Nitrogen and Phosphorus Fertilizers Affect Flavonoids Contents of St. John's Wort (*Hypericum perforatum L.)*

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Abstract
Saint John's wort (*Hypericum perforatum* L.) is a valuable medicinal plant that has been used since ancient time due to producing a wide range of secondary metobolites with significant pharmaceutical effects such as wound healing and antidepressant properties. There are 60 herbal drugs that originate from the plants. In Iran there are 3 herbal drugs that contained St. John's wort. It contains several important secondary metabolites such as naphthodianthrones (Hypericin and Pseudohypericin), phloroglucionols (Hyperforin and Adhyperforin), flavonoids (chlorogenic and isochlorogenic acid, apigenin, biapigenin, rutin, quercetin, isoquercetin, amentoflavon) and essential oils. In this research we conducted the field trial in two successive years for studying the effects of three levels of nitrogen (zero, 75, 125 KgN/ha) and three levels of phosphorus fertilizers (zero, 50 and 100 KgP_2O_5/ha) on flavonoids content of the plants by HPLC-DAD method. Statistical design was RCBD with three replicates. We analyzed chlorogenic and isochlorogenic acid , apigenin , biapigenin , rutin , quercetin , isoquercetin and amentoflavon of the samples. Our results show that nutrition of *Hypericum perforatum* with the fertilizers can improved drug quality by changing of flowering habits and flavonoids content of the plant in comparison with control treatment. Optimum fertilizer treatment for production of high dry herb yield and flavonoids content was 125 Kg/ha nitrogen and 50 Kg P_2O_5/ha phosphorus fertilizer.

Key Words index : Fertilizer, Flavonoids,HPLC-DAD, St.John's wort

Introduction

St. John's wort (*Hypericum perforatum* L.*)* is considered an important source of pharmaceutical and dietary supplements. It occurs naturally in the Northern of Iran, Asia Minor, Europe and Northern Africa (Bombardelli and Morazzoni,1995; Cellarova et al 1995). It has pharmaceutical effects such as wound healing and antidepressant properties(Chatterjee et al 1998;ESCOPE,1996). The most important secondary metabolites in *Hypericum perforatum* are naphthodianthrones (hypericin and pseudohypericin), acylphloroglucinols (hyperforin and adhyperforin), essential oils and flavonoids (American Herbal Pharmacopoeia, 1997; Anoun, 1991; Erdelmeier et al, 1998; Maisenbacher and Kovar,1992). Culture extension of medicinal plants need to optimizing cultural practices such as nutrition, irrigation, harvest time and etc (Azizi,2001;Mathe,1988). The formation of typical secondary metabolites as well as physiological and biochemical processes depend on the actual environmental conditions. Other than genetic and ontogenetic factors, the environmental factors take only quantitatively modifying effect. The edaphic factors, among the environmental factors, are of specific importance with regards to plant production (Franz,ch,1983). Bernath(1986) also illustrated that relationship between the nutrient elements and metabolic processes leading to the formation of special products in medicinal plants is certainly important. In this research we studied the effect of nitrogen and phosphorus fertilizers on the flavonoids content of *Hypericum perforatum* L.

MATERIAL AND METHODS

Field experiment and biological material. The research was conducted in the Agricultural Research Station of Tarbiat Modares University (North of Tehran) during 2000-2002. Seeds of "Topas" cultivar of St. John's wort were washed overnight in tap water and air-dried. They were sown in outdoor bed and irrigated regularly. Seedlings were transplanted to the field when they were 25 centimeter length . The statistical design used in the research was randomized complete block design(RCBD) with nine treatments and four replicates. Fertilizers treatments included three levels of nitrogen (zero, 75, 125 Kg N/ha) and three levels of phosphorus fertilizers (zero, 50 and 100 Kg P_2O_5/ha). Each plot was 160×125 centimeter and seedlings were planted in 40×25 centimeter distances. In the next year the flowering plants were harvested (top 10 centimeter) and dried in dark condition at 30 ± 5 degree centigrade for 3-5 days.

HPLC-DAD analysis. Extracts were prepared from dried biomass (0.2-0.3 g) by sonication at room temperature with 10 ml of a methanol-water solution (80:20). Solutions were filtered through a 0.2-µm filter and were analyzed by HPLC-DAD as described elsewhere (Dias et al. 1998, 1999). The secondary metabolites quantification were done by the external standard method using a standard solution. Identified compound with their respective retention time and selected elution gradient time shown in Table 1 and 2 respectively.

Results and Discussion

Our results presented in Table 2 and 3. As shown in Table 2, nitrogen and phosphorus treatments had a significant effect (α=0.01) on number of stem/plants in successive years. The highest number of stem/plant in the both year (16.55 and 18.13 respectively) belong to N125P100 treatment and the lowest one (8.89 and 10.35 respectively) belong to check treatment (Table2). Nitrogen and phosphorus treatments also had a significant effect on dry herb yield. Our results shown that the lowest dry herb yield produced in control plot in both years (745.883 and 683.46 g/m^2 respectively). The highest dry herb yield in the first year (984.567 g/m^2) produced in plots received medium and high level of nitrogen. The lowest dry herb yield produced in check treatments in both year (745.883 and 683.46 g/m^2 respectively)(Table2). Fertilizer treatments also affect biochemical properties of *Hypericum perforatum*. Chlorogenic acid content significantly affected by nitrogen fertilizers(Table 3). The highest content of chlorogenic acid produced in N0P100 and the lowest one belong to N125P100. Nitrogen application decreased the chlorogenic acid content. The higher the nitrogen concentration the lower the chlorogenic acid content. Analysis of variance of data showed that NP supply had not significant effect on isochlorogenic acid content. Such as the chlorogenic acid, the highest isochlorogenic acid content detected in N0P100 treatment and the lowest one in control plots(4899.644 and 3708.073611µg/gD.W. respectively). Quercetin analysis of the samples did not show significant differences between the treatments (Table3). Our data about the rutin content suggest that the lowest rutin content (10556.56611µg/gD.W.) produced in N0P50 and the highest content(52809.13611µg/gD.W.) in N75P50. Biapigenin analysis of treated Hypericum perforatum shown significant effect of NP supply, as phosphorus fertilizers decreased biapigenin but nitrogen fertilizers increased it. ANOVA of the results shown an interaction between nitrogen and phosphorus fertilizers in biapigenin production. The highest biapigenin content (408.611µg/gD.W.) belong to N125P0 and the lowest content (162.383 µg/gD.W.) belong to N75P0. There is not significant differences between amentoflavon content of samples (exception N125P50 that shown higher amentoflavon compare to control(Table3). Sulisbury and Ross(1992) presented that nitrogen fertilizers increased plant sensitivity to pathogen by increasing chlorogenic acid content . Davis and et al (1988) shown that chlorogenic acid has an important role in adventitious root formation in cutting. Omidbaigi and Azizi(2000) shown that other than environmental factors , harvest time has an important effects on biochemical properties of the plants. They shown that full flowering time is the best harvest time for St.John's wort production. In conclusion , phytochemical properties of medicinal plants affected by several factors such as cultural practices, environmental condition, genetic properties and developmental stage. Martonfi and Repcak(1994) and Repcak and Martonfi(1997) shown that flowers parts are the main organs that produced active principles in Hypericum perforatum therefore each treatments that changed the ratio of flower/herb affected active substances properties of St.John's wort. Chemical and physical properties of soil affect directly or indirectly active substances content of medicinal plants(Bernath,1986;Franz,1983). In this research NP supply affect flavonoids content of St.John's wort indirectly by changing of the flower/herb ratio. Our results comparision with other research (Umek et al, 1999) shown that active substances of cultivated *Hypericum perforatum* in Iran are in a good situation suitable for production herbal drugs.

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Table 1- Compound identified in the methanolic extracrts of *Hypericum perforatum* L. biomass with their respective retention time

Compound	Retention time(minute)
Isochlorogenic acid	3.13
Chlorogenic acid	4.19
Biapigenine	63.88
Rutin	11.74
Quercetin	20.85
amentoflavon	31.21

Table2-Effects of nitrogen and phosphorus fertilizers on yield parameters in *Hypericum perforatum*

Treatments	No of Flowering stem/plant		Dry herb yield (g/m^2)	
	First year	Second year	First year	Second year
N0P0	8.89 d	10.53 e	745.883 c	683.46 c
N0P50	13.44 bc	11.23 e	829.817 bc	832.92 bc
N0P100	11.64 c	14.13 d	789.25 c	751.26 bc
N75P0	13.8 bc	16.05 bc	984.567 a	872.82 bc
N75P50	14.25 ab	17.5 ab	832.133 bc	1105.45 ab
N75P100	15.46 ab	14.76 cd	961.483 a	865.44 bc
N125P0	13.19 bc	15.1 cd	932.883 a	1020.06 ab
N125P50	15.13 ab	17.43 ab	894.133 ab	1155.34 a
N125P100	16.55 a	18.13 a	964.85 a	917.86 abc

Means in each column compared using Duncan’s multiple range test (0.05)

Table3-Effects of nitrogen and phosphorus fertilizers on flavonoids content of Hypericum perforatum

Treatments	Flavonoids contents(Microgram/g.dry weight basis)					
	Chlorogenic acid	Isochlorogenic acid	Rutin	Quercetin	Amentoflavon	Biapigenine
N0P0	1516.516ab	3708.073b	11239.81b	2259.037a	25.9970b	372.907ab
N0P50	1528.743ab	4164.929ab	10556.56b	2266.935a	24.0636b	294.381abc
N0P100	***1583.742a***	***4899.644a***	12259.67b	1969.244a	13.974b	167.616c
N75P0	1203.287bc	4054.709b	9716.715b	1552.837a	18.6700b	162.383c
N75P50	1401.248abc	3979.56b	***52809.13a***	1255.928a	12.4287b	203.015bc
N75P100	1166.602c	4173.752ab	10819.8b	1570.459a	11.8520b	188.500bc
N125P0	1361.381abc	4445.472ab	11029.46b	1345.891a	15.9895b	***408.611a***
N125P50	1247.542bc	3983.881b	12154.84b	2168.608a	***29.6191a***	302.151abc
N125P100	1156.402c	3808.619b	11186.56b	2066.704a	14.7941b	215.712bc