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Full Length Research Paper

Application times and concentration of humic acid impact on aboveground biomass and oil production of hyssop (*Hyssopus officinalis*)

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The objective of this study was to find the influence of different humic acid concentration and foliar application times on aboveground biomass and essential oil production of hyssop (*Hyssopus officinalis* L.) in 2008 and 2009 at experimental field of Ferdowsi University of Mashhad in Iran. The field experiment was a factorial based on randomized complete block design with three replications. Two times of foliar applications of humic acid (one time in first cut, two times in both cuts) were applied along with four humic acid concentrations (0, 100, 200 and 300 mg/l) in this experiment. Aboveground and leaf biomass and essential oil production, harvested at flowering were measured as annual production of hyssop (*H. officinalis* L.). Essential oil percentage did not response to different treatments in both years of experiment. Different application times did not change harvested aboveground biomass and essential oil production. Humic acid concentration showed great influence on aboveground and leaf biomass and essential oil production. Highest concentration of humic acid (300 ml/L) resulted in highest amounts of aboveground (+88%) and leaf (+77%) biomass and essential oil production (+71%) compared to control treatment. Hyssop produced higher biomass (+4%) and oil (+3%) in highest concentration of humic acid (300 ml/L) in the second year of the experiment compared to the first year.

Key words: Biomass, essential oil, foliar application, humic acid, *Hyssopus officinalis.*

INTRODUCTION

Environmental pollution due to excessive application of chemical fertilizer such as nitrogen fertilizer to agricultural lands, surface waters and groundwater is one of the most important environmental and social concerns throughout the world especially in developing countries (Parr et al., 1992). Soil organic matter (SOM) has beneficial effects on soil quality and positive effects on crop productivity and also has the potential to sequester carbon. In addition, organic matters could reduce the application of industrial fertilizers in long term (Rasmussen and Parton, 1994). Iran's agricultural soils organic matter with less than 0.3% is quite low in semi-arid environments (Ayoubi and Alizadeh, 2007). However, standard amount of soil organic matter is between 1.5 to 2% (Woodwell, 1984). Lamiaceae plant family has distributed all over the world and is in use as medicinal resources, horticultural ornaments and food additives (Murakami et al., 1998). *Hyssopus officinalis* (L.) of family Lamiaceae is a perennial semi-shrub which grows wild and is cultivated in temperate regions of Asia, Europe and America (Garg et al., 1999). Its dried flowering shoots are used in tea blends for cough relief, for antispasmodic effects, and to relieve catarrh (Kizil et al., 2008).

It has erect branched stems up to 60 cm long covered with fine hairs at the tips, with narrow oblong 2 to 5 cm long leaves. It has small blue flowers borne on the upper part of the branches during summer (Kizil et al., 2008). Hyssop oil greatest use is in flavoring alcoholic beverages, meat products, and seasonings (Leung and Foster, 2003). This plant is a typical xerophyte and is well adapted to drought and low input conditions (Khazaie et al., 2008).

Humic acids are complex compounds that exist in soils with high levels of organic matters and quinine functional groups, which are formed by microbial decay of plant

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tissues and are resistant to microbial degradation (Thygesen et al., 2009). These materials occurring widely in soils, sediments, and water, contain lots of functional groups such as carboxylic, phenolic, and methoxy groups (Xu et al., 2005). Humic acids are negatively charged colloid recalcitrant to biodegradation so it can be stored in soil for a long time (Qualls, 2004).

Direct impacts of humic acids on plants are realized by its effects on root and shoot processes and increasing nutrients absorption (Lobartini et al., 1997). Furthermore, humic acid could enhance seedling growth of tomato and cucumber plant (Atiyeh et al., 2002). Some studies indicated that humic acids could be used as growth regulator such as gibberlic acid, to improve plant growth and enhance stress tolerance (Piccolo et al., 1992). Albayrak and Camas (2005) found that increasing application of humic acid up to 1200 (ml/ha) has significantly promoted root and leaf yield of forage turnip (*Brassica rape* L.), however, yield of forage turnip indicated descending trend beyond 1200 (ml/ha) application levels.

Shoot and root growth of corn plants also promoted by humic acid until 640 ppm (Tan and Nopamornbodi, 1979). Ayas and Gulser (2005) reported the increase of yield and nutrient content of spinach (*Spinacia oleracea*, var. spinoza) by application of humic acid at medium levels (250 g/m^2). They concluded that increased nitrogen uptake caused by humic acid application was the main reason of enhanced vegetation growth of spinach. Application of humic acid increased head weight of lettuce (*Lactuca sativa* L. var. longifolia) by increasing the availability of phosphorus and nitrogen (Cimrin and Yilmaz, 2005).

Timing of fertilizer application highly influences the yield and yield components of many crops (Loecke et al., 2004). This is in interaction with other management factors such as, irrigation, application of pesticides and harvesting times (Bush and Austin, 2001). There is no precise investigation of application time impacts of humic acid on different plants growth, however other fertilizers had direct influences on plants growth. Time of application of nitrogen had strong correlation with yield and yield component of corn (Binder et al., 2000) and wheat (Jan and Khan, 2000) and could have effect on leaching of nitrogen (Rozas et al., 2000). Medicinal plants essential oil production has directly influence with fertilization materials (Moradi et al., 2010). In addition, some reports shown syntactic fertilizers interrupted on essential oil production of medicinal plants by imbalance of essential macro elements absorption and concentration of these materials (Azizi and Omid-Beigi, 2001; Ameri and Nasiri, 2009). Therefore, using organic fertilizers improved quality and quantity of medicinal plants.

The main aims of this study were to investigate the impacts of different levels of humic acid concentration and application times on yield and essential oils of *Hyssopus officinalis* (L.).

MATERIALS AND METHODS

Study area

This investigation was performed in 2007 and 2008 growing seasons at the experiment station of the College of Agriculture, Ferdowsi University of Mashhad (latitude: 36°15' N, longitude: 59°28' Elevation: 999 m) in the central part of Khorasan province of Iran. Climatic conditions of Khorasan province varied between semi-dry and locally humid in the north to dry in the Southern parts. Annual precipitation rate in Khorasan province is 250 mm for Central parts (study location). Precipitation amount was 83 mm for first year (2008) and 122 mm for second year (2009) of study. The soil of the experimental field was silty loam with pH 7.5, contains total N (200 ppm), total P (9.4 ppm), and total K (120 ppm) with an EC of 0.11 dsm^{-1}.

Experimental design

This experiment was carried out by using a factorial based on randomized complete block design with three replications. Two times of foliar applications of humic acid (Humax, JH Biotech, Inc., Ventura, CA) in first cut (T1) (38 days after transplanting), and in both first and second cut (T2) (38 and 85 days after transplanting) were applied along with four humic acid concentrations (0, 100, 200 and 300 mg/l). All plots were fertilized uniformly with cow manure at 20 tha^{-1}, in autumn of each year. Cow manure contained total N (790 ppm), total P (97 ppm), and total K (241 ppm). Hyssop seeds were planted in nursery and transplanted in the field on 27 March of both study years. The size of each plot was 4 × 3 m. Irrigation schedule was performed in 7 days intervals immediately after planting. Weeds were controlled by hand when needed. Plants were cut in full flowering stage as first and second cut on 5 June and 16 September respectively in 2008 and on 22 May and 11 September respectively in 2009.

Plant material

Plant measurements in this study was in different yield factors, which includes aboveground biomass (g.m^{-2}), leaf biomass (g.m^{-2}), essential oil percentage (%) and essential oil yield (g.m^{-2}), aboveground biomass and leaf biomass measured by collecting plants materials from 0.5 m^{-2} of each plot. Collected materials were weighted after drying in a heater at 75°C for 72 h. Essential oils were extracted from dried aerial parts of the collected samples of each treatment by hydrodistillation for 3 h, using a Clevenger-type apparatus (British Pharmacopeia, 1998).

Statistical analysis

In order to evaluate the treatments impacts on study parameters, analysis of variance (ANOVA) was performed as standard procedure for factorial randomized block designs. The t-test was used to find significant differences among treatments. The significant differences between treatments were compared by Duncan's multiple range tests at 5% probability level.

RESULTS

Application times

Different application times of humic acid did not show any

Table 1. Effects of different application times and humic concentrations on aboveground biomass, oil production and oil percentage of *Hyssopus officinalis*.

Application time	Aboveground biomass ($g.m^{-2}$)		Total essential oil yield ($g.m^{-2}$)		Essential oil (%)		Leaf biomass ($g.m^{-2}$)	
	2008	2009	2008	2009	2008	2009	2008	2009
T_1	1768.90^{ns}	1885.50^{ns}	12.26^{ns}	11.79^{ns}	1.35^{ns}	1.36^{ns}	1176.00^{ns}	1224.20^{ns}
T_2	1977.80^{ns}	1946.20^{ns}	12.54^{ns}	13.38^{ns}	1.32^{ns}	1.32^{ns}	1216.40^{ns}	1203.20^{ns}
Humic acid concentration (ml/L)								
0	1439.8^{b}	1503.20^{b}	12.36^{b}	$9.79b^{c}$	1.10^{ns}	1.12^{ns}	952.40^{b}	959.80^{b}
100	1580.7^{b}	1532.30^{b}	8.03^{b}	8.83^{c}	1.39^{ns}	1.39^{ns}	1068.40^{b}	1045.10^{b}
200	1722.8^{b}	1822.80^{b}	10.97^{b}	12.70^{b}	1.45^{ns}	1.45^{ns}	1138.0^{b}	1158.50^{b}
300	2750.1^{a}	2805.10^{a}	18.24^{a}	19.04^{a}	1.39^{ns}	1.39^{ns}	1626.5^{a}	1691.50^{a}

First cut (T1), second cut (T2).

significant impact (P > 0.05) on aboveground and leaf biomass production in both years of the experiment. However, aboveground and leaf biomass of hyssop showed higher values in both years at two times (T2) applications (Table 1). Total essential oil yield of hyssop was not different in response to different application times in 2008 and 2009 (Table 1). The highest and lowest oil production obtained at two times application (13.38 $g.m^{-2}$) and at one time application (11.79 $g.m^{-2}$) in 2009. Essential oil percentage did not response to different application times in both years of experiment (Table 1). Generally, hyssop plants produced higher biomass and essential oil yield in the second year compared to the first year across all application times.

Humic acid concentration

The typical growth parameters such as biomass production especially by increased in plant growth hormones production of plants which treated by humic substances (Arancon et al., 2006). In this experiment, averaged across all humic acid concentration levels, both aboveground biomass and leaf weight of hyssop indicated significant difference (P < 0.05). Our results illustrated that the highest aboveground and leaf biomass production was obtained at highest humic acid concentration (300 ml/L) in both years of experiment (Table 1). Highest change in aboveground (+59%), leaf (+42%) biomass production and total essential oil yield (+66%) are obtained at highest concentration of humic acid application in 2008 (Figure 1). Similar results obtained on aboveground (+53%), leaf (+49%) biomass production and total essential oil yield (+46%) in 2009 (Figure 1). Hyssop aboveground and leaf biomass production were gradually increased by increasing humic acid concentrations (Table 1). Furthermore, hyssop aboveground biomass in first year of experiment is significantly more than second year of experiment (Table 1).

Different levels of humic acid concentration did not significantly influence essential oil percentage in both years of experiment (P > 0.05). On the other hand, there was statistically significant difference among various levels of humic acid concentrations effect on total essential oil yield in both years of experiment (Table 1). Higher total essential oil obtained at (300 ml/L) humic acid concentration in 2008 (18.24 $g.m^{-2}$) and in 2009 (19.04 $g.m^{-2}$). There was positive trend among humic acid concentration levels increase and essential oil production increase in 2009 (Table 1).

Interactive effects of application times and humic acid concentration

Aboveground and leaf biomass production

Increasing humic acid concentration from 0 to 300 ml/L induced highest interaction of the two treatments on aboveground and leaf biomass at two application times of hyssop in both years of experiment (Table 2). The lowest above ground biomass, 1330 $g.m^{-2}$, was obtained from control treatment (without humic acid application), and the highest, 3047 $g.m^{-2}$ from two application times (T2) together with highest humic acid concentration (300 ml/L) in 2008 (Table 2). Similar results obtained in second year of experiment

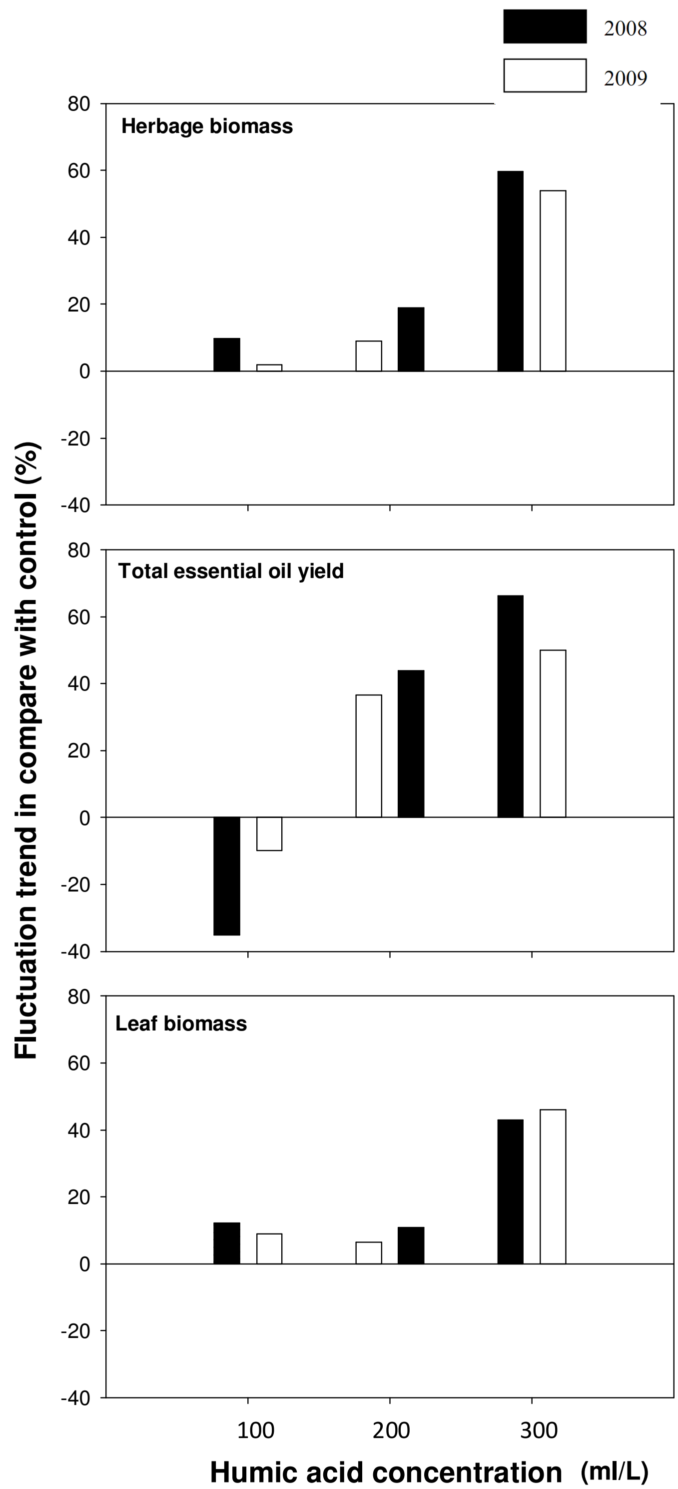

Figure 1. Percentage of aboveground biomass, leaf biomass and total essential oil change at different humic acid concentrations in compare with control in 2008 and 2009.

(1545 and 3217 g/m^{-2} as highest and lowest values respectively) (Table 2). Increasing the humic acid concentration from 0 to 300 ml/L generally resulted in higher production in both years compared to 100 and 200 ml/L (Table 2). Aboveground and leaf biomass production was lower in first year of study. It was found that the

Table 2. Interactive effects of application times and humic acid concentrations on essential oil percentage and essential oil yield in 2008 and 2009.

Application time	Humic acid concentration (ml/L)																				
	Aboveground biomass (g.m^{-2})				Ratio			Total essential oil yield (g.m^{-2})				Ratio			Leaf biomass (g.m^{-2})				Ratio		
2008	**0 (C1)**	**100 (C2)**	**200 (C3)**	**300 (C4)**	**C2/C1**	**C3/C1**	**C4/C1**	**0 (C1)**	**100 (C2)**	**200 (C3)**	**300 (C4)**	**C2/C1**	**C3/C1**	**C4/C1**	**0(C1)**	**100 (C2)**	**200 (C3)**	**300 (C4)**	**C2/C 1**	**C3/C 1**	**C4/C 1**
T1	1330	1582	1709	2452	1.18	1.28	1.84	11	10	12	14	0.85	1.04	1.27	896	1154	1115	1538	1.28	0.96	1.37
T2	1548	1578	1735	3047	1.01	1.12	1.96	12	6	9	21	0.46	0.74	1.65	1008	982	1160	1714	0.97	1.15	1.70
2009	**0 (C1)**	**100 (C2)**	**200 (C3)**	**300 (C4)**	**C2/C1**	**C3/C1**	**C4/C1**	**0 (C1)**	**100 (C2)**	**200 (C3)**	**300 (C4)**	**C2/C1**	**C3/C1**	**C4/C1**	**0 (C1)**	**100 (C2)**	**200 (C3)**	**300 (C4)**	**C2/C 1**	**C3/C 1**	**C4/C 1**
T1	1545	1694	1909	2392	1.09	1.23	1.54	9	9	12	15	1.73	1.33	1.73	896	1154	1115	1538	1.28	1.24	1.71
T2	1460	1370	1735	3217	0.93	1.18	2.2	10	7	13	22	0.77	1.26	2.13	1008	982	1160	1714	0.97	1.15	1.70

In first cut (T1) and in both cuts (T2).

effects of different humic acid concentrations were more than application times, on aboveground and leaf biomass in both years of experiment (Table 2). However, higher values of leaf biomass obtained at 300 ml/L humic acid concentration for all application times in 2008 and 2009.

Total essential oil yield

Increasing humic acid concentration from 0 to 100 ml/L resulted in lower production of total essential oil yield in both years compared with 0 to 200 ml/L (Table 2). Upturning humic acid concentration from 0 to 300 ml/L resulted in higher production of total essential oil yield at two times application in both years of the experiment (Table 2). Highest total essential oil obtained at two application times (prior to both cuts) together with highest humic acid concentration (300 ml/L) (21 g.m^{-2}) in 2008 (Table 2). Similar results obtained in 2009 (22 g.m^{-2}) (Table 2).

DISCUSSION

Humic acid could be used as organic fertilizer and growth regulator to adjusted hormone level, for plant growth improvement and enhance stress tolerance along with increasing shoot and root growth (Nardi et al., 2002). Generally, application times of humic acid did not show a significant influence except on total essential oil yield in 2009. However, two application times showed higher values compared to one time application (Table 2). Humic acid concentration represented direct impacts on aboveground and leaf biomass and total essential oil yield but different levels of humic acid showed no effects on essential oil percentage. Highest humic acid concentration (300 ml/L) illustrated maximum values of above ground and leaf biomass and total essential oil yield in both years of experiment. Some studies have reported the ability of humic substances to increase shoot growth in different plant species cultivated under diverse growth conditions. However, the mechanism responsible for this effect of humic substances is poorly understood (Brewitz et al., 1995). On the other hand, Mora et al. (2010) reported increased of root nitrate reductase activity acid was main reason of improvement of shoot growth of plants in high concentration of humic. It seems that essential oil percentage are controlled by some other factors. Humic acid applied to the plant growth medium at 1000 mg kg^{-1} concentration increased seedling growth and nutrient contents of plants. Humic acid not only increased macro-nutrient contents, but also enhanced micro-nutrient contents of plant organs (Turkmen et al., 2004).

Conclusion

In conclusion, interaction between humic acid concentration and application times showed that two times application and highest humic acid concentration (300 ml/L) provided the highest values in studied plant parameters in both years of experiment. It seems that increase in

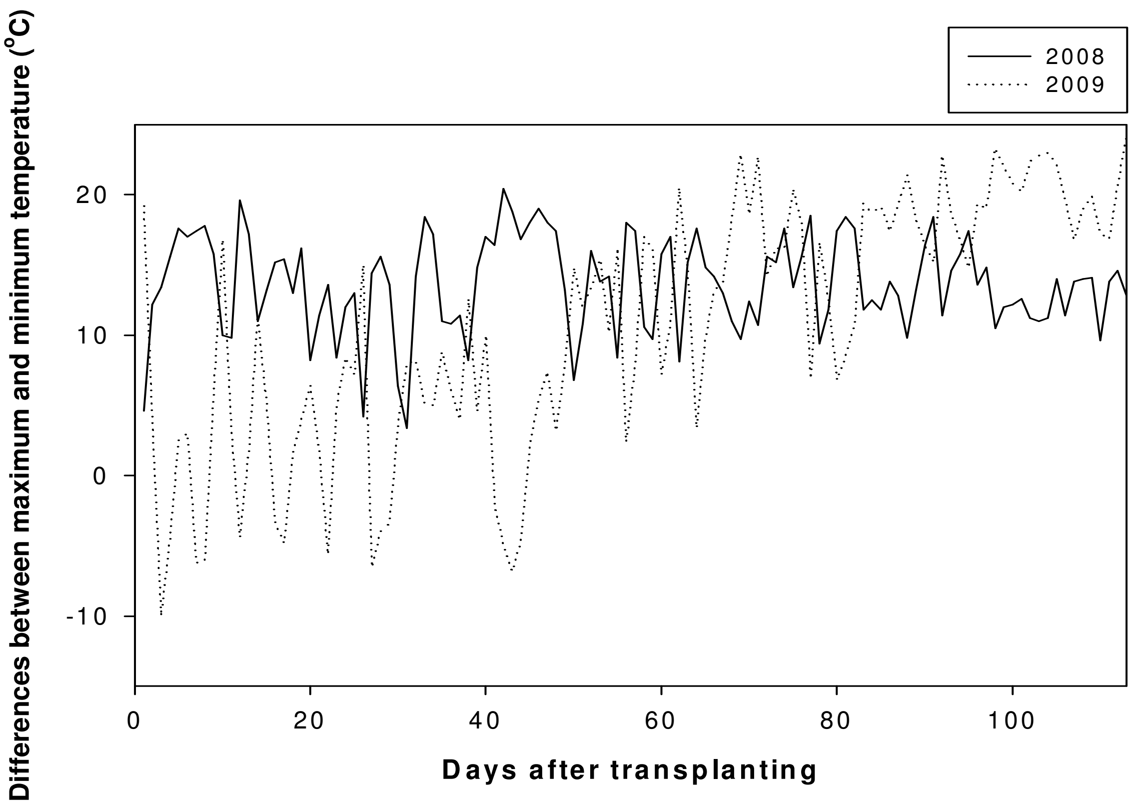

Figure 2. Differences between maximum and minimum temperatures during growth period of *Hyssopus officinalis* in 2008 and 2009.

aboveground and leaf biomass and total essential oil yield on these treatment increase nitrate uptake rate and promoted vegetative growth of hyssop in this experiment (Mora et al., 2010). The weather conditions of two years of the experiment were different than each other, especially in maximum and minimum temperature and rainfall during growth period (Figure 2). It seems that lower yield of hyssop in the first year of experiment related to higher maximum temperature and high difference between maximum and minimum temperature in first year of experiment compared to the second year (Figure 2). Higher temperature especially more than 30°C may have negative impacts on leaf production which contains more essential oil than other parts of plants in first year of experiment.

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