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ABIOCHEMICAL STUDY OF *LUPINUS LUTEUS* L. UNDER BORON TOXICITY WITH POSSIBILITY OF CULTIVATION IN THE SOUTHERN REGION OF IRAQ

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Abstract:
A study was conducted in aie by the diversity of boron ratios in their lands, in addition to examine to test the cultivation of this plant in the southern region of Iraq and within two regions that were characterized the toxicity effect of Boron (B) in *Lupines luteus* L. plant with four concentrations (0, 2.5, 5 and 10 mg/kg) to test the plant tolerant of boron toxicity. Boron spread in agricultural soil in two regions: Al- Hutta in the north of Basra city and Abu-Alkhasib in the west. It was concluded from the experiment that the plant toxicity symptoms of Boron appeared on the leaves at 5 mg/kg, with necrosis, chlorosis, and blackness in the edges of the leaves and increased in 10 mg/kg B. The accumulation of B in the leaves with increasing their concentration. Total biomass were decreased to 45% while plant tissue-B increased. Photosynthesis pigments (chlorophyll and carotenoids) were reduced 45% with increased B treatments; soluble carbohydrates and proteins were decreased by 72% and 78%, respectively, with excess B. The conclusion from the experiment that the first region (Al-Hutta) is the best in planting a *L. luteus* plant, and Abu-Al-khasib is not suitable for cultivating this plant. The *L. luteus* plant is one of the plants that tolerant the toxicity of B at a level less than 5 mg/kg.

Keywords: Boron toxicity, biomass, photosynthesis pigments, carbohydrate, soluble proteins, southern of Iraq .

INTRODUCTION

observed in the southern lands of Iraq in general due to the rise in the salt tongue extending from the Arabian Gulf region, as well as the increase in the level of boron in these in gerenal due to the high level of top soil on the hand and on the other hand poor drainage of sewage and washing soil before planting not good ,and frequent There are many agricultural areas scattered in the southern region of Iraq,especially in the province of Basra, including Al-Hutta and Abu-Al-Khasib.Recently,an increase wa fertilization led to an high level of boron in these areas .Boron (B) is a trace element considered essential for higher plants due to its physiological and metabolic functions (Shireen *et al.*2018), but B toxicity is an agriculture problem; being productive crops and vegetables in many regions of the world. Increasing B concentrations in the natural soil may occur with agriculture in the arid areas. Irrigation can also be added B. Countries such as Iraq, Syria, Egypt, Libya, Turkey, and South Asia are indicated by low rainfall B cannot be leached and may accumulate on the topsoil and become toxic to plant growth. In contrast, high fall levels of groundwater cause significant water evaporation and accumulation of B on the topsoil. This element's absorption via roots may accumulate in plant tissues, especially in the leaves, during transpiration (Maria *et al.*, 2017). The *Lupinus luteus* from legume crop, annual plant and native in the Mediterranean region (Polit et al., 2019), planted in milled sandy and volcanic soils, is grown in 600 areas of Arab land (Juzon *et al.*, 2019). The *L. luteus* plant is essential in agriculture as a source of nitrogen and sustainable agriculture. *L. luteus* contains the amount of protein in seeds; this protein has the best amino acid composition. Therefore, seeds are a used food source for humans and livestock (Lucas *et al.* 2015; Wilmowicz, 2019). Seeds protein have pharmaceutical qualities that affect blood pressure, metabolism, and glucose (Ogura *et al.*, 2014; Juzon *et al.*, 2017; Polit *et al.*, 2019). Yellow *L. luteus* is considered a cheap feed protein (Podlesna *et al.*, 2014). For agriculture purposes, yellow *L. luteus* has fewer requirements among other *L. luteus* plants and biodiversity in crop rotations and permanent grassland (Tavoletti *et al.*, 2011). Production of legumes is increasingly threatened by various environmental stress, such as B toxicity, salinity, temperature, and drought (Daryanto *et al.*, 2015).

The *L. luteus* plant includes a low level of oils, carbs, and alkaloids, in addition to a high content of protein, fibers, minerals, and numerous important phytochemicals (Abraham et al. 2019; Vollmannova *et al.* 2021). In general, legumes are plants that do not require a lot of fertilizer in the soil because their roots have symbiotic bacteria in the nodding where they grow, such as Rhizobium bacteria, which fix atmospheric nitrogen (Zahran,1999). These plants are employed in a rotation system to boost other crops' yield and restore degraded land (Watson *et al*., 2017: Atnaf *et al*., 2020). Lupin can be used as a substitute for soybean, which is known for its high protein content and several medical applications (Sedlakova *et al*., 2018). Lupin plant prefers free-draining soils with low lime concentration as an environmentally sustainable protein crop (below 3 %). This plant cannot take iron from the soil because of the fine clay and silt fractions (Arncken *et al*., 2020). This *L. luteus* is more resistant to abiotic and biotic stressors (Romeo *et al*., 2018). Because of its ability to accumulate Cd, Zn, and other heavy metals in the nodulated roots, it can be utilized as a phytoremediator (Fernandez et al., 2007). Because of the high alkaloid content, the seeds of the *L. luteus* plant have been employed for both feeding and medicinal purposes (Prusinski, 2017). Gluten-free bakery products made with Lupin flour and protein concentrate, including bread, biscuits, pasta, and cakes, are safe for celiac disease sufferers (Grela *et al*., 2017).

Plants provide a large portion of man's diet, and he typically seeks out sources of plants that are high in nutrients, such as proteins. Because of the alkaloid content, the *L. luteus* plant is one of the most protein-rich plants that can supply people and their animals with a plant with great nutritional value and medical applications. However, to achieve great output, this plant must be grown under optimum conditions. Agricultural soils in Iraq's southern region, particularly in the province of Basra, have a high level of salt. B is essential to higher plants because of its physiological and metabolic activities (Fareeha 2018). Because the range between the plant's needs for B and its toxicity is so narrow, it's one of the most important factors in determining plant growth and production, especially since B can rise in ideal agricultural soils that aren't affected by salinity through excessive salinity or the rise of atmospheric water, which causes B to accumulate on the surface of the soil through the evaporation process, especially in the province of Ontario (Marina *et al*.,2017). The transmission of B to the human or animal body when eating this plant, health problems such as weight loss, diarrhea, anorexia nervosa, and its effect on the heart and blood vesselsmay appear, the *L. luteus* plant must be grown in a balanced soil, the mineral elements, especially for boron (Nielsen 2014). There has never been any research done on the effects of boron on the Lupine plant in Iraq. There has been no previous research on the cultivation of *L. luteus* in Basra province. It was focused on monitoring heavy metals and some effects on some plants in Iraq (Hamza *et al*. 2020, Al-Abbawy *et al*. 2021; Azeez 2021), so the goal of this study is to show the effect of boron toxicity on the metabolic processes of the *L. luteus* plant and their tolerance to B concentrations to show the possibility of cultivating this plant in the southern regions of Iraq, specifically in the Basra province, which has varying degrees of boron concentrations, the proposed areas for testing cultivation ware the Abu al-Khasib area, west of Basra, and the Al-Huttta area, north of Basra.

MATERIALS AND METHODS

The experiment was conducted in semi greenhouse conditions under natural light. The mixture soil was dried and smashed in a sieve of 2mm, characteristics of soil are shown in Table 1. The experiment's soil layout was completely randomized design (CRD) with 16 treatments and three replication of a *L. luteus* plant. A solution used as a nutrient was mixed with soil content which has 0.5 gm/kg NH_2NO_3 and, KH_2PO_4, for one kg of soil, 5mg/kg $MnSO_4.H_2O$, $CuSO_4.5H_2O$, $ZnSO_4$ for one kg of soil and 0.4mg/kg H_3BO_3 for one kg of soil. As for the treatment, 0, 2.5, 5 and 10 mg/kg boron concentrations were selected. Mixing the amount of boric acid 17.48% B. with the soil by spraying method.

Fifteen seeds were sterilized by 2% hypochlorite and then doubled DW .grown in plastic pots 30 cm, in diameter and containing 3 kg from the soil, seeds were grown for three weeks, after seeding, with various treatments.

Table 1. Soil characteristics of the experiments

FC% DM	PWP% DM	pH	CEC(cm/kg)	EC(dS/m)	N%	P mg/kg soil	K mg/kg soil
22.0	9.0	6.5	1.3	11.0	0.04	13.0	59.0

FC: Field capacity, PWP: Permanent wilting point, EC: Electrical conductivity of mixture soil, CEC: Action exchange capacity, N: nirogen, P: Phosphorus, K: potassium.

The soil in the two study areas was analyzed momthly from October to April to determine the percentage of boron in the soil according to the following method:

Boron concentration

According to the method of Curcomine (APHA, 2017), by 0.5 mg ash collected from leaf digested by 4 mL Hydred-Florid and complete to 10 mL DW and by ion exchange (1.3*20cm), by adding curcumin stain and put the test tube in the bathroom (55 ºC) for 80 min. The spectrophotometer was used with 540nm, and the Equation (1) was used to measure boron concentration:

$$B=A2*C/A1*S \quad (1)$$

where

A1=Absorption standard sample, A2: Absorption the samples, C= Boron concentration in standared sample, S= the volume of samples.

Photosynthesis pigments (total chlorophyll and carotenoid) content

Extraction of chlorophylls and .carotenoids .2 gm of leaves .ground with 10ml of cold acetone 80%, the supernatant was measured, at 663,646 nm for chlorophyll and 470nm, for carotenoid were calculated following (Asare –Boamah *et al.*(1986).

Chlorophyll b = 22.9 (OD 645) − 4.68 (OD 663) ×V×W (2)
Total Chlorophyll = 20.2 (OD 645) − 8.02(OD 663) ×V×W (3)
Carotenoid = [17.1-(OD 480) (Chl a + chl b)-(9.47/119.26] (4)

Soluble carbohydrate content

Extraction according to Watanabe *et al.* (2000) method, 0.5 g ground dry leaves with 10 mL 80% ethanol alcohol then centrifuged at 1500 g for 15 min, supernatant with 80% ethanol alcohol were mixed to 20 mL, 1 mL from alcoholic extract transfer to test tube with 0.3 ml enthrone reagent, the sample measurement at spectrophotometer 620 nm and 1 mL DW for standard, soluble carbohydrate was evaluated by carbohydrate standard curve.

Soluble protein content

Extraction processes were conducted according to (Bavei et al., 2011), 0.5 g dried leaves was ground with liquid nitrogen and mixed with 0.1 M phosphate puffer (pH=7) with PMSF(phenyl metmansulfonyl fluoride) at 4ºC, then centrifuged in 20min,supertenute was used to evaluate soluble protein according to (Bradford,1976) by Bradford reagent, the spectrophotometer was to measurement at 595nm and DW. Used as and albumin was employed to the camper.

RESULTS

The result was observed by the analysis of soil in the two area within the period from October to april to study the extent of boron concentration in these two regions,the mean had been between 0.5-3 mg/kg in Houtta region and 5-7 mg/kg in Abu-Al Khasib region. By experience in the *L. luteus* plant with different levels of boron 2.5, 5, 10 mg/kg ,the signs of boron toxicity started to appear at 5 mg/kg by twinning the margins of leaves and batches in the blade at the end of week to the second.10 mg/kg was faster in appearance the toxicity signs from 5 mg/kg, has been positive correlation with increase the B concentration in the leaves. We notice that the total amount of biomass changes by changing the concentrations of treatment, the total sum of the biomass increased significantly in the low treatment of B and decreased significantly in the high treatment of B (Fig. 1); this is evidenced by the positive correlation with B treatments (Table 4).

In Fig. 1 we noted that the sum of the biomass has been affected significantly with increased concentrations of treatments.

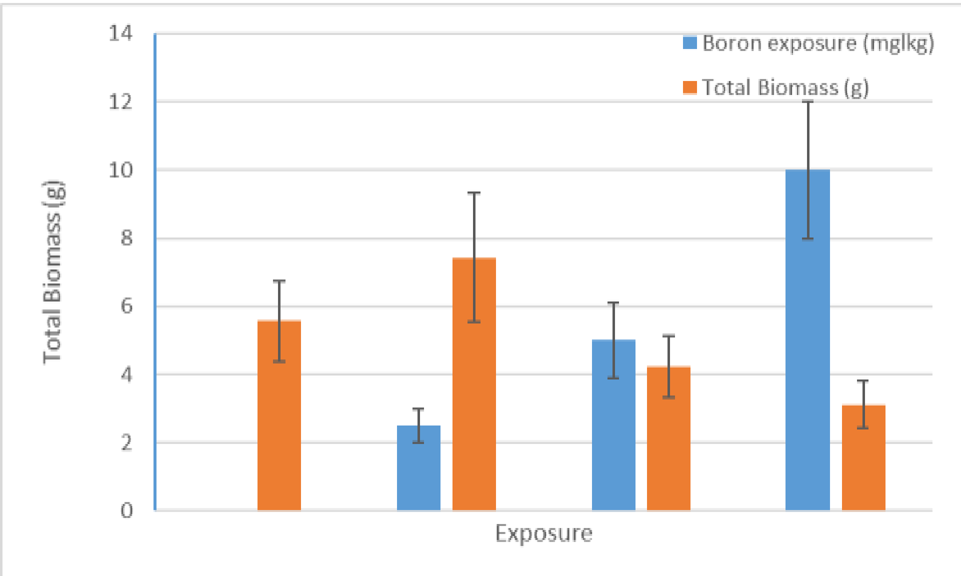

Fig. 1. Boron concentration (mg/g) and total biomass (g/plant) of *L. luteus* plant as effected by boron treatment in $p \leq 0.05$.

The increased boron concentration and toxicity signs appeared positively correlated with reduced photosynthesis pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid) as shown in Table2. The results showed a positive correlation with B treatments, (Table4).

The carbohydrate was observed significantly reduced in soluble carbohydrates with increased B concentration in *L. luteus* plant, (Table 3), this was illustrated by a positive correlation with boron treatments, so the minor content in soluble carbohydrate was observed in 10mg/kg B, but increase significantly at low boron treatment 2.5mg/kg,

Table 2. *L. luteus* plant's photosynthesis pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids content mg/g) as affected by B treatments in $p \leq 0.05$

Boron concentraion (mg.L⁻¹)	chl a (mg/g)	chl b (mg/g)	total chl (mg/g)	carotenoid (mg/g)
0.0	45.4±0.76	21.4±0.33	62.1±0.02	0.72±87
2.5	47.8±1.61	22.31±0.43	69.10±0.12	0.89±77
5	40.22±10	19.11±0.02	58.32±0.32	0.56±09
10	22.93±51	14.21±0.002	37.29±0.44	0.47±005

From table 3 it is obvious that an increasing of the content of proteins by the influence of the treatments, it was observed that they were significantly reduced with increased concentrations of Boron, this followed by a positive correlation with boron treatments, It found the lowest rate of soluble proteins in the treatment 10 mg/kg compared with the standard treatment while low treatments 2.5 mg/kg significantly increased the content of soluble proteins.

Table 3. soluble carbohydrates and soluble proteins of *L. luteus* plant as affected by B treatments in $p \leq 0.05$.

Boron concentration ($mg.L^{-1}$)	Soluble Carbohydrates (mg/g)	Soluble Proteins (mg/g)
0.0	20.11±0.09	18.21±0.08
2.5	26.22±1.0	22.09±019
5	17.03±0.05	15.008±0.11
10	12.43±0.008	11.16±0.55

Table (4) Correlation of Boron concentration and Total chlorophyll, Cartenoid, soluble carbohydrates, soluble proteins.

Correlations

	Total chlorophyll	cartenoid	Soluble carbohydrates	Soluble proteins
Total chlorophyll	1	.885**	.892**	.946**
cartenoid	.885**	1	.995**	.974**
Soluble carbohydrates	.892**	.995**	1	.969**
Soluble proteins	.946**	.974**	.969**	1

**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

The southern area of Iraq located in the territory which has risen by the Boron level with high level of salt the output of irrigation is watered with the shatt-al Arab river and associated with the Arabian gulf and because of the seawater levels from the shatt Al-Arab river, which is considered to be the source of many agricultural land in the governor of the sight. In this study started with an analysis of agricultural soil in two urban area, Abu-Al khasib and Houtta which are located in west and north of west of Basra respectively. Also Grive *et al*.(2010) ,was observed the appearance of B marks in cotton plant at 5 mg/kg and mentioned that the height of the B leads to an accumulated at the edges of the leaves through the stream of the transpiration and this agreed with prior study was conducted by Cervilla *et al*.(2012) in barley and tomato plant respectively .

B accumulates in the tissues of the plant, making it toxic to the organisms that eat it because the water pathway continues to flow from the roots to the leaves, carrying the B from the soil and spreading in the leaves, thus evaporating the water with transpiration and the accumulation of B at the end of the transpiration pathway, causing the black and burn marks at the edges of the leaves (Tayyaba *et al*. 2018).

According to Bardees et al. (2019) on the Fuengreek plant and Ghosh *et al*. (2016) on the mungbean plant, exposure of these plants to abnormal conditions such as increased boron or salinity causes physiological and biochemical changes in the plant, and the concentrations of these compounds change with the surrounding conditions.

These internal alterations have an impact on the plant's appearance, particularly the leaves. It has been observed that in dicotyledonous plants with reticulated veins, an excess of B over the normal limit causes blackening in the edges of the leaves and spots. This was seen in the *L. luteus* plant leaves at concentrations of 5 mg/kg and 10 mg/kg, which agrees with Doğru *et al* (2020) on the wheat plant.

, biomass measurements are considered a physiological indicator of the plants' response to environmental stresses and the reduction of biomass by the effect of stresses indicates the removal of nutrient arrival due to the effect of stressful elements (Lata *et al.,*2017), it was observed during the study in the *L. luteus* plant that the total biomass was affected by increasing concentrations of treatments, its indicator to the tolerance of the plant for environmental stress (Khursheda *et al*., 2015)

We note that the relationship is positively correlated with increased B concentrations, consistent with the study of Cervilla *et al.* (2012) in the tomato plant. They observed that increased boron concentration leads to a reduction in the biomass of the tomato plant; the increase in boron concentrations enhances the formation of ROS, which intern damages the cell membranes and accrues programming of cell death by reducing cell division.

, that agreed with Naiz *et al.*(2008) in the Gossypium plant but the low B not affected on chlorophyll and carotenoids pigments. The *L. luteus* plant can be tolerant this level of Boron; also Hassni *et al.(*2009) observed the Citrus plant could grow with boron toxicity appeared on leaves, the B is important in the concentration of cellular wall, but increase boron concentration that overlaps in the cell wall is meaningful and continued cellular activities, (Riad *et al*.,2004). In addition, the plasma membrane from within the boron targets so that they are associated with OH of glycoproteins and glycolipids in plasma membrane concentration, so that cellular disrupts especially plastids through chlorophyll and carotenoids, Tavallali (2018). The changes in soluble carbohydrate essential in the study of stress it has a direct relationship with photosynthesis Saad-Allah (2015) from the results, we not an increase of coefficients, which relation positively with low concentrations of Boron in leaves of *L. luteus* .

It is considered one of the most important aims that are attracted to ROS, and this leads to changes in the protein composition in addition to rapid oxidation and disruption of cellular membranes Gill and Tuteja (2010); thus, the activity of the proteolysis protease enzyme increases compared to the enzyme that builds the proteins, this is agreed with Badawy *et al.*(2017).

Low levels of treatment 2.5 mg/kg significantly increased the content of soluble proteins, indicating a balance in protein content. This level of B has enhanced the process of photosynthesis and the availability of CO_2, improving the metabolism of nitrogen and proteins, which is consistent with what Bestias *et al.* (2004) reported in the Zea mays plant.

CONCLUSIONS

In conclusion, increase B in the soil is toxic to the *L. luteus* plant, and signs of toxicity appeared at the concentration of 5 mg/kg of B. It was found during the experimental that the study indicators of biomass and even the photosynthetic pigments and the content of soluble carbohydrates and soluble proteins were significantly reduced with an increase in B and showed improvement at the low level of B, so the first region Al-Hutta is better in planting Lupine plant than the second region Abu- Al-khasib and the Lupine plant can provide tolerant B elevation at least 5 mg/kg of B.

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