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Original Article

Effect of Seed Priming and Soil Application of Humic Acid on Growth and Yield of Roselle (*Hibiscus sabdariffa* L.)

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ABSTRACT

A factorial field experiment based on a randomized complete block design with three replications was conducted to investigate the effect of seed priming and soil application of humic acid on the growth and yield of Roselle (*Hibiscus sabdariffa* L.). The research was carried out in Farm Research of Shirvan Faculty of Agriculture in 2020. Experimental factors included seed priming with three levels of humic acid (0 (P_1), 2.5g/l (P_2) and 5g/l (P_3)) and two levels of humic acid soil application (0 (S_1) and 6 liters per hectare humic acid (S_2)). The results of the statistics analysis showed that the application of humic acid (both seed priming and soil application) significantly increased the growth traits and yield of Roselle plants compared to non-humic acid-treated plants (P_1S_1). Plants treated with P_3S_2 significantly had the highest plant height (167.3 cm), number of branches per plant (9.8), number of leaves per plant (81), leaf dry weight (34 g/m^2), leaf area index (5.2), calyx yield (53.3 g/m^2), number of seeds per plant (882), seed yield (1281 kg/ha) and biological yield (4230 kg/ha). However, non-humic acid-treated plants (P_1S_1) had the lowest plant height (133.5cm), number of branches per plant (6.7), number of leaves per plant (46), leaf dry weight (18.7 g/m^2), leaf area index (2.9), calyx yield (37.3 g/m^2), number of seeds per plant (554), seed yield (977 kg/ha) and biological yield (2900 kg/ha). The results also showed that soil application of humic acid had a more positive effect on the growth traits of Roselle plants than seed priming. However, leaf chlorophyll content and weight of thousand seeds (g) were not affected by both seed priming and soil application of humic acid.

INTRODUCTION

Roselle is an annual tropical and subtropical medicinal plant belonging to the Malvaceae family. It is successfully grown in tropical and subtropical climates [1]. The calyx is a commercially important part of the *H. sabdariffa* commonly used in food and cosmetic industries as a source of natural coloring agents to prepare refreshing beverages and jellies [2,3]. The calyx of the flower is rich in secondary metabolites, which have medicinal properties [4,5]. The calyces have large quantities of organic acids (citric, malic, oxalic, and tartaric acids) and vitamin C [6]. Two anthocyanins, namely cyanidin-3-sambubioside (gossypicyanin) and delphinidin-3-sambubioside (hibiscin) are dominant in calyces. Two minor anthocyanins, delphinidin- 3-glucoside and cyanidin-3-glucoside are also present [7,8,9]. Roselle fruits contain many essential nutrients such as vitamins A and C, minerals, carotene, and dietary fiber [10]. In addition, various medicinal applications such as anti-oxidation effects as well as treatment of diseases like hypertension, pyrexia, cancer, kidney stones, and blood pressure have been reported for this plant [11]. The application of organic acids to improve the quality and quantity of agricultural products has been widely prevalent because of having harmonic compounds having useful effects on crop yield and improving agricultural products. As an ecofriendly fertilizer, humic acid is one of the most important organic fertilizers that can positively affect plants' growth and increase nitrogen, potassium, calcium, magnesium, and phosphorus absorption by the plant [12]. Humic acid increases the population of soil organisms, improves soil physical conditions, and adjusts the pH of the media. It also has enzymatic and hormonal effects on plant growth. Humic substances enhance plant resistance to environmental stresses such as drought and salinity, enhance seed germination and root growth, and consequently improve crop quantity and quality. These effects were more pronounced in soil application than foliar spraying [13]. The application of humic acid as a powder in the soil increased the length and weight of carrot roots and eventually overall full plant growth [14]. Canellas *et al*. (2012) reported that humic acid improved the formation of lateral roots and increased crop yield due to increasing nutrient uptake and hormone-like properties [15].

Some studies also have shown humic substances increase plant growth by increasing water and nutrient absorption, availability of nutrient elements, development of root system, plant chlorophyll content, and alterations in plant enzyme activity [16-18]. Humic acid is a part of the humus compounds, which contains most of the known trace minerals and plays a significant role in plant nutritional balances [19]. Former studies have proven that humic substances can affect plant physiology via hormone-like effects, influence photosynthesis, and activate certain enzymes [20]. It has been reported that humic acid can act as a growth regulator because enhances the auxin, cytokenin, and gibberllins, which cause elongation of the stem and improve plant growth [14]. Humic acid also increases soil porosity and root growth, which leads to an increase in the shoot system [21]. It has been shown that humic acid is a suitable nutritional source for improving Roselle growth, yield, and nutrient uptake under drought-stress conditions [22]. In another study on Roselle, enhancement of chlorophyll *a* and *b*, carotenoids, and leaf carbohydrates and a decrease in proline content of Roselle under stress conditions were observed [23].

The purpose of this study is to determine the effect of seed priming and soil application of humic acid on the growth traits and economic yield of Roselle.

MATERIALS AND METHODS

This study was conducted in the Research Field of the Shirvan Faculty of Agriculture located in Khorasan Shomali province, northeast of Iran in 2020. The experimental site is characterized by a semi-arid climate with average annual precipitation and mean annual temperature of 290 mm and 14.4 ºC, respectively.

A factorial field experiment based on a randomized complete block design with three replications was conducted to investigate the effect of seed priming and soil application of humic acid on the growth and yield of Roselle (*Hibiscus sabdariffa* L.). Experimental factors included seed priming with three levels of humic acid (0 (P1), 2.5g/l (P2), and 5g/l (P3)) and two levels of soil application of humic acids (0 (S1) and 6 liters per hectare of humic acid (S2)). Therefore, the experimental treatments were as follows: P1S1 (non-humic acid-primed seeds + non-humic acid soil application), P1S2 (non-humic acid-primed seeds + 6 L/ha humic acid soil application), P2S1(seeds were primed with 2.5 g/L+ non-humic acid soil application), P2S2 (seeds were primed with 2.5 g/L humic acid + 6 L/ha humic acid soil application), P3S1 (seeds were primed with 5 g/L humic acid + non-humic acid soil application) and P3S2 (seeds were primed with 5 g/L humic acid + 6 L/ha humic acid soil application). Humic acid (humax) powder containing 90% humic acid, 10% folic, and potassium sulfate was used. For priming, Roselle seeds were exposed to the desired pre-treatments (0, 2.5g/l, and 5g/l) for 6 hours in a dark room at 15 ºC and then dried at room temperature and then sowed on the farm. Applications of soil humic acid were done three times 20, 40, and 60 days after emergence. The amount of humic acid according to treatment and plot area was calculated and added to 10 liters of water, and then the solution was poured into the plant's root area. The space between the planting points was 30 cm, and the row spacing was 50 cm, which resulted in 6.6 plants m-2. Weeding was done by hand twice during the growing season when needed. Plants were irrigated at 10-day intervals. For measuring growth parameters and yield components of Roselle plants, five plants were randomly selected from each plot on November 10. The selected plants were cut from above ground, and the growth parameters, such as the number of leaves per plant, plant height (cm), number of branches per plant, leaf dry weight (g/m^2), leaf area index, calyx yield, seed yield, and biological yield were recorded. Plant height was measured from the base to the shoot tip of the plant using a measuring tape.

Table 1 The analysis results of farm soil in 0-30 cm depth.

Fe (ppm)	Mn (ppm)	K (ppm)	P (ppm)	N (%)	pH	EC (dS/m)	Soil depth (cm)	Soil texture
3.8	6.7	219	10	0.092	8	1.62	30	Clay

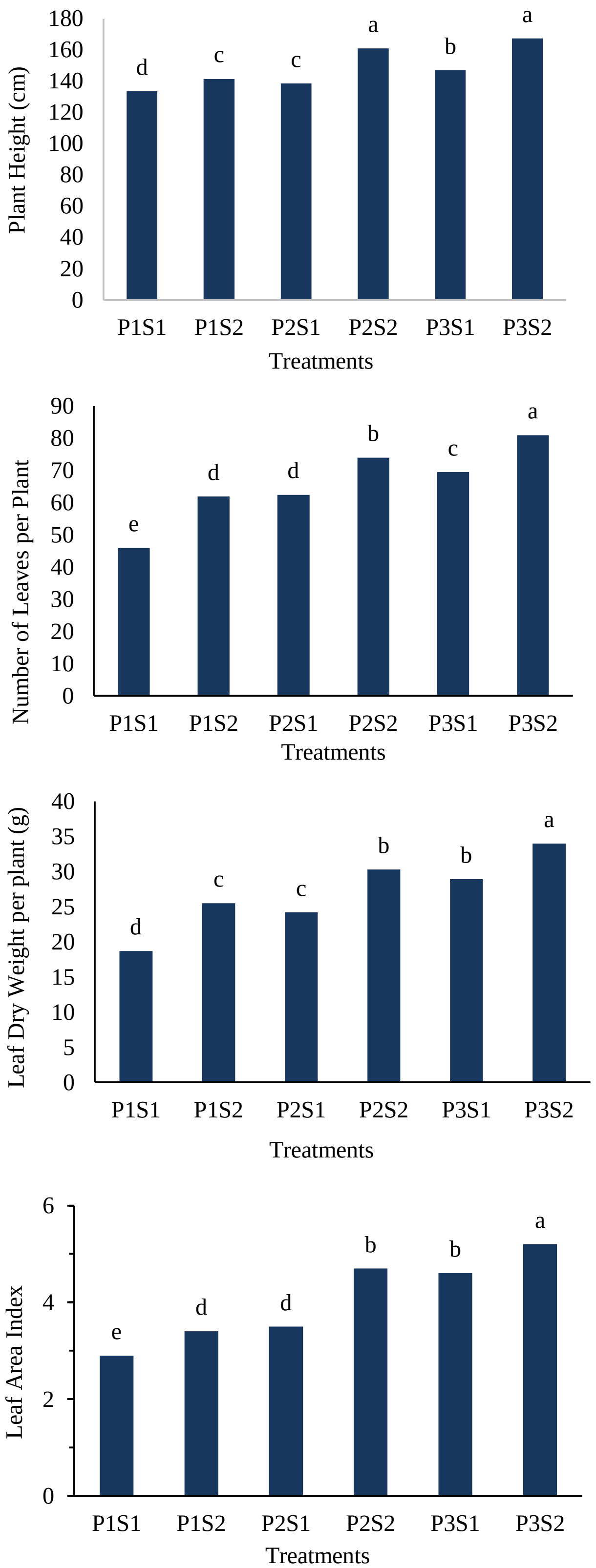

Fig. 1 Effect of different humic acid treatments on the plant height, number of leaves per plant, leaf dry weight, and leaf area index of Roselle plants. The mean of each column with similar letters is not significantly different (p≤0.01) according to Duncan's test.

The total leaf area per plant was measured with an automatic area meter (*Delta-T Device MK2, UK*). The data for all characteristics were analyzed using the analysis of variance procedure of Statistical Analysis System (SAS) software, version 6.12. Means were compared by Duncan's multiple range tests at the 0.05 probability level for all comparisons.

RESULTS AND DISCUSSIONS

The results of the ANOVA showed that the main effects of seed priming, soil application of humic acid, and also the interactions between the priming and soil application of humic acid on most growth traits were significant ($p<0.01$) (Table 2). However, leaf chlorophyll content and weight of a thousand seeds were not affected by treatments. Plants treated with P3S2 significantly had the highest plant height (167.3 cm), number of leaves per plant (81), leaf weight per plant (34.5 g/plant), and leaf area index (5.2) compared to non-treated plants (P1S1) (control) (Fig.1). However, there was no significant difference in plant height of plants that were treated with P2S2 and P3S2 (Fig. 1).

Calyx yield, number of seeds per plant, seed yield per hectare, and biological yield also increased by application of humic acid, so that plants treated with P3S2 produced 53.3gm-2, 882 seeds per plant, 1281 kg/ha and 4230 kg/ha, respectively compared to non-humic acid-treated plants (P1S1) (37.3 g/m^2, 554 seeds, 977 kg/ha, and 2500 kg/ha, respectively) (Fig. 2). However, there was no significant difference in calyx yield and seed yield of plants were treated with P2S2 and P3S2 (Fig. 2). Higher seed yield in the plants treated with P2S2 and P3S2 can be attributed to higher seed production per plant does not seed weight because the weight of one thousand seeds was not affected by humic acid (data not shown).

Based on the results, the plant growth traits were significantly affected by humic acid application. It has been shown that seed priming is a technique that allows seeds to readily absorb water without root growth and helps them germinate at an early stage. Germination is an important and sensitive stage in the plant life cycle and is a main process in seedling emergence, the time between planting and seedling establishment. Therefore, rapid and uniform germination is essential to increase plant growth traits, and consequently plant yield [24].

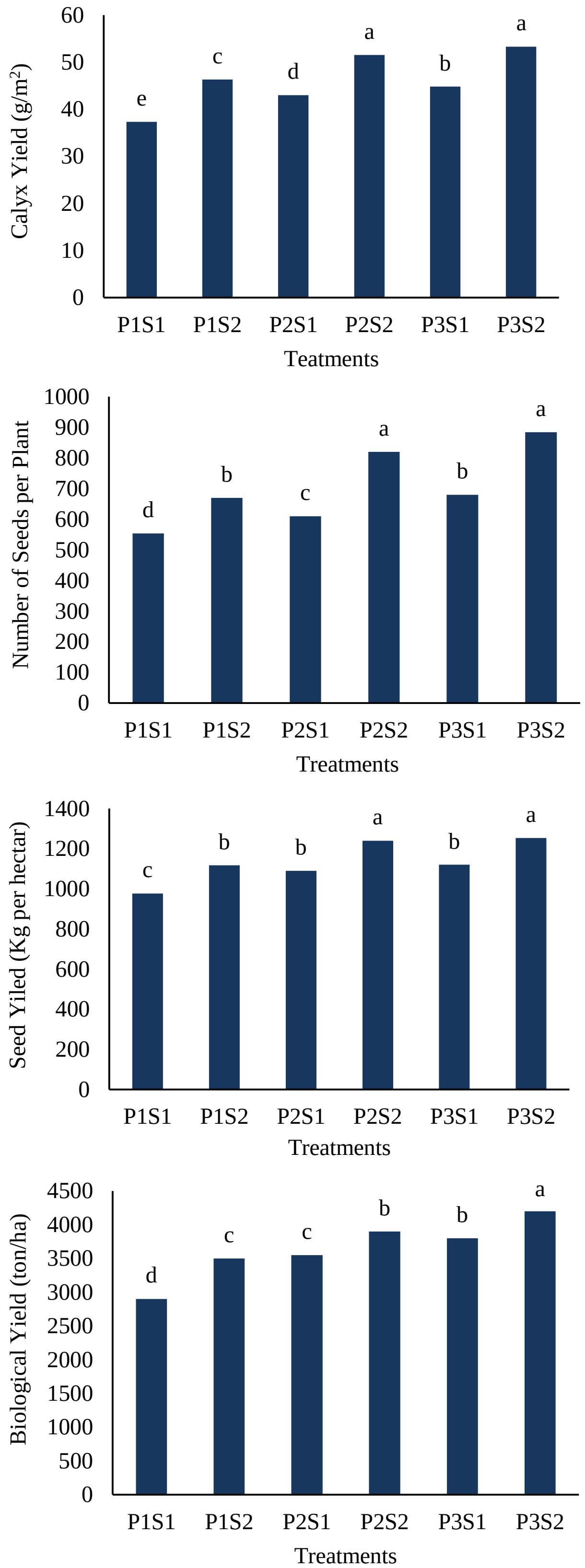

Fig. 2 Effect of different humic acid treatments on the calyx yield, number of seeds per plant, seed yield and biological yield of Roselle plants. The mean of each column with the similar letters are not significantly different ($p \leq 0.01$) according to Duncan test.

Former research has shown that priming with humic acid can improve seed germination and relevant features [25, 26]. Humic acid also causes rapid and uniform germination in seeds by increasing their metabolic activity [27]. It has been suggested that humic acid has a harmonic characteristic that results in root volume elevation, and eventually increases nutrient absorption [28]. Humic substances pretreatment-seed cause physiological changes, such as changes in the amount of sugar, organic compounds, and ions accumulated in the seeds, roots, and finally the leaves of the seedlings. These lead to an increased germination rate and greater resistance to adverse conditions in the established seedlings. It has been illustrated that the application of humic acid increased the germination percentage of Roselle seeds under drought-stress conditions [25].

Humic substances increase the plant growth parameters and stress tolerance of plants by increasing water and nutrient absorption, availability of elements, development of root system, plant chlorophyll content, and alterations in plant enzyme activity [16-18]. Khalil and Yousef (2014) reported that humic acid is a part of the humus compounds, which contain the most known trace minerals and play a main role in plant nutritional balances [19]. Previous studies have shown that humic substances can affect plant physiology via hormone-like effects, influence photosynthesis, and activate certain enzymes [20].

Humic acid plays an important role in the development of leaf area by reducing pH and increasing iron uptake [29]. It has been explained that the application of 1200 mg/l humic acid caused further development of leaf area in the forage Turpin [30]. Some studies have shown that humic acid can act as a growth regulator because it enhances the auxin, cytokenin, and gibberllins, which cause elongation of the stem and improve plant growth [14]. Humic acid also increases soil porosity and root growth, which leads to an increase in the shoot system [21]. It has been reported that humic acid is a suitable nutritional source for improving Roselle growth, yield, and nutrient uptake under drought-stress conditions [22].

Table 1 Analysis of variance of seed priming and soil application of humic acid on growth triads and yield of Roselle plants

S.O.V	d.f	Plant height	No. of branches	No. of leaves per plant	Leaf dry weight	Leaf area index	Calyx yield	No. of seeds per plant	Seed yield
Block	2	296.1 ns	4.04 ns	92.95 ns	13.6 ns	0.23 ns	88.8 ns	27760.3 *	201416 *
Humic seed priming (P)	2	2270.7 **	18.75 *	2115 **	412 **	2.16 **	4555 **	134840 **	305449 **
Humic soil application (S)	1	2.892 b	4.82 *	2436 **	452 **	3.27 **	156557 **	306520 **	630767.5 **
P * S	2	60.60 **	0.40 *	376.05 **	264.30 *	0.88 **	389.98 **	8105.2 *	37133 *
Error	10	99.94	2.6	38	11.4	0.03	16398	149294	11199
CV	17	7.13	4.56	6.60	12.70	5.30	14.55	10.37	8.80

*, ** and ns: significant at 5 and 1 % probability level, and no significant respectively.

Generally, the results of this study showed that humic acid seed priming and soil application both had a suitable effect on the plant growth parameters and yield of Roselle plants. It has revealed that among the various humic acid treatments, P3S2 treatment had the highest effect on the plant growth properties. In the present study, the seed priming and soil application of humic acid were studied in farm conditions; further studies could be done to investigate the effect of humic acid on medicinal plants. Due to the unsuitable management of medicinal plants in Iran, one of the most markable problems is the lack of information about the use of humic acid to increase fertility in the soil. Therefore, more studies are necessary about the role of humic acid in mitigating the low fertility in the soil. Moreover, regarding very few studies about the positive effect of humic acid on the growth of medical plants, it is recommended to investigate the effect of this material on other medical plants.

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