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Phytochemical and Morphological Attributes of St. John's Wort (*Hypericum perforatum*) Affected by Organic and Inorganic Fertilizers; Humic Acid and Potassium Sulphate

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Abstract

This experiment was designed to evaluate the effects of organic (liquid humic acid) and inorganic (potassium sulphate) on phytochemical and morphological attributes of St. John's Wort (*Hypericum perforatum*). Thus, a research was conducted in a factorial experiment (3×3) based on completely randomized design with three replications. Treatments consisted of potassium sulphate (K_x) at three concentrations (0, 60 and 100 $Kg.h^1$) which were treated before flowering and humic acid (H_x) at three concentrations (0, 20 and 40 $L.h^{-1}$) which were fertigated four times of 15-days intervals. Results showed that the plant stem height, number of flowering stems and number of flowers were significantly affected by simple effect of each fertilizers ($p<0.01$), while their interaction effect was not significant for the plants height. The highest contents of fresh and dry weight were achieved under the highest amounts of fertilizers (K_{100} and H_{40}). The highest stem height, number of flowers and number of flowering stems also belonged to these treatments. Increment of applied fertilizers led to increase of obtained essential oils, so that application of these fertilizers simultaneously increased the essential oil content up to 6-fold. Regarding the antioxidant activity, applied fertilizers at their high levels showed significant effects on decrease of EC_{50}, which means the increment of antioxidant activity of *H. perforatum*.

Keywords: antioxidant activity, essential oil content, yield

Introduction

Medicinal and aromatic plants can play an important role in commercial crops, which also represent a safe alternative for chemical pharmaceutical industries. St. John's Wort (*Hypericum perforatum* L.) due to its therapeutic efficacy has been used for decades in folk medicine (Ang *et al*., 2002; Barnes *et al*., 2001) and is considered as a promising medicinal plant with valuable potential as a source of hypericin, essential oils and antioxidants (Cellarova *et al.,* 1995). In recent years, the consumption of these plant-derived products has increased dramatically and presently it is one of the most consumed medicinal plant worldwide (Wills *et al.,* 2000). *Hypericum* extracts show significant antioxidant activity both in vitro and in cell systems, inhibiting free radical generation and lipid peroxidation (Almeida *et al.,* 2009; Benedi *et al.,* 2004; Zheleva-Dimitrova *et al.,* 2010). It also has been used in herbal medicine, externally for the treatment of skin wounds, eczema, burns and, internally, for disorders of the central nervous system, alimentary tract and other purposes (Barnes *et al.,* 2001; Gioti *et al.,* 2009).

Fertilizer treatment is known to be one of the main factors increasing the yield of plants (Gul *et al.,* 2011). It affects the accumulation, mineralization of organic matter added to the soil and determines plant production potential. Fertilization also determines the concentration of bioactive plant chemicals (Said-Al Ahl *et al.,* 2007). Thus, the improvement of plant nutrition can contribute to increased morphological and phytochemical attributes of plants.

Reports have shown that humic acid is one of the humic fractions of the soil organic matter that is responsible for the generic improvement of soil fertility and improved its productivity (Fortun *et al.,* 1989). Accordingly, this material possesses many beneficial agricultural properties, so that it can participate actively in the decomposition of organic matters and minerals. In addition, it improves soil structure and changes physical properties of soil, promote the chelation of many elements and make these available to plants (Said-Al Ahl *et al.,* 2009). Its positive effects were also reported *in vitro* and *in vivo* by Yildirim (2007) which resulted in increase of quality and quantity of potato. Cacilia *et al.* (2011) ferigated different levels of humic acid on *Thymus vulgaris* and found that applied treatment increased wet and dry weight of plant and antioxidant activity of *Thymus vulgaris* leaves extract. They also showed that essential oils content decreased under the mentioned treatment. Increase of humic acid levels from 0 to 40% led to increment of wet and dry weight of *Ocimum santum* and finally increased the plant yield (Prabhu *et al.,* 2010).

Potassium fertilization is also one of the methods which have great influence on all agronomic variables of yield and chemical constituents of aromatic and medicinal plants. This element (K) is involved in the growth of meristematic tissue and is indispensable for the maintenance of cell turgor pressure, which is required for cell expansion (Rogalski, 1994; Özgüven *et al.,* 2008). The use of K is especially important when high yield is expected (Defan *et al.,* 1999). The effect of this element has been reported by many researchers on onion (Thabet *et al.,* 1994), *Pyrethrum spp.* (Sastry and Singh, 1990), *Achillea millefolium* (Lieres *et al.,* 1994) and *Calendula officinalis* (Lieres *et al.,* 1994). Their results showed a positive yield response to potassium fertilizer application. Increasing evidence also suggests that mineral nutrients play a critical role in plant stress resistance. Out of all the mineral nutrients, potassium (K) plays a particularly critical role in plant growth and metabolism, and it contributes greatly to the survival of plants. The importance of K fertilizer for the formation of crop production and its quality is also known (Wang *et al.,* 2013).

However, studies on agronomic factors such as application of potassium and humic acid as well as nitrogen fertilization on yield, essential oils and antioxidant activity of *H. perforatum* have not been investigated thoroughly until now. This study was designed to study the effect of using humic acid and potassium sulphate on the morphological and phytochemical characteristics of *Hypericum perforatum* medicinal plant.

Materials and methods

Plant material

The plant material was prepared from Science and Technology Park in Khorasan-e Razzavi–Mashhad. In spring, the plants were transplanted into the field of Faculty of Horticulture, Ferdowsi University of Mashhad, for fertilization treatments.

The experimental layout was factorial in a complete randomized design (CRD), with three replications. Potassium sulphate (K_2SO_4) was applied at the rates of 0.0 (K_0), 60 (K_{60}) and 100 (K_{100}) kg.ha^{-1}. The other treatment was humic acid, which was applied at three rates of 0 (H_0), 20 (H_{20}) and 40 (H_{40}) L.ha^{-1}. Plants were treated at two stages, before flowering by potassium fertilizer and fertigated four times of 15-days intervals with humic acid.

Besides the wet and dry weight, plant number of flowers, stem height and number of flowering stems were determined at the end of the growing stage. To extract and quantify the essential oils, a weight of 100 g of dried herb were subjected to hydro-distillation for 3 hours using a Clevenger apparatus, according to British Pharmacopoeia (1963). Essential oil percentage of each treatment was determined and expressed as (%), while essential oil yield per plant can be expressed as ml.plant^{-1}. The essential oils of each treatment were collected and dehydrated over anhydrous sodium sulphate.

Preparation of methanol extract

The plant materials (20 g of powdered material) were extracted with 200 ml of methanol. Containers stayed on the shaker for 24 hours. This was repeated three times. The obtained methanol extracts passed through Whatman filter paper No. 1. The filtrates obtain Determination of antioxidant activity.

The antioxidant activity of *Hypericum perforatum* extracts was determined by neutralizing properties of free radical scavenging DPPH method (Liu, 2004). Thus, 100 ml of the extract was centrifuged and 2900 ml of DPPH solution was added. Mixture was quickly stirred and maintained at room temperature for 15 min in the dark. Decrease in absorption was determined at a wavelength of 515 nm by a spectrophotometer (model uv-1800 PC, Japan). In this study, results were evaluated as relative activities against α-tocopherol, such served as positive control. Therefore, the antioxidant capacity of the extracts was calculated and expressed as the percent inhibition of DPPH.

$$\%DPPHsc = (A_{cont} - A_{samp}) \times 100/A_{cont} \qquad (a)$$

$$A_{samp} = \text{DPPH + Absorption of the sample} \qquad (b)$$

$$A_{cont} = \text{Absorption of DPPH} \qquad (c)$$

$$\text{Percentage of inhibition} = \%DPPHsc \qquad (d)$$

Results and discussion

The ANOVA indicated that most of measured attributes of *Hypericum perforatum* plant were significantly affected by both treatments. Data presented in Tab. (1) revealed that the highest stem (91.6 cm) was recorded at the highest level of potassium sulphate (K_{100}) treatment, while the smallest height (60.4 cm) was recorded in the lowest level of potassium sulphate (K_0) treatment. On the other hand, the highest stem (80.33 cm) was achieved with the highest level of humic acid treatment. No significant difference was observed between the highest level of humic acid (H_{40}) and the second level (H_{20}), while both mentioned levels showed a significant difference with the control plants (H_0). This means that the control plants possessed the lowest stem height (66.7 cm).

Simple effect of potassium sulphate and humic acid fertilizers were significant for the number of flowering stems, but their interaction effect was not significantly different. The highest number of flowering stems (106 stems per plant) under potassium sulphate treatment was obtained with the highest level of this treatment, while the lowest number (31.00 stems per plant) was recorder for the control plants. Regarding the humic acid treatment, the most number of flowering stems (68.22 stem per plant) and the least number (50.00 stems per plant) were recorded in the levels of H_{40} and H_0, respectively. Our results were in agreement with results reported by Badawy *et al.* (2009). They stated that levels of potassium fertilizer significantly increased the stem height and number of flowering stems in *Artemisia annua* plant. Mert (2008) also reported that increment of potassium fertilizer significantly led to increase of stem height and number of flowering stems in all *Artemisia annua* ecotypes. Arancon *et al.* (2006) also showed that application of humic acid affected the strawberry stems' height. Results of this experiment also showed a significant difference between levels of potassium sulphate and humic acid for measured number of flowers (Tab. 1).

Tab. 1. Effect of potassium sulphate and humic acid fertilizers on morphological attributes

Treatments	Stem height (cm)	Flowering stem	Number of flowers
K_{100}	91.6^{a}	106^{a}	24.33^{a}
K_{60}	69.6^{b}	44.00^{b}	18.3^{b}
K_0	60.4^{c}	31.00^{c}	15.53^{b}
H_{40}	80.33^{a}	68.22^{a}	34.33^{a}
H_{20}	74.6^{a}	62.77^{a}	17.92^{b}
H_0	66.7^{b}	50.00^{b}	5.64^{c}

Means with the same letters are not significantly different

Results indicated that potassium sulphate and humic acid significantly affected wet and dry weight of *H. perforatum* in the first and second cuttings ($p<0.01$). In the first cutting, the highest (440 g per plant) and the lowest (44.6 g per plant) wet weight were recorded in the $K_{100}H_{40}$ treatment and control plants, respectively (Fig. 1). Regarding the dry weight of the first cutting, the same result was also observed for the $K_{100}H_{40}$ (84.4 g per plant) and control (8.90 g per plant) plants (Fig. 2). The highest wet (570 g per plant) and dry (105.5 g per plant) weight of the second cutting were obtained from plants treated with $K_{100}H_{40}$ and the lowest wet (110.9 g per plant) and dry weight (21.50 g per plant) from control ones (Fig. 3 and 4).

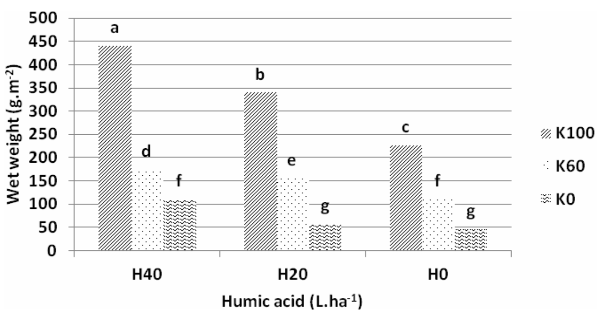

Fig. 1. Effect of humic acid and potassium sulphate on wet weight in the first cutting

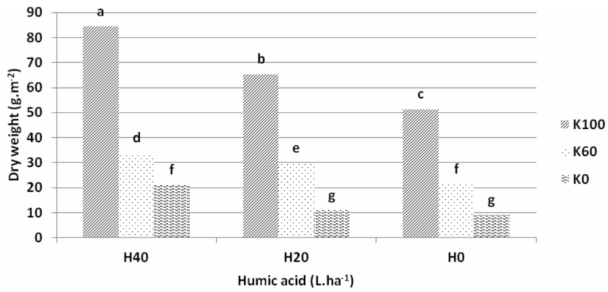

Fig. 2. Effect of humic acid and potassium sulphate on dry weight in the first cutting

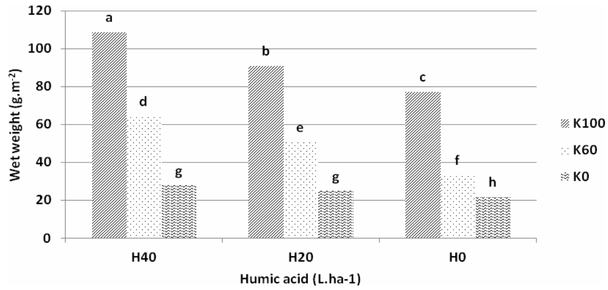

Fig. 3. Effect of humic acid and potassium sulphate on dry weight in the second cutting

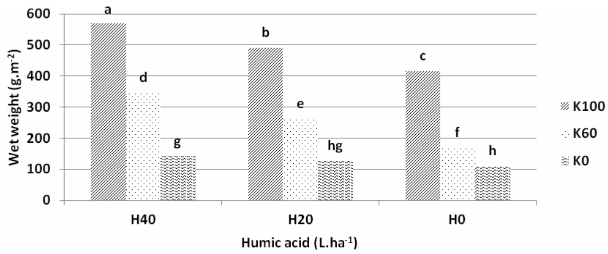

Fig. 4. Effect of humic acid and potassium sulphate on wet weight in the second cutting

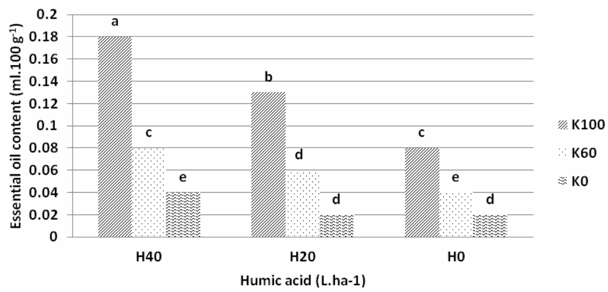

Fig. 5. Effect of humic acid and potassium sulphate on essential oils content

Antioxidant activity

The DPPH method for evaluation of antioxidant activity reflects the ability of the present compounds to scavenge hydrophilic free radicals (Zheng and Wang, 2001). DPPH inhibition was investigated and the results were evaluated as relative activities against α-tocopherol as positive control (Tab. 2).

A lower EC_{50} indicates a higher antioxidant activity (Maisuthisakul and Pongsawatmanit, 2007). As shown in Tab. 2, the least EC_{50}, which indicates the highest antioxidant activity, belonged to plants treated with potassium sulphate at level of 100 $kg.ha^{-1}$. Control plants possessed the least antioxidant activity and showed no significant difference compared with plants treated with 60 $kg.ha^{-1}$ of this fertilizer.

Humic acid had a significant effect on antioxidant activity of *H. perforatum*. The lowest value of EC_{50} was related to the highest level of humic acid, while the highest value of EC_{50} was obtained from control plants. There was also significant difference between control and two other levels (20 and 40 $l.ha^{-1}$) of humic acid.

Thus, applied fertilizers at their high levels showed significant effects on decrease of EC_{50}, which means the increment of antioxidant activity of *H. perforatum*. The high antioxidant activity of this medicinal plant is the reason of flavonoids and phenols existence (Butterweck, 2003). It seems that application of these fertilizers have led to increment of the mentioned secondary metabolites and followed by an increase of antioxidant activity in comparison with control plants. Totally, *H. perforatum* possessed a high antioxidant activity. Results of this study were in accordance with reports of Gioti *et al.* (2009). One of the destructive effects of free radicals production in human body is severe damage to nervous system (Behl and Mosmann, 2002). Thus, high antioxidant activity of this plant extract can inhibit most of diseases and more works to increase antioxidant activity power of this important medicinal plant seems to be necessary.

Tab. 2. Calculated EC_{50} and its comparison with α-tocopherol EC_{50} as positive control

Treatment	Calculated EC50 (mg.ml-1)	α-tocopherol EC50	Calculated EC50/ α-tocopherol EC50
0	0.05	0.0038	13.158
0×20	0.043	0.0038	13.579
0×40	0.041	0.0038	10.789
60×0	0.041	0.0038	7.895
60×20	0.037	0.0038	9.737
60×40	0.033	0.0038	6.842
100×0	0.032	0.0038	8.421
100×20	0.005	0.0038	1.316
100×40	0.005	0.0038	1.316

Essential oil content

As shown in Fig. 5, increment of applied fertilizers led to an increase of the obtained essential oils. Although the essential oils content of this plant is low, application of these fertilizers simultaneously increased the essential oils content up to 6-fold. The highest essential oils content was achieved at the highest levels of fertilizers, potassium sulphate and humic acid. Control plants possessed the lowest essential oil content under both treatments. Recorded essential oils content of this study is in accordance with the results of Schwob *et al.* (2004). Hendway and Khalid (2009) investigated the effect of simultaneous application of macro and inorganic elements on *Matricaria chamomilla*. They found that simultaneous application of those fertilizers led to a more significant increase of essential oil content, rather than separately applicated fertilizers. They suggested that increase of flower number and dimensions led to an increase of essential oil content. Effect of potassium and nitrogen fertilizers on *Tanacetum balsamita* was also investigated by Hasanpour *et al.* (2008) and they reported that the highest essential oil content was achieved under higher levels of fertilizers. They stated that suitable nutrition would result in promotion of involved secondary metabolites synthesis pathways. It seems that potassium is consumed in enzymes structure involving biochemical pathways of secondary metabolites synthesis. They also reported that imbalance of fertilizers would have similar effects as nutrition shortage on yield, followed by secondary metabolites production, such as essential oil content.

Conclusions

Based on recorded data of the present study, application of the mentioned fertilizers separately or simultaneously caused the increase of this important medicinal plant wet and dry weight, and finally the yield. Number of flowers, stem height and flowering stems reached the highest value at the highest level of both fertilizers ($K_{100}H_{40}$). As potassium plays an important role in the development of reproductive parts of plants, it might be one of the reasons of the above mentioned increase. The highest essential oils content was recorded at up levels of both fertilizers ($K_{100}H_{40}$). Since the most essential oils content accumulates in the flowering stems, an increase of these stems' height and a larger number of flowers would lead to an increase of essential oils content of *H. perforatum*. Antioxidant activity of this plant is flavonoids contributed. It seems that application of these fertilizers increased the antioxidant activity by an increase of this pigment content.

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