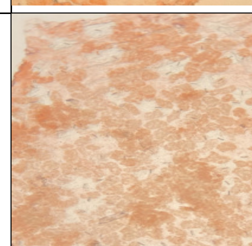
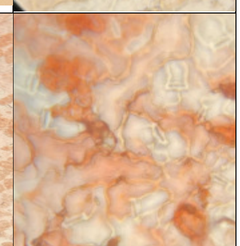
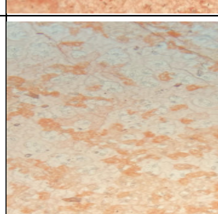
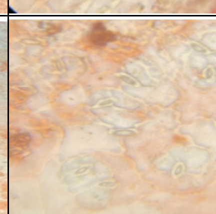
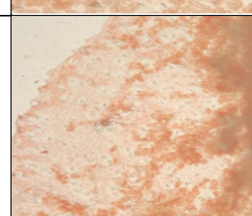
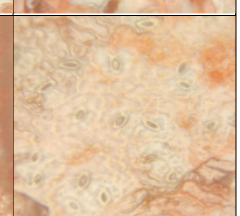
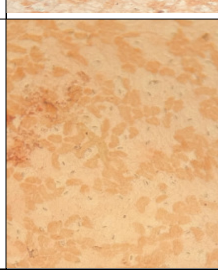
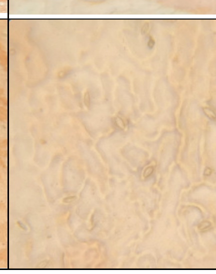
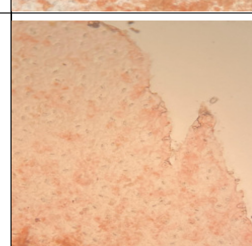
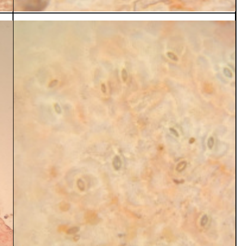
The results of leaf epidermal anatomical attributes showed significant differences in epidermal cell thickness and stomata between various treatments (Figure 10). T_3 had the maximum stomata thickness (Figure 9) at 34.1 µm as compared to the control group's stomata thickness at 10.17 µm. Similar to this, there was a significant difference in epidermal cell thickness across treatments (Figure 9). T_1 had the largest thickness of 27.73 µm, whereas the control group had the lowest thickness of 13.367 µm. Unlike other anatomical parameters, stomata and leaf epidermis thickness have increased in stress conditions and decreased in SA treatments.



(A) (B)

Figure 9. Leaf epidermis anatomical parameters, where (A) is stomatal thickness, (B) is epidermal cell thickness. The error bar graphs showing the effect of different treatments on the leaf epidermis anatomical parameters of *Brassica napus* L. plants

The data is the mean with ± SE error bars are denoting standard errors.. Control = water only, $ToSA_1$ = 0.5 mM SA, $ToSA_2$ = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Treat.	10X	40X	Treat.	10X	40X
Control			T_1SA_1		
$ToSA_1$			T_2SA_1		
$ToSA_2$			T_3SA_1		
T_1			T_1SA_2		

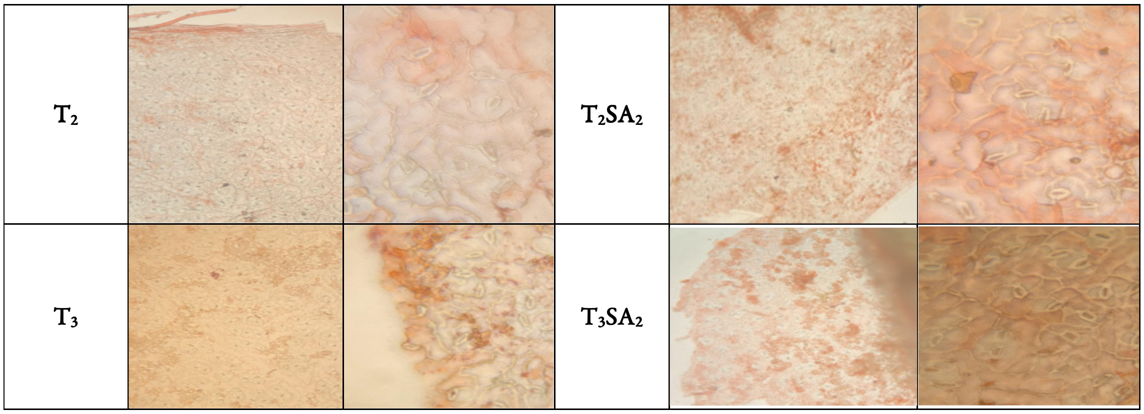


Figure 10. Comparison of leaf epidermis anatomy between different levels of lead nitrate, salicylic acid and lead nitrate + salicylic acid when applied at different time. Treat.: treatment, epc: epidermal cells, st: stomata

Control = water only, $ToSA_1$ = 0.5 mM SA, $ToSA_2$ = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

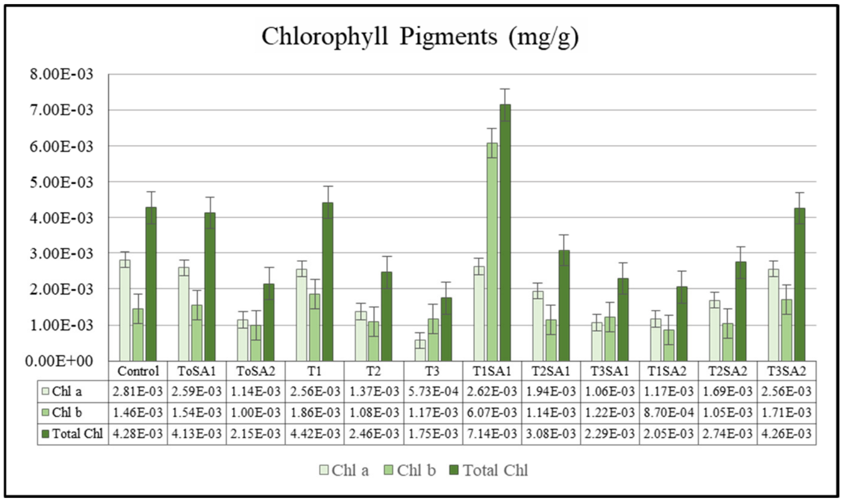
Chlorophyll pigments estimation

The analysis of chlorophyll pigments revealed that chlorophyll contents are significantly influenced by Pb and SA treatments. Chlorophyll a b and total chlorophyll (Figure 11) were decreased under the stress of Pb with most pronounced damage occurred in higher stress level (T_3). SA not only reduced this damage but also enhanced the contents of these pigments by 60-95 %. The ANOVA outcomes (Table 3) reveal that treatments had a significant impact on chlorophyll content in the plants of *Brassica napus* L., with highly significant effects on chlorophyll a ($F = 10.0$, $p = 0.0000$), chlorophyll b ($F = 3.34$, $p = 0.0066$), and total chlorophyll contents ($F = 5.67$, $p = 0.0002$). These results offer strong suggestion that treatments caused meaningful modifications in chlorophyll levels.

Table 3. ANOVA for physiological attributes of *Brassica napus* L. under the treatment of different concentrations of Lead nitrate and Salicylic acid and Lead nitrate + Salicylic acid

Chlorophyll content	Sum of squares (SS)	Mean square (MS)	F-value
Chlorophyll a	1.960E-05	1.782E-06	10.0***
Chlorophyll b	6.584E-05	5.985E-06	3.34**
Total Chlorophyll	7.756E-05	7.051E-06	5.67***

ns = non-significant ($P > 0.05$); * = significant at $P < 0.05$; ** = highly significant at $P < 0.01$; *** = very highly significant at $P < 0.001$



	Control	ToSA1	ToSA2	T1	T2	T3	T1SA1	T2SA1	T3SA1	T1SA2	T2SA2	T3SA2
Chl a	2.81E-03	2.59E-03	1.14E-03	2.56E-03	1.37E-03	5.73E-04	2.62E-03	1.94E-03	1.06E-03	1.17E-03	1.69E-03	2.56E-03
Chl b	1.46E-03	1.54E-03	1.00E-03	1.86E-03	1.08E-03	1.17E-03	6.07E-03	1.14E-03	1.22E-03	8.70E-04	1.05E-03	1.71E-03
Total Chl	4.28E-03	4.13E-03	2.15E-03	4.42E-03	2.46E-03	1.75E-03	7.14E-03	3.08E-03	2.29E-03	2.05E-03	2.74E-03	4.26E-03

Figure 11. Effect of different treatments on the chlorophyll pigments of *Brassica napus* L. plants

The data is the mean with ± SE error bars are denoting standard errors; Control = water only, $ToSA_1$ = 0.5 mM SA, $ToSA_2$ = 1 mM SA, T_1 = 1 mM $Pb(NO_3)_2$, T_2 = 2 mM $Pb(NO_3)_2$, T_3 = 4 mM $Pb(NO_3)_2$, T_1SA_1 = 1 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_2SA_1 = 2 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_3SA_1 = 4 mM $Pb(NO_3)_2$ + 0.5 mM SA, T_1SA_2 = 1 mM $Pb(NO_3)_2$ + 1 mM SA, T_2SA_2 = 2 mM $Pb(NO_3)_2$ + 1 mM SA, T_3SA_2 = 4 mM $Pb(NO_3)_2$ + 1 mM SA

Discussion

The conducted study suggest that lead (Pb) stress adversely affected all the morphological, anatomical and physiological attributes of *Brassica napus* L. plants, whereas the foliar application of salicylic acid (SA) substantially alleviated these undesirable effects.

A remarkable reduction was observed in growth parameters such as the length of root and shoot, their fresh as well as dry weights, number of leaves and leaf area (Figure 2) by lead (Pb) stress. Maximum reduction in root length and shoot length was occurred in highest Pb level (T_3). This reduction in root and shoot length (cm) was happened because heavy metals reduce the division and expansion of cells especially in the zone of elongation and also cause formation of lateral roots in many plants. The development of lateral roots is the first sign of heavy metal toxicity because these lateral roots hinders water absorption, ultimately reduces the amount of photosynthates transportation to the roots, which leads to lower root length (Riyazuddin *et al*., 2022). The reduction in root length was also due to the excessive absorption of Pb by roots, which impairs the tonoplast's and plasmalemma's selective permeability and barrier function (Shehzad *et al*., 2023). This type of root length reduction was stated by Ali *et al*. (2015) in *B. napus* under Pb toxicity. Pb interacts immediately with the shoot's cellular metabolism, resulting in a decrease in shoot length (Riyazuddin *et al*., 2022). Similar results for shoot length reduction were reported by Shehzad *et al*. (2023) in three species of *Brassica* i.e., *B. campestris*, *B. napus* and *B. juncea*, Pratima and Pratima (2016) in *B. juncea*, Sheetal *et al*. (2016) in mustard and Kaur (2018) in *Brassica juncea*. The utilization of SA helped in mitigating the detrimental effects of lead toxicity on root and shoot length. Salicylic acid (SA) manages the antioxidant system during stressed conditions due to heavy metals (Zengin, 2015). Externally supplying salicylic acid increases plant development and production and the antioxidant system, decreases oxidative damage, and boosts the antioxidant system in *Brassica campestris* (mustard) under lead (Pb) stress which in turn helped in maximizing root and shoot growth (Hasanuzzaman *et al*., 2019). Ghani *et al*. (2015) observed similar alleviating role of SA against Pb toxicity in *B. napus's* root and shoot length.

Fresh and dry weights (g) of both roots and shoots of plants were also dropped under Pb stress with most of the decrease occurring in T_3. Pb reduces mineral uptake of roots which ultimately disturb the metabolism of plant, causing decrease in root and shoot biomass (Shehzad *et al.*, 2023). The photosystem II, carbon reduction cycle enzymes, and photosynthesis may all be inhibited by HMs stress, which ultimately leads to a decrease in biomass (Gill *et al.*, 2015). Similar findings were suggested by Shehzad *et al.* (2023), who also noticed a sharp reduction in fresh weights as well as dry weights of roots and shoots of *Brassica* species under Pb stress. A study by Hattab *et al.* (2016) on *Medicago sativa* revealed that when seedlings are exposed to lead nitrate, their fresh weight decrease after 7 days. Also, Venkatachalam *et al.*, (2017) found that in *Acalypha indica,* similar reduction in root and shoot fresh and dry weight was observed after 12 days of exposure to lead stress. Other related studies by Kohli *et al.* (2018) and Khan *et al.* (2018b) showed the simillar decline in fresh and dry biomass under Pb stress in Indian mustard and castor oil beans respectively. These parameters were also improved considerably when salicylic acid (SA) was introduced. SA has a unique ability to combat toxicity brought on by heavy metal stress by breaking down ROS (Reactive Oxygen Species). With the stimulation of antioxidant enzyme synthesis, which lowers ROS levels, exogenously given SA increases the plant's resilience to heavy metal stress (Emamverdian *et al.*, 2020). These findings are consistent with the observations made by Arshad *et al.* (2017), who also indicated that Pb-induced toxic effects on fresh weights as well as dry weights of barley genotypes were alleviated by the application of SA.

The leaf area (cm^2) of *B. napus* increased under the lower concentration of lead (Pb) i.e., T_1 (1mM Pb) but as the levels of Pb stress increased the leaf area reduced with the highest reduction in T_3 (4mM Pb). This enhancement in leaf area at lower Pb level was due to the activation of plant defensive system and more production of secondary (natural) compounds that can cope with the stress conditions (Piršelová *et al.*, 2021). Higher metal (Pb) stress alters the relationship between plants and nutrients and disrupts the ratio of nutrients in plant tissues (Alamri *et al.*, 2018), which in turn retards cell division either directly or indirectly and cause reduced leaf area (Dalyan *et al.*, 2020). The same enhancement in leaf area was noticed by Zunaidi *et al.* (2024) in two *Brassica* species i.e., *B. chinensis* (leaf area was increased by 23.5% as compared to control) and *B. rapa* (leaf area was increased by 11.8% as compared to control) under Pb stress showing the tolerance mechanism of these species to Pb toxicity. On the other hand, Ahmed *et al.* (2023) observed reduced leaf area in *Brassica rapa* L. under Pb stress. Salicylic acid application reversed these toxic effects of Pb. Taghizadeh *et al.* (2017) also found similar mitigating role of SA in leaf area of *Brassica oleracea* under the stress of Pb.

Similarly, the decline in the number of leaves of *Brassica napus* L. plants under Pb treatments noticeably reversed at the application of salicylic acid as previously stated by Ghani *et al.* (2015), who found that in pea plants, the growth parameter like the number of leaves has been increased after 44 days of combine application of lead and salicylic acid. The harmful effects of heavy metals on gas exchange attributes, photosynthetic rate, stomatal conductance, and chlorophyll levels are responsible for the decline in leaf number (Shehzad *et al.*, 2023). The yield characteristic i.e. number of siliques and yield per plant (g) were increased at lower Pb levels and decreased at the treatment of higher Pb levels. This toxicity of Pb was suppressed by adding SA, showing similarity with Hasanuzzaman *et al.* (2019) who demonstrated that lead (Pb) stress dramatically decreased the growth and productivity of mustard plants. Nevertheless, these negative effects were lessened by using salicylic acid as a foliar spray, which enhanced plant development and productivity. According to reports, the overall yield drop under Pb treatment is caused by a decrease in yield-measuring parameters (Ashraf and Tang, 2017) and its enhancement at SA application resulted in reduced damage, showing SA's role in overcoming the oxidative stress induced by contamination of lead.

The anatomical results of the present study highlighted that the cell diameter, area, and thickness of both epidermis and vascular bundles of root, stem, and midrib of *Brassica napus* L. were decreased under the treatments of different levels of Pb. HM poisoning causes the growth of thinner mesophyll tissue and the decrease of xylem vessels and parenchymatous tissues. These findings support the earlier data reported by Wafee *et al.* (2018), who looked into how lead (Pb) toxicity affects the anatomical characteristics of the economically and medicinally significant plant periwinkle (*Catharanthus roseus* L.). Pb buildup was indicated by reductions in cell diameters following introduction to increased Pb concentrations, especially in vascular tissue of stems and roots. However, the application of SA proved beneficial in reducing these toxic effects caused by Pb. SA maintains the structural integrity of cells by enhancing the antioxidant defense system and restores the thickness of epidermal and vascular tissues in plants that have been damaged by stress (Agnihotri *et al.*, 2018). In case of leaf epidermis anatomy, the stomatal and epidermal cell thickness increased under Pb stress and decreased at the supplementation of SA. This increase in their thickness under stress is due the reason that heavy metals cause malformation and distortion in the shape of guard cells which results in the disturbance of stomata size (Guo *et al.*, 2023).

The physiological parameter, chlorophyll pigments (chlorophyll a, chlorophyll b and total chlorophyll) decreased at the higher levels of Pb stress (T_2, T_3) as shown in Figure 11. It was proposed that Pb substitutes iron (Fe) ions for magnesium (Mg) present in the center of porphyrin ring of chlorophyll which could slow down the synthesis of chlorophyll pigments by suppressing the photosynthetic activity (Saman *et al.*, 2022). The foliar application of SA minimized these toxic effects of Pb. By boosting antioxidant activity in the plants, SA treatment increased the amount of chlorophyll a and chlorophyll b, in plants under Pb stress by boosting antioxidant activity because it could blocked the Ca channels that aid in Pb translocation in the roots (Sharma *et al.*, 2020). Similar conclusions were made by Alamer and Fayez (2020) in parsley that there occurred a decline in the contents of chlorophyll a along with chlorophyll b under the toxicity of lead nitrate. This decrease has been reduced when salicylic acid was sprayed on the Pb-treated plants. The findings of Arshad *et al.* (2017) similarly indicated that Pb stress decreased the total chlorophylls, chlorophyll a, as well as chlorophyll b in barley genotypes. The utilization of salicylic acid (SA) helped mitigate the detrimental effects of lead toxicity on these chlorophyll levels.

The evidences from this research have great importance in agricultural management. As we found that salicylic acid (SA) has the potential to reduce the detrimental effects of lead on plant morphology, and anatomy, it could be a helpful method for enhancing crop resilience in regions where heavy metal (HM) pollution is an issue. With a deeper understanding of the physiology of plant stress, these findings offer practical methods for lessening the negative impacts of heavy metal contamination on agricultural productivity and plant health. SA's potential as a long-term solution can enhance food security and environmental health in places contaminated by heavy metals.

Conclusions

This research provides an in-depth examination of the impact of lead (Pb) exposure on canola (*Brassica napus*) as well as the capability of salicylic acid (SA) as a mitigating factor. The study's findings demonstrate that exposure to lead has a negative impact on the morphological anatomical and physiological attributes of rapeseed plants. Lower level of SA (0.5mM) mitigated the harmful effects of lead (Pb) more efficiently than higher value (1mM). However, the protective benefits of SA decrease and may even become unproductive if concentrations rise above an ideal threshold. This emphasizes how crucial it is to properly optimize SA concentration in order to maximize its benefits without facing the risk of adverse impacts on plant productivity and overall health. The results obtained highlight the salicylic acid's potential to protect plants from heavy metal stress and present a viable strategy for enhancing crop yield and health in contaminated areas. This is of special significance for sustainable farming in heavy metal-polluted areas, where increasing crop resistance is essential for the security of food.

Authors' Contributions

Conceptualization: AS, AH; Writing - original draft: AS, AH, IQ; Validation and Visualization: AH, AG; Software; AS, IQ, DI; Funding acquisition: DI, AG; Writing - review and editing: AS, AH, DI, AG.
All authors read and approved the final manuscript.

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Conflict of Interests

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

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