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RESEARCH ARTICLE

Phytochemical analysis of canola (*Brassica napus* L.) Cultivars: Mitigating lead stress effects through the foliar spray of potassium chloride

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ABSTRACT

The current work was performed to examine the effect of the foliar spray of potassium for lessening the toxicity of heavy metal in three varieties of Canola (*Brassica napus* L.) viz; Exceed, Cyclone and Legend. Seeds were grown in pots. They were arranged in a Completely Randomized Design (CRD). When seeds germinated, some were kept without treatment (control) and others were treated with 15mg/kg and 30mg/kg of Lead along with a Potassium spray. Plants were harvested and subjected to phytochemical studies. The role of phytochemical studies (alkaloid, flavonoid, and phenolic) showed an increase in response against heavy metal stress. While the plants treated with Potassium revealed a decreased response in phytochemical studies. Plants treated with both Pb metal and K expressed a lesser increase in phytochemical compared to plants only treated with metal. This study sheds light on the effectiveness of Potassium in improving growth and dealing with metal stress to decrease the harmful effect of heavy metals. However, further studies are required to identify the mechanisms of Potassium mediated alleviation of toxicity of Pb and other heavy metals.

Keywords: Alkaloid; Canola; Lead; Potassium; Phytochemicals

INTRODUCTION

Global food production is facing formidable challenges as a result of climate change effects like warming, drought, flooding, and other extreme events. *Brassica napus* is a crucial element of global trade and agro-economics due to its contribution to the oilseed sector. The increasing prevalence of multiple abiotic stresses is leading to significant economic losses in the B. napus crop. Therefore, it is essential to enhance the crop's resilience and productivity by equipping it with the capacity to withstand and thrive under multiple abiotic stresses (Lohani et al., 2020).

Certain adaptation processes are displayed by some plant species, referred to as hyperaccumulators, to survive in extremely polluted settings. Phylogenetically, several Brassica species flourish on contaminated soil for rehabilitation because they are hyper-metal accumulators (Sytar et al., 2021) Because of how much less expensive *B. napus* is to utilize in phytoremediation than more traditional techniques, this practice is becoming more popular. This plant's roots are more efficient at absorbing Cu, Cd, Pb, and Zn. The importance of *B. napus* as a prospective crop has increased recently due to its great capacity for absorbing metals, rapid growth, high above-ground biomass, and abundant oil contents in seeds (Suman et al., 2018).

Brassica napus L., commonly known as rapeseed, is a valuable source of protein and oil that can be utilized for human and animal consumption. The oils derived from this crop are commonly used in food preparation as they offer both improved taste and nutrition. Unlike solid animal fats, vegetable oils are generally considered to be more beneficial for human health. However, the growth and productivity of rapeseed are often restricted by ecological factors (Mostafavi, 2012). Annual forage is another perspective of canola. Traditionally, it was used as a forage for poultry and field-raised animals. Dry matter of 1.0 to 2.0 tons per acre in a single season can produce by

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canola (Sarı, 2006). In recent years, rapeseed has emerged as a promising alternative to olive oil, as it possesses a similar proportion of monounsaturated fatty acids and a favourable overall fatty acid composition. This has led to an increase in the use of rapeseed oil, also known as canola oil, in non-Mediterranean countries due to its lower cost and perceived health benefits, which are comparable to those of olive oil (Lin et al., 2013).

In soils, two types of metals are crucial for optimal plant growth, namely Zn, Mn, Cu, Fe, Mg, Mo, and Ni, which are considered essential micronutrients. On the other hand, there are non-essential elements, such as Ag, Cd, Co, As, Sb, Cr, Pb, Se, and Hg, whose physiological and biological functions are still unclear. The contamination of heavy metals in the atmosphere, resulting from natural processes and human activities, poses a significant and widespread threat. Heavy metals, also known as trace metals, are generally considered potentially toxic elements. These metals can exist in different forms in the soil, depending on their solubility. The term "heavy metal" refers to elements with an atomic mass greater than 20 (excluding alkali metals) and a specific gravity above a certain threshold (Hirzel et al., 2020).

Canola is being broadly grown worldwide, because of its high-quality oil. However, due to the existence of excessive quantities of toxic metals, its yield is harshly influenced commonly through irrigation water contaminating arable lands and vehicles. Lead exists in various forms in the environment and is one of the most commonly found heavy metals on a large scale (Hirzel et al., 2020). The contamination of lead is a significant issue, particularly in agricultural areas. It is a highly toxic element that can cause harm through toxicity, migration, and transformation. Different cultivars of canola may vary in their ability to transport and accumulate lead in various organs and tissues, leading to harmful alterations in the uptake and accumulation of other essential inorganic nutrients. As a toxic heavy metal, lead has a prolonged residence time in the soil and is now considered a "chemical of very high concern." Several studies have been conducted to examine the accumulation pattern of nutrients, including lead, from the roots to the shoots of four top canola cultivars and their effects on the cultivars' development (ATSDR, 2017).

Lead toxicity can disrupt various biochemical and physiological pathways in plants, leading to inhibited growth, nutrient inhibition, chlorophyll degradation, and lipid peroxidation, as well as the inhibition of ion homeostasis and the production of active oxygen species (Zhou et al., 2018). Plants respond to the oxidative stress induced by lead by stimulating their antioxidant defence mechanisms, including both enzymatic and non-enzymatic antioxidants (Ashraf et al., 2017). Potassium is a crucial macronutrient that plays essential roles in plant growth and development, including protein synthesis, enzyme activation, and carbohydrate metabolism, as well as cation-anion balance, energy transfer, osmoregulation, water movement, and stomatal regulation (Wang et al., 2013). However, under stress, the osmotic effect and ion toxicity can inhibit plant root growth, decreasing nutrient uptake and translocation, particularly that of potassium. Plants have long been a rich source of potential medicinal products containing bioactive compounds that offer health benefits to humans. Phytochemicals are naturally occurring, organically active chemical compounds found in plants that protect them from environmental stress, UV exposure, pollution, and pathogenic attacks, as well as contribute to their aroma, colour, and flavour (Ghasemzadeh et al., 2015). Carotenoids, vitamin E compounds, lignans, phenolics, β-glucan, and inulin are among the most important groups of phytochemicals found in healthy grains (Liu, 2007).

Phytochemicals are bioactive compounds found in plants that have been shown to have potential health benefits. In a study of canola, selecting phytochemicals as independent variables would allow researchers to investigate their effects on various aspects of canola, such as growth, yield, and nutritional quality. Additionally, studying phytochemicals in canola could help to identify potential health benefits associated with consuming canola products, which could have implications for human health. Phytochemicals refer to a diverse group of secondary metabolites present in plants, including alkaloids, tannins, saponins, terpenoids, steroids, and flavonoids (also known as phenolic compounds). Flavonoids have been shown to possess antioxidant properties, which contribute to their anti-inflammatory, anti-allergenic, antiviral, anticarcinogenic, and anti-ageing effects (Agati et al., 2012). Antioxidants are commonly used in food and medicine to prevent oxidation, and they can be categorized into two groups: natural antioxidants and artificial antioxidants. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) have been widely utilized in the food, pharmaceutical, and cosmetic industries (Lobo et al., 2010).

Phenolics apply positive impacts on human health. They are the results of secondary metabolism in plants (Fraga, 2009). Phenolics are the most abundant group of phytochemicals found in whole grains, and they function as natural antioxidants by scavenging free radicals and protecting large biological molecules such as lipids, DNA and proteins from oxidative damage (Slavin, 2004). Among the phenolics, flavonoids are a low-molecular-weight compound that is present as phytonutrients in the human diet and is ubiquitous in the plant kingdom.

They are valuable ingredients for a wide range of health-promoting activities, including pharmaceutical, medicinal, and cosmetic products, due to their antioxidative, anticarcinogenic, anti-inflammatory, and antimutagenic properties that can modify important cellular enzymatic functions. Additionally, they act as inhibitors of numerous enzymes (Walker et al., 2000).

Tannins constitute the third significant group of phenolic compounds, characterized by their high contents of polymers and oligomers. These compounds have the ability to form complexes with minerals, cellulose, proteins, and starch. Tannins can be classified into two types: condensed and hydrolysable tannins, which have molecular weights ranging from 600 to 3000. They act as toxins that can impede the growth and survival of most herbivores, serving as repellents to many grazing animals (Walker et al., 2000). Tannins are pivotal in plants' resistance to various diseases. The accumulation of tannins in plants can be drastically affected by environmental factors, such as high temperature, light conditions, excessive CO2 in the atmosphere, and nutrient variation (Fine et al., 2006).

Alkaloids are nitrogen-based chemical compounds. They are produced by plants for protection functions against predation by organisms, such as insects, herbivores, microorganisms and sometimes, even other plants. Alkaloids are also produced to damage the growth and development of the offending organisms through allelopathic action (Tedeschi et al., 2021). Their lethal effect depends on their type and the amount eaten by the animal. But its main purpose is to prevent eating by visual or olfactory signals (Sestak, 1996). In the plant kingdom, saponins (a group of secondary metabolites) are widely distributed. The saponins group is composed of compounds such as glycosylated steroids, triterpenoids, and steroid alkaloids, which can form a stable foam in aqueous solutions such as soap, hence their name "saponin" (Bohlmann et al., 1998). Saponins are present in plants, particularly in families such as Agavaceae, Amaryllidaceae, Asparagaceae, Bromeliaceae, Dioscoreaceae, Liliaceae, Palmae, and Scrophulariaceae, and possess diverse biological activities due to their surface active or detergent properties (Marahatha et al., 2021). These compounds exhibit various pharmacological activities, including anti-inflammatory, antidiabetic, hepatoprotective, and immunomodulatory activities (El Aziz et al., 2019).

Understanding the effects of climate change on canola phytochemicals and the ultimate impact of these effects on canola productivity remains challenging as integrated interactions are quite complex. The amount of light, humidity and diurnal variables on canola constitutive and induced phytochemical defence have not been investigated. Therefore, further study is required for investigation.

AIMS AND OBJECTIVES

The objective of this study is to assess the efficacy of potassium chloride foliar spray in mitigating the adverse effects of lead stress on canola (Brassica napus L.) cultivars. The study aims to achieve the following objectives:

- To evaluate the impact of lead metal stress on plant growth parameters and determine if the foliar spray of potassium can alleviate these effects on three different canola varieties, namely Exceed, Cyclone, and Legend.
- To investigate whether low or high concentrations of lead metal affects the morphological and physiological parameters of the canola plant varieties differently.
- To determine if potassium treatment in the form of a foliar spray can play a positive role in reducing the toxic effects of metals on the three varieties of B. napus.

The findings from this research will expand our knowledge of the effects of potassium and lead, both individually and in combination, on plant secondary metabolites.

MATERIALS AND METHODS

This work was conducted in the Botanical Garden and Research Laboratory of the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. This work was performed to analyze the production of phytochemicals when heavy metal (Lead) applies on three varieties of Canola (*Brassica napus* L.) viz Exceed, Cyclone, and Legend.

Material

The material required for this study was plastic Pots with a diameter of 65cm. Pots filled with soil, which was collected from the Botanical Garden of Bahauddin Zakariya University, Multan. Amount of 4 kg soil is put in each pot. From Ayub Agriculture Research Institute, Faisalabad, Canola seeds were bought.

Methodology

The methods consisted of the steps given below:

- Plant Seeds sowing and layout plan.
- Collecting data
- Thinning of plants
- The application of fertilizer
- The application of treatments
- Collection of data

Soil analysis was performed in the laboratory of the Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan. The soil was first air-dried and then filtered through a 2 mm sieve to eliminate any significant contaminants before being used in experiments. To characterize the soil properties, we analyzed the soil for pH, EC and saturation percentage.

Canola seeds of threel varieties were sown. After seven days of sowing, 80% of the seeds germinated. Then after twelve days, the plants were thinned, and only three plants were left in each pot. By following agronomical ways fertilizers like urea were applied to all plants. To ensure that the fertilizer did not influence the unequal production of phytochemicals in the study, standardized methods of fertilization were used. The organization of the plant was Completely Randomized Design (CRD). Lead in the form of $PbCl_2$ solution was applied to plants after twenty-three days. To prepare the lead solution, lead chloride was dissolved in distilled water, resulting in a stock solution with a concentration of 1000 mg/L. The desired concentration was then achieved by diluting the stock solution with distilled water. Similarly, the potassium chloride solution was made by dissolving potassium chloride in distilled water to produce a stock solution of 1000 mg/L, which was then diluted to the required concentration, as described by Zhang et al. (2019). Then after seven days, Potassium chloride (KCl) foliar spray was applied. The foliar method involves spraying the solution directly onto the leaves of the plant, allowing for quick absorption. The concentration of the solution was measured, and the amount was applied to ensure that the plants are receiving the desired amount of potassium chloride. By monitoring the growth and health of the plants to ensure that they were responding appropriately to the treatment. All Canola plants were treated with six distinct treatments. In all varieties control plants (T_1) were without any treatment. The T_2 plants were treated only with the foliar application of Potassium chloride. While T_3 plants were treated with 15mg/kg Pb in three varieties. The T_4 plants were subjected to a foliar spray of potassium chloride along with 15mg/kg of lead treatment, while the T_5 plants received only 30mg/kg of lead treatment. The T_6 plants, on the other hand, were treated with a combination of 30mg/kg of lead and a foliar spray of potassium chloride.

Plant phytochemical analysis

The following standard methods were used to determine various plant compound contents in this study:

- phenolic contents: The Folin-Ciocalteu method was used as described by Zardo (2020), with minor modifications.
- Flavonoid Contents: The method described by Bhom and Kocipai Abyazon (1994) was used with slight modifications.
- alkaloid contents: The Harborne method was used, as described in the literature (Harborne, 1973).
- Tannin Contents: The Folin-Denis method was used as described in the literature (Folin and Denis, 1915).
- Saponin Contents: The burner method was used, as described by Barlie et al. (2007).

Statistical analysis

The laboratory experimental data and absolute growth studies were statistically evaluated by implementing analysis of variance (ANOVA) techniques based on a completely randomized design (CRD) with two factorial arrangements. A significance level of ($P<0.05$) was set for the study. To interpret the data, Duncan's new multiple-range test was utilized at a probability level of 5% (Duncan, 1955). The mean and standard deviation of each parameter were calculated and compared to assess the variation among a variety of means, treatment means, and their interactions. The results were considered significant if the obtained p-value was less than 0.05.

RESULTS

Total alkaloid contents in stem

Table 1 presents the statistical analysis of variance results for the alkaloid contents in stem. The findings revealed a significant difference in the alkaloid contents in stem across treatments, but not between the two varieties. Comparisons between treatments and varieties indicated no significant differences.

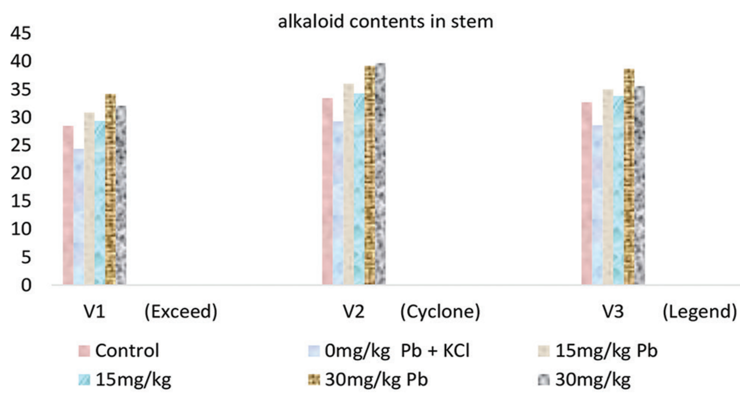
ANOVA for the effect of different heavy metals (Pb) at different concentrations on alkaloids, flavonoids, phenolics, tannins and saponins contents in stem and their amelioration by Potassium Chloride.

The application of low and high doses of Lead in three varieties increased the alkaloid contents in stem, with

Sources	df	MSS									
		Alkaloids in stem	Alkaloids in root	Flavonoids in stem	Flavonoids in root	Phenolics in stem	Phenolics in root	Tannins in stem	Tannins in root	Saponin stem	Saponin in root
Treatments (T)	5	110.40	107.44	16.40	5.290	5.301	8.561	5.799	9.326	1.247	0.524
Variety (V)	2	144.59	14.101	0.840	1.303	0.153	0.022	0.0057	0.0451	0.0172	0.062
T X V	10	1.5635	0.0124	0.133	0.205	0.098	0.054	0.0177	0.0172	0.003	0.005
Error	36	44.609	13.794	0.163	0.098	0.084	0.022	0.0311	0.039	0.015	0.057

Table 1: Mean value (±SE) Effect of the foliar application of Potassium Chloride on alkaloid contents in Canola the stem under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=6.38)
V_1 (Exceed)	28.5±3.83	24.4±3.48	30.9±4.14	29.4±3.59	34.2±3.57	32.1±3.97	29.9±4.49[a]
% age Difference		-14.3%	8.42%	3.15%	20%	12.6%	
V_2 (Cyclone)	33.46±8.06	29.3±8.12	36.03±7.71	34.3±8.19	39.23±8.36	39.7±5.71	35.3±7.45[ab]
% age Difference		-12.2%	7.69%	2.51%	17.24%	18.64%	18.72%
V_3 (Legend)	32.7±7.67	28.6±7.59	35.03±7.91	33.83±7.80	38.7±7.70	35.6±7.58	34.1±7.21[b]
% age Difference		-12.5%	7.12%	3.45%	18.3%	8.86%	14.04%
Mean LSD= (4.51)	31.5±6.32[a]	27.4±6.27[a]	34.01±6.34[ab]	32.5±6.38[ab]	37.4±6.41[ab]	35.8±6.10[b]	33.13±6.81
% age Difference		-13%	7.96%	3.17%	18.7%	13.65%	



a greater increase observed at higher concentrations. However, the foliar application of Potassium Chloride caused a decline in the alkaloid contents in stem, particularly a 13% decrease observed in the three varieties. The application of Potassium Chloride helped reduce the harmful effects of low concentrations of Lead, resulting in a lesser increase of 3.1% in the alkaloid contents in stem, compared to 7.9%, whereas a higher concentration of Lead resulted in a greater increase of 13.6%. Varieties and treatments comparison showed that the foliar application of Potassium Chloride led to a reduction in the alkaloid contents in stem of $V_{1.}$

(Exceed) by 14.3%, V_2 (Cyclone) by 12.2%, and V_3 (Legend) by 12.5%. In contrast, the application of Lead increased the alkaloid contents in stem, particularly in V_1 (Exceed) with an increase of 8.4% and 20% for 15mg/kg and 30mg/kg respectively. Similarly, V_2 (Cyclone) showed an increase of 7.6% and 17.2% with 15mg/kg and 30mg/kg, respectively, whereas V_3 (Legend) showed an increase of 7.1% and 18.3%. The application of Potassium Chloride helped reduce the toxicity of Lead, resulting in a lower increase of 3.1% compared to 8.4% and 12.6% in V_1 (Exceed). In V_2 (Cyclone), Potassium Chloride nullified the toxicity of Lead increasing by 2.5% and 18.6% with 15mg/kg and 30mg/kg, respectively, whereas in, V_3 (Legend), an increase of 3.4% and 8.8% was observed.

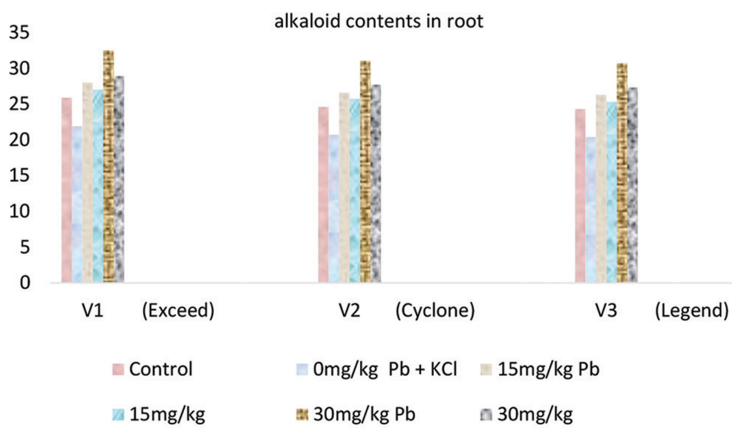
Total alkaloid contents in root

The analysis of variance presented in Table 2 demonstrated a highly significant difference in the alkaloid contents in root among treatments, with no significant difference observed between the two varieties.

A comparison of varieties and treatments also revealed no significant differences. When comparing the three varieties, V_2 (Cyclone) exhibited a higher alkaloid contents in root than V_1 (Exceed) by a value of 4.81, while V_3 (Legend) showed a decrease in alkaloid contents by 6.2%. The application of Potassium Chloride was found to reduce the effects of low concentrations of Lead and increase the alkaloid contents in root by 4.5% instead of 8.03%, and with high concentrations of Lead, it increased the alkaloid contents in root by 12.4% instead of 26%A comparison of varieties and treatments revealed that the application of Potassium Chloride decreased the alkaloid contents in root of V_1 (Exceed) by 15.4%, in V_2 (Cyclone) by 15.8%, and in V_3 (Legend) by 16%. Supplying 15mg/kg and 30mg/kg Lead resulted in an increase of 8.1% and 25.4% respectively, in the alkaloid contents in root of V_1 (Exceed). However, the

Table 2: Mean value (±SE) Effect of the foliar application of Potassium Chloride on alkaloid contents in Canola the root under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb+KCl	15mg/kg Pb	15mg/kg Pb+KCl	30mg/kg Pb	30mg/kg Pb+KCl	Mean (LSD=3.55)
V_1 (Exceed)	25.9±5.03	21.9±4.98	28±5.12	27.03±5.13	32.5±5.62	28.9±5.03	27.4±5.44
% ageDifference		-15.4%	8.10%	4.3%	25.4%	11.5%	
V_2(Cyclone)	24.6±3.23	20.7±3.14	26.6±3.27	25.76±3.24	31.06±3.62	27.7±3.40	26.08±4.27
% ageDifference		-15.8%	8.13%	4.7%	26.2%	12.6%	4.81%
V_3(Legend)	24.3±1.95	20.4±2	26.3±2.05	25.3±1.95	30.7±1.9	27.33±1.95	25.7±3.59
% ageDifference		-16%	8.23%	4.11%	26.3%	12.3%	-6.20%
MeanLSD= (2.51)	24.9±3.23[a]	21.01±3.18[ab]	26.9±3.28[b]	26.03±3.28[b]	31.45±3.54[b]	28±3.27[c]	26.4±4.47
% ageDifference		-15.6%	8.03%	4.53%	26%	12.4%	



application of Potassium Chloride reduced the alkaloid contents in root of V_1 (Exceed) by a value of 4.3% and 11.5% when low and high doses of Pb were applied, respectively. In V_2 (Cyclone), the alkaloid contents in root increased by 4.7% and 12.6% with low and high doses of Pb, respectively, but was reduced by KCl by a value of 4.1% and 12.3%. Similarly, in V_3 (Legend), the alkaloid contents in root increased by 4.1% and 12.3% with low and high doses of Pb, respectively, but was reduced by KCl by a value of 4.1% and 12.3%.

Total flavonoid contents in stem

Table 3 shows the results of ANOVA for the flavonoid contents in stem. It indicates that the flavonoid contents in stem differed significantly among treatments and the three varieties. However, there were no significant differences observed between treatments and varieties.

In V_1 (Exceed), V_2 (Cyclone), and V_3 (Legend), the application of low and high doses of Lead increased the flavonoid contents in stem by 34.4% and 107%, respectively. Conversely, the application of Potassium Chloride led to a decrease in flavonoid contents in stem by 17%. The application of KCl along with 15mg/kg and 30mg/kg Lead reduced the increase in flavonoid contents in stem to 8.2% and 68%, respectively. Furthermore, the flavonoid contents in stem increased by 42% and 99% in V_1 (Exceed) when supplied with 15mg/kg and 30mg/kg Lead, respectively. In V_2 (Cyclone), the increase was 25.4% and 98%, and in V_3 (Legend), it was 41.3% and 122.7%. The application of foliar Potassium Chloride along with low and high doses of Pb increased the flavonoid contents in stem by 7.2% and 61.5% in V_1 (Exceed). In V_2 (Cyclone), the increase was 2.3% and 62.7%, while in V_3 (Legend), it was 14.7% and 82.7%.

Total flavonoid contents in root

Table 4 presents the results of the analysis of variance for flavonoid contents in root. Table indicates that there is a highly significant difference in flavonoid contents in root among treatments, as well as among the three varieties. However, the interaction between treatments and varieties was not significant.

Comparing the three varieties, it was found that the flavonoid contents in root was present equally in both V_1 (Exceed) and V_2 (Cyclone) varieties, but V_3 (Legend) variety expressed a higher level of flavonoid contents by 18.2% compared to V_1 (Exceed) variety. In contrast, V_2 (Cyclone) variety had a 9.11% decrease in flavonoid contents in root compared to V_1 (Exceed) variety. Application of low and high doses of Lead in V_1 (Exceed), V_2 (Cyclone), and V_3 (Legend) varieties increased the flavonoid contents in root by 151% and

Table 3: Mean value (±SE) Effect of the foliar application of Potassium Chloride on flavonoid contents in Canola the stem under Lead Toxicity (*Brassica napus* L) (LSD = 0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=0.38)
V_1(Exceed)	2.76±0.47	2.33±0.32	3.93±0.25	2.96±0.20	5.5±0.72	4.46±0.73	3.66±1.19[a]
% ageDifference		-15.5%	42%	7.2%	99%	61.5%	
V_2(Cyclone)	3.03±0.47	2.6±0.3	3.8±0.5	3.1±0.25	6.0±0.25	4.93±0.56	3.9±1.29[ab]
% ageDifference		-14.1%	25.4%	2.3%	98%	62.7%	6.5%
V_3(Legend)	2.9±0.26	2.43±0.32	4.1±0.4	3.33±0.20	6.46±0.20	5.3±0.17	4.08±1.46[b]
% ageDifference		-16.2%	41.3%	14.8%	122.7%	82.7%	13.3%
MeanLSD= (0.27)	2.9±0.37[a]	2.45±0.29[b]	3.9±0.36[c]	3.14±0.25[d]	6.01±0.57[d]	4.9±0.59[e]	3.89±1.31
% ageDifference		-17%	34.4%	8.2%	107%	68%	

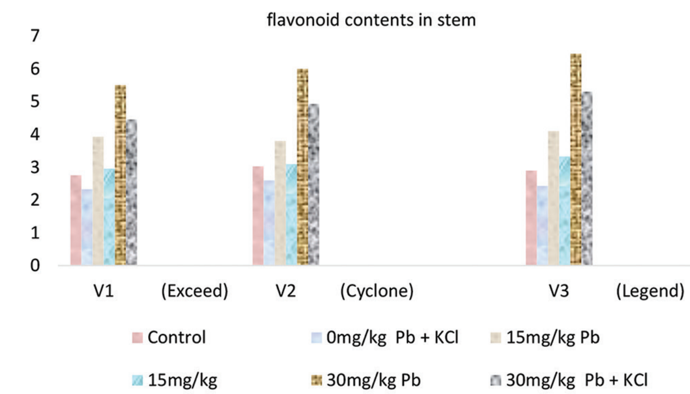
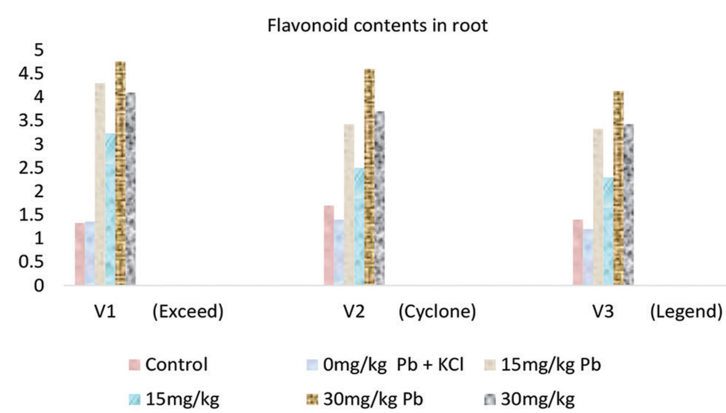


Table 4: Mean value (±SE) Effect of the foliar application of Potassium Chloride on flavonoid contents in Canola the root under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=0.30)
V_1 (Exceed)	1.33±0.05	1.36±0.25	4.30±0.66	3.23±0.47	4.76±0.48	4.1±0.2	3.18±1.45[a]
% age Difference		2.25%	223%	142%	257%	208%	
V_2 (Cyclone)	1.7±0.45	1.4±0.34	3.43±0.20	2.5±0.17	4.6±0.48	3.7±0.1	2.89±1.20[b]
% age Difference		-17.6%	101%	47%	170%	117%	-9.11
V_3 (Legend)	1.4±0.2	1.2±0.1	3.33±0.05	2.3±0.15	4.13±0.0.20	3.43±0.05	2.6±1.11[c]
% age Difference		-14.2%	137%	64.2%	195%	145%	18.2%
Mean LSD= (0.21)	1.47±0.30[a]	1.32±0.23[b]	3.69±0.57[b]	2.7±0.48[c]	4.51±0.46[d]	3.74±0.31[d]	2.90±1.26
% age Difference		-10.2%	151%	83.6%	206%	136%	



206%, respectively. The foliar application of Potassium Chloride resulted in a decrease in the flavonoid contents in root by 10.2% in all three varieties. Regarding the relationship between varieties and treatments, the flavonoid contents in root of the V_1 (Exceed) variety increased by 2.2% due to the foliar application of

Potassium Chloride. In V_2 (Cyclone) variety, the flavonoid contents in root decreased by 17.6% due to the application of Potassium Chloride, while in V_3 (Legend) variety, it decreased by 14.2%. Supplying 15mg/kg and 30mg/kg Lead increased the flavonoid contents in root by 223% and 257% in V_1 (Exceed), 101% and 170% in V_2 (Cyclone), and 137% and 195% in V_3 (Legend), respectively. The foliar application of Potassium Chloride decreased the effects of 15mg/kg and 30mg/kg doses of Pb, which increased the flavonoid contents in stem by 142% and 208% in V_1 (Exceed). In V_2 (Cyclone), the foliar applied Potassium Chloride decreased the effects of 15mg/kg and 30mg/kg doses of Pb, which increased the flavonoid contents in stem by 47% and 117%, while 64.2% and 145% increase were observed in V_3 (Legend).

Total phenolic contents in stem

The analysis of variance results for flavonoid contents in root is summarized in Table 5, which shows a highly significant difference in the contents among treatments and varieties, but no significant interaction between them.

Comparing the three varieties, it was found that V_3 (Legend) had a significantly higher level of flavonoid contents in root, by 18.2% compared to V_1 (Exceed), while V_2 (Cyclone) had a 9.11% decrease compared to V_1. The application of low and high doses of Lead increased flavonoid contents in root by 151% and 206%, respectively, in all three varieties. However, foliar application of Potassium Chloride resulted in a 10.2% decrease in flavonoid contents in root for all three varieties. Regarding the relationship between varieties and treatments, V_1 (Exceed) had a 2.2% increase in flavonoid contents in root due to the foliar application of Potassium Chloride, while V_2 (Cyclone) and V_3 (Legend) had a decrease of 17.6% and 14.2%, respectively. Supplying 15mg/kg and 30mg/kg Lead increased flavonoid contents in root by 223% and 257% in V_1 (Exceed), 101% and 170% in V_2 (Cyclone), and 137% and 195% in V_3 (Legend), respectively. Additionally, the foliar application of Potassium Chloride decreased the effects of 15mg/kg and 30mg/kg doses of Pb, which increased the flavonoid contents in stem by 142% and 208% in V_1 (Exceed), 47% and 117% in V_2 (Cyclone), and 64.2% and 145% in V_3 (Legend).

Total phenolic contents in root

According to Table 6, the phenolic contents in root varied significantly among treatments and did not differ significantly among the three varieties. However, the comparison between treatments and varieties showed significant results. In contrast, the phenolic contents in stem were present equally in all three varieties.

Supplying low and high doses of Lead to V_1 (Exceed), V_2 (Cyclone), and V_3 (Legend) varieties increased the phenolic contents in root by 30.6% and 103.7%, respectively. On the other hand, the foliar application of Potassium Chloride resulted in a decrease in phenolic contents in root by 43.5%. The foliar application of KCl had a compensatory effect on the toxicity of low and high doses of Lead, which increased the phenolic contents in root by 19.3% and 73.6%, respectively. In V_1 (Exceed), the phenolic contents of root increased by 37% and 124% with supplying 15mg/kg and 30mg/kg Lead respectively. In V_2 (Cyclone), supplying low and high doses of Pb increased the phenolic contents in root by 17.3% and 76.5%, while in V_3 (Legend), supplying low and high doses of Pb increased the phenolic contents in root by 39.7% and 115.9%. The effects of low and high doses of Lead on phenolic contents in root were compensated by KCl by a value of 2.1% and 3.3% in V_1 (Exceed), 6.1% and 42.2% in V_2 (Cyclone), and 28.4% and 89.2% in V_3 (Legend).

Total tannin contents in stem

Table 6 revealed a highly significant difference in tannin contents in stem among treatments, while no significant difference was observed among the three varieties. However, significant results were observed when comparing treatments and varieties.

The tannin contents in stem were uniform across all three varieties. When low and high doses of Lead were supplied to V_1 (Exceed), V_2 (Cyclone), and V_3 (Legend) varieties, it resulted in an increase in tannin contents in stem by 30.6% and 103.7%, respectively. In contrast, the foliar application of Potassium Chloride caused a decrease in tannin contents in stem by 43.5%. KCl compensated for the toxicity of low and high doses of Lead and increased tannin contents in stem by 19.3% and 73.6%, respectively. The supply of 15mg/kg and 30mg/kg Lead to V_1 (Exceed) increased tannin contents in stem by 37% and 124%, respectively. In V_2 (Cyclone), supplying low and high doses of Pb increased the tannin contents in stem by 17.3% and 76.5%, while in V_3 (Legend), supplying low and high doses of Pb increased tannin contents in stem by 39.7% and 115.9%. KCl compensated for the effects of low and high doses of Lead on phenolic contents in root by 2.1% and 3.3% in V_1 (Exceed), 6.1% and 42.2% in V_2 (Cyclone), and 28.4% and 89.2% in V_3 (Legend).

Total tannin contents in root

Table 8 presents the results of the analysis of the variance of the tannin contents in root. The data showed that there was no significant difference among treatments, but there was a highly significant difference between the two varieties. The interaction between varieties and treatments did not yield significant results either.

Table 5: Mean value (±SE) Effect of foliar on the application of Potassium Chloride on phenolic contents in Canola the stem under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=0.27)
V_1 (Exceed)	2.56±0.46	2.13±0.49	3.73±0.15	2.86±0.30	4.23±0.05	3.93±0.15	3.24±0.83
% age Difference		-16.7%	45%	11%	65.2%	53.5%	
V_2 (Cyclone)	2.46±0.30	2.2±0.35	3.2±0.1	3.96±0.20	3.53±0.37	2.3±0.4	3.1±0.66
% age Difference		-10.5%	30%	60%	43.4%	-6.5%	-4.32%
V_3 (Legend)	2.3±0.4	2.03±0.3	3.26±0.11	2.7±0.3	4.26±0.05	3.66±0.11	3.07±0.81
% age Difference		-11.7%	41%	17.3%	85%	56%	-5.24%
Mean LSD= (0.19)	2.44±0.36[a]	2.13±0.35[b]	3.4±0.32[c]	2.98±0.27[d]	4.15±0.18[e]	3.7±0.27[f]	3.13±0.76
% age Difference		-12.7%	39.3%	22.1%	70%	51.6%	

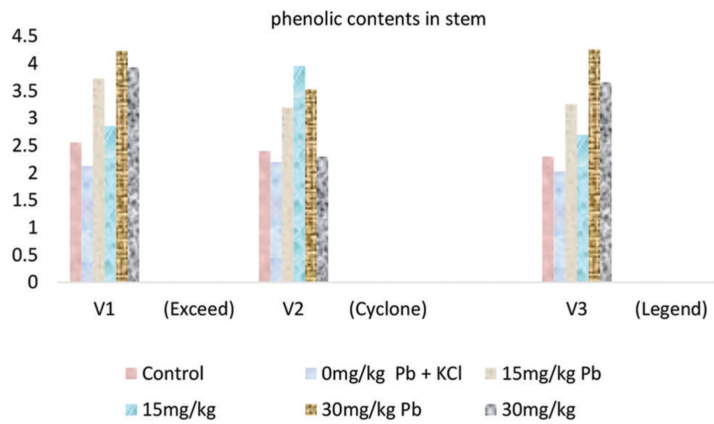
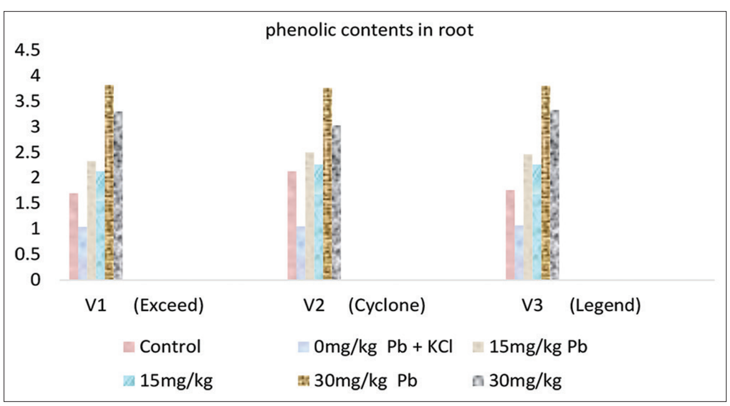


Table 6: Mean value (±SE) Effect of the foliar application of Potassium Chloride on phenolic contents in Canola the stem under Lead Toxicity (*Brassica napus* L) (LSD=0.05).

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=0.14)
V_1 (Exceed)	1.7±0.1	1.04±0.01	2.33±0.05	2.13±0.05	3.82±0.23	3.3±0.23	2.39±0.97
% age Difference		-38%	37%	25.2%	124%	94.1%	
V_2 (Cyclone)	2.13±0.25	1.05±0.01	2.5±0.1	2.26±0.15	3.76±0.15	3.03±0.208	2.45±0.86
% age Difference		-50.7%	17.3%	6.1%	76.5%	42.2%	2.5%
V_3 (Legend)	1.76±0.15	1.07±0.15	2.46±0.05	2.26±0.05	3.8±0.26	3.33±0.15	2.45±0.94
% age Difference		-39.2%	39.7%	28.4%	115.9%	89.2%	2.4%
Mean LSD=(0.10)	1.86±0.25[a]	1.05±0.01[b]	2.43±0.1[c]	2.22±0.1[d]	3.79±0.19[e]	3.23±0.22[f]	2.43±0.91
% age Difference		-43.5%	30.6%	19.3%	103.7%	73.6%	



Compared to the V_1 (Exceed) variety, the tannin contents in root was higher in the V_2 (Cyclone) variety by 4.47%, while in V_3 (Legend), it increased by 1.47%. The application of Potassium Chloride resulted in a

Table 7: Mean value (±SE) Effect of the foliar application of Potassium Chloride on tannin contents in Canola the stem under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb+KCl	30mg/kg Pb	30mg/kg Pb+KCl	Mean (LSD=0.16)
V_1 (Exceed)	0.54±0.15	0.22±0.07	1.6±0.2	4.06±0.15	2.4±0.1	1.87±0.29	1.33±0.75
% age Difference		-59.2%	196.2%	651.8%	344%	246.2%	
V_2 (Cyclone)	0.62±0.25	0.35±0.17	1.59±0.10	1.39±0.09	2.27±0.14	1.82±0.06	1.34±0.69
% age Difference		-43.5%	206.4%	124%	266%	193.5%	0.75%
V_3 (Legend)	0.47±0.14	0.19±0.09	1.7±0.17	1.53±0.20	2.41±0.03	1.9±0.36	1.36±0.82
% age Difference		-59.5%	261%	225.5%	412%	304%	2.25%
Mean LSD=(0.11)	0.54±0.17[a]	0.25±0.12[b]	1.63±0.15[c]	1.43±0.15[d]	2.36±0.11[e]	1.86±0.23[f]	1.34±0.75
% age Difference		-53.7%	201.8%	164.8%	337%	244.4%	

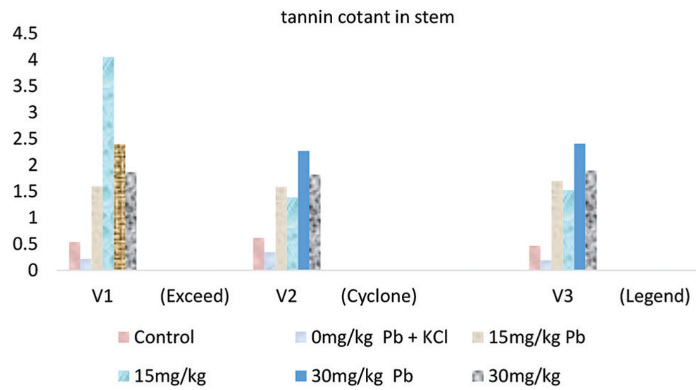
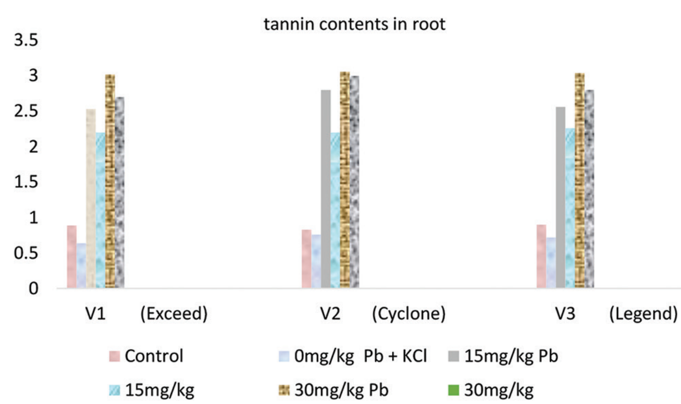


Table 8: Mean value (±SE) Effect of the foliar application of Potassium Chloride on tannin contents in Canola the root under Lead Toxicity (*Brassica napus* L) (LSD=0.05).

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb+KCl	Mean (LSD=0.18)
V_1 (Exceed)	0.89±0.09	0.64±0.12	2.53±0.25	2.2±0.1	3.02±0.11	2.7±0.23	2.01±0.95
% age Difference		-28%	184%	147%	239.3%	203%	
V_2 (Cyclone)	0.83±0.07	0.76±0.06	2.8±0.1	2.2±0.26	3.06±0.14	3±0.55	2.10±1.02
% age Difference		-8.43%	273.3%	165%	268.6%	261.4%	4.47%
V_3 (Legend)	0.9±0.05	0.72±0.13	2.56±0.15	2.26±0.20	3.04±0.15	2.8±0.1	2.04±0.94
4 % age Difference		-20%	184.4%	151.1%	237.7%	211.1%	1.47%
Mean LSD=(0.13)	0.87±0.07[a]	0.70±0.11[a]	2.63±0.2[b]	2.22±0.17[c]	3.04±0.11[d]	2.85±0.32[d]	2.05±0.95
% age Difference		-19.5%	202.2%	155.1%	249.4%	227.5%	



decrease in tannin contents in root by 19.5% across all three varieties. However, the application of Potassium Chloride compensated for the low concentration of Lead, which increased the tannin contents in root by

155.1%, and the high concentration of Lead, which increased the tannin contents in root by 227.5%. In the Exceed variety, supplying 15mg/kg and 30mg/kg Lead increased the tannin contents in root by 184% and 239.3%, respectively. In Cyclone, the tannin contents in root increased by 273.3% and 268.6% with the same doses of Lead. In Legend, the tannin contents in root increased by 184.4% and 237.7%. When foliar Potassium Chloride was applied, it reduced the toxicity of low and high doses of Pb, resulting in an increase in tannin contents in root of 147% and 203% in Exceed. In Cyclone, low and high doses of Pb increased the tannin contents in root, which was alleviated by KCl, resulting in an increase of 165% and 261.4%. In Legend, the increase was observed to be 151.1% and 211.1%.

Total saponin contents in stem

Table 9 presents the ANOVA results for saponin contents in stem. Table shows that saponin contents in stem differed highly significantly among treatments but not significantly among the three varieties. Additionally, interactions between varieties and treatments did not reveal significant differences.

A comparison of all varieties indicated that V_2 (Cyclone) had a higher saponin contents in stem compared to V_1 (Exceed) by 2.18, while Legend had a saponin contents of 2.91%. Low and high doses of Lead in the three varieties increased the saponin contents in stem by 25.8% and 45.8%, respectively. However, foliar application of Potassium Chloride resulted in a decrease of saponin contents in stem by 40% in all three varieties. A low concentration of Lead increased saponin contents in stem by 16.6% due to compensation by KCl, while a high concentration of Lead increased it by 36.6%. In V_1 (Exceed), saponin contents in stem increased by 18.6% and 43% by supplying 15mg/kg and 30mg/kg Lead, respectively. In V_2 (Cyclone), saponin contents in stem increased by 26.8% and 43% by supplying 15mg/kg and 30mg/kg Lead, respectively. In V_3 (Legend), saponin contents in stem increased by 32.7% and 53% by supplying 15mg/kg and 30mg/kg Lead, respectively. Application of Potassium Chloride alleviated the toxicity of low and high doses of Pb, resulting in an increase of saponin contents in stem by 10.5% and 34.9% in V_1 (Exceed), and by 16.2% and 34.9% in V_2 (Cyclone), while an increase of 23.5% and 41.8% were observed in V_3 (Legend).

Total saponin contents in root

According to Table 10, the ANOVA results indicated a highly significant difference in saponin contents in root among treatments and between the two varieties. However, comparisons between treatments and varieties did not show significant differences.

When comparing the three varieties, V_2 (Cyclone) showed a 7.3% increase in saponin contents in root compared to V_1 (Exceed). V_3 (Legend) exhibited a 26.8% increase in saponin contents in root compared to V_1 (Exceed). Low and high doses of Lead increased saponin contents in root in all three varieties by 200% and 310%, respectively. When a low concentration of Lead was combined with foliar application of KCl, the combined effect led to a 110% increase in saponin contents in root, while the high concentration of Lead led to a 205% increase. The interaction between varieties and treatments showed that in V_1 (Exceed), the saponin contents in root decreased by 69.2% due to the foliar application of Potassium Chloride. In V_2 (Cyclone), the saponin contents in root decreased by 44.8%, and in V_3 (Legend) by 51.6% due to the application of Potassium Chloride. When foliar Potassium Chloride was applied with low and high doses of Pb, their combined effect led to a 50% and 119.2% increase in saponin contents in root of V_1 (Exceed). In V_2 (Cyclone), the combined effect led to a 37.9% and 89.3% increase, while in V_3 (Legend) the increase was 54.8% and 138.7%.

DISCUSSION

The research work on the determination of various phytochemicals showed an increase in the establishment of flavonoids, alkaloids, phenols, and tannins in stem and the root of plants that were treated with various amounts of Lead. Our results are similar to the findings of a past researcher who described an increase of phenolics and flavonoid contents in plants that were treated with heavy metals (Márquez-García et al.).

Our results described that metal stress (Lead) causes harmful effects on plant growth by might be producing ROS species, but the plant has adapted to nullify its effects by the production of various non-enzymatic antioxidants like alkaloids, terpenoids, tannins, phenolics, flavonoids and saponins. Related results reported by Hasanuzzaman showed that abiotic stresses are the main reducing factors. They are influencing plant growth and development, universally. I am interested in exploring the biochemical, molecular, physiological, and cellular mechanisms that underlie responses to abiotic stress and developing effective techniques to enhance sustainable agricultural production. Abiotic stress can lead to the accumulation of ROS, which can cause oxidative damage in plants. However, the metabolism of ROS is critical for plant growth, development, adaptation, and survival in stressful environments. ROS production and scavenging are essential components of plant defence mechanisms. Overexpression of candidate genes that

Table 9: Mean value (±SE) Effect of the foliar application of Potassium Chloride on Saponin contents in Canola the stem under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=0.11)
V_1 (Exceed)	1.23±0.05	0.76±0.15	1.46±0.05	1.36±0.05	1.76±0.05	1.66±0.05	1.37±0.34
%age Difference		-38.2%	18.6%	10.5%	43%	34.9%	
V_2 (Cyclone)	1.23±0.15	0.73±0.20	1.56±0.05	1.43±0.11	1.76±0.05	1.66±0.05	1.4±0.36
%age Difference		-40%	26.8%	16.2%	43%	34.9%	2.18%
V_3 (Legend)	1.13±0.20	0.66±0.25	1.5±0.1	1.4±0.1	1.733±0.05	1.6±0.1	1.33±0.38
%age Difference		-41.5%	32.7%	23.8%	53%	41.5%	-2.91%
Mean LSD=(0.08)	1.2±0.14[a]	0.72±0.18[a]	1.51±0.07[b]	1.4±0.08[b]	1.75±0.05[c]	1.64±0.07[d]	1.37±0.359
%age Difference		-40%	25.8%	16.6%	45.8%	36.6%	

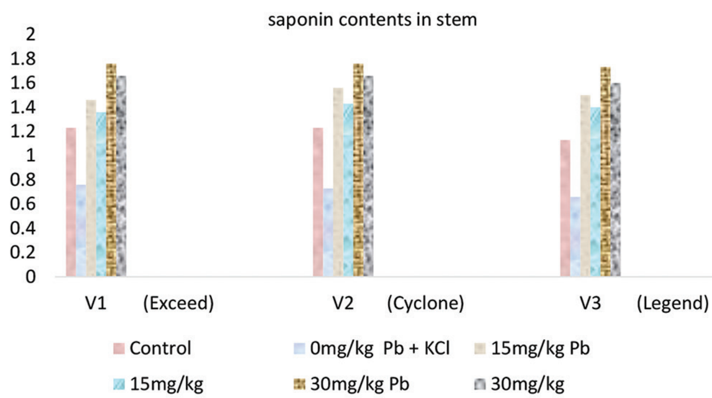
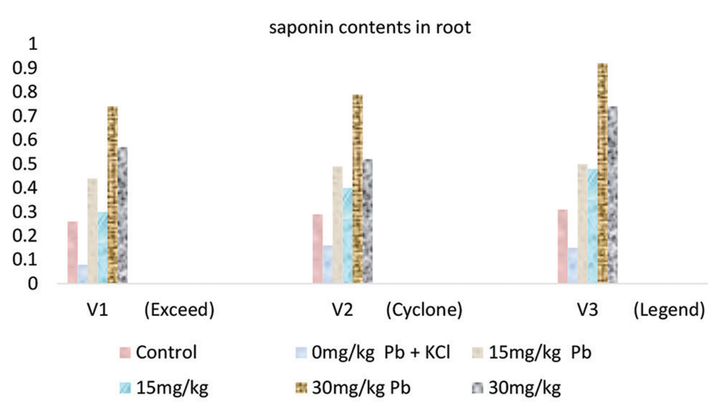


Table 10: Mean value (±SE) Effect of the foliar application of Potassium Chloride on Saponin contents in Canola The roots under Lead Toxicity (*Brassica napus* L) (LSD=0.05)

	Control	0mg/kg Pb + KCl	15mg/kg Pb	15mg/kg Pb + KCl	30mg/kg Pb	30mg/kg Pb + KCl	Mean (LSD=0.07)
V_1 (Exceed)	0.26±0.05	0.08±0.03	0.44±0.10	0.39±0.05	0.74±0.07	0.57±0.052	0.41±0.22[a]
%age Difference		-69.2%	69%	50%	184.6%	119.2%	
V_2 (Cyclone)	0.29±0.04	0.16±0.03	0.49±0.03	0.4±0.04	0.79±0.17	0.52±0.10	0.44±0.21[b]
%age Difference		-44.8%	68.9%	37.9%	172.4%	79.3%	7.3%
V_3 (Legend)	0.31±0.04	0.15±0.06	0.57±0.05	0.48±0.1	0.92±0.06	0.74±0.10	0.52±0.27[b]
%age Difference		-51.6%	83.8%	54.8%	196.7%	138.7%	26.8%
Mean LSD=(0.05)	0.20±0.04[a]	0.13±0.05[b]	0.60±0.08[c]	0.42±0.07[d]	0.82±0.12[e]	0.61±0.12[f]	0.46±0.23
%age Difference		-35%	200%	110%	310%	205%	



encode ROS-detoxifying enzymes has been widely used to improve tolerance to various abiotic stresses. Nonetheless, the balance between ROS generation and detoxification is maintained through both enzymatic and non-enzymatic antioxidant systems under stress conditions (Hasanuzzaman et al., 2020).

This study showed that phytochemicals of non-enzymatic antioxidant nature were decreased by the foliar spray of nutrients. A decrease in phytochemical contents by the application of nutrients in metal-treated plants might be its involvement in GSH synthesis(Ramírez et al., 2013). Similarly, its involvement in glutathione (GSH) and phytochelatin (PCs) production could also be the cause. The role of nutrients in ameliorating mental stress through the production of enzymatic antioxidants might reduce the need and production of non-enzymatic antioxidants (Livak & Schmittgen, 2001). The observed decrease in phytochemical contents when potassium chloride is added can be explained by the reason that potassium competes with other minerals, such as calcium and magnesium, for uptake by the plant. When there is an excess of potassium, it can interfere with the absorption of these other minerals, which are necessary for the synthesis of certain phytochemicals. Therefore, the addition of potassium chloride may lead to a decrease in the overall production of certain phytochemicals.

CONCLUSION

The study conducted at the Botanical Garden and Research Laboratory of the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan suggests that the foliar spray of potassium can reduce the toxicity of heavy metals in Canola plants. The experiment showed that the phytochemical studies, which include alkaloid, flavonoid, and phenolic, indicated an increase in response against heavy metal stress. However, the plants treated with both lead metal and potassium showed a lesser increase in phytochemical studies than plants treated with only metal. The findings of the study suggest that the use of potassium can improve the growth of Canola plants and reduce the harmful effects of heavy metals. However, further studies are necessary to identify the mechanisms by which potassium mediates the alleviation of heavy metal toxicity. Overall, this study provides valuable insight into the potential role of potassium in managing heavy metal stress in plants.

Authors contribution

Ghulam Yasin and shahzadi Saima designed experiment. Aqsa Ahmad and Farah Akmal conducted experiment and participated in manuscript drafting and proof reading. Adeela Altaf and Ikram ul Haq analysed data and edited manuscript.

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