

Effect of Humic Acid on Seed Germination and Seedling Growth of *Borago officinalis* and *Cichorium intybus*

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ABSTRACT: The study was conducted to investigate the effect of humic acid on the germination properties of medicinal plants *Borago officinalis* and *Cichorium intybus* in a completely randomized design with five replications. Experimental treatment comprised the following dosages 0, 15, and 30 g l⁻¹. The results revealed that effect of humic acid on the germination properties of *C. intybus* was significant ($p < 0.01$) except for the germination percentage and mean germination time ($p < 0.01$). In addition, humic acid was effective on the morphological properties of *C. intybus* except for radical and pedicel dry weight. Humic acid was also effective on the germination properties of *B. officinalis* except for the germination percentage ($p < 1\%$); it was only effective on morphological properties viz. radical fresh weight, seedlings' pedicel length and allometric coefficient. The most effect was obtained in the 30 g l⁻¹ humic acid. The control treatment had the least effect on the plant's properties. In conclusion, the results showed that application of 30 g l⁻¹ humic acid was effective in germination of the plant species and stimulated the plants germination.

Keywords: Allometric coefficient, Medical plants, Seed management

1 INTRODUCTION

In recent years, the application of medicinal plants in order to cure and prevent diseases has dramatically increased worldwide, especially in Iran (Mohajeri *et al.*, 2007). Iran is a country with different climate conditions and rich in plant flora. Despite having a long history in use and production of medicinal plants, the report of Iran medicinal plant production and consumption is not suitable (Rassam *et al.*, 2014). Failure in treating many chronic diseases, chemical medicines' side effects, and microorganisms' increasing

resistance against many medications, particularly antibiotics are reasons for considering herbs (Mirzaei and Jaberi Hafshajani, 2010). One of the medicinal plants is *Cichorium intybus*, which is a rich source of phenolic compounds giving the plant a specific anti-oxidant characteristic (Cavin *et al.*, 2005). *C. intybus* leaves contain apigenin flavonoids, quercetin, and luteolin (Sareedenchai and Zidorn, 2010), and are used as a gastric nourishing, diuretic, laxative, bile clearing, and antipyretic drug in traditional medicine (Samsam Shariat, 2005). The plant,

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Borago officinalis, is also one of the important medicinal plants whose flowers and leaves are used as a sudoriferous, pacifier, and blood purifier material (Zargari, 2004). *B. officinalis* is recommended in traditional medicine in treating inflammations, coughs, and other respiratory problems (Osborne, 1999).

As far as medicinal plants production is concerned, true value is dedicated to the product quality and sustained production, and product quantity is in the second place of prominence (Darzi *et al.*, 2011), in such a way that suitable and right application of foods and nutrients during the implant, grow, and harvest of medicinal plants not only plays a significant role in increasing the performance but is really influential on the quality and quantity of their ingredients too (Vildova *et al.*, 2006). Therefore, using a suitable fertilizer is a main factor in successful planting of medicinal plants (Carrubba *et al.*, 2002).

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 production rise and improving agricultural products. Among these acids is humic acid that forms an insoluble stable complex with the micro-nutrients (Piccolo *et al.*, 1993; Michael, 2001; Rubio *et al.*, 2009). 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 (Sabzevari *et al.*, 2009).

Regarding the useful effects of humic compounds on improving plants' growth, Xuenyuan *et al.* (2001) reported that low concentrations of humic acid significantly increased the growth of *Triticum aestivum*. Verlinden *et al.* (2010) studied the effect of humic acid on several rangeland species. They reported that the application of humic acid led to increase the plant's production. Moreover,

Sheriff (2002) showed that the application of 100 mg kg⁻¹ humic acid led to increase stem dry weight (23%) and root dry weight (32%) of *Zea mays*. It further showed a significant elevation in soil nitrogen and the nitrogen stored in the plant.

In spite of numerous studies on the effect of humic acid on garden and agricultural plants, very few studies have been carried out about its effect on medicinal plants, thus the present study aims to investigate the effect of acid humic's different concentrations on the germination and morphological characteristics of the medicinal plants' seeds (*C. intybus* and *B. officinalis*) in order to determine the best response of these species to the applied concentrations.

2 MATERIALS AND METHODS

The present study was carried out in the laboratory of Agriculture Research Center, affiliated with University of Zabol. The minimum and maximum temperature of the lab was 18 and 22 °C, respectively. The experimental design was completely randomized with five replications. First, the seeds were disinfected by 5% sodium hypochlorite for three minutes, and then they were rinsed with distilled water (Sabzevari *et al.*, 2009). Next, the petri dishes and seedbeds (Watman filter paper, No. 1) were sterilized in autoclave (RT-2, Reihan Teb, Iran). Then 25 seeds of the plant were set evenly throughout each petri dish. Humic acid (humax powder (60%) containing 60% humic acid and 15-20% folic acid) at three levels (0, 15 and 30 g l⁻¹) was applied to the seeds in petri dishes (Sabzevari *et al.*, 2009). In order to eliminate negative effects of evaporation, the humic acid solution was added to each petri dish during the test period (for six days) so that the seeds were in contact with the solution but were not submerged (Sabzevari *et al.*, 2009). The first counting of the germinated seeds occurred 24 hours after transferring them into the petri dishes; the seeds

whose radicals were observable, were counted as germinated and brought out of the petri dishes (Wiese and Larry Binning, 1987). Germination seeds were counted and recorded daily (Farajollahi *et al.*, 2012). This was done each 24 hours until the germination was completed. On the last counting day (the 6th day), the radicle and pedicle length (using a caliper) and the dry and fresh weights of the seedlings were measured (using digital balance with 0.0001 g precision). After six days, having fixed the number of the germinated seeds and having finished the growth period, the germination properties, including the germination rate, germination percentage, germination time, and seed vigor index were measured according to Eqs. 1-4):

$$SG = \sum Ni / Di \quad (1)$$

Where, SG, Ni and Di are germination rate, number of germinated seeds in each day, and counted day, respectively (Mereddy *et al.*, 2000).

$$GP = (n/N) 100 \quad (2)$$

Where, GP is germination percentage, n is total number of the germinated seeds during counting, and N is total number of the germinated seeds in each petri dish (Behbodian *et al.*, 2005).

$$MGT = \frac{\sum nd}{\sum n} \quad (3)$$

Where, MGT, n, d and $\sum n$ are mean germination time, number of germinated seeds during the day, number of days since the beginning of germination, and total number of germinated seeds, respectively (Ellis and Roberts, 1981).

It is to be mentioned that the needed mean time for germination is counted as an index of germination rate and speed (Ellis and Roberts, 1981).

$$SVI = \text{mean of initial stem length} + \frac{\text{the mean of initial root length}}{\text{viability}} \quad (4)$$

The seedling vigor index (SVI) was determined at the end of growing trial after calculating the pedicle and radicle lengths (Saravanakumar *et al.*, 2007). In this respect, viability is the final germination percentage. Allometric coefficient was obtained through calculating the radical length to pedical length ratio based on Eq. 5:

$$\text{Allometric coefficient} = \frac{\text{radicle length}}{\text{pedicle length}} \quad (5)$$

In each petri dish, 10 seedlings were randomly chosen, and the radicle and pedicle lengths were calculated by a caliper. Then in order to determine the dry weight, first, the samples were washed with distilled water, and after having removed the radicle and pedicle, they were placed in the oven (Dena-Iran) in 70 °C for 48 hours. Then the dry weight and fresh weight of both radicle and pedicel were measured.

2.1 Data analysis

Statistical analyses of the experimental data were performed using the SPSS software (ver. 18.0). All reported results are the means of five replicates, and deviations were calculated as the standard error of the mean (SEM). The statistical processing was mainly conducted by analysis of variance (ANOVA). Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as significant level.

3 RESULTS

3.1 Effect of humic acid on germination properties

The results of analyzing variances of *C. intybus* seeds' germination properties (Table 1) revealed that humic acid had significant effect on the germination rate and seed vigor index ($p < 1\%$), while it had no significant effect on the germination rate and germination mean time. The maximum and minimum germination rates of *C. intybus* were observed at concentration of 30 g l^{-1} and the control treatment, respectively (Table 2). The highest seed vigor index was observed at 30 g l^{-1} dose; however, regarding this index, there was no significant difference in comparison with 15 g l^{-1} concentration, while the lowest seed vigor index belonged to the control treatment. Humic acid led to significant increase in the germination rate in comparison with the control treatment. The highest germination rate was measured in 30 g l^{-1} dosage, and the lowest germination rate was calculated in the control treatment.

In case of *B. officinalis*, humic acid had significant effect ($p < 1\%$) on the germination rate and germination mean time (Table 1), while it had no meaningful effect on the germination rate and seed vigor index. Both concentrations (30 and 15 g l^{-1}) increased the germination rate of the plant significantly; however, the maximum germination rate (20.29%) was calculated at 30 g l^{-1} of humic acid (Table 3), and the lowest level (3.77%) belonged to the control treatment. Germination mean time also significantly increased upon humic acid concentration elevation. The maximum germination mean time belonged to 30 g l^{-1} , and the lowest average was observed for the control treatment. However, the germination percentage and seed vigor index were not considerably affected, and there was

no significant difference among the treatments (Table 3).

3.2 Effect of humic acid on morphological properties

Regarding the morphological indices of *C. intybus*, the results of variance analysis revealed that the effect of humic acid on pedicel length, allometric coefficient, and radicle length ($p < 1\%$) was significant, while it did not have a significant effect on the dry and fresh weights of radicle and pedicel. Maximum radicle length and minimum radicle length of *C. intybus* were obtained at 30 g l^{-1} (6.91 mm) and the control treatment (2.6 mm), respectively (Table 2). Also maximum (5.65 mm) and minimum (4.68 mm) pedicel length were related to 30 g l^{-1} concentration and the control treatment, respectively. Moreover, maximum allometric coefficient was obtained at 30 g l^{-1} concentration of humic acid, which was not significantly different from 15 g l^{-1} humic acid, and the minimum allometric coefficient was related to the control treatment (Table 2).

The same trend was seen for *B. officinalis*. Humic acid had a significant effect on the radicle length, pedicel fresh weight, and allometric coefficient ($p < 1\%$) (Table 1). The maximum radicle length was obtained at 30 g l^{-1} concentration (1.35 mm), and the minimum was obtained in the control treatment (Table 3). The maximum radicle fresh weight turned out to be in 30 g l^{-1} concentration (0.2 g), and the minimum radicle fresh weight was observed in the control treatment (0.05 g). The maximum of allometric coefficient was obtained at 30 g l^{-1} treatment such that the maximum and minimum allometric coefficient of *B. officinalis* belonged to the concentration of 30 g l^{-1} and the control treatment, respectively (Table 3).

Table 1: Results of analysis variance of the germination and morphological properties of *C. intybus* and *B. officinalis* seeds under different humic acid applications

Plant	Source of variation	df	Mean square										
			Germination present	Germination rate	Allometric coefficient	Radical length	Pedical length	Pedical dry weight	Radical dry weight	Pedical fresh weight	Radical fresh weight	Seed vigor index	Germination time
<i>C. intybus</i>	Humic acid	2	12.44 ^{n.s}	2075.69**	1.05**	85.98**	1398.96**	0.002 ^{n.s}	0.002 ^{n.s}	0.002 ^{n.s}	0.034 ^{n.s}	1.98**	0.001 ^{n.s}
	Error	6	64	58.49	0.47	8.82	18.38	0.003	0.001	0.002	0.035	174.01	0.007
<i>B. officinalis</i>	Humic acid	2	144 ^{n.s}	223.23**	1.26**	192.02 ^{n.s}	3572.04**	0.004 ^{n.s}	0.002 ^{n.s}	0.001 ^{n.s}	0.045**	1.41 ^{n.s}	123.87**
	Error	6	138.66	20.57	0.14	140.2	303.37	0.002	0.002	0.003	0.003	63.136	7.19

** and * show significance at the 0.01 and 0.05 probability level, respectively, and n.s means non-significant.

Table 2: Comparison of the germination and morphological properties of *C. intybus* under different humic acid applications

Humic acid (g l ⁻¹)	Seed vigor index	Allometric coefficient	Radical length (mm)	Pedical length (mm)	Germination rate
0	68.2 ± 9.90 ^b	0.55 ± 0.08 ^b	2.60 ± 4.38 ^c	4.68 ± 1.09 ^b	23.16 ± 2.02 ^c
15	89.03 ± 7.40 ^b	1.06 ± 0.10 ^a	5.07 ± 2.4 ^b	4.77 ± 2.75 ^b	52.33 ± 9.29 ^b
30	119.90 ± 1.90 ^a	1.22 ± 0.07 ^a	6.91 ± 5.49 ^a	5.65 ± 4.20 ^a	75.66 ± 7.63 ^a
Sig	0.009**	0.00**	0.00**	0.01**	0.00**
F	11.42	47.66	76.07	9.74	41.86

**Values within a column followed by the same letters are not significantly different (p<0.05, means±SE). Significant at the 0.01 probability level.

Table 3: Comparison of the germination and morphological properties of *B. officinalis* under different humic acid applications

Humic acid (g l ⁻¹)	Germination rate	Radical fresh weight (g)	Allometric coefficient	Radical length (mm)	Germination time
0	3.77 ± 1.43 ^b	0.05 ± 0.03 ^b	0.93 ± 0.10 ^b	0.98 ± 0.12 ^b	1.20 ± 0.749 ^b
15	11.16 ± 0.02 ^b	0.14 ± 0.04 ^b	1.52 ± 0.62 ^{ab}	1.26 ± 0.18 ^a	8.70 ± 1.48 ^a
30	20.97 ± 7.45 ^a	0.29 ± 0.07 ^a	2.29 ± 0.21 ^a	1.35 ± 0.24 ^a	13.97 ± 2.74 ^a
Sig. F	0.01**	0.00**	0.01**	0.00**	0.00**
	10.84	14.06	8.50	11.77	17.22

**Values within a column followed by the same letters are not significantly different (p<0.05, means±SE). Significant at the 0.01 probability level.

4 DISCUSSION

In this study, the effect of humic acid on stimulating plants' germination properties was studied. The results showed that applying humic acid led to a significant increase in germination rate of the plants. Mosa-pour *et al.* (2014) reported that humic acid affected the germination rate of *Calendula officinalis* positively. Sabzevari *et al.* (2009) found that 54 mg l⁻¹ concentration of humic acid resulted to increase the germination rate. Due to its low molecular weight, humic acid was absorbed quickly by the seed, which increased the absorption of elements such as nitrogen and phosphorus (Asenjo *et al.*, 2000), and consequently, caused the plants' germination stimulation (Piccolo *et al.*, 1993).

The germination percentage of the seeds was not affected by different concentrations of humic acid. The reason that humic acid was not significant on the seed germination percentage is that the seed of the study plants had no problem regarding germination percentage so that, in the control treatment, germination percentage of all seeds was equal to 100%. Piccolo *et al.* (1993) reported that humic acid (at 40-5000 mg l⁻¹) had not effective impact on the germination percentage of tomato (*Solanum lycopersicum*) and lettuce (*Lactuca sativa*) seeds. The results of mean comparison indicated that applying humic acid had a significant effect on the germination mean time of *B. officinalis* seeds, while it was not significant on *C. intybus*. The results of Iswaran *et al.* (1971) revealed that the application of humic acid in soya bean (*Glycine max*) increased its water absorption, germination rate and respiration, so the increased germination rate in the seeds decreased the germination mean time. Sabzevari *et al.* (2009), studying the effect of four concentrations of humic acid (0, 54, 36, 72 mg l⁻¹) on the germination of spring wheat, demonstrated that medium humic acid decreased germination mean time in comparison with the control treatment, so that

the application of humic acid (36 and 72 mg l⁻¹) led to decreasing the germination mean time in comparison with the control treatment.

The reasons for lack of humic acid's positive response at the required time for the germination of *C. intybus* seeds can be the type of the applied humic material, or lack of the plant's reactivity to humic acid (Shahsavani Markade and Chamani, 2014). In the present study, seed vigor index increased at 30 g l⁻¹ concentration of humic acid. Sabzevari *et al.* (2009) demonstrated that 54 mg l⁻¹ humic acid increased the seed vigor index of *Triticum aestivum* significantly. The mechanism of effect of humic material on seed germination is not clearly defined; however, in some resources, two effects are pointed out as humic acid's direct (production and function of plant hormones) (Nardi *et al.*, 2002) and indirect (better absorption of nutrients) influence on germination (Chen and Aviad, 1990).

The application of humic acid increased the length as well as the fresh and dry weight of the root of the plants. Heidari and Minaei (2014) studied the effect of humic acid on production of *Borago officinalis*. They suggested that humic acid increased the dry weight of flower in comparison with the control treatment. Cordeiro *et al.* (2011) studied the effect of humic acid on corn's root growth and found that 3 mmol of humic acid mixed with NO₃ could lead to the corn root's development and increased its root's fresh weight and dry weight ratio. The reason for the increased dry weight of the underground organ by applying humic acid is known to be that humic acid raises chlorophyll pigments, and consequently, increases photosynthesis and the plant production. In other words, it can be said that having hormone influences, humic acid promotes root growth, and accordingly, enhances root's dry weight (Ghasemi *et al.*, 2012). Ayman *et al.* (2009) applied amino acids and humic acid as the catalyst of growth, function, and resistance to fungal diseases in

fabia bean planted in clay soils. All morphological traits (except branching and leaves) and functions (except sheath and weight of 100 seeds) were improved by humic acid application.

The results demonstrated that applying humic acid increased the pedicle length, dry and fresh weights of the plants. Eyheraguibel *et al.* (2008) investigated the effect of humic acid on *Zea mays*' seed germination. They found that humic acid promoted the growth of the plant's stem. The study of Gulser *et al.* (2010) on the effect of humic acid on pepper revealed that the fresh and dry weights of the leaf and stem increased under the effect of humic acid. Ulukan (2008) reported that stem height showed the highest response to humic acid. In a survey, Sheriff (2002) reported that dry weight of *Zea mays* significantly increased through applying 150 mg of humic acid. Tejada and Gonzalez (2003) also observed that performance, chlorophyll content, carotenoids and macro- and micro-nutrients increased in *Asparagus officinalis*. The catalyst effect of humic materials on stem growth in the first place may be due to the influence on root's H⁺-ATPase activity as well as the distribution of nitrates in root and stem, which in turn lead to changes in certain distribution of cytokines, polyamines, and abscisic acid (ABC). Thus, they affect stem growth (Rubio *et al.*, 2009).

The results of variance analysis in both plants' seeds indicated the significant effect of humic acid on the allometric coefficients. Kauser and Azam (1985) found that humic acid spraying on the seed of *Triticum aestivum* at 54 mg l⁻¹ concentration accompanied 50% root increase and 22% increase in dry weight. Jones *et al.* (2004) stated that humic acid has a harmonic characteristic and results in root volume elevation, and consequently, increases nutrient absorption. On the other hand, it can be claimed that because of high cation exchange of humic acid, it provides useful elements and

excretes toxic elements and heavy metals in the plants' roots. It can be said that humic acid was not influential on some morphological traits of the plants because humic materials can decrease the plants' growth due to over-production of plant hormones. In this case, Cooper *et al.* (1988) mentioned that the applied concentrations of humic acid (0, 300, 200, 100, and 400 mg l⁻¹) had no effect on the plants' growth, which corresponds to the findings of this study.

5 CONCLUSION

Generally, the results of this study showed that humic acid had a suitable effect on seed germination of *B.officinalis* and *C.intybus*. It was revealed that among the various concentrations of humic acid, 30 g l⁻¹ dosage had the highest effect on seed germination properties. The application of humic acid can qualitatively and quantitatively improve the plant efficiency, and also reduce the use of chemical fertilizers and environmental pollution. Regarding the increase of seed germination indices by the application of up to 30 g l⁻¹, probably higher concentrations of humic acid would provide better results. In the present study, application of humic acid was studied in the lab conditions; further studies could be done to investigate the effect of humic acid in the soil conditions. Due to the unsuitable management of medicinal plants in Iran, one of the most important problems is 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 to mitigate 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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تأثیر اسید هیومیک بر جوانه‌زنی بذر و رشد گیاهچه *Cichorium intybus* و *Borago officinalis*

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چکیده به‌منظور بررسی اثر اسید هیومیک بر ویژگی‌های جوانه‌زنی بذر گیاهان دارویی گل‌گاوزبان و کاسنی در قالب طرح کاملاً تصادفی با پنج تکرار انجام شد. تیمارهای آزمایشی شامل سه غلظت صفر، ۱۵ و ۳۰ گرم بر لیتر اسید هیومیک بود. نتایج نشان داد اثر اسید هیومیک بر تمامی ویژگی‌های جوانه‌زنی بذر کاسنی به‌جز درصد جوانه‌زنی و میانگین زمان جوانه‌زنی در سطح یک درصد معنی‌دار بود. هم‌چنین نتایج نشان داد که اسید هیومیک بر شاخص‌های مورفولوژی کاسنی به‌جز وزن تر و خشک ریشه‌چه و ساقه‌چه اثر معنی‌دار داشت. اثر اسید هیومیک بر تمامی ویژگی‌های جوانه‌زنی بذر گل‌گاوزبان به‌جز درصد جوانه‌زنی در سطح یک درصد معنی‌دار بود و تنها بر ویژگی‌های مورفولوژی شامل وزن تر ریشه‌چه، طول ساقه‌چه و ضریب آلومتری تأثیر معنی‌دار داشت. از بین غلظت‌های مختلف اسید هیومیک بیش‌ترین تأثیر در غلظت ۳۰ گرم بر لیتر محاسبه گردید و تیمار شاهد کم‌ترین تأثیر را بر کلیه صفات گیاهان مورد مطالعه داشت. به‌طور کلی نتایج نشان داد که کاربرد اسید هیومیک در غلظت ۳۰ گرم بر لیتر بر جوانه‌زنی گیاهان موثر بوده و جوانه‌زنی گیاهان مورد مطالعه را بیش‌تر تحریک کرد.

کلمات کلیدی: ضریب آلومتریک، گیاهان دارویی، مدیریت بذر