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Influence of textile dyes on some morphological, biochemical and physiological characteristics of broad bean (*Vicia faba* L.)

Ata Bahojb-Almasi[1], Sirous Hasannejad[1*], Hassan Aghdasinia[2] and Fariborz Zare Nahandi[3]

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[1]Department of Plant Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[2]Faculty of Chemical and Petroleum Engineering, University of Tabriz, Tabriz, Iran.
[3]Department of Horticultural Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
*Corresponding author; Email: sirous_hassannejad@tabrizu.ac.ir

Abstract
An experiment was conducted to evaluate the effect of textile dyes on some morphological and biochemical traits of board bean at a greenhouse of University of Tabriz, Iran, in 2016. Three types of dye (Acid Yellow, Acid Red, Direct Blue) and five dye concentrations (0, 30, 50, 70 and 90 mg/L) were studied using a factorial experiment based on randomized complete block design with three replications. Treatments were applied at seedling, pre-flowering and flowering stages. In this experiment, seed number, leaf number, peroxidase (POX) and catalase (CAT) activities, decolorization percentage and protein content were measured at all three stages of development. Results indicated that the effect of dye concentrations was significant on POX, CAT, leaf number, seed number and decolorization percentage at pre-flowering and flowering stages, and on protein content at all stages, whereas type of dye only had significant effect on seed number, protein content at pre-flowering and on CAT activity at both pre-flowering and flowering stages. Interaction of dye type with dye concentration was only significant for decolorization percentage at pre-flowering and flowering stages. The greatest increase in POX and CAT activities, and protein content were observed with 90 mg/L dye concentration at both pre-flowering and flowering stages; however, highest seed number were observed with the control treatment (0 mg/L dye concentrations) at pre-flowering and flowering stages. We may conclude that although absorbing dye by plants imposed a stress on them, but they were able to survive under this stress. Therefore, we might consider broad bean as one of the efficient plants for phytoremediation.

Keywords: Broad bean; Decolorization; Dye; Phytoremediation; Seed number.

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Introduction

Textiles and their wastes causes important environmental hazards; the main problem is the high concentration of organic matter and dyes. The majority of the synthetic dyes have been reported to be harmful for human health and are even toxic for plants. According to Ravi *et al.* (2014), many dyeing industries release their sewage into the environment either, after partial treatment or no treatment at all. They expressed that this wastewater, which is strongly alkaline, harms the plants and soil microflora when used for irrigational purposes and also decrease the soil fertility through its toxic compounds.

Conventional biological methods, and also chemical and physical treatments are usually used to remove the dye from textile effluent. However, physicochemical treatment processes could not be suitable due to the high chemical and operating costs and dangerous byproducts; thus biological

treatments are proper alternatives to these problems (Olejnik and Wojciechowski 2012). Recently, biological methods that use living organisms, including plants, have been developed because they are ecofriendly choices (Vafaei *et al*. 2013) with low-cost.

Asamudo *et al*. (2005) have described the bioremediation of textile effluent using the fungus *Phanerochaete chrysosporium.* Kabra *et al*. (2012) have used *Glandularia pulchella* plant species for the phytoremediation of textile wastewaters and different dyes mixtures. Also, Anjana and Salom Gnana Thanga (2011) used *Eichhornia* sp., *Salvinia* sp. and *Pistia* sp. for the phytoremedition of synthetic textile dyes. Jayanthy *et al* (2013) stated that *Leucaena leucocephala* can play a role as phytostabilizer for reactive dyes of different colors and can rectify polluted soils up to 50%. Watharkar and Jadhav (2014) indicated that the use of *Petunia grandiflora* and *Gailardia grandiflora* would be very esthetically gratifying, ecofriendly and energy saving technology for the treatment of textile dyes. Lokhande *et al.* (2015) reported that using hairy roots of *Sesuvium portulacastrum* L. can be efficient to degrade textile dye, Reactive Green 19A-HE4BD. Khandare *et al.* (2011) revealed the potential of *Aster amellus* to decolorize Remazol Red and textile effluent to a considerable extent. The plant, through its enzymatic machinery was able of to phytotransform the dyes to less toxic compounds. In another study by Khataee *et al*. (2012), the considerable potential of *Lemna minor* L. for phytoremediation of Acid Blue 92 was indicated; although, dye removal efficiency was dependent on different operational parameters.

The hazardous chemical compounds in industrial wastewater have detrimental impacts on germination and growth of plants when the plants are irrigated by this water (Ravi *et al.* 2014). However, under environmental stresses, some enzymes, such as CAT, are highly activated to protect the plants from these stresses. CAT is one of the main antioxidant enzymes (Scandalios *et al*. 1997) necessary for the elimination of H_2O_2 (Noctor *et al*. 2000) through breaking H_2O_2 into water and oxygen (Scandalios *et al*. 1997).

In this study, an attempt was made to investigate the influence of textile dyes on some morphological, biochemical and physiological traits of broad bean.

Materials and Methods

This study was carried out at the experimental greenhouse of University of Tabriz, Iran, during 2016 growing season. The experiment was laid out as factorial based on randomized complete block design with three replications. The treatments were three types of dye (Acid Yellow, Acid Red, Direct Blue) and five dye concentrations (0, 30, 50, 70 and 90 mg/L). Each experimental pot was sown with three local broad bean seeds in 3-cm depth. Pots were placed in the greenhouse with 28-26 °C temperature and 35-40% humidity. After seedling establishment, the plants were thinned to one plant per pot. Plants at seedling, pre-flowering and flowering stages were transported to bottles which were filled with tap water. Then, each plant was placed in a separate

bottle. After transporting the plants, different dye concentrations were treated into the bottles. During the vegetative growth, Hoagland solution was applied to the bottles. Oxygen was pumped by pipes into bottles for avoiding plants from lower oxygen tension. The plants were allowed to complete their growth to maturity. After taking the plants out of the bottles, dye solutions were placed in tubes separately, then, decolorization percentage was measured by the spectrophotometer. Decolorization was determined by monitoring the absorbance at the maximum wavelength for each dye, and by the reduction of the major peak area in the visible region for that dye. Then, the decolorization percentage was calculated. After bringing the plants to the lab, seed number and leaf number were measured. Protein percentage of the plants was determined by the method of Bradford (1976) with bovine serum albumin as the standard. CAT activity was measured by the method described by Aebi (1984). Also the method described by Herzog and Fahimi (1973) was followed to determine the peroxidase (POX) activity. After testing the normality of data, analysis of variance was carried out using MSTAT-C software. Then, the means were compared by Duncan's Multiple Range Test ($p \leq 0.05$). Figures were drawn by Excel 2010.

Results

POX

There were no significant differences among dye types in terms of POX activity at all three stages (Tables 1, 2 and 3); however, dye concentration had significant effect on POX of the plants at pre-flowering (Table 2) and flowering (Table 3) stages. POX increased significantly with increasing dye concentration as compared to the control (Table 5).

CAT

There were significant differences among dye types for CAT activity at pre-flowering (Table 2) and flowering (Table 3) stages. Direct Blue showed significantly higher CAT activity than Acid Red and Acid Yellow dyes at both pre-flowering and flowering stages (Table 4). Dye concentration had also significant effect on CAT at pre-flowering (Table 2) and flowering (Table 3) stages. Results indicated that use of dye significantly increased CAT over the control and amount of CAT elevated by increasing dye concentration (Table 5).

Decolorization percentage

Dye type and dye concentration and their interaction had significant effect on decolorization percentage at pre-flowering (Table 2) and flowering (Table 3) stages. Application of dye increased decolorization percentage strongly as compared to the control at pre-flowering (Figure 1a) and flowering (Figure 1b) stages. The maximum decolorization percentage was observed for Direct Blue at 30 mg/L concentration at both pre-flowering and flowering stages (Figures 1a and 1b), and very low values were obtained at 0 mg/L dye concentration, regardless of the dye type (Figures 1a and 1b). The small amount of decolorization percentage in the controls can be attributed to the components of Hoagland solution.

Table 1. Analysis of variance of all characteristics as affected by type of dye and dye concentration at seedling stage.

SOV	df	Proxidase	Catalase	Decolorization percentage	Seed number	Leaf number	Protein content
Replication	2	0.001^{ns}	0.001^{ns}	56^{ns}	0.022^{ns}	17.27^{ns}	0.035^{ns}
Type of dye (A)	2	0.047^{ns}	0.006^{ns}	916^{ns}	0.089^{ns}	15.00^{ns}	0.081^{ns}
Dye concentration (B)	4	0.049^{ns}	0.045^{ns}	2173^{ns}	1.422^{ns}	18.09^{ns}	5.738^{*}
A × B	8	0.002^{ns}	0.003^{ns}	43^{ns}	0.172^{ns}	15.72^{ns}	0.221^{ns}
Error	28	0.028	0.025	1062	0.737	22.08	2.16

ns: not significant; *: significant at 5% and 1% probability levels, respectively.

Table 2. Analysis of variance of all characteristics as affected by type of dye and dye concentration at pre-flowering stage.

SOV	df	Proxidase	Catalase	Decolorization percentage	Seed number	Leaf number	Protein content
Replication	2	0.055^{**}	0.002^{ns}	453^{**}	3.67^{**}	27.8^{**}	0.36^{*}
Type of dye (A)	2	0.001^{ns}	0.012^{*}	1981^{**}	1.07^{*}	1.1^{ns}	0.24^{*}
Dye concentration (B)	4	0.250^{**}	0.224^{**}	8392^{**}	5.39^{**}	100.3^{**}	18.69^{**}
A × B	8	0.001^{ns}	0.001^{ns}	232^{*}	0.21^{ns}	1.5^{ns}	0.09^{ns}
Error	28	0.002	0.002	80	0.29	4.2	0.07

ns: not significant; *, **: significant at 5% and 1% probability levels, respectively.

Table 3. Analysis of variance of all characteristics as affected by type of dye and dye concentration at flowering stage.

SOV	df	Proxidase	Catalase	Decolorization percentage	Seed number	Leaf number	Protein content
Replication	2	0.056^{**}	0.005^{ns}	412.6^{*}	0.089^{ns}	33.76^{*}	0.179^{ns}
Type of dye (A)	2	0.001^{ns}	0.018^{**}	2096.4^{**}	0.289^{ns}	0.36^{ns}	0.087^{ns}
Dye concentration (B)	4	0.251^{**}	0.220^{**}	8238.4^{**}	6.589^{**}	80.72^{**}	19.384^{**}
A × B	8	0.002^{ns}	0.002^{ns}	240.2^{*}	0.289^{ns}	7.02^{ns}	0.174^{ns}
Error	28	0.002	0.003	82.9	0.232	10.07	0.092

ns: not significant; *, **: significant at 5% and 1% probability levels, respectively.

Table 4. The effect of dye type on seed number, Catalase (CAT) and protein content of broad bean (*Vicia faba* L.) plants.

Dye type	Seed number	CAT (mg protein^{-1} min^{-1})		Protein content (%)
	Pre-flowering	Pre-flowering	Flowering	Pre-flowering
Acid Red	3.27a	0.711b	0.652b	25.06b
Direct Blue	3.00ab	0.725a	0.721a	25.31a
Acid Yellow	2.73b	0.701b	0.688ab	25.25ab

Means with different letters in each column are significantly different, based on Duncan's Multiple Range Test at $p \leq 0.05$.

Table 5. The effect of dye concentration on peroxidase (POX), catalase (CAT), protein content, seed number and leaf number of broad bean (*Vicia faba* L.) plants.

Dye concentration	POX (mg protein^{-1} min^{-1})		CAT (mg protein^{-1} min^{-1})		Protein content (%)		
	Pre-flowering	Flowering	Pre-flowering	Flowering	Seedling	Pre-flowering	Flowering
0 mg.L^{-1}	0.60e	0.58e	0.52e	0.49e	24.62b	23.33e	23.99e
30 mg.L^{-1}	0.70d	0.68d	0.61d	059d	25.87ab	24.23d	24.75d
50 mg.L^{-1}	0.81c	0.79c	0.71c	0.70c	25.68ab	25.37c	26.21c
70 mg.L^{-1}	0.90b	0.88b	0.80b	0.77b	25.09b	26.19b	26.86b
90 mg.L^{-1}	1.03a	1.01a	0.92a	0.89a	26.72a	26.90a	27.46a

Means with different letters in each column are significantly different based on Duncan's Multiple Range Test at $p \leq 0.05$.

Table 5 continued

Dye concentration	Seed number		Leaf number	
	Pre-flowering	Flowering	Pre-flowering	Flowering
0 mg.L^{-1}	4.11a	3.22a	38.56c	42.22a
30 mg.L^{-1}	3.33b	2.11b	40.67b	40.56ab
50 mg.L^{-1}	3.00b	1.44c	44.33a	38.44bc
70 mg.L^{-1}	2.22c	1.11c	46.33a	37.00cd
90 mg.L^{-1}	2.33c	1.33c	45.56a	34.56d

Means with different letters in each column are significantly different based on Duncan's Multiple Range Test at $p \leq 0.05$.

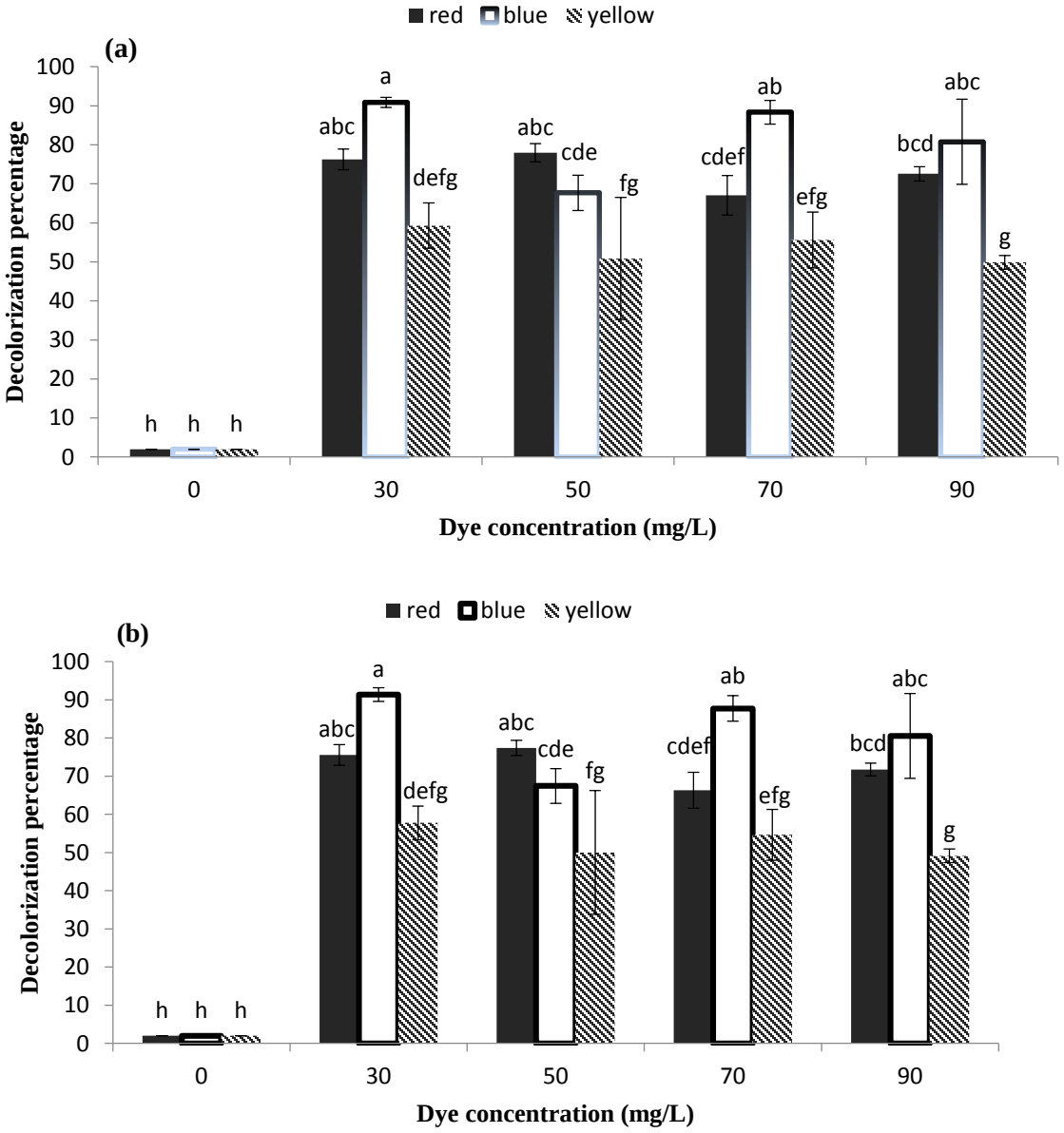

Figure 1. The interaction effect of dye type with dye concentration on decolorization percentage at pre-flowering (a) and flowering (b) stages; means with different letters are significantly different, based on Duncan's Multiple Range Test at $p \leq 0.05$.

Seed number

Type of dye had significant effect on seed number only at pre-flowering stage (Table 2). Acid Red showed the highest seed number followed by Direct Blue (Table 4). Dye concentration affected seed number significantly at pre-flowering and flowering stages (Tables 2 and 3). By increasing dye concentration seed number decreased at both stages. The lowest seed number was observed under the two highest dye concentrations (70 and 90 mg/L) at both pre-flowering and flowering stages (Table 5).

Leaf number

Effect of dye type was not significant on leaf number (Tables 1, 2 and 3). Effect of dye concentration was significant on leaf number at both pre-flowering and flowering stages (Tables 2 and 3). Leaf number increased as dye concentration increased at pre-flowering stage; however, it decreased at flowering stage by increasing dye concentration (Table 5).

Protein content

Type of dye affected protein content significantly only at pre-flowering stage (Table 2). Direct Blue showed the highest leaf number followed by Acid Yellow (Table 4). Effect of dye concentration on protein content was significant at all stages (Tables 1, 2 and 3). Results indicated that increasing dye concentration increase protein content at these stages. The highest protein content was observed under 90 mg/L dye concentration at seedling, pre-flowering and flowering stages (Table 5).

Discussion

CAT and POX activity was elevated by increasing of dye concentration in our study. CAT and POX are among the antioxidant enzymes found in plants (Mittler 2002). Several reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radical, are produced when plants are exposed to harmful stress conditions (Sudhakar *et al*. 2001); however, plants eliminate ROS by their antioxidant defense systems (Mittler 2002). It has been indicated that plants with higher antioxidants levels, are mainly more resistant to the oxidative damage (Sudhakar *et al*. 2001).

When plants are faced with low dye concentration, the amount of dye in the area is not enough to disrupt basic biological functions, so plants activate tolerance mechanisms and try to decolorize dye from the area; however, when concentration reaches to a toxic level, plants are not able to tolerate the stress and, therefore, their growth is reduced. In the present study, seed number at both per-flowering and flowering stages and leaf number at flowering stage reduced significantly as compared to the control; the reduction was especially higher at 70 mg/L and 90 mg/L dye concentrations. Contrary to the flowering stage, the increase in dye concentration enhanced leaf number at pre-flowering stage. The different results about the effect of dye concentration on leaf number between pre-flowering and flowering stages, can be attributed to the difference in metabolic processes at these stages of plant development. The reduction of morphological

attributes due to dye stress could be related to the existence of large amounts of heavy metals and dissolved salts in the dyes. The high concentration of these pollutants in irrigation water can affect the growth metabolism and enzymatic activity especially at flowering stage, which results in lower number of seeds per plant. According to Ravi *et al*. (2014), textile dye wastewater adversely affected growth parameters of soybean crop because of containing higher amount of harmful chemicals. Others have also reported similar findings (Swaminathan *et al*. 1992; Dutta and Boissya 2000; Sivakumar *et al*. 2001).

Although dyes reduced seed number of broad bean in this research, especially at higher concentrations, but still it managed to survive even under higher dye concentration. The study of Torbati *et al*. (2014) about the bioremediation ability of watercress (*Nasturtium officinale*) demonstrated the capacity of watercress to upregulate its antioxidative defense system to mitigate the oxidative stress caused by C.I. Basic Red 46 dye treatment. Khataee *et al*. 2012) reported the significant decolorization of Acid Blue 92 by duckweed (*Lemna minor*) grown in a hydroponic medium. Khataee *et al*. (2013) also indicated the biodegradation of C.I. Acid Blue 92 by aquatic fern (*Azolla filiculoides*) under hydroponic conditions. Patil and Jadhav (2012) reported the potential of *Tagetes patula* L. in decolorization of Reactive Blue 160. Vafaei *et al* (2013) revealed that *Hydrocotyle vulgaris*, as an aquatic higher plant, is able to effectively remove a textile dye (BR46) from the contaminated water. Similar results were reported by Mahmood *et al*. (2014) in relation to bioremediation potential of cattail (*Typha angustifolia*) for the treatment of the dye Reactive Blue 19.

Increasing the dye concentration, increased protein percentage of broad bean plants, despite the fact that the dyes decreased seed number. The induction of the stress response may lead to expression of a group of proteins referred to as stress proteins, which are thought to protect the plant cells (Gabara *et al*. 2003). The reason for the increase in protein content may be attributed to the presence of nitrogenous compounds in dye (Yasmeen *et al*. 2014). Salakinkop and Hunshal (2014) also reported an increase in the protein percentage of the wheat using sewage irrigation as compared to bore well-irrigation; however, they also reported an improvement in dry matter production and grain yield under sewage irrigation. On the other hand, Ramya *et al*. (2017) showed a decrease in the protein content of *Arachis hypogea* at all concentrations of dye effluent compared to the control. Yasmeen *et al*. (2014) observed that untreated wastewater significantly decreased total soluble proteins, plant chlorophyll contents and CAT activity as compared with treated wastewater. Fertigation with biologically treated wastewater increased N and P moderately, but untreated wastewater enhanced these elements to the toxic levels. They showed that biologically treated wastewater had no deleterious effects on the studied traits, as compared with untreated wastewater and clean irrigation water, and significantly increased the plant biomass and yield.

They concluded that wastewater should be treated before its application to agricultural fields.

Conclusions

Results indicated that increasing dye concentration increased protein content at all stages, and POX and CAT activities at pre-flowering and flowering stages, but it decreased seed number at pre-flowering and flowering stages and leaf number at flowering stage. Type of dye only affected seed number and protein content at pre-flowering stage, and CAT activities at both pre-flowering and flowering stages and didn't have any significant effect on other characteristics. We may conclude that treating dye imposes stress on broad bean plants, but they can survive under stress and may be recommended for bioremediation purposes.

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تاثیر مواد رنگزا بر برخی صفات مورفولوژیک، بیوشیمیایی و فیزیولوژیک باقلا (*Vicia faba* L.)

عطا باحجب الماسی[1]، سیروس حسن نژاد[2]*، حسن اقدسی نیا[3] و فریبرز زارع نهندی[4]

۱- دانشجوی دکتری بوم شناسی زراعی، گروه اکوفیزیولوژی گیاهی، دانشکده کشاورزی، دانشگاه تبریز، تبریز.
۲- گروه اکوفیزیولوژی گیاهی، دانشکده کشاورزی، دانشگاه تبریز، تبریز.
۳- دانشکده مهندسی شیمی و نفت، دانشگاه تبریز، تبریز.
۴- گروه علوم باغبانی، دانشکده کشاورزی، دانشگاه تبریز تبریز.
*مسئول مکاتبه؛ Email: sirous_hassannejad@tabrizu.ac.ir

چکیده

به منظور بررسی گیاه پالایی پساب رنگی توسط باقلا و اثرات رنگ بر این گیاه آزمایشی به صورت فاکتوریل در قالب طرح بلوک‌های کامل تصادفی با سه تکرار در سال زراعی ۱۳۹۵ در گلخانه تحقیقاتی دانشکده کشاورزی دانشگاه تبریز، به اجرا در آمد. فاکتور اول نوع رنگ در سه سطح، شامل رنگ زرد اسیدی (Acid Yellow)، قرمز اسیدی (Acid Red) و آبی دایرکت (Direct Blue) و فاکتور دوم غلظت رنگ در پنج سطح شامل صفر، ۳۰، ۵۰، ۷۰ و ۹۰ میلی گرم بر لیتر ماده رنگی بود. تیمارها در سه مرحله گیاهچه‌ای، قبل از گلدهی و گلدهی ارزیابی شدند. در این پژوهش برخی صفات شامل درصد تجزیه رنگ، تعداد دانه، تعداد برگ، میزان پروتئین و فعالیت پراکسیداز و کاتالاز مورد اندازه‌گیری قرار گرفتند. اثر غلظت رنگ در مراحل قبل از گلدهی و گلدهی بر میزان پراکسیداز، کاتالاز، تعداد برگ، تعداد دانه و درصد تجزیه رنگ و در هر سه مرحله روی میزان پروتئین معنی‌دار بود، در حالی که نوع رنگ فقط بر تعداد دانه و میزان پروتئین در مرحله قبل از گلدهی و فعالیت کاتالاز در هر دو مرحله قبل از گلدهی و گلدهی تأثیر معنی‌داری داشت. اثر متقابل نوع رنگ با غلظت رنگ فقط در مورد درصد تجزیه رنگ، در مراحل قبل از گلدهی و گلدهی، معنی‌دار شد. بیشترین میزان فعالیت POX و CAT و درصد پروتئین با غلظت رنگ ۹۰ میلی گرم در لیتر در هر دو مرحله قبل از گلدهی و گلدهی مشاهده شد؛ با این حال، بیشترین تعداد دانه در غلظت صفر میلی گرم در لیتر رنگ در مراحل قبل از گلدهی و گلدهی به دست آمد. می‌توان نتیجه گرفت که اگرچه جذب رنگ توسط باقلا مقداری تنش بر آن تحمیل کرد، ولی این گیاه توانست تحت این تنش زنده بماند. بنابراین، ممکن است گیاه باقلا را به عنوان یکی از گیاهان کارآمد برای گیاه‌پالایی توصیه کرد.

واژه‌های کلیدی: باقلا؛ تعداد دانه؛ رنگزا؛ رنگ‌زدایی؛ گیاه‌پالایی.