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Research Article

Physiological and biochemical responses of lettuce plant to the allochemical compound of paraxanthine

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Abstract

In this study, the effects of paraxanthin on lettuce were investigated. Paraxanthin is a group of purine alkaloids, secondary metabolites of plants. This compound is made up of the caffeine metabolism found in plants such as tea, coffee and cocoa. There have been previous reports of paraxanthine antihypertensive properties. Three different concentrations of paraxanthine as 10, 50 and 100 µg/ml were treated on lettuce seedlings and then some physiological and biochemical parameters were measured at 7 leave stage of the plants. The results showed that seed germination, radicle and plumule growth of lettuce plant decreased at different concentrations of the paraxanthine in a dose dependent manner. At the concentration of 100µg/ml of paraxanthine, all of the growth parameters were completely inhibited. Some measured parameters such as wet and dry weights of the shoots, photosynthetic rate, photochemical efficiency of photosystem II, leaf relative content and total protein decreased in the paraxanthine treated plants compared to the control group. However, some other parameters such as proline content, hydrogen peroxide and the activity of polyphenol oxidase, ascorbate peroxidase, catalase and protease in the treated plants increased. On the other hand, a significant change in electrophoretic pattern of the plant leaf proteins was observed. Some electrophoretic bands in the treated plants with 100µg/ml of paraxanthine removed in comparison with the control group. The present study demonstrated that the paraxanthine as an allelochemical causes some physiological and biochemical responses in the lettuce which are much similar to those appears under abiotic stress.

Key words: Allelochemicals, Allelopathy, Lettuce, Paraxanthine

Introduction

In the recent decades, allelopathy and its related mechanisms has been increasingly subjected to study by biologists. The main purpose of this study has been aimed to designate bioherbicides that combats weeds effectively with more less harmful envirmental effects.

Allelopathy has been described as a positive or negative interaction between plants through chemicals released into the environment. The chemicals named as allelochemicals that release from source plant to suppress surrounding plants (Iqbal and Fry, 2012; Ashihara, 2006; Wang, 2012). An allelochemical can inhibit some plant functions such as respiration; photosynthesis; stomata function; cell membrane permeability; cell proliferation; chlorophyll content; protein synthesis and alteration of enzyme activity as described by Narwal (1996).

Purine alkaloids are regarded as plant secondary metabolites derived from purine nucleotides. Paraxanthine as a purine alkaloid is produced from caffeine metabolism pathway in some plant such as Tea, coffee and cacao (Ashihara, 2008). It is a dimethyl derivative of xanthine known as a caffeine structurally related compound. In addition to paraxanthine, other caffeine-like compounds found in human or animals urine consuming tea or caffea (Rodriguez-Gil *et al*., 2018). Explaned that paraxanthin is also commonly used anthropogenic marker and release from urbane sewage to environmental waters.

A previous report revealed that paraxanthin along with some other purin alkaloids such as caffein and theophyllin indicate considerable allelopathic potential. Some previous reports demonstrated allelopathic potential of a number of purine alkaloids such as paraxanthin on lettuce plants. It was shown that this compound inhibit seed germination and seedlind growth of lettuce plants (Muchtaridi *et al*., 2021). It was also described that purine alkaloids in seed coat of tea plants indicated authotoxicity and stunt germination of contained seeds (Chen-Yang *et al*., 2011). Sasamoto *et al*. (2015) demonstrated that purin alkaloids inhibit cell division and colonization of lettuce cells.

The current study aims to study the effects of various concentration of paraxanthine on some physiological and biochemical process of lettuce such as growth attributes, photosynthetic parameters, antioxidant machinery and osmo protectant systems.

Material and methods

Treatments and measurement of growth

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parameters: All of the planting process and experiments were performed at the University of Mohaghegh Ardabili, Faculty of Science. Allelopathic test was performed on *Lactuca sativa* L cv. Siahoo. Four doses of paraxanthine were prepared by dilution of the paraxanthine with pure water. Seeds were uncontaminated with NaClO (5%) for 5 min and then washed five times with pure water. Four replications of tests comprised of 25 seeds were prepared four each treatment using sterile petri dishes (90 mm) lined with a single filter paper (whatman, number 2). Five ml of different paraxanthine was added to each of petri dishes then prepared plate were placed in Incubator at 25℃. Each day the number of germinated seeds was counted and after 5 days germination percentage in treatment and control groups was calculated. After one weeks the lettuce seedlings were transplanted to pots containing peat and were irrigated with Hoagland nutrient solution containing paraxanthine with the concentration of 0.01, 0.05 and 0.1 mg/ml. After 28 days of applying the treatments, the plants were harvested for performing the experiments. The relation elongation ratio of root and shoot were calculated. In the case of cultured seedlings in above concentrations, the fresh and dry weight of shoots, in the treated group were compared to the control group.

Leaf relative water content (RWC): The youngest mature leaves were determined for RWC evaluation. After measurement of fresh weight, the leaves were hydrated by floating in distillated water for 48 h. The leaves were subjected to weighting again to get turgid weight (TW) and then incubated for 48h at 80℃. Finally dry weight (DW) was measured. (Deef and Fattah, 2008).

RWC (%) = [F(W-DW) / (TW-DW)] × 100

W: Fresh weight of leave sample

TW: Turgid weight of leave sample

DW: Dry weight of leave sample

Chlorophyll and carotenoids contents: One hundred mg of fresh leaves were extracted by 5 ml acetone 80%. Pigments content were measured using the spectroscopic method. Amounts of chlorophyll *a*, chlorophyll *b* and total chlorophyll and carotenoids were determined at A645, A663 and A440 using the following formula (Lichtenthaler and Buschmann, 2001).

Chl*a* = (12.7.A663-2.69.A654) V/1000W

Chl*b* = (22.9.A645-4.68.A663) V/1000W

[Chls *a*+*b*] = 20.21.A645+8.62.A663

Car = 100(A470)-3.27(mg chl.*a*)-104(mg chl.*b*)/227

SPAD unit of chlorophyll, photosynthesis rate and chlorophyll fluorescence: Three lettuce plants from each treatment were chosen from different pots. Gas exchange parameter measurements were performed on youngest leaves. After 28 days, the photosynthesis rate was measured with an LI 6400 portable infrared gas analyzer IRGA. The chlorophyll content along with photosynthesis rate of expanded leaves were measured based on method of Lavresunior *et al.* (2012). 28 days after initiating the treatment, relative chlorophyll content was determined in SPAD units, by chlorophyll meter. Prior to measurement of chlorophyll florescence, the leaf samples were dark-adapted at room temperature. Three leaves from each group were used. After adaptation in dark condition, each leaf sample was put in FMS leaf clip (7 mm aperture) and then Fv/Fm was measured using Fluorescence Monitoring System (FMS, Hansatech).

Free amino acids content: 0.5 g frozen leaves were homogenized in 50 mM phosphate buffer solution and then centrifuged in 3000 g for 20 min, then to the supernatant was added 350 mg ninhydrin solution and placed in warm water bath at 70-100℃ and then the reaction was abolished using ice bath and the absorption read at 570nm. The total free amino acids content was evaluated using a standard curve prepared with known concentration of glycine (Wagner, 1979).

Proline content: Frozen leaves (0.5 g) were homogenized in 10 ml of sulphosalicyilc acid (3%) and then centrifuged 10000 g. the supernatant (0.5 ml) was mixed with 1ml of ninhydrin (2.5%). The mixture was kept at 100℃ for 1 h and then the reaction was terminated by cooling the mixture in ice bath. The reaction mixture was extracted by 2 ml of toluene and finally the absorption was recorded at 520 nm. The proline concentration was calculated by using a standard curve (Bates e*t al*., 1973).

Glycine betaine content (GB): Analysis of glycine betaine were determined according to the method of Grieve and Grattan (1983). 0.5 gr frozen leaves were powdered with liquid nitrogen and were homogenized with 20 ml of pure water. The homogenate was incubated for 24 in an incubator at 25℃. 0.5 ml of filtrated extract was blended with 2 N H_2SO_4 solution and 0.1 ml of potassium tri-iodide solution (containing 3.72 g Iodine and 10 g potassium iodide in 50 ml of 1 N HCl) and then was shaken in an ice-cold water bath for 30 min. Finally, 14 ml of dichloroethane was added to the mixture and were quaked for 48 h at 25℃. The concentrations of glycine betaine were calculated using standard curve prepared with particular concentration of GB (Grieve and Grattan, 1983).

Hydrogen peroxide (H_2O_2) content: Hydrogen peroxide level were identified according to Velikova *et al.* (2000). Frozen leaves (0.5 g) homogenized in an ice bath using 1 ml 0.1% TCA. The homogenate mixture was centrifuged at 10000 g for 15 min. Then to each tube were added 10 mM of potassium phosphate buffer (pH=7.0) and 100 µL of 1 M KI. The absorption was recorded at 390 nm. Hydrogen peroxide content of leaves was calculated by using a standard curve prepared with various concentration of H_2O_2 (Moradi *et al.*, 2018).

Lipid peroxidation: To estimate of malondialdehyde (MDA) content of leaves, as a measure of the amount lipid peroxidation, the thiobarbituric acid (TBA) assay was used (De voce, 1997). 0.3 g fresh leaves were homogenized with 5 ml

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of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 g for 15 min and 0.5 ml of the supernatant was mixed with 2 ml of TBA (0.5%) in TCA (20%). The mixture was incubated for 1 h at 100℃. When reaction stopped, the mixture was centrifuged at10,000 g for 5 min. The absorbance of the supernatant was screened at 532 and 600 nm (Moradi *et al*., 2018). The level of lipid peroxidation was calculated by using the related extinction coefficient (155 mM^{-1} cm^{-1}).

Catalase (CAT) activity assay: Catalase activity assay was recorded by disappear H_2O_2 at 240 nm, the reaction mixture contains 50 mM K-phosphate buffer (pH=7) and 0.3 ml H_2O_2 3%. The enzyme activity is determined by Beer Lambert Law with extinction co efficiency of 39.4 $mM^{-1}.cm^{-1}$ (Cakmak and Marschner, 1992).

Polyphenol oxidase (PPO): The enzymatic activity of polyphenol oxidase, pyrogallol was used as a substrate. The reaction mixture consisted 2.5 mM potassium phosphate buffer (pH=6.8) and 0.2 mL of 4% pyrogallol. The mixture was incubated at 40˚C and then the reaction was added 0.2 ml enzyme extraction and absorbance was recorded at 430 nm. The enzyme activity was determined by Beer Lambert Law with extinction co efficiency of $\boldsymbol{\varepsilon}$:2.47 $mM^{-1}.cm^{-1}$ (Moradi *et al*., 2018).

Ascorbate peroxidase (APX): Ascorbate-peroxidation activity was measured according to the method of Nakano and Asada (1981) by reducing concentration of ascorbate at 290 nm. The reaction mixture contained 0.05 M potassium phosphate buffer (pH 7.0), 0.6 mM ascorbate and 0.2 ml of 3% hydrogen peroxide in a total volume of 6 ml. The reaction was initialized by adding hydrogen peroxide and the change in absorbance was measured at 290 nm with extinction co efficiency of 2.8 $mM^{-1}.cm^{-1}$ (Moradi *et al*., 2018).

Protease active assay: The procedure of Brik *et al*. (1962) as used for the determination of protease activity. The reaction mixture consisted of 1 ml of 1% Casein (prepared in 0.05 M phosphate buffer) and then incubated at 45 °C, for 1 h. The reaction was terminated by addition of 0.1 mL of 40% trichloro acetic acid (TCA). Absorbance was recorded 280 nm. The activity of protease enzyme was calculated by using the extinction coefficient of 21.5 $mM^{-1}.cm^{-1}$ (Moradi *et al*., 2018).

Total protein content: Protein concentration in the enzyme extract was evaluated using Folin Ciocalteu reagent by the method of Bradford (1976). Bovine Serum Albumin was used for preparation of standard curve. The absorption was measured at 540 nm using spectrophotometer (Moradi *et al*., 2018).

SDS-PAGE procedure: Protein profiling studied on 10% SDS-PAGE. By adding sample buffer (5X) and protein samples in 1:4 ratio, the samples were prepared for electrophoresis. The same preparation method was also repeated for standard protein marker. Samples were subjected to boiling water bath for 2 to 3 min and then were injected using a micro syringe into wells through electrode buffer. Also, the standard marker was injected into the gel to identify the molecular weight of the bands.

Each sample well was loaded with 100 µg of protein in 10 µl of sample buffer containing bromophenol blue as tracking dye. The prepared gel was run at 50 mA current and voltage of 150 V. Protein bands were detected by coomassie brilliant blue staining. Finally, the stained gel was then transferred to de staining solution (Methanol and glacial acetic acid in 5:1 proportion). SDS-PAGE destain solution is to de stain Coomassie dye from the gel (Ladizinsky and Hymowitz, 1979).

Statistical analysis: All of experiments were set at a complete randomized design with three replications. All of data were analyzed using ANOVA followed by a *Duncan* test (SPSS Version 16) at $P \leq 0.05$.

Results

Growth parameters: The effect of paraxanthine on the growth parameters of lettuce showed that, paraxanthin significantly ($P \leq 0.05$) reduced seed germination, root and shoot growth as well as shoot fresh-dry weights of lettuce (Table 1, Fig. 1). Paraxanthine decreased the seed germination by 5.26%, 10.5% and 18.7% compared to the control with the concentration of 10, 50 and 100 µg/ml, respectively. Significantly negative correlation existed between paraxanthine and root and shoot lengths, as well. The maximum inhibition in shoot and root length was found at 100 µg/ml of paraxanthine. The shoot and root length were minimized to 42.8% and 16.6% at same concentration of paraxanthine, respectively. Also, our results indicated that dry and fresh weight shoot of lettuce seedlings were significantly lowered in the all concentration of paraxanthine. The highest of concentration (100 µg/ml) reduced fresh and dry weight of shoot by 47% and 43% compared with the control, respectively.

Leaf relative water content (RWC): The RWC of lettuce plants was significantly decreased from %74 in the control group to 60, 55 and 51% at 10, 50 and 100 µg/ml paraxanthine, respectively (Table 1, Fig. 2).

Photosynthetic pigments: Our results revealed that chl*a*, chl*b*, total chlorophyll and carotenoids content of lettuce plants were significantly diminished at paraxanthine treatments at $P \leq 0.05$(Table 1). At the highest concentration of the paraxanthine (100 µg/ml) the level of chl*a*, chl*b*, total chlorophyll and carotenoids reduced by 91.5%, 38.5%, 57.5% and 74.4% compared with the control, respectively (Fig. 3).

SPAD, photosynthesis rate, chlorophyll florescence: The results demonstrated that relative chlorophyll content (SPAD) significantly decreased in paraxanthine treated groups of lettuce plants than control ones (Table 1). At the concentration of 10, 50 and 100 µg ml^{-1} of paraxanthine SPAD unit of chlorophyll showed a decrease to 14.7%, 27.2% and

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Table 1. Analyses of variance table for physiological parameters

Parameters / SV	Shoot Length	Root Length	Shoot fresh weight	Root Fresh weight	RWC	Chl a	Chl b	SPAD	P.R.	Fv/Fm
Paraxanthin Treatment	13.30*	5.11*	0.37**	0.30**	7850.11*	34577.32*	807341.65*	0.86*	0.00086*	0.00033*
Error	2.501	0.820	0.021	0.025	603.812	2659.700	53822.712	0.091	0.000043	0.00003

****, * indicate significant at 1 and 5 % levels of probability, respectively**

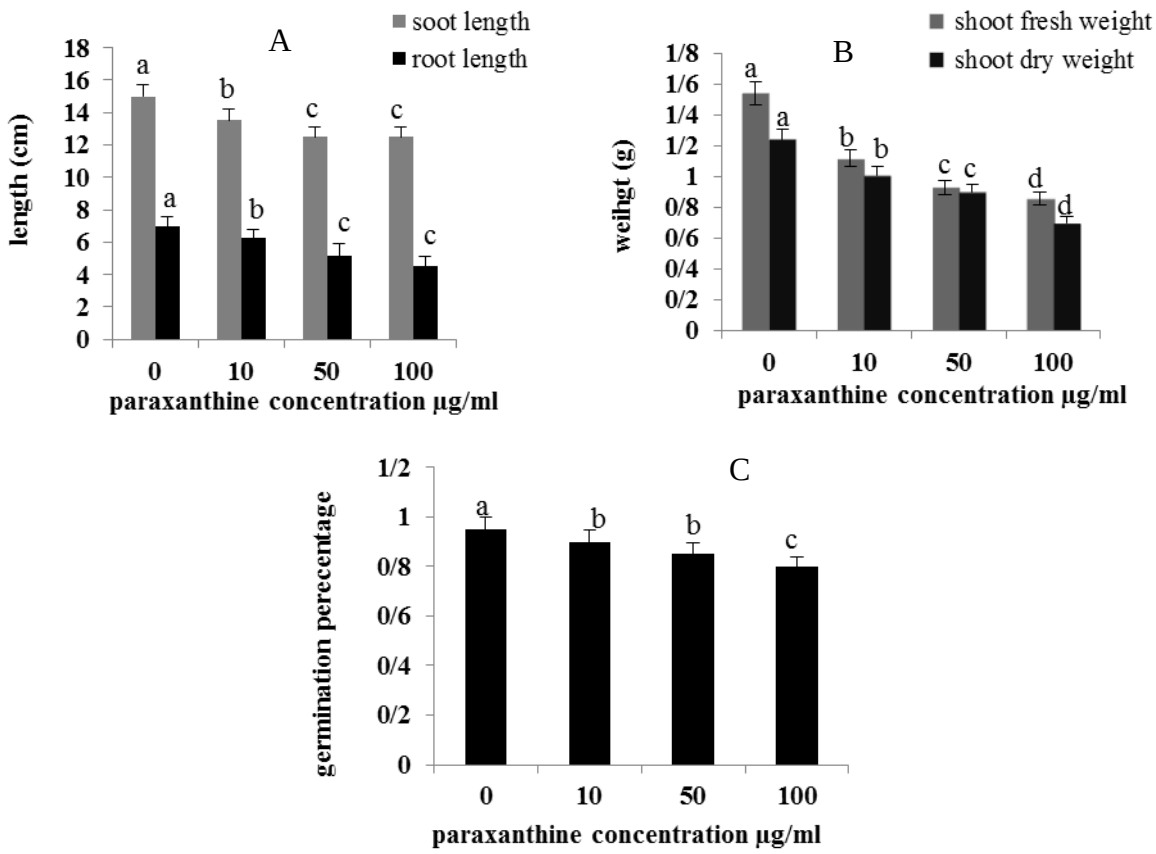


Figure 1. The effect of paraxanthine on root and shoot length (A). dry weight and fresh weight (B). Seed germination percentage (C) of lettuce. Columns with at least one similar letter are not statistically significant differences (P≤0.05).

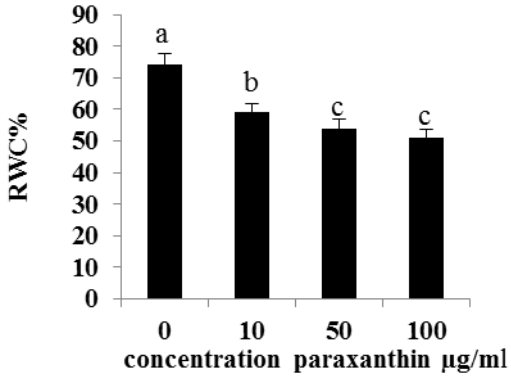


Figure 2. The effect of paraxanthine on the relative water content (RWC). Columns with at least one similar letter are not statistically significant differences (P≤0.05).

33.3% % in comparison to the control group (Fig. 4A).

Significant relation was also observed between the lettuce plants in photosynthetic rate. Photosynthetic rate was reduced after application of paraxanthine at 10, 50 and 100 µg ml^{-1} of the compound about 60%, 60% and 80% of the control value, respectively (Fig. 4B).

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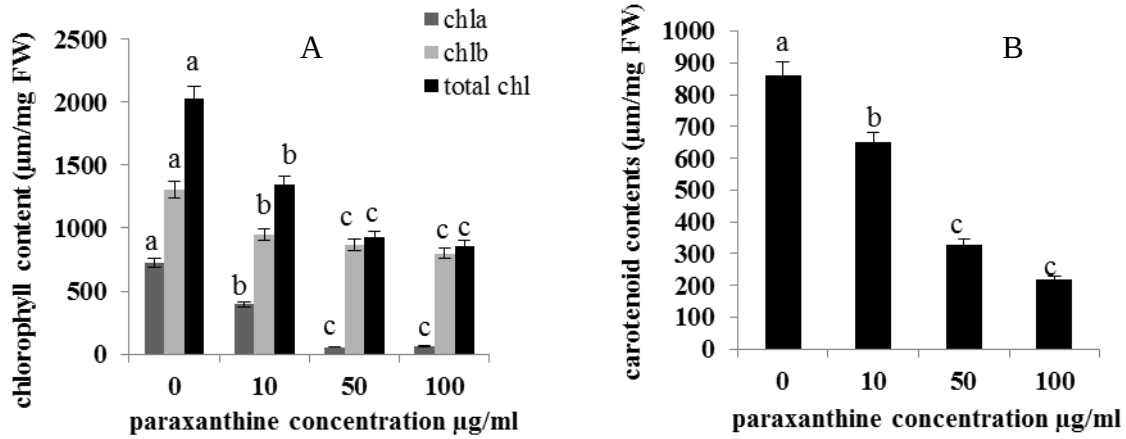


Figure 3. The effects of paraxanthine on (A) chlorophyll content (mg/g FW) and (B) carotenoid content (mg/g FW) of lettuce. Columns with at least one similar letter are not statistically significant differences (P≤0.05).

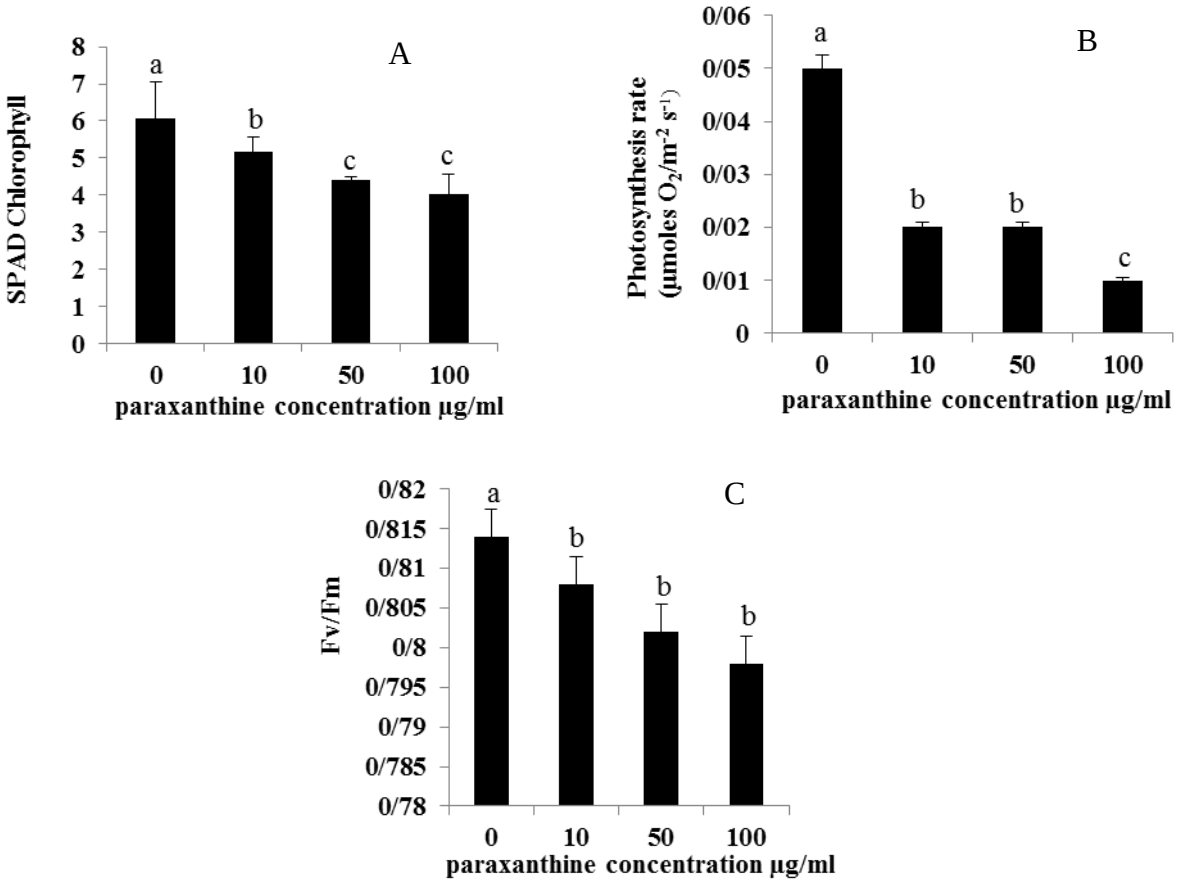


Figure 4. Effect of paraxanthine on chlorophyll content in SPAD units (A), Photosynthesis rate (µmoles O_2/m^{-2} s^{-1}) (B) and Fv/Fm (C) in lettuce plants treated with xanthotoxin. Columns with at least one similar letter are not statistically significant differences (P≤0.05).

Our results also displayed that paraxanthine diminished the photochemical efficiency of photosystem II (Fv/Fm values) in treated lettuce plants. The rate falls below to 0.8 in the plant treated with 100 µg $ml^{-1}$of paraxanthine implied to a stressful condition (Fig. 4C).

Proline, total free amino acids and glycine betaine content: Glycine betaine (GB) level and total free amino acids, in all the tested treatments are presented in Figure 5. All of the three measured amino acids content were significantly increased in all treatment at P≤0.05 (Table 2). Paraxanthine increased the GB content values in lettuce by 7.09%, 14.3% and 28.8% at 10, 50 and 100 µg ml^{-1}, respectively. The influence of paraxanthine on total free amino acids and proline is showed in Figure 5. The total free amino acid

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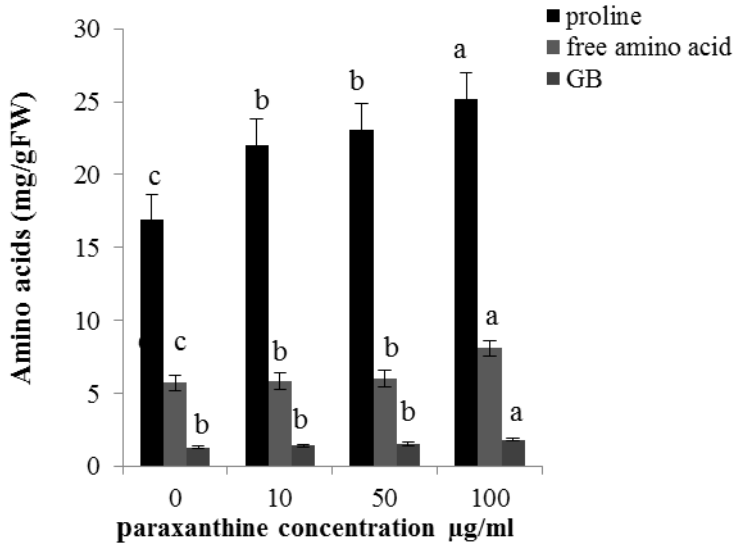


Figure 5. Proline content, total free amino acids and glycine betaine content in response to paraxanthine. Columns with at least one similar letter are not statistically significant differences (P≤0.05).

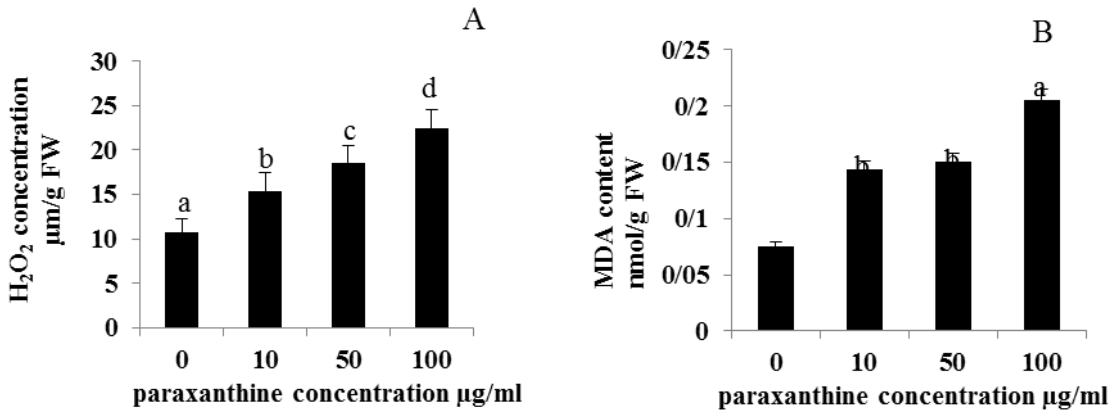


Figure 6. Hydrogen peroxide in response to paraxanthine (A). MDA content in response to paraxanthine (B). Columns with at least one similar letter are not statistically significant differences (P≤0.05).

content was increased after application of paraxanthine by 2.62%, 4.98% and 29.2% than control at 10, 50 and 100 µg ml^{-1} of paraxanthine, respectively.

There was a significant increase in free proline accumulation with increasing of paraxanthine at P≤0.05. Proline content was increased after application of paraxanthine at 10, 50 and 100 µg ml^{-1} as 23.3%, 26.7% and 32.9 compared with control group, respectively.

Hydrogen peroxide content and lipid peroxidation: Figure 6 shows the changes in H_2O_2 and malondialdehyde (MDA) contents in lettuce after paraxanthine treatments. H_2O_2 content and the lipid peroxidation marker (MDA content) were significantly increased by paraxanthine treatment at P≤0.05 (Table 2). The MDA concentration at treatment of 100 µg ml^{-1} of paraxanthin was higher than the control plants about 100%.

Treatment of paraxanthine increased H_2O_2 content in lettuce plants by 29.4%, 41.6% and 90.9.% for 10, 50 and 100 µg/ml respectively, as well.

Antioxidant enzymes assay: The effect of paraxanthine on specific activity of some antioxidant enzymes and protease is shown in Figure 7. The results indicated that the activity of catalase (CAT), polyphenol oxidase (PPO), ascorbate peroxidase (APX) increased in response to paraxanthine treatment (Table 2). The highest activity of CA, PPO and APX were recorded at 100µg/ml. CAT activity increased by 25%, 80% and 86.6% at 10, 50 and 100 µg/ml of the control, respectively. Treatment with paraxanthine increased the activity of PPO by 29.3%, 53% and 72.3% for 10, 50 and 100 µg/ml concentration, respectively, as well. Also, paraxanthine increased the activity of APX by 48.5%, 68.9% and 77.3% for 10, 50 and 100 µg/ml concentration respectively. The level of protease activity in the leaves were altered in response to paraxanthine (Fig. 7). Treatment with paraxanthine increased the activity of protease by 8.75%. 27.5% and 39.15% in the plant treated with at 10, 50 and 100 µg/ml of paraxanthine, respectively.

Protein content: The paraxanthine induced a significant change in concentration of total proteins

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Table 2. Analyses of variance table for biochemical parameters

SV \ Parameters	Proline Content	Free Amino acid	GB	Hydrogen Peroxide	MD	Protease	Ascorbate Peroxidase	Poly phenol oxidase	Catalase	Protein
Paraxanthin Treatment	45.13*	1.89*	0.17*	86.66**	0.016*	0.19**	0.004*	0.10*	0.076*	0.0033*
Error	3.210	0.211	0.021	4.330	0.901	0.012	0.003	0.010	0.006	0.0004

****, * indicate significant at 1 and 5 % levels of probability, respectively**

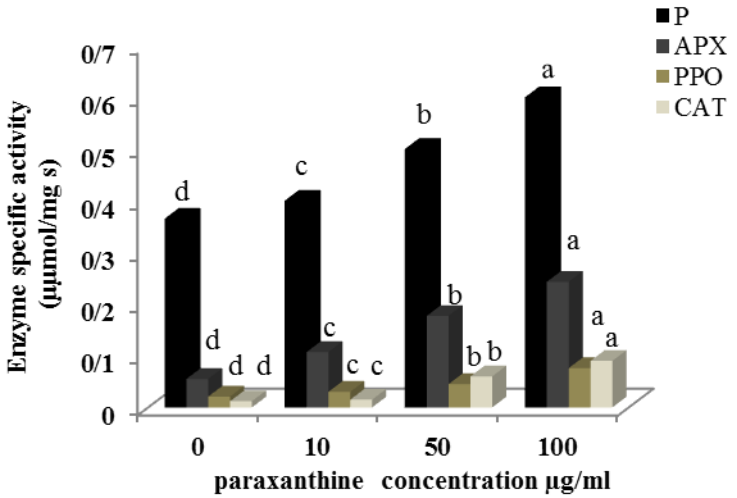


Figure 7. Specific activity of various enzymes, catalase (CAT), polyphenol oxidase (PPO), ascorbate peroxidase (APX) and protease (P) in respond to different concentration of paraxanthine in lettuce. Columns with at least one similar letter are not statistically significant differences (P≤0.05).

content in treated lettuce plants (Figure 8). The content of total proteins in 50 and 100 µg/ml of paraxanthine had a significant difference with respect to the control at P≤0.05. In general, the total protein content in different concentration of paraxanthine was decreased as 23.9% than control at 100 µg/ml.

SDS-PAGE protein profile: The results of SDS-PAGE analysis of lettuce leaves proteins showed that there was a considerable difference in electrophoretic patterns of protein bands in paraxanthine treated plants with control group. As it is shown in figure 9, some electrophoretic bands with molecular wight of 25-60 KD presented in control group, were disappeared at the treated group with 100 µg/ml of paraxanthine.

Discussion

The results of present work demonstrated that paraxanthine reduced seed germination, shoot-root lengths and shoot weight of lettuce plants. It might be attributed to the effects of paraxanthine in diminishing mitotic divisions of plant cells. A previous work implied on role of purine alkaloids on proliferation of lettuce cells obtained from protoplasts. (Sasamoto *et al.*, 2015). It can be assumed that purine alkaloids such as paraxanthine with a purine base skeleton resembles to cytokinins and may exhibit a possible interact with this hormones receptors. It was shown that plant cells may be promoted to synthesis purine alkaloids at stressful conditions like intense light that might be related to metabolism shifting from cytokinin biosynthesis to the alkaloids (Ashihara *et al*., 2011; Ashihara *et al*., 2013).

It was previously described that allelochemicals cause decreasing in all of growth parameters (Sarkara *et al.*, 2012; Liu *et al.*, 2021). It may be due to a hormonal balance disturbance between ABA and ethylene in germination seeds, inhibition of water uptake and a reduction in nutrient uptake in plant roots resulting a decrease in leaves relative water contents (Alam and Islam, 2002; Cheng and Cheng, 2015).

It is obvious from the results that chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content significantly decreased in lettuce plants effected with paraxanthine. Chlorophyll and carotenoid as photosynthetic pigments was previously reported that exhibit a reduction under allelochemical stress (Singh *et al.*, 2009). It could be related to blocking of chlorophyll biosynthesis or induction of chlorophyll degradation through allelochemical (Kapoor *et al.*, 2016). Decrease in photosynthetic pigments in allelochemical stress tends to a low photosynthetic rate, as well as. These alternations could be caused a reduction in plant growth at allelochemical stress condition (Babu and Kandasamy, 2008). Hence, it could be inferred that the high concentrations of paraxanthine have inhibitory effect on growth and photosynthesis through decrease in chlorophyll synthesis (Li *et al*., 2020). The reduction in chlorophyll content and growth parameters was also observed by Srivastava (2010) in cowpea under condition stress of allelochemicals.

It was also found that paraxanthine induces a

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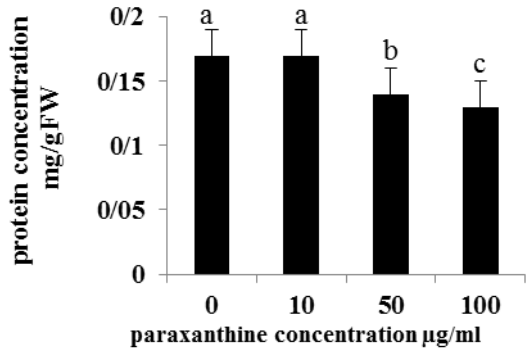


Figure 8. Effect of paraxanthine on total proteins of lettuce. Columns with at least one similar letter are not statistically significant differences (P≤0.05).

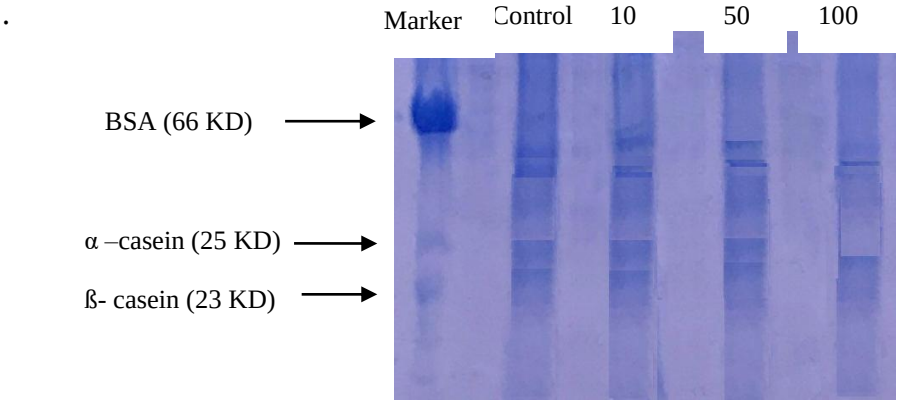


Figure 9. SDS-PAGE analysis of lettuce proteins treated with different concentration of paraxanthine. Lanes from left side represents marker, control and treated groups with 10, 50 and 100 µg/ml of paraxanthine, respectively.

secondary stress named oxidative stress in lettuce plants characterized by high level of reactive oxygen species. Hydrogen peroxide as a reactive oxygen species raised in the paraxanthine treated plants. It may lead to lipid peroxidation process resulting serious damage to organelle and cytoplasmic membranes. The membrane disruption in thylakoid membrane can be responsible for diminish in photochemical efficiency of photosystems II index (Fv/Fm). This reduction, in turn, can be regarded as another reason for photosynthesis rate falling. Measurement of chlorophyll fluorescence parameters such as Fv/Fm index is a powerful technique to estimate the rate of linear electron transport and recognize a stressful condition for plants (Dayan and Zaccaro, 2012).

Our results showed that free amino acids content and osmo protection markers proline and glycine betaine level raised in lettuce plants after paraxanthine treatment. The accumulation of this compatible osmolytes is a certain response in different plants to endure water loss under various environmental stresses. In addition to osmotic adjustment, these solutes may serve a protection role in plants at stress conditions (Demiral and Turkan, 2006). These compatible solutes may be protecting plants against reactive oxygen species and their harmful damages on cellular membranes and cell components such as DNA, proteins and sugars (Scandalios, 1993; Song *et al.*, 2013).

Plant has its own defense mechanisms in response to the generation of ROS by induction of antioxidant enzymes and nonenzymatic antioxidants (weir *et al.*, 2004; Rocio *et al*., 2007; Sweetlove, 2002). The present work revealed that some antioxidant enzymes such as catalase (CAT), polyphenol oxidase (PPO), ascorbate peroxidase (APX) increased in response to paraxanthine that might be tend to minimize paraxanthine created oxidative stress.

The SDS-PAGA analysis of the lettuce plants treated with paraxanthine indicated that some proteins bands disappeared in the plant group treated with 100 µg/ml of the compound. The missed bands with molecular weights of 25-60 KD may be related to photosystem II complex proteins. The proteins that have molecular weights of 25 to 70 KD and are damaged by reactive oxygen species (Fujii *et al.*, 2016). Further investigation is need to confirm the hypothesis. Rice *et al*. 1984 demonstrated that allelochemicals diminished the incorporation of certain amino acids into proteins and free amino acids increased due to proteins degradation and reduction rate of proteins.

Briefly, it can be speculated that paraxanthine induced an alleloochemical stress in lettuce plants causing some damages and promote a responses in the plants resemble some other environmental stress such as

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drought, salinity and so on. The plants responses aim to eliminate ROS and free radicles, osmoregulation and osmoprotection process.

Conclusion

It can be concluded that paraxanthine might be regarded as a potent allelochemical to induce some physiological and biochemical changes on lettuce as a model plant in allelopathic studies. It may release from tea, coffee or cacao leaches to environment and effect surrounding plants. The substance may also leak from some urban sewage to farms through surface, underground or irrigation waters and effects farming or native plants.

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