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Role of Salicylic Acid, Biochar, and Their Combinations in Improving Physiological Characteristics of Broad Bean *(Vicia faba* L.*)* and Decreasing Cadmium Stress.

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ABSTRACT

The present study conducted at the Biology Department, Faculty of Science, Soran University in Soran city, in the Iraq-Kurdistan Region, using pot experiment to investigate the influence of salicylic acid, Biochar, and their combined effect on the broad bean physiological characteristics exposed to cadmium stress. The experiment was included three factors, five levels of cadmium (0, 0.5, 1, 2 and 4) mg Cd kg^{-1}soil, two levels of Biochar (0, 250)g pot^{-1} and two concentrations of salicylic acid (0, 100) mg L^{-1} and their interactions on certain growth, yield components, and overall yield of the broad bean plant cultivated in calcareous soil. The factorial pot experiment was conducted utilizing a complete randomized design (CRD) with 5 replications. Results show that cadmium, Biochar, and their interactions substantially influence plant height, the maximum mean height was recorded from Cd2, B1, and Cd2B0 with the mean values of (21.64, 29.23, 22.85) cm and the minimum values obtained from Cd5, B0, and Cd4B0 with the mean of (18.47, 20.51, and 17.98) cm. Moreover, the effect of treatments and their interactions significantly influenced pod weight, with maximum values of 127.92, 129.10, 150.66, and 162.50 g for B1, B1SA1, Cd5B1, and Cd5B1SA1, respectively. The minimum values were 102.09, 101.36, 89.03, and 84.53 at B0, B0SA0, Cd1B0, and Cd1B0SA0, respectively. Furthermore, singular treatments and their interactions had significant effect of seed protein content the maximum values were obtained from Cd5, Cd5B1, Cd5SA1, B1SA1, and Cd5B1SA1 with the mean of (27.16, 27.23, 27.59, 25.31, and 28.70)% respectively , while the minimum values(23.07, 21.20, 21.74, 22.28, and 19.83) % were noted from Cd1, Cd1B0, Cd1SA1, B0SA1, and Cd1B0SA1) respectively.

***Keywords*:** Biochar, Cadmium stress, Salicylic acid, Broad bean yield, Thermal stress.

INTRODUCTION

Broad bean is a prevalent crop within the Fabaceae family and an important cool-season food legume. Initially cultivated in the Mediterranean Crescent, it is extensively cultivated globally. In contrast to soybeans, it may be grown in many regions [1]. China dominates the worldwide faba bean production scene, leading with an annual output of 1,723,598 tonnes. Ethiopia ranks as the second-largest producer, with 1,070,637 tons per year [2].

Currently, environmental stressors, including abiotic and biotic factors, have emerged as a primary concern for scientists globally due to their detrimental impact on the lifespan of plants, from germination of seeds to crop survival and performance [3]. Heavy metals provide a considerable risk to biota and the food chain, as they are detrimental to living organisms even at trace levels. Essential heavy metals, including copper, iron, manganese, and zinc, are requisite for plant development and physiological processes, but non-essential heavy metals such as cadmium (Cd), arsenic, and lead are not [4]. Heavy metals possess three characteristics: toxic effects, tenacity, and biological accumulation. Owing to their non-degradable characteristics, metal ions endure in the environment [5]. Approximately 30,000 tons of Cd are emitted into the atmosphere each year, with 4,000 to 13,000 tons attributable to anthropogenic activity [6]. Cadmium can undergo biomagnification in terrestrial environments via the trophic levels of chains of food [7]. In humans, around 3% of the Cd in Cd-contaminated soils can be transferred to individuals through the ingestion of rice grown in such soils [8]. Typically, less-specialized transporters facilitate Cd absorption in the plasma membrane of plant root cells, then translocating it to the aerial sections of the plant (from shoots to grains) [9]. Plants may readily absorb Cd from the soil through their roots via passive or active mechanisms. Cadmium negatively impacts plant

morphology, resulting in diminished agricultural productivity and disturbances in growth and metabolic processes. It disrupts photosynthesis, transport mechanisms, and enzymatic functions, diminishes chlorophyll concentrations, and elevates oxidative stress indicators. These consequences lead to pollution of the soil, water contamination, health issues, and even carcinogenic threats [10, 11, 12].
Salicylic acid (SA) is generated from chorismite via two different mechanisms in plants: the isochorismate pathway and the phenylalanine ammonia-lyase pathway [13]. Numerous studies demonstrated that exogenous plant hormones and other antioxidant compounds may stimulate the defense mechanisms of plants to mitigate Cd toxicity [14, 15]. Recent studies demonstrate that salicylic SA is pivotal in plant responses to diverse abiotic stimuli, including freezing, drought, thermogenesis, osmotic stress, and metal toxicity, and that pre-treatment with suitable SA concentrations might elicit acclimation effects. Symptoms of Cd plant poisoning encompass leaf yellowing and chlorosis, stunted plant growth, constrained root development, obstruction of photosynthesis, alterations in chloroplast ultrastructure, lipid peroxidation, and disturbances in nitrogen metabolism [16].
Biochar (B), a carbon-dense substance generated via pyrolysis, is essential for preserving ecological equilibrium, mitigating soil contamination, and promoting sustainable agricultural advancement [17]. It is generated via the thermal breakdown of biomass, enhancing soil quality, productivity, and carbon sequestration, while mitigating greenhouse gas emissions. Biochar can absorb 12% of yearly anthropogenic carbon dioxide emissions, enhance soil microbial activity, and increase physical qualities such as pH, cation exchange capacity, & water retention capacity. It also mitigates nutrient depletion, enhances nutrient bioavailability, and sequesters toxic compounds in polluted soils [18]. The precise method by which rice husk-Biochar or SA reduces heavy metal levels in plants and the environment has been elucidated; however, their synergistic impact remains undetermined [19]. Since there are few or no studies in this area about the combination effects of Cd, Salicylic acid, and -B- on physiological characteristics, growth and decrease Cd stress for this reason the objective of this study focus on:

1. Influence of Salicylic acid and biochar on decrease cadmium stress
2. Improving physiological characteristics growth and growth components of broad bean plant.

Materials and Methods:

This experiment was commenced during October 2024 to April 2025 at the research station of the Biology Department, Faculty of Sciences, Soran University, in the Iraq-Kurdistan Region, situated at (36º 41' 49" N and 44º 31' 44" E), to investigate the impact of salicylic acid, Biochar, and five concentrations of cadmium on some growth characteristics and yield of broad bean plants in calcareous soil. The plants were grown in plastic pots measuring 29 cm in diameter and 32 cm in depth, filled with soil gathered from the surface layer (0-30 cm depth) of an agricultural farm located behind the Soran Independent Administration.The soil physical and chemical properties were shown in table (1) and the metrological data were recorded in table (2). The collected soil was air dried and then sieved with a 4 mm diameter sieve. Then, fifty pots were filled with 15.3 kg of sieved soil without Biochar, while the other fifty pots were filled with 13 kg soil mixed with 250 g of B, five concentrations of Cd (0, 0.5, 1, 2, and 4 mg Cd kg^{-1} soil using cadmium chloride ($CdCl_2$) as a source of Cd. This was done prior to planting on October 10, 2024, then five seeds of broad bean were sown in each pot. Following that, the pots were irrigated using well water. After two weeks the germinated seeds were thinned to three plants pot^{-1}.
The studied factors were summarized as follows:
First factor: five levels of cadmium (0, 0.5, 1, 2, and 4 mg Cd kg^{-1}), as denoted by (Cd1, Cd2, Cd3, Cd4, Cd5)
Second factor: Two levels of biochar (0 and 250 g pot^{-1} (B0 and B1).
Third factor: Two concentrations of Salicylic acid (SA) (0, 100 mg L^{-1}, SA0 and SA1).
The completely randomized design with 5 replicates was used; the total number of experimental units was equal to: levels of Cd*levels of B * Levels of SA*Replication= *5*2*2*5 = 100 experimental units.
The studied factors included:
The following parameters are a part of the MSc research which were selected for this article:

1- Plant height(cm): Plant height (cm) was calculated from the soil top to the apex of the plant using a ruler,
2- Leaf number per plant. The number of leaves per plant was recorded by counting the leaves on one randomly chosen plant.
3- Leaf area (cm^2)

$Leaf\ Area\ (cm^2) = 0.919 + 0.6821 \times Leaf\ Length\ (cm) \times Leaf\ Width(cm)$ [20]

4- Number of pods: The number of pods counted from randomly selected plant in each pot.
5- Weight of pod: The pods per pot were weighted per using a sensitive balance.
6- Chlorophyll intensity or content: Chlorophyll intensity (SPAD), was assessed using a portable chlorophyll meter (model Lyander, LD-YD, China).
7- Nitrogen concentration in leaves and seeds was determined using a modern nitrogen meter, a new non-

distractive method according to [21, 22]

8- Protein% % in seeds was determined using the following equation:

Protein %= nitrogen%*6.25 [23]

The data were statistically examined using IBM SPSS 25, employing analysis of variance (ANOVA) to ascertain significant differences across treatments, with Duncan's multiple range test applied at P≤ 0.01.

Table 1. Some physico-chemical properties of the Soran soil used in the pot experiment. *

Soil properties	Units	Soran
Particle size distribution (PSD)		
Sand	(g kg^{-1})	370
Silt	(g kg^{-1})	250
Clay	(g kg^{-1})	380
Soil texture		clay loam
Soil pH		7.53
Bulk density	g cm^{-3}	1.44
Soil water content at		
Saturation point (S.P.)	%	52,00
Field capacity (F.C.)	%	26.60
Wilting point (W.P.)	%	13.80
ECe	dS m^{-1}	0.37
CEC	$Cmol_C$ kg Soil	26.96
Organic matter content		11
Calcium carbonate	`(g kg^{-1} soil)	309.20
Active calcium carbonate		48.90
Available nitrogen	(mg kg^{-1}soil)	59.67
Available Phosphorous	(mg kg^{-1}soil)	2.01
Available potassium	(mg kg^{-1}soil)	64.10
Available Fe		2.00
Available Zn		0.49
Available Cd		0.40

*The soil was analyzed at department of natural resources, faculty of agriculture, Suleimani university.

Table 2. Climatic conditions during the growing season, which recorded by the Department of Agriculture of Soran

Months	Year	Air Temp. C°		Relative moisture %	Rainfall (mm)
		Minimum	Maximum		
October	2024	6	39	76.9	0.20
November	2024	-1	25	89.5	80.2
December	2024	-4	21	90.0	32.4
January	2025	-5	18	86.3	38.2
February	2025	-8	19	93.5	59.5
March	2025	0	30	79.6	15.1
April	2025	5	36	74.8	83.9

*The negative values were recorded in some days within the period of the study.

Results and Discussion:

Plant height (cm)

Plant height is a crucial measure of vegetative development, which, in turn, relies on cell division and elongation [24]. Statistical analysis in table 3 conducted that Cd levels had a significant effect on plant height, with the highest mean plant height recorded at Cd_2 (21.64) cm, whereas the lowest was seen at Cd_5 (18.47) cm, demonstrating a statistically significant decrease. As revealed by [25], plant height diminished as Cd content increased across all treatments. This illustrates that Cd stress impedes plant growth. [26], revealed that Cd stress results in reduced plant height, fresh weight, transpiration rate, and photosynthetic activity. However, statistical analysis shows that SA singularly had a non-significant effect on plant height. Moreover, B shows that have significant effect on plant height with maximum value at B1 (29.23) cm, and minimum value was at B0 (20.51) cm, as revealed by [27], The maximum height rate was seen in the treatment with 10 g of B , yielding heights between 64.73 and 68.90 cm. In addition, the two-way interaction of Cd–B statistically significantly plant height, with the highest value at Cd2B0 (22.85cm) and the lowest value at Cd4B1 (17.98 cm), as disclosed by [28], Certain research indicated that B did not diminish Cd bioavailability in polluted soils. The majority indicated reduced bioavailability, while others reported no impact, and some noted enhanced bioavailability. Furthermore, triple-way interaction between Cd, B and SA was statistically non-significant.

Table 3. Shows the influences of levels of Cd, B ,SA, and their combinations on plant height.*.

Levels of Cd (mg kg^{-1})	Levels of Biochar application	Level of SA (mg L^{-1}) application		Means value				
		SA_0	SA1	Cd-Biochar		Cd-SA	Cd	
Cd_1	B_0	21.16^{a}	21.1^{a}	21.13^{ab}	SA_0	20.57^{a}	Cd_1	20.49^{ab}
Cd_2		23.1^{a}	22.6^{a}	22.85^{a}		22.27^{a}		
Cd_3		22.1^{a}	18.9^{a}	20.5^{ab}		20.2^{a}	Cd_2	21.64^{a}
Cd_4		20.67^{a}	18.07^{a}	19.37^{ab}		19.15^{a}		
Cd_5		20^{a}	17.47^{a}	18.68^{ab}		19.23^{a}	Cd3	19.42^{ab}
Cd_1	B_1	19.97^{a}	19.73^{a}	19.85^{ab}	SA_1	20.41^{a}		
Cd_2		21.43^{a}	19.43^{a}	20.43^{ab}		21.02^{a}	Cd4	18.68^{ab}
Cd_3		18.3^{a}	18.4^{a}	18.35^{ab}		18.65^{a}		
Cd_4		17.63^{a}	18.33^{a}	17.98^{b}		18.2^{a}	Cd5	18.47^{b}
Cd_5		18.57^{a}	17.97^{a}	18.27^{ab}		17.72^{a}		

SA- Biochar interaction

Level of SA (mg L^{-1}) application	B_0	B_1	SA means
SA_0	21.39^{a}	19.18^{a}	20.29^{a}
SA_1	19.63^{a}	18.77^{a}	19.2^{a}
Biochar means	20.51^{b}	29.23^{a}	

*Mean for the factors and their combination separately having the same letter or letters, means there is no significant difference among them at $p \leq 0.01$, and vice versa.

leaf number

According to the analysis of variance (ANOVA) of the data presented in table 4, the number of leaves on broad bean plants was not substantially affected by the individual factor Cd, B, SA and their interactions. Despite slight numerical variations among treatments, the descriptive data indicated that these discrepancies were neither consistent nor significant.

Table 4. Shows influences of levels of Cd, B, (SA) and their combinations on leaf number

Levels of Cd (mg kg^{-1}) Levels of B application	Level of SA (mg L^{-1}) application		Means value		
	SA0	SA1	Cd-B	Cd-SA	Cd

Cd$_1$		57.4^{a}	63.6^{a}	60.5^{a}		57.5^{a}	Cd1	59.15^{a}
Cd$_2$		69^{a}	53.4^{a}	61.2^{a}		67.4^{a}		
Cd$_3$	B0	65.4^{a}	54.6^{a}	60^{a}	SA$_0$	63.5^{a}	Cd2	62.15^{a}
Cd$_4$		56.4^{a}	61.4^{a}	58.9^{a}		57.5^{a}		
Cd$_5$		64^{a}	59.6^{a}	61.8^{a}		67.6^{a}	Cd3	61.7^{a}
Cd$_1$		57.6^{a}	58^{a}	57.8^{a}		60.8^{a}		
Cd$_2$		65.8^{a}	60.4^{a}	63.1^{a}		56.9^{a}	Cd4	60.35^{a}
Cd$_3$	B1	61.6^{a}	65.2^{a}	63.4^{a}	SA$_1$	59.9^{a}		
Cd$_4$		58.6^{a}	65^{a}	61.8^{a}		63.2^{a}	Cd5	63.1^{a}
Cd$_5$		71.2^{a}	57.6^{a}	64.4^{a}		58.6^{a}		

SA- Biochar interaction

Level of SA (mg L^{-1}) application	B0	B1	SA means
SA$_0$	62.44^{a}	62.96^{a}	62.7^{a}
SA$_1$	58.52^{a}	61.24^{a}	59.88^{a}
B means	60.48^{a}	62.1^{a}	

*Mean for the factors and their combination separately having the same letter or letters, means there is no significant difference among them at $p \leq 0.01$, and vice versa.

leaf area (cm^{2})

The statistical analysis in table 5 shows that the effect of Cd, B, and SA singularly and the interaction of Cd-B and B-SA had a non-significant effect on leaf area. However, the interaction of Cd-SA and Cd-B-SA had statistically significant effect on leaf area/plant. The highest value of leaf area at Cd5SA0 and Cd5B1SA0 were (878.50 and 912.07 cm²), respectively, and the lowest value of leaf area at Cd2SA1 and Cd2B0SA1 were (588.57 and 541.57 cm²), respectively. This is similar [29], revealed that leaf area, shoot fresh biomass (SFB), and root fresh biomass (RFB), were increased in the synergistic application of plant growth regulators (such as IAA and GA) + Biochar (5% w/w) treatments, respectively, than the control. The reduction effect of the interaction of Cd-SA may be due to very low (cold) temperature (-8 ^{0}C) as recorded in the field. [30] revealed that reduced temperatures also influence leaf development. Plants cultivated under chilling circumstances exhibited smaller leaves compared to plants produced in normal or elevated temperature settings. This indicates that the combined application of B and SA might produce a synergistic stress response, particularly in plants previously subjected to Cd exposure. This type of adverse synergy may occur when many treatments impose excessive stress on identical physiological systems, disrupting the normal metabolic and hormonal signals in plants.

Table 5.Shows the influences of levels of Cd, B, SA and their combinations on the plant leaf area. *.

Levels of Cd (mg kg^{-1})	Levels of B application	Level of SA (mg L^{-1}) application		Means value				
		SA0	SA1	Cd- B		Cd-SA	Cd	
Cd$_1$		689.33^{bc}	780.99^{b}	735.16^{a}		621.11^{ab}	Cd$_1$	673.18^{a}
Cd$_2$		855.28^{ab}	541.57^{c}	698.43^{a}		812.03^{ab}		
Cd$_3$	B0	772.89^{b}	645.86^{bc}	709.37^{a}	SA$_0$	727.03^{ab}	Cd$_2$	700.3^{a}
Cd$_4$		641.93^{bc}	671.57^{bc}	656.75^{a}		636.57^{ab}		
Cd$_5$		844.93^{ab}	639.55^{bc}	742.24^{a}		878.5^{a}	Cd3	704.19^{a}
Cd$_1$		552.88^{c}	669.52^{bc}	611.2^{a}		725.26^{ab}		
Cd$_2$		768.78^{b}	635.58^{bc}	702.18^{a}		588.57^{b}	Cd4	664.33^{a}
Cd$_3$	B1	681.16^{bc}	716.85^{b}	699^{a}	SA$_1$	681.35^{ab}		
Cd$_4$		631.2^{bc}	712.63^{b}	671.92^{a}		692.1^{ab}	Cd5	746.61^{a}
Cd$_5$		912.07^{a}	589.87^{c}	750.97^{a}		614.71^{ab}		

SA- Biochar interaction

Level of SA (mg L^{-1}) application	B0	B1	SA means
SA$_0$	760.87^{a}	709.22^{a}	735.05^{a}

SA_1	655.91^{a}	664.89^{a}	660.40^{a}
B means	708.39^{a}	687.05^{a}	

*Mean for the factors and their combination separately having the same letter or letters, means there is no significant difference among them at $p \leq 0.01$, and vice versa.

Number of pods (Pod plant^{-1})

This characteristic is one of the basic components of yield in determining the amount of economic yield [24]. Table 6 shows that some of the studied factors were affected significantly at P-value≥ 0.01 on the number of pods, except levels of Cd, SA, and their interaction affected non-significantly. The levels of B significantly affected the highest value and the lowest value (9.3 and 7.64) of pod plant^{-1}, which was recorded from B1 and B0, respectively. Moreover, the interaction between SA-B and Cd-B and Cd-B-SA were affected significantly on number of pods the maximum value (9.72, 10.20 and 11.60) pods plant^{-1}.were from interaction treatments of (SA1B1, Cd5B1 and Cd5B1SA1) respectively, while the lowest value was recorded (7.76, 7.00 and 6.00) pods plant^{-1} respectively. The results are similar to [31], in the case of using B, demonstrating that there are several findings about the beneficial effects of B on legume growth and physiological characteristics. The utilization of Biochar enhanced both the root and shoot dry mass, in addition to increasing the quantity of pods in common bean. [32], revealed that the use of SA has been documented to enhance plant height, branch quantity per plant, pod count per plant, seed count per pod, 100-seed weight, and seed weight per plant.

Table 6. Shows the influences of levels of Cd, B, SA, and their combinations on a number of pods per pot.*

Levels of Cd (mg kg^{-1})	Levels of B application	Level of SA (mg L^{-1}) application		Means value				
		SA0	SA1	Cd-B		Cd-SA		Cd
Cd_1	B0	7.00^{abc}	8.60^{abc}	7.80^{bc}	SA_0	7.60^{a}	Cd_1	7.80^{a}
Cd_2		7.00^{abc}	8.40^{abc}	7.70^{bc}		8.40^{a}		
Cd_3		8.00^{abc}	6.00^{c}	7.00^{c}		8.70^{a}	Cd_2	8.65^{a}
Cd_4		7.80^{abc}	8.00^{abc}	7.90^{b}		8.00^{a}		
Cd_5		9.00^{abc}	6.60^{bc}	7.80^{b}		8.90^{a}	Cd3	8.10^{a}
Cd_1	B1	8.20^{abc}	7.40^{abc}	7.80^{b}	SA_1	8.00^{a}		
Cd_2		9.80^{abc}	9.40^{abc}	9.60^{a}		8.90^{a}	Cd4	8.80^{a}
Cd_3		9.40^{abc}	9.00^{abc}	9.20^{b}		7.50^{a}		
Cd_4		8.20^{abc}	11.20^{ab}	9.70^{a}		9.60^{a}	Cd5	9.00^{a}
Cd_5		8.80^{abc}	11.60^{a}	10.20^{a}		9.10^{a}		

SA- Biochar interaction

Level of SA (mg L^{-1}) application	B0	B1	SA means
SA_0	7.76^{b}	8.88^{ab}	8.32^{a}
SA_1	7.52^{b}	9.72^{a}	8.62^{a}
B means	7.64^{b}	9.30^{a}	

*Mean for the factors and their combination separately having the same letter or letters, means there is no significant difference among them at $p \leq 0.01$, and vice versa.

Weight of pods (g plant^{-1})

The data presented in table 7 prove that singular Cd, SA, and Cd-SA interaction were statistically non-significant. In contrast, individual B, B-SA interaction, Cd-B interaction, and Cd-B-SA interaction had significant effect on weight of pods, which the maximum values were (127.92, 129.10, 150.66 and 162.50 g plant^{-1}) at B1, B1SA1, Cd5B1 and Cd5B1SA1, respectively. The minimum values were (102.09, 101.36, 89.03, and 84.53 g plant^{-1}) at B0, B0SA0, Cd1B0, and Cd1B0SA0, respectively. The beneficial impact of SA on growth and yield may be ascribed to its modulation of many plant hormones, namely altering the balances of auxins, gibberellins, cytokinin's, and abscisic acid, hence enhancing growth and yield under both optimal and stress circumstances. And using SA increase weight of pods compare to control [33]. Furthermore, [34], revealed that B levels significantly increased fresh pod production from the control treatment, with 15 *M*g ha^{-1} (Mega gram ha^{-1}) yielding the maximum value of 81.53 *M*g ha^{-1}. However, The prolonged manifestation of B enhances soil nutrient availability and absorption efficiency, becoming it a vital nutrient source for soil microbes and plants [35]. Moreover, The utilization of B and compost enhanced polluted soil by diminishing Cd toxicity and facilitating its immobilization, hence promoting plant development [36].

Table 7. Shows the influences of levels of Cd, B, (SA) and their combinations on pods weight. *

Levels of Cd (mg kg^{-1})	Levels of B application	Level of SA (mg L^{-1}) application		Means value				
		SA0	SA1	Cd- B		Cd-SA	Cd	
Cd_1	B0	84.53^{b}	93.53^{ab}	89.03^{b}		98.24^{a}	Cd_1	98.05^{a}
Cd_2		99.09^{ab}	105.48^{ab}	102.29^{b}		115.38^{a}		
Cd_3		102.33^{ab}	98.75^{ab}	100.54^{b}	SA_0	118.00^{a}	Cd_2	115.14^{a}
Cd_4		98.20^{ab}	121.75^{ab}	109.97^{ab}		107.86^{a}		
Cd_5		122.65^{ab}	94.58^{ab}	108.61^{ab}		130.74^{a}	Cd3	115.61^{a}
Cd_1	B1	111.95^{ab}	102.20^{ab}	107.08^{ab}		97.87^{a}		
Cd_2		131.67^{ab}	126.19^{ab}	128.93^{ab}		115.84^{a}	Cd4	116.57^{a}
Cd_3		133.67^{ab}	131.56^{ab}	132.61^{ab}	SA_1	115.15^{a}		
Cd_4		117.52^{ab}	123.08^{ab}	120.30^{ab}		122.41^{a}	Cd5	129.64^{a}
Cd_5		138.83^{ab}	162.50^{a}	150.66^{a}		128.54^{a}		

SA- Biochar interaction			
Level of SA (mg L^{-1}) application	B0	B1	SA means
SA_0	101.36^{b}	126.73^{a}	114.04^{a}
SA_1	102.82^{ab}	129.10^{a}	115.96^{a}
B means	102.09^{b}	127.92^{a}	

*Mean for the factors and their combination separately having the same letter or letters, means there is no significant difference among them at $p \leq 0.01$, and vice versa.

Chlorophyll intensity

The significance on chlorophyll content, and there is a significant difference between Cd1 and Cd3 only which increase Cd to a certain level, causing an increase chlorophyll in broad bean leaves. The maximum level was found at Cd3 level, denoted by the letter a (45.64 SPAD), and the minimum value was Cd1, denoted by the letter b (42.78 SPAD). This may be a dilution effect since the leaf area in Cd3 is higher than Cd1, as presented in table 5. Regarding the impact of B, SA, and their interaction, was statistical analysis in figure 1a shows a non-significant effect on leaf chlorophyll content. The combined effect of Cd-B, Cd-SA, B-SA, and Cd-B-SA, as shown in figures (1 b and c), on leaf chlorophyll was statistically significant at P-value ≥ 0.01. which the highest value was recorded from Cd3B1, Cd3SA1, B1SA1, and Cd3B1SA1 were (47.34, 46.76, 44.29, and 48.34 SPAD), respectively. While the lowest value at Cd4B0, Cd4SA1, B1SA1, and Cd1B0SA0 were (42.18, 42.27, 42.92, and 40.79 SPAD) respectively. [3], demonstrated that the combined application of B and SA led to an increase in chlorophyll content. However, both B and SA effectively sustained elevated chlorophyll levels under Cd stress, particularly when used in conjunction at moderate Cd concentrations, underscoring their potential to enhance plant tolerance and production under heavy metal stress settings. Salicylic acid enhances the synthesis of chlorophyll, carotene, and anthocyanin, as well as photosynthesis, by promoting grana plate formation, facilitating chlorophyllase development, and inhibiting chlorophyllase enzymes, so positively influencing photosynthesis [37]. The application of RH BC to the soil, either independently or in conjunction with exogenous SA treatment (spraying), significantly enhanced photo synthetic pigments relative to the control treatment [19, 11].

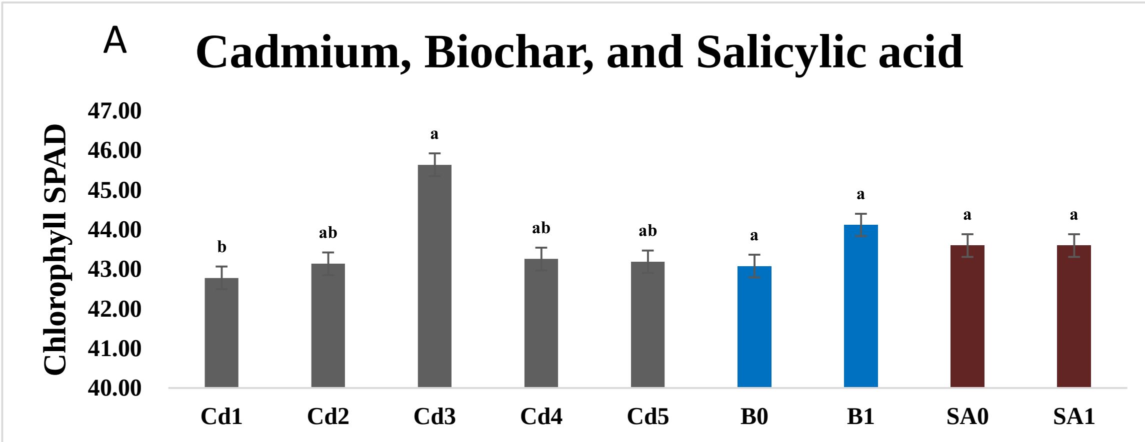

Figure 1a: Shows individual influences of levels of Cd, B, levels of SA on leaves chlorophyll intensity.

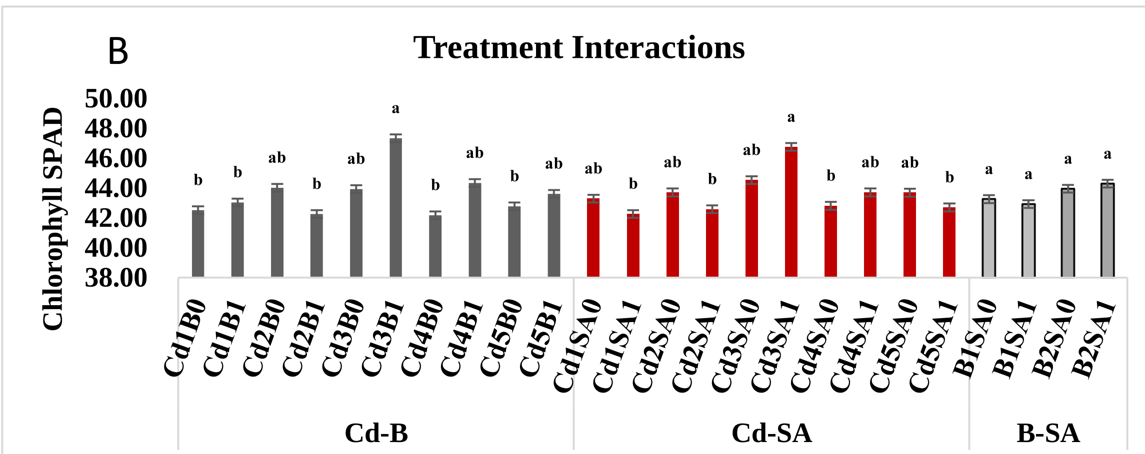

Figure 1b: Represents Cd, B, and SA interaction combinations on leaves chlorophyll content.

1. **Nitrogen content** (mg g^{-1})

the statistical analysis in figure 2a shows that the levels of (Cd) significantly influence nitrogen concentration, with a

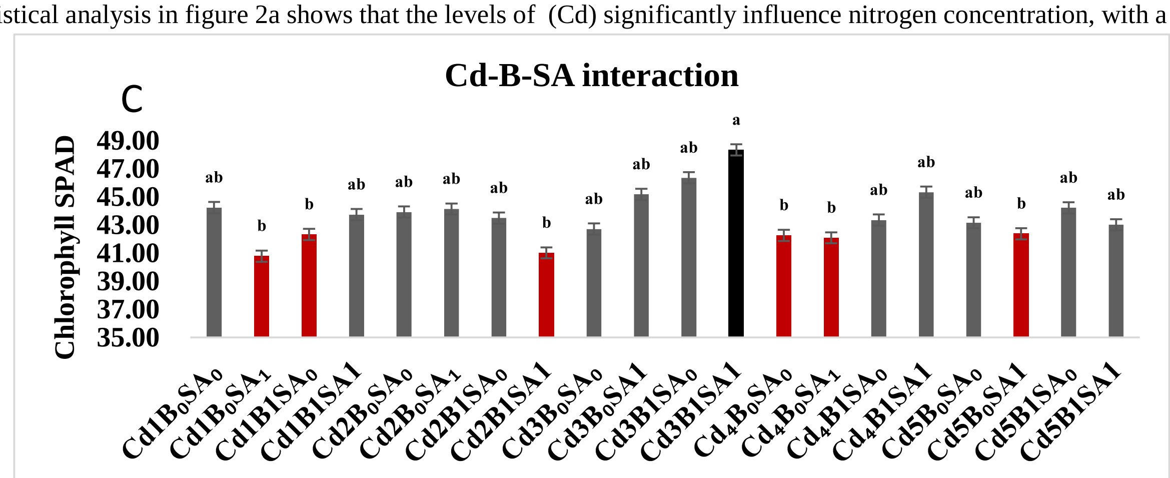

Figure 1c: Shows Cd-B-SA interaction on leaf chlorophyll content.

notable difference seen between Cd1 and Cd3. An increase in Cd to a certain level result in elevated nitrogen content. The maximum nitrogen value at Cd3 (17.12 mg g^{-1}), whereas the minimum value was at Cd1 (16.26 mg g $^{-1}$). Nitrogen metabolism has been demonstrated to be influenced by the existence of Cd in many plant species [38]. About Cd4 and Cd5, as revealed by [39], demonstrated that Cd not only impeded the absorption and transport of nitrate but also adversely impacted nitrate assimilation by decreasing the activity of nitrate reductase. However, Statistical analysis in Figure 2a indicates that varying B application rates and SA singularly and their interaction had no significant influence on plant nitrogen availability. Moreover, statistical analysis revealed that the interaction between Cd-B, Cd-SA and Cd-B-SA was significant with maximum value at Cd3B1, Cd3SA1, and Cd3B1SA1 (17.72, 17.5 and 18.10 mg g^{-1}) respectively and minimum value at Cd4B0, Cd1SA1, Cd1B0SA1 were (16.03, 16.03, and 15.57 mg g^{-1}) respectively shown in figure 2b and 2c. The combination of B-SA on Cd stress resulted in higher nitrogen levels compared to the application of each treatment alone. This indicates the presence of an extra or synergistic mechanism that facilitates the body's absorption or retention of nitrogen. [19], show that the incorporation of B with sprayed SA resulted in the most significant enhancement of all plant parameters in comparison to the individual applications of B or SA. In addition, Foliage treatment with SA and growth-promoting bacteria enhances plant tolerance, while soil amendments enhance morphophysiological and biochemical characteristics such as stem length, leaf development, and chlorophyll concentration [40]. Furthermore, [41], documented that B modifies the biochemical properties of plants due to its composition of essential nutrients, including nitrogen, potassium, phosphorus, and zinc, which are crucial for many plant species.

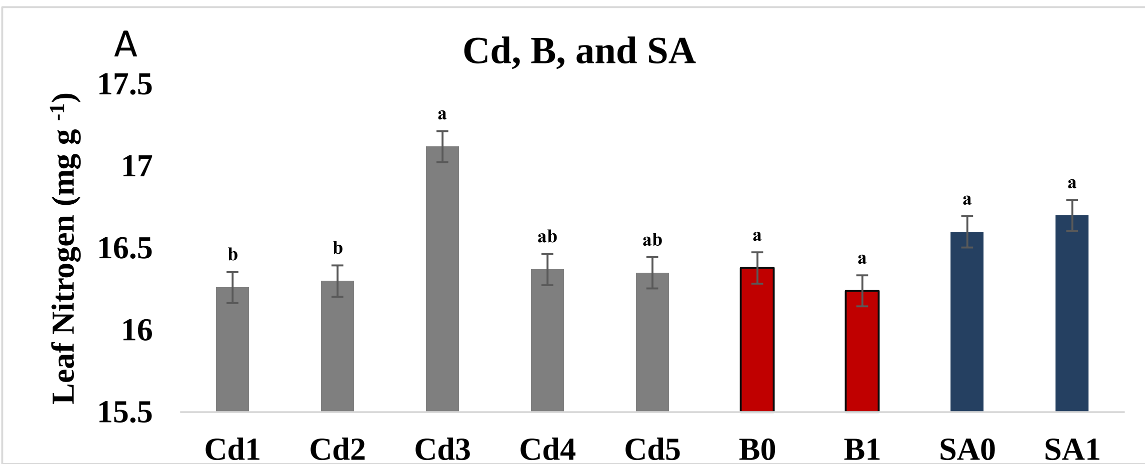

Figure 2a: Shows the influences of levels of Cd, B, levels of SA, on leaf nitrogen content.

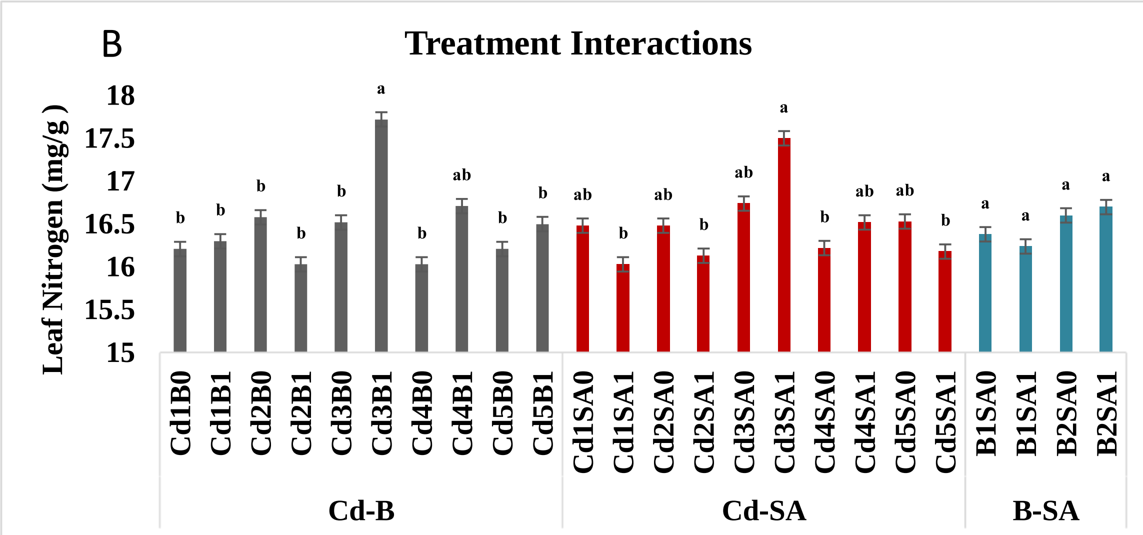

Figure 2b Shows interaction effect of Cd and B on leaf nitrogen content.

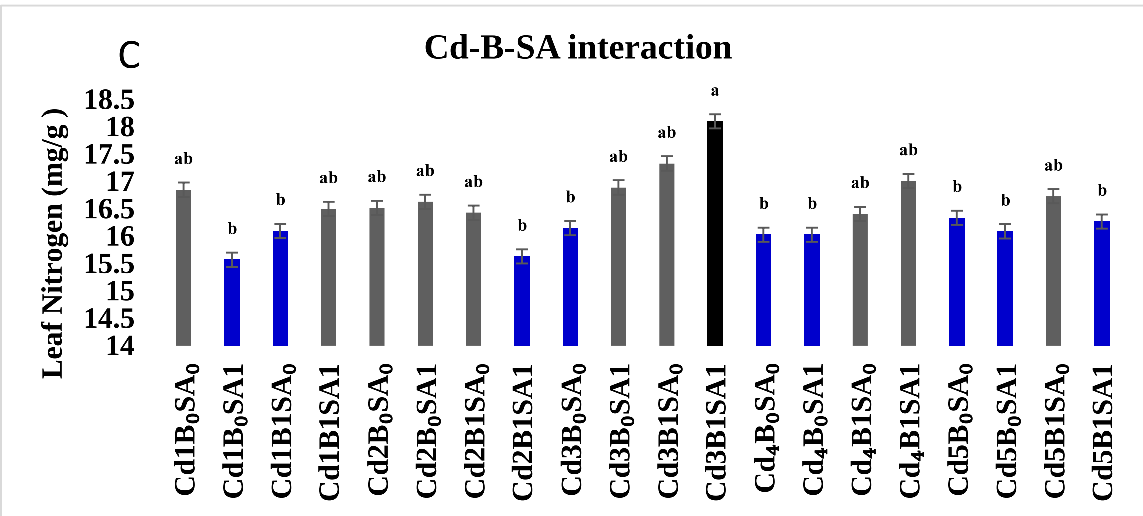

Figure 2c: Shows the interaction effect of Cd-B-SA on leaf nitrogen content.

Seed protein (%)

In table 8 the statistical analysis shows that Cd had a significant effect on seed protein, with Cd5 having the maximum value (27.16 %) and Cd1 having the minimum value (20.6 %). This may be stress-induced protein synthesis as revealed by [25], Cadmium stress can influence the synthesis of common proteins and the development of stress proteins. Proteins may enhance the quantity of functional proteins to sustain normal physiological metabolic processes in cells, hence improving plant resilience to stress. However, individual B and SA were statistically non-significant on the characteristic of plant seed protein content.

In addition, data in table 8 shows that the interaction of Cd-SA, Cd-B and B-SA had a statistically significant effect on seed protein, where protein went up at Cd5SA1, Cd5B1, and B1SA1 their value were (27.59%, 27.23%, and 25.31%) respectively, and protein went down at Cd1SA1, Cd1B0 and B0SA1 their value were (21.74%, 21.20% and 22.28%) respectively. However, Salicylates are designated as plant-derived signals that activate defense genes in response to stress. The amplification of these signals triggers an increase in the manufacture of allelochemicals and defense proteins, therefore safeguarding the plant [42, 43]. Salicylic acid may influence the production of defenserelated enzymes and specific proteins that help plants adapt and mitigate Cd toxicity [44]. In the interaction of Cd-B-SA, statistical analysis in Table 6 revealed that Cd-B-SA had a significant effect on seed protein content, with a maximum value at Cd4B0SA0 (31.68) and a minimum value at Cd1B1SA1 (18.28). as we mention before our plant may have effect from cold stress (-8 c), these are too much stress to our plant that may effect on physiology of plants. As [45], show, the adaptation process of plants to cold stress encompasses extensive physiological and molecular regulation networks. Physiologically, it presents as reduced enzyme activity, membrane system degradation, decreased capacity for photosynthesis, and cellular dehydration.

Table 8. shows the influences of levels of Cd, B, (SA), and their combinations on seed protein% *.

Levels of Cd (mg kg^{-1})	Levels of B application	Level of SA (mg L^{-1}) application		Means value				
		SA_0	SA_1	Cd- B		Cd-SA	Cd	
Cd_1	B_0	22.56^{de}	19.83^{g}	21.20^{d}	SA_0	24.41^{b}	Cd1	23.07^{b}
Cd_2		22.46^{de}	20.66^{f}	21.56^{cd}		24.29^{b}		
Cd_3		22.53^{de}	22.02^{e}	22.27^{c}		23.28^{bc}	Cd_2	23.33^{ab}
Cd_4		25.73^{bc}	22.42^{de}	24.08^{bc}		24.72^{b}		
Cd_5		27.71^{ab}	26.48^{b}	27.10^{ab}		26.74^{ab}	Cd3	23.38^{ab}
Cd_1	B_1	26.26^{b}	23.64^{d}	24.95^{ab}	SA_1	21.74^{d}		
Cd_2		26.11^{b}	24.10^{cd}	25.11^{ab}		22.38^{c}	Cd4	24.26^{ab}
Cd_3		24.04^{cd}	24.95^{cd}	24.50^{b}		23.48^{bc}		
Cd_4		23.70^{d}	25.17^{c}	24.44^{b}		23.8^{bc}	Cd5	27.16^{a}
Cd_5		25.76^{bc}	28.7^{a}	27.23^{a}		27.59^{a}		

Level of SA (mg L^{-1}) application	SA- Biochar interaction B_0	B_1	SA means
SA_0	24.2^{ab}	25.17^{ab}	24.68^{a}
SA_1	22.28^{b}	25.31^{a}	23.79^{a}
B means	23.24^{a}	25.24^{a}	

*Mean for the factors and their combination separately having the same letter or letters, means there is no significant difference among them at $p \leq 0.01$, and vice versa

Conclusion:

This study clearly demonstrates that Cd stress diminishes many physiological and growth characteristics of broad bean plants grown in calcareous soil. However, the use of SA and B , both alone and synergistically, significantly mitigated these adverse effects. Under elevated Cd stress, the synergistic application of B (250 g pot^{-1}) and SA (100 mg L^{-1}) demonstrated the most significant enhancement in plant height, leaf area, pod quantity and weight, chlorophyll concentration, nitrogen accumulation, and seed protein percentage compared to other treatments. This indicates that the combined application of B and SA might produce a synergistic stress response, particularly in plants previously subjected to Cd exposure.

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دور حامض الساليسيليك والفحم الحيوي والتداخل بينهما في تحسين الخصائص الفسيولوجية لنبات الباقلاء وتقليل إجهاد الكادميوم.

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الخلاصة

أجريت هذه الدراسة في قسم علوم الأحياء، كلية العلوم، جامعة سوران ، محافظة أربيل بهدف تقييم التأثيرات الفردية والمشتركة لحامض الساليسيليك ***(SA)*** والفحم الحيوي ***(B)*** على الاستجابات الفسيولوجية لنبات الباقلاء ***(Vicia faba L.)*** تحت إجهاد الكادميوم ***(Cd)***. نفذت البحث باستخدام خمس تراكيز من الكادميوم (0، 0.5، 1، 2، و4) ملغم.كغم$^{-1}$، ومستويين (0 و250) غرام.سندانة$^{-1}$ من الفحم الحيوي و مستويين (0 و100 ملغم.لتر$^{-}$ من حامض الساليسيليك والتداخل بينهم بأستخدام التصميم العشوائي الكامل ولخصت أهم النتائج بمايلي: أن للكادميوم والفحم الحيوي ر والتداخل بينهما تأثيرًا معنويا على ارتفاع النبات، حيث سُجل أعلى متوسط لارتفاع النبات عند المعاملات ***Cd2*** ، ***B1***، و ***Cd2B0*** و بمعدل (21.64، 29.23، و22.85) سم على التوالي، في حين سُجل أدنى الارتفاع في ***Cd5*** ، ***B0***، و ***Cd4B0*** و بمعدل ***(18.47,*** 20.51، و17.98) سم على التوالي**.** أثرت المعاملات و التداخل بينها معنويا على وزن القرون، حيث سُجلت أعلى القيم عند***B1*** ، ***B1SA1***، ***Cd5B1***، و ***Cd5B1SA1***(127.92، 129.10، 150.66، و162.50***)*** على التوالي، في حين سُجلت أدنى القيم عند***B0*** ، ***B0SA0***، ***Cd1B0***، و ***Cd1B0SA0***، ***(102.09*** 101.36، 89.03، و84.53) على التوالي**.** كما أثرت العوامل الفردية والتداخلات بينهما معنويا على محتوى البروتين في البذور، حيث سُجلت أعلى القيم عند ***Cd5***، ***Cd5B1***، ***Cd5SA1***، ***B1SA1***، و***Cd5B1SA1*** (27.16، 27.23، 27.59، 25.31، و28.70)٪ على التوالي، في حين سُجلت أدنى القيم عند ***Cd1***، ***Cd1B0***، ***Cd1SA1***، ***B0SA1***، و***Cd1B0SA1*** (23.07، 21.20، 21.74، 22.28، و19.83***)*** % على التوالي.

الكلمات المفتاحية: الفحم الحيوي، إجهاد الكادميوم، حامض الساليسيليك، إنتاج لنبات الباقلاء ، اجهاد الحراري.