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RESEARCH ARTICLE

# Biochar-mediated mitigation of dishwashing detergent-contaminated water effects on *Vicia faba* L.: Physiological and gene expression analysis

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## Abstract

The increasing population and ongoing modernization exert significant pressure on the environment, particularly affecting water and soil quality. The increasing population and ongoing modernization exert significant pressure on the environment, particularly affecting water and soil quality. Biochar (BC) is increasingly recognized as a sustainable solution for enhancing plant growth and mitigating environmental stress, due to its distinctive properties, including improving soil permeability and retaining nutrients and water. The study aimed to assess the effect of detergents at high levels (25, 50 and 75 %) and subsequently evaluate the potential role of BC in reducing the adverse impact of the water contaminated with detergents on growth, productivity and gene expression. The morphological parameters analysed were (height, number of leaves, number of pods, number of seeds, leaf area and chlorophyll contents). Control treatments have used tap water for irrigation. Gene expression analysis was performed using the RT-qPCR technique to detect the expression of stress-related genes (Unigene077619 calcium-binding protein, Unigene047182 zinc ion-binding protein and Unigene072411 alpha/beta fold hydrolase). The results indicated that the effect of detergents were concentration-dependent and statistically significant, except for leaf area, that showed an inverse correlation with increasing detergent concentration. The 75 % showed the highest negative impact on growth parameters and gene expression pattern. BC treatments were evaluated against 75 % detergent concentration at application rates of 3 %, 5 % and 10 %. The 5 % BC amendment demonstrated that the most effective mitigation was followed by 3 % and 10 %, respectively. The study concluded that BC revealed a positive effect on cope with abiotic stresses such as water contamination with detergents.

**Keywords:** biochar; dishwashing detergents; gene expression; rT-qPCR; *Vicia Faba* L.

## Introduction

Biochar is a solid, carbon-rich material produced through the pyrolysis of biomass residues in the absence of oxygen to prevent their complete oxidation into $CO_2$. It is commonly derived from forestry waste, agricultural byproducts and plant biomass remnants. (1, 2). BC possesses beneficial properties such as high surface area, elevated pH and enhanced cation exchange capacity, which contribute to improved soil fertility, greater availability of organic matter and efficient water retention. The BC was significantly enhancing chlorophyll synthesis, photosynthesis and gene expression activity and maintains the hormonal balance and osmolytes which improve plant tolerance against ionic and osmotic stress (3, 4). Developing countries suffer from untreated industrial and municipal wastewater used for crop irrigation. Household detergents have a detrimental impact on crops, as they impair plant growth when wastewater containing these substances which used for irrigation. The discharge of undesirable compounds, such as trace elements, heavy metals and domestic waste into the environment further exacerbates the issue by negatively affecting plant growth and productivity (4, 5). The expanding population has burdened global food security and agriculture is the main source to boost food shortage problems. Safeguarding water from contamination enhances crop growth and productivity and ensures a safe food role (6, 7). Chemical detergents are small ingredients such as bleaching agents, surfactants and enzymes that remove stains and dirt from fabrics effectively (8, 9). Faba bean (*Vicia faba* L.) is recognized as a multipurpose legume crop, offering high-protein food for humans, forage for animals and ecological benefits through its role in atmospheric nitrogen fixation. Faba bean is a rich legume considered a source of carbohydrates, proteins, vitamins, fiber and minerals. (10-12). Given the widespread use and improper disposal of detergents, which results in their infiltration into soil and groundwater and subsequently affects domesticated crops and considering the limited research on the potential of BC to mitigate these effects, this study aimed to evaluate the impact of dishwashing and laundry detergent-contaminated water on *Vicia faba* L. at both morphological and molecular levels.

## Materials and Methods

## Experimental Design

The experiment was conducted in the greenhouse of the College of Science at Tikrit University (34°40'41"N, 43°39'20"E). Seeds of *Vicia faba* L. were obtained from the College of Agriculture at Tikrit University and used for this study during (September 2023 to May 2024). Seeds immersed in disinfectant in 10 % sodium hypochlorite solution and running tap water used for rinse seeds. It was germinated in trays containing peat moss soil. Seedlings were transplanted into 10 L pots containing sterilized soil. Transplanted seedlings were kept well irrigated in the greenhouse for 3 months. Three levels of irrigation water were used (25 %, 50 % and 75 %) then were contaminated with liquid dishwashing detergents and three treatments were performed using BC (3 %, 5 % and 10 %) in addition to control treatment using tap water. The experimental model was a completely randomized block design and five replicates were performed for each treatment. Physiological measurements taken and molecular measurements were tested 3 months after the sowing date.

## Morphological traits

The harvested samples were separated into leaves, pods, stems and seeds. Measurements recorded three months after planting included plant height (cm), number of leaves, number of pods, number of seeds, leaf area ($cm^2$) and total chlorophyll content. Plant height was measured from the soil surface to the tip of the apical shoot using a measuring tape. The number of leaves was counted along the main stem from the base at the soil surface up to the apical shoot.

## Chlorophyll content

Chlorophyll a, b and total chlorophyll were measured by grinding 250 mg of plant sample in 20 mLof 80 % acetone, the sample was centrifuged and the absorbance was measured at (645-663) nm using spectrophotometer (OPTIZEN Alpha-double-beam UV-vis spectrophotometer 2018) (K-LAB Co. LTD No. S23/Korea) (13).

$$\text{Chl a} = (12.7\ (D663) - 2.69\ (D645) \times V) / (1000 \times W)$$

$$\text{Chl b} = (22.9\ (D645) - 4.68\ (D663) \times V) / (1000 \times W)$$

$$\text{Total Chlorophyll} = \text{Chl-a} + \text{Chl-b} \qquad \text{Eqn 1)}$$

(V - volume; D - absorption; W - fresh weight)

## Biochar preparation

Paper production wastes (artificial lignin) were used as an eco-friendly source of BC that was provided from Al-Taji Paper Factory, Baghdad city. The BC was synthesized by melting artificial lignin in a sodium sulfate ($Na_2SO_4$) solution prepared with deionized water and the pH was subsequently adjusted using hydrochloric acid (HCl) to be pH7. The solution subjected to 160 °C for 2 hrs. The solid matrix was soaked in $MgCl_2$ and then dried to eliminate the humidity. The final preparation step was subjecting the sample to 500 °C in the absence of oxygen and then washed in unionized water (14).

## Gene expression analysis

Gene expression analysis was conducted using the RT-PCR (Reverse Transcription Polymerase Chain Reaction) technique. The procedure was carried out in the Molecular Biology Laboratory, Department of Biology, College of Science at Tikrit University.

## RNA extraction

Transzol Up plus (RNA Kit) was supplied from TRANS company to extract RNA followed by converting RNA into cDNA. Primers designed to detect the gene expression for (Unigene072411 alpha/beta fold hydrolase, Unigene 047182 zinc ion-binding protein and Unigene077619 calcium-binding EF-hand protein) (Table 1).

## Converting RNA into cDNA

**Table 1.** Primers used to perform gene expression analysis of some stress-related genes

| Primer | | 5'- Sequence-3' |
|---|---|---|
| actin gene (housekeeping gene) | F | CGTCTTCCCCTCCATCG |
| | R | CTCGTTAATGTCACGCAC |
| Unigene 072411 alpha/beta fold hydrolase | F | ACATCATAACTCGCACAGCA |
| | R | CTGCAACATCAGTGCCAAGT |
| Unigene 047182 zinc ion-binding protein | F | TGGTAGGATGTAAGCACTGAAGC |
| | R | GAGAATGGAGATGTAGATCACGCT |
| Unigene 077619 calcium-binding EF-hand protein | F | GCGAATCGGTTACTCACAGTG |
| | R | GCGAATCGGTTACTCACAGTG |

Folding= $2^{-\Delta\Delta CT}$

$\Delta CT$= CT Gene - CT House Keeping Gene

$\Delta\Delta CT$= $\Delta CT$ (treated) - $\Delta CT$ (control)

RT-qPCR technique was used to measure the gene expression that was performed by converting the single-strand RNA into cDNA using Easy Script First-Strand cDNA Synthesis (TRANA).

## Real-time polymerase chain reaction (RT-qPCR)

The RT-qPCR reaction was performed using a Bio Molecular System (made in Australia). The reaction mixture was prepared using the Perfect Start Green qPCR Super Mix kit, supplied by TRANS. Pre-prepared cDNA samples were prepared from the reverse transcription reaction and the specific primers prepared in advance at a concentration of 10 pmol/µL. They were placed in a Freezing Rack Tube. The reaction mixture was then prepared according to the manufacturer's instructions, as shown in Table 2.

## Statistical analysis

The physiological parameters statistically analysed using JASP (Jeffreys's Amazing Statistics Program) - one-way ANOVA. Significant differences among values of all treatments were determined at $P \leq 0.05$.

# Results

**Table 2.** rt-qPCR reaction mix that was used in the gene expression analysis of samples

| Additives | Amount |
|---|---|
| cDNA template | 1 µL |
| F-Primer (Forward) | 0.5 µL (10 pmol concentration) |
| R- Primer (Reverse) | 0.5 µL (10 pmol concentration) |
| Perfect start green qPCR super mix | 10 µL |
| RNase free water | 8 µL |
| Total size | 20 µL |

### Morphological parameters under detergent

Plant growth under different concentrations of liquid detergents (25 %, 50 %, 75 % and control) was evaluated using five replicates per treatment. morphological parameters including plant height, number of leaves, number of pods, number of seeds, leaf area and total chlorophyll content were recorded three months after planting. The data of liquid detergent concentrations is illustrated in Table 2. The statistical analysis of the data showed the correlation between detergent concentration and growth parameters were inversely correlated and the negative effect of detergents was concentration dependent. Statistical analysis of all measured parameters revealed significant differences among the detergent concentrations in all treatments compared to the control plants irrigated with tap water, except for chlorophyll content, which showed no significant difference and was negatively affected by increasing detergent concentration (Table 3).

### Morphological parameters under BC

The results of treating soil with different BC percentages at 3 %, 5 % and 10 % were recorded after 3 months of planting. The obtained results compared with detergent treatment at 75 % which has the highest negative impact on growth. The BC results showed increased growth at all parameters compared with soil without BC and 5 % was the highest growth indicator among other percentages. The statistical analysis revealed the significance of BC treatments between percentages and 5 % was significant at $P \leq 0.05$. The growth indicators per parameter illustrated in Table 4.

### Gene expression

As shown in Table (5, 6), the results of gene expression analysis using RT-qPCR for (Unigene072411 alpha/beta fold hydrolase, Unigene 047182 zinc ion-binding protein and Unigene077619 calcium-binding EF-hand protein) genes for detergent treatments showed that the expression of Unigene072411 alpha/beta fold hydrolase decreased at 25 % and increased at 50 % and 75 % compared to the control. As for Unigene 047182 zinc ion-binding protein, decreased at 25 % and increased at 50 % and 75 %. Unigene077619 calcium-binding EF-hand protein was expressed at all treatments and directly correlated with concentration increments. These genes have a role in coping with abiotic stresses therefore it expressed greatly with stress increase and decreased with low concentration may be because the detergents pose a minor effect to elicit gene expression.

## Discussion

People are increasingly concerned about the environmental effects of household cleaners, particularly their harmful impact on garden-grown crops. The study aimed to assess the potential effect of BC to mitigate the detergents on plant growth, development and gene expression levels. Detergent is a remarkable pollutant that poses great risks to natural environment. Its elements can penetrate soil and water from

**Table 3.** Morphological traits of *Vicia faba* L. subjected to different concentrations of dishwashing detergent (25 %, 50 %, 75 % and control) (plant height, number of pods, number of seeds, number of leaves, leaf area and chlorophyll content)

| Detergent concentration | Height cm | Number of leaves | Number of pods | Number of seeds | Leaf area cm² | Chlorophyll content |
|---|---|---|---|---|---|---|
| Control | 74.50±0.5 | 53.8±1.3 | 7.2±1.09 | 35.2±0.8 | 4.52±0.1 | 48.28±0.4 |
| 25 % | 67.9±1.1$^a$ | 46.8±1.7$^a$ | 6±1.2$^a$ | 23±1.2$^a$ | 4.5±0.1$^a$ | 50.11±0.5$^a$ |
| 50 % | 59.57±0.5$^b$ | 44±1.2$^b$ | 5±0.7$^b$ | 11.2±1.09$^b$ | 5.48±0.2$^b$ | 49.89±0.5$^a$ |
| 75 % | 55.35±0.9$^c$ | 38±1.8$^c$ | 5±1.2$^c$ | 10.4±0.8$^c$ | 5.21±0.1$^c$ | 39.47±1.6$^a$ |

*Values are the mean of five replicates ± SD. The data analysed using JASP Software-One way Anova *P≤0.05. The identical letters within a column means no significant differences.

**Table 4.** Morphological traits of *Vicia faba* L. sowed in different percentages of BC (3 %, 5 % and 10 %). Compared with 75 % dishwashing detergent on plant height, number of pods, number of seeds, number of leaves, leaf area and chlorophyll content were measured

| Biochar % | Plant height cm | Number of leaves | Number of pods | Number of seeds | Leaf Area cm² | Chlorophyll content |
|---|---|---|---|---|---|---|
| 75 % dish washing detergent | 55.35±0.9 | 38±1.8 | 5±1.2 | 10.4±0.8 | 5.21±0.1 | 39.47±1.6 |
| 3 % | 68.9±1.7$^a$ | 47.4±2.4$^a$ | 7±0.7$^b$ | 31.8±2.0$^a$ | 5.55±0.3$^a$ | 50.22±1.07$^b$ |
| 5 % | 79.21±2.4$^b$ | 66.4±2.3$^b$ | 7.4±0.5$^a$ | 36.6±2.6$^b$ | 7.21±0.4$^b$ | 60.85±1.3$^b$ |
| 10 % | 62.36±3.2$^a$ | 58.2±3.4$^a$ | 6.6±0.5$^c$ | 36±1.5$^c$ | 6.45±0.3$^c$ | 46.48±0.9$^b$ |

*Values are mean of five replicates ± SD. The data analysed using JASP Software-One way Anova *P≤0.05. The identical letters within a column means no significant differences.

**Table 5.** RT-qPCR results and Genes used for control and detergent treatments and expression of genes as detected

| Folding sample | Unigene 072411 alpha/beta fold hydrolase | Unigene 047182 zinc ion-binding protein | Unigene 077619 calcium-binding EF-hand protein |
|---|---|---|---|
| Control | 1.000 | 1.000 | 1.000 |
| 25 % | 0.015 | 0.733 | 26.655 |
| 50 % | 20.549 | 55.831 | 92.728 |
| 75 % | 13.168 | 15.136 | 96.905 |

**Table 6.** RT-qPCR results and Genes used for control and Biochar treatments and gene expression analysis

| Folding sample | Unigene 072411 alpha/beta fold hydrolase | Unigene 047182 zinc ion-binding protein | Unigene 077619 calcium-binding EF-hand protein |
|---|---|---|---|
| Control | 1.000 | 1.000 | 1.000 |
| 3 % | 2.214 | 11.451 | 17.413 |
| 5 % | 6.324 | 35.478 | 126.728 |
| 10 % | 7.685 | 4.913 | 44.216 |

multiple sources. It can alter important parameters such as salinity, temperature, turbidity and pH (15). One of the sources of detergent is sewage which is being used for the irrigation of crops. Our results reported that dishwashing liquid detergents were negatively impacted the growth, development and productivity of *Vicia faba* L. that irrigated with different concentrations of contaminated water with detergents, especially at high concentrations. However, some parameters inversely directly correlated with detergent concentrations (leaf area) while other parameters were directly inversely correlated with detergent concentration (height, pod number, leaves number and seeds number and chlorophyll content). High concentrations of laundry detergent decreased the leaf relative water content (16). Household detergent waste significantly deteriorates plant cells and overall plant health by disrupting various physiological and biochemical processes. The presence of these compounds in the soil and irrigation water can lead to detriment the effects on germination, growth and cellular integrity (7,17,18). Some studies indicated that detergents can extremely reduce germination rates and growth performance in plants such as maize, wheat, faba bean and chickpea even at low concentrations (0.01 %) causing remarkable declines in seed vigor, root length and increase cell leakage. Liquid detergents have been shown to impair catalase activity indicating oxidative stress and decrease photosynthesis (4,19).

Genotoxic effects have been observed, with some detergents causing chromosomal aberrations in plant cells, leading to potential long-term genetic damage. Detergents can induce oxidative damage, affecting cell membranes and viability. Certain domestic detergents were found to cause significant cell membrane damage and elevate ion accumulation in plant tissues (20). Detergent pollution decreases nitrogen and potassium availability in soil, soil salinity increases with higher detergent concentrations (8,18,19). The detergents increase cell leakage and reduced cell survival. Exposure to detergents can increase accumulation of elements such as $Na^{+}$, $K^{+}$ and $Ca^{+2}$ in seedlings. It can be concluded availability of detergent components in irrigation water at higher concentrations can adversely affect seedlings by impairing light-harvesting pigments and cell viability (16). BC reduces the uptake of heavy metals by forming complexes and precipitating contaminants, thus decreasing their mobility and availability to plants. BC enhances soil resilience by mitigating heavy metal contamination and drought stress through increased water-holding capacity, heavy metal absorption and modification of soil properties, ultimately improving soil fertility and plant growth (21-26).

## Conclusion

The study concluded that BC exhibits a positive and promising role in reducing the negative effect of high levels of detergents that are used domestically on *Vicia faba* L. growth. The increased use and improper disposal of detergents have placed a significant burden on the environment, leading to contamination of irrigation water and groundwater and resulting in negative effects on all plant growth parameters. BC presents a potential solution for mitigating these impacts by improving soil and water quality, thereby enhancing crop production. Results demonstrated that the application of BC at 5 % effectively counteracted the detrimental effects of 75 % detergent concentration, which had caused statistically significant reductions in all assessed physiological parameters of *Vicia faba* L.

## Authors' contributions

RZAS carried out the molecular genetic studies in particular DNA extraction and gene expression analysis using real time PCR. MAI carried out the preparation of biochar and recorded the experiment results. RKH and AIAO participated in the writing and formatting of the manuscript. IOS participated in the proof reading and checking the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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