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DETERMINATION OF THE BEST HERBAGE YIELD AND HYPERICIN CONTENT OF ST. JOHN'S WORT (*Hypericum perforatum* L.) UNDER SEMI ARID CLIMATIC CONDITIONS

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ABSTRACT

St. John's Wort (*Hypericum perforatum* L.) has been used as a medicinal herb since ancient times, it contains several natural products with noteworthy biological activities. There is no clear information about harvesting time yield and yield components of St. John's Wort as the plants are collected from wild. Therefore, this research aimed to determine ontogenetic (pre-flowering, full flowering and post-flowering periods) and morphogenetic (bottom, middle and top parts) variations in herb yield and hypericin content of St. John's Wort under Diyarbakır ecological conditions during the 2004-05, 2005-06 and 2006-07 growing seasons. Fresh and dry herb yield, dry leaf yield and hypericin content were recorded. Ontogenetic x morphogenetic interaction resulted in statistically significant effects on yield characteristics and hypericin contents. The plant was not harvested during the seedling year; whereas fresh herb yields in second and third year ranged 2721 to 5607 kg ha^{-1} and 2196 to 3955 kg ha^{-1} respectively; while dry leaf yield in the second year varied ranged 323 to 1555 kg ha^{-1} and in the third year 161 to 928 kg ha^{-1}, hypericin content in the second and third year varied between 0.022 to 0.093% and 0.018 to 0.065% depending on parts of the plant. Hypericin content varied according to different parts of the plant, and the maximum value of 0.093% was obtained from the top part of the plants at the full flowering period. The results showed that there is a relationship between dry leaf yield and hypericin content of the plant parts and development stages of the plant.

Keywords: St. John's Wort; development stages, plant parts, dry herbage yield, hypericin

INTRODUCTION

Hypericum genus (belonging to Guttiferae family) is distributed in the tropical and subtropical regions of Africa, South America, Asia and Europe, and it is also found in Turkey. It is represented by 46 genus and 1000 species in world. There are 80 species showing the natural distribution in Turkey (Leung and Foster, 2003; Potoglu and Tokur, 2004).

St. John's Wort (*Hypericum perforatum* L.) was used in the treatment of wounds, women' diseases, hemorrhoid and kidney stones all over the world for two thousand years (Shapiro, 1998). Nowadays, the use of St. John's Wort as anti-depressant is fairly common, several galenic preparations and pharmaceutical products prepared from this plant exceed $570 million worth sales value worldwide (Cirak et al., 2007). In addition, its usage as tea is also popular to calm nervous, depression, insomnia, relaxing, expectorant, pain relieve, digestive, worm reducer and wound healing. Also, it is used in increasing urine and therapy of gastritis. When flowers and plant oils are mixed together, it is used for treatment of hemorrhoids and skin-redness relief (Baytop, 1984; Patocka, 2003; Leung and Foster, 2003; Tolonen, 2003; Spinelle, 2004). All over the world, demand of St. John's Wort is met by its cultivation or collection from the nature. Although St. John's Wort contains flavonoids, phenolic acids, essential oils, high activity routine, pectin, choline, sitosterol compounds as well as vitamin A and C, the main components of active principles remain hypericin, 1,3,6,7-tetrahyroxy-xanthone and hyperforin (Ebadi, 2002; Schwob et al., 2002; Poutaraud and Girardin, 2004). Hypericin, also known as red pigment, is best known component of the plant, its content varies between 0.02 – 2.5% (Burdock, 1997). These plant components could be used for treatment of anti-retroviral and AIDS against HIV without any known side effects (Leung and Foster, 2003).

The formation and accumulation of active components in medicinal plants is influenced by environmental factors

(Franz, 1983); which play an important role on yield, yield continuity and composition of the active ingredients. Ecological factors and cultivation techniques are very important factors on cultivation of medicinal plants. Successful cultivation of the medicinal plants depends on determination of appropriate harvest time (Chatterjee, 2002), as well as on the genetic and environmental factors resulting in variation of hypericin productions vary in St. John's Wort depending on plant parts, age and development stages (Buter et al., 1998; Poutaraud and Girardin, 2005). Differences in active ingredient contents of herb parts of St. John's Wort are important in determination of the appropriate harvest time. Moreover, as plant part contain different rates of secondary metabolites and herbage yield, cutting height also affects the quality of harvested materials. Therefore, this study aimed to determine herbage yield and hypericin content in different parts (morphogenetic variation) of St. John's Wort at different growing stages (ontogenetic variation) and different parts of the plant at Diyarbakir ecological conditions.

MATERIALS AND METHODS

The study was carried out at the experimental station of Faculty of Agriculture at Dicle University for three growing seasons (2005, 2006, 2007) Diyarbakir province located at a 37˚53' N latitude, 40˚16' E longitude and 680 m above sea level. St. John's Wort seeds were provided by Field Crops Department, Faculty of Agriculture at Ege University.

Soils of the experimental location contained low organic matter (1.77%) and phosphorus (33 kg P_2O_5 ha^{-1}), pH of 7.7 and the texture was clay. Meteorological data for the growing seasons are shown in Table 1. According to the long-term meteorological record, between January and June period, the mean temperature and total precipitation were 12.5°C and 327.5 mm, respectively. The average temperature values of 2005, 2006 and 2007 for the period of January-June were 16°C, 16°C and 10.8°C, respectively, relative humilities were 44.8, 53.8 and 74.6%, respectively. The amounts of total precipitations were 408.2 mm, 592.5 mm and 333 mm, respectively.

Table 1. Means temperature, humidity and precipitation values at the study area for long term and 2005, 2006 and 2007 years.

Year/Month		J	F	M	A	M	J	JL	A	S	O	N	D	Mean
Temp. (ºC)	Long	1.6	3.5	8.2	13.7	19.7	29.1	31.0	30.3	24.8	17.1	9.5	4.0	16.0
	2005	2.3	3.0	8.4	14.1	19.6	25.8	32.4	0.0	25.0	16.2	7.5	5.3	16.0
	2006	0.4	4.3	9.2	14.5	19.4	28.5	31.4	32.6	25.0	17.6	7.8	0.7	16.0
	2007	-5.4	3.0	8.8	10.3	20.6	27.2	-	-	-	-	-	-	10.8
Hum. (%)	Long	76	71	65	63	55	35	26	26	31	48	66	75	50.8
	2005	66	62	53	52	44	25	11	20	31	40	60	73	44.8
	2006	77	71	62	69	53	23	25	16	36	71	73	69	53.8
	2007	89	79	73	79	76	52	-	-	-	-	-	-	74.6
Total Precipit. (mm)	Long	73.1	68.5	65.9	70.2	42.0	7.8	0.7	0.5	2.6	30.9	54.3	70.2	470.2
	2005	58.7	46.8	58.4	36.8	26.5	33.1	0.0	0.0	0.7	14.9	38.0	94.3	408.2
	2006	121.3	121.0	26.6	77.9	38.4	0.0	6.1	0.0	3.5	104.5	67.3	25.9	592.5
	2007	44.5	79.8	55.5	88.2	45.5	19.5	-	-	-	-	-	-	333.0

Source: State Meteorology Institute (Diyarbakir, Turkey).

St. John's Wort plant seeds were sown on seedbed tubes containing soil, sand and burnt farmyard manure (1:1:1) mixture, and placed in a greenhouse on 05 January 2005. On a regular basis, maintenance works such as weed control and irrigation were applied. When the seedlings reached to 10-15 cm plant height, they were transferred to the trial on April, 18, 2005. Each plot consisted of four rows (45 x 20 cm) covering 5.4 m^2. The plots were arranged in a randomized complete block design with three replications. The plants were not harvested during first year because of weak and slow growth. The plants were harvested at *pre flowering*, *flowering* and *post flowering stages* (Table 2). Harvested area in the each plot was 1.35 m^2. In the field experiment against to summer drought, plants were irrigated using sprinkling for three times during each growing season in July, August and September. No fertilizer application was applied during field trials.

Table 2. Harvest dates of St. John's Wort in two years.

Development Stages	2006	2007
Pre flowering	12 May	25 May
Full Flowering	25 June	01 June
Post flowering	06 June	18 June

After harvest, whole plants were equally divided into three parts, as bottom, middle and top (leaves + flowers) to determine the morphogenetic variability of different growth stages and then weighted for fresh herbage yield. The plant materials were dried at 35 °C for 48 hour in the drying cabinet until they attained constant weight. Dry herbage yield was determined. In the study, plant height, fresh and dry herb yield, dry leaf yield and hypericin content of the separated parts of the plant were measured for each three harvest periods.

Hypericin Analysis

DAC (1986) method was used to determine the total hypericin content of the separated plant parts. Hypericin content was determined using the following equation.

$C = E590 \times (£ \times e)^{-1} \times 50$

Where, E590 is the value read at 590 nm in spectrophotometer; £, 718 extraction coefficient; e, amount of sample (g); C, percentage of hypericin (%).

Statisticsal Analysis

The data were analyzed by the computer MSTAT-C package program. The data was analyzed using split-plot design: main plot was the development stages, sub-plot was the plant parts. Significant differences among the mean values are given separately for each year of the trial (Table 3). Results of plant height were also analyzed according to the Randomized Complete Block Design.

Table 3. Results of analysis of variance and F values of the investigated characteristics.

Source of Variance	Fresh herbage yield (kg ha^{-1})		Dry herbage yield (kg ha^{-1}		Dry leaf yield (kg ha^{-1})		Hypericin content (%)	
	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07
Develop. stage	2.09ns	0.54ns	3.00ns	0.09ns	2.92ns	0.18ns	34.19**	0.92ns
Plant parts	3.18ns	1.00ns	2.76ns	0.92ns	78.16**	29.52**	26.31**	34.03**
Dev. stage x Plant parts	44.72**	5.64**	41.14**	2.19ns	23.33**	4.01*	4.46*	2.17ns

* indicates the significance at 0.05 probability level
** indicates the significance at 0.01 probability level
ns indicates the non significance

RESULTS AND DISCUSSION

Plant growth is slow with few flowers, branches and low yield during first year (Pluhar et al., 2002; Kacar and Azkan, 2007). St. John's Wort that is 3-5 years perennial plant is intensively grown for two-three years, such that most productive period is the second year of growth (Pluhar et al., 2002). Therefore, the secondary metabolite of the first year is low (Buter et al., 1998). Accordingly, the data for second and third year of field experiment were evaluated that included plant height, fresh & dry herbage yield, dry leaf yield and hypericin content (Tables 3, 4, 5 and 6).

Plant Height

The differences between the mean values of plant height were statistically significant in terms of developmental stages (Table 3). The longest plants were obtained at full flowering (78.7 cm) and post flowering period (76.8 cm) during second year, and also the maximum value was obtained at full flowering period as 77.9 cm in the third year (Table 4). Plant height was similar to the findings of Ceylan et al. (2002) who indicated that plant height varies between 45 and 49 cm at flowering stage during second year.

Table 4. The means of plant height obtained in different development stages in the trial years.

Development Stages	Plant height (cm)	
	2005-06	2006-07
Pre flowering	69.6 b	74.3 ab
Full Flowering	78.7 a*	77.9 a
Post flowering	76.8 a	75.9 ab
LSD (5 % Develop. stages)	6.34	

*Means followed by the same letter are not significantly different from each at 5% probability level ($p < 0.05$).

Fresh and Dry Herbage Yields

During 2005-06 growing season, the maximum fresh herbage yield (5607 kg ha^{-1}) was obtained from the top part of the plant after the flowering period, but in the 2006-07 growing season fresh herbage yield of bottom parts of plants pre flowering and flowering stages were recorded as 3892 and 3955 kg ha^{-1}, respectively (Table 5). The harvest time x plant part interaction was statistically significant ($p<0.01$) (Table 3). Among the three plant parts, fresh herb yields during the flowering period in both trial years were not significantly different. Amount of rainfall in the second year was higher than third year (Table 1), for this reasons fresh herbage yields showed differences between the second and third years. When mean values of the plant parts are considered during second harvest year, there were not statistically significant differences, but, fresh herbage yield values obtained from top part of plant (4465 kg ha^{-1}) was higher compared to the bottom part of the plants (4156 kg ha^{-1}). In the third year, fresh herbage yield values of the bottom and top parts of the plant were higher compared to the middle part's. The bottom leaves close to soil turned to yellow and dropped. Accordingly fresh herbage yield of top part of plants that increased with the development stages in both trial years. Fresh herb yield was between those reported by Bayram et al. (2002) (2540–15030 kg ha^{-1}), and was consistent with top herbage yield values (734-4690 kg ha^{-1}).

In the first year, the maximum dry herbage yield values were obtained from the top part of the plant at post flowering stage (2136 kg ha^{-1}), full flowering period in bottom (2112 kg ha^{-1}) and middle parts of plants (2007 kg ha^{-1}). The lowest value was obtained from top part of the plant during pre flowering stage (Table 5). Effect of harvest period x plant parts interaction on dry herbage yield mean had statistically significant effect during 2005-06 growing season, it was not significant during 2006-07

growing season (Table 3). Dry herbage yields of St. John's Wort varied according to the development stage, part of plants and plant age. In the second year, the values were higher compared to third year. St. John's Wort is a perennial plant, usually 3-5 years old, according to cultivation practices, intensive farming is done for two-three years, and most productive period is found to be the second growing season (Pluhar et al., 2002). Similarly, Osinska and Werlarz (2002) reported that yield of herb of *Hypericum* species was higher in the second year of vegetation.

Table 5. The means of fresh, dry herbage and leaf yield obtained from bottom, middle and top parts of St. John's Wort at different development stages in the trial years.

	2005-06			2006-07		
Development Stage	**Bottom**	**Middle**	**Top**	**Bottom**	**Middle**	**Top**
			Fresh herbage yield (kg ha^{-1})			
Pre-flowering	4138 c	4197 c	2721 e	3892 a	3433 abc	2874 bcd
Full-flowering	5284 ab	4980 b	5066 b	3955 a	3233 abc	3585 ab
Post-flowering	3047 de	3313 d	5607 a	2196 d	2585 cd	3711 ab
Mean	4156	4163	4465	3347	3083	3390
LSD (5%)		528 (int.)			884 (int.)	
			Dry herbage yield (kg ha^{-1})			
Pre-flowering	1473 c	1461 c	863 d	1417	1205	861
Full-flowering	2112 a	2007 a	1730 b	1346	1304	1242
Post-flowering	1471 c	1511 c	2136 a	1168	1213	1384
Mean	1685	1660	1576	1310	1241	1162
LSD (5%)		183 (int.)			ns	
			Dry leaf yield (kg ha^{-1})			
Pre-flowering	488 ef	809 d	653 de	416 b	590 b	596 b
Full-flowering	668de	1006 c	1205ab	387 bc	587 b	852 a
Post-flowering	323 f	697 d	1555 a	161 c	557 b	928 a
Mean	493	837	1138	655	578	792
LSD (5%)		195 (int.)			232 (int.)	

*Means followed by the same letter are not significantly different from each at 5% probability level ($p< 0.05$).

Dry Leaf Yield

In the second and third harvesting years, the highest dry leaf yields were obtained during post flowering stage at the top part of plant as 1555 kg ha^{-1} and 928 kg ha^{-1}, respectively (Table 5). The lowest values were detected at the bottom part of the plants as 323 kg ha^{-1} in the second year and 161 kg ha^{-1} in the third harvest year. In general, the highest dry leaf yields were obtained from top part of plant during the flowering and post flowering stages. In regard to the both developmental stages there was no statistical difference among mean values. Effects of plant parts and harvest period x plant parts interaction on dry leaf yield in both years were statistically significant (Table 3). These results could not be compared with the previous literature due to lack of literature about dry leaf yield.

Hypericin Content

During second growing season, the highest mean value of hypericin content (0.093%) was obtained from top part of the plants during full flowering stage (Table 6). The lowest one (0.022%) was obtained from bottom part of plant during pre flowering stage. During 2006-07 growing season, in regard to mean value of hypericin, although it was not statistically significant difference; the highest value was obtained from top part of plant during full flowering period (0.065 %). With the highest hypericin content at top part of the plants, the lowest one in bottom part of the plants were understood in St. John's Wort plant morphogenetic variation is present (Table 6). In 2006-07 growing season the highest mean content of hypericin by 0.056 % was obtained from top part of the plants and the lowest value as 0.023% was obtained from bottom part of the plants. Effects of plant parts in both years, developmental stage and harvest period x plant parts interaction of second year on hypericin content were statistically significant (Table 3). During 2005-06 growing season, effect of development stages and morphogenetic on hypericin content was statistically significant. Hypericin content in second year was higher compared to the third year results. In general, hypericin content of plants at all development stages was increased from bottom part to upper part.

Comparing Hypericin content of St. John's Wort, it was high at time of flower opening (0.50%) and lower when the flowers were fully open (0.33%). Therefore, selection of the appropriate harvest time is very important (Buter and Buter, 2002; Poutaraud and Girardin, 2004). These results showed that there is a close relationship between chemical content of the plant parts and the development stages. For medicinal purposes, St. John's Wort should be harvested during flowering period due to

the highest content of hypericin contents at that level of growth. The petal margins of flowers and leaves of *H. perforatum* L. are characterized by the presence of dark-colored glades, that are the accumulation sites of hypericin (Raina et al, 2005). Banyai et al. (2002) reported that the hypericin content of generative part of the plant (0.152-1.19 %) was higher compared to the vegetative parts (0.06-0.39%). Similar results were reported by Bayram et al. (2002) and Kacar and Azkan (2005; 2007) who obtained high proportion of hypericin (0.124-0.223%) from top drug parts of St. John's Wort during beginning of the flowering and full flowering periods.

Table 6. The means of hypericin content obtained from bottom, middle and top parts of St. John's Wort at different development stages in the trial years.

	Hypericin content (%)					
	2005-06			2006-07		
Development Stage	**Bottom**	**Middle**	**Top**	**Bottom**	**Middle**	**Top**
Pre-flowering	0.022 d	0.031 cd	0.036 cd	0.018	0.020	0.061
Full-flowering	0.036 cd	0.044 c	0.093 a	0.024	0.024	0.065
Post-flowering	0.040 c	0.041 c	0.071 b	0.028	0.022	0.042
Mean	0.033	0.039	0.066	0.023	0.022	0.056
LSD (5%)	0.018 (int)			ns		

*Means followed by the same letter are not significantly different from each at 5% probability level ($p < 0.05$).

According to Pluhar et al. (2002), the highest rate of hypericin is obtained in second growing season. In another study, it was reported that hypericin rate may vary by genotype, growing conditions, plant growth period at harvesting time (Buter and Buter, 2002). German Codex (DAC) indicates that the content of hypericin of *H. perforatum* L. should be between 0.05-0.3%. It was reported that hypericin rates varies between origins; in European origin 0.05-0.3% (Holzl and Ostrowski, 1987) in American origin plants 0.04-0.19% (Walker et al., 2001) plants originated in Australia, 0.004-0.215% (Southwell and Campbell, 1991) in Turkey, 0.205% (Meral, 2000), 0.132-0.308 % (Cakmak and Bayram, 2003) and 0.164-0.203 % (Kacar and Azkan, 2007). Our results for hypericin contents ranged 0.018 to 0.093% depending on the development stages, part of plants and plant ages, they are in agreement with Holzl and Ostrowski (1987); Southwell and Campbell, (1991) and Walker et al., (2001) and are lower than that reported by Meral, (2000); Cakmak and Bayram, (2003); Leung and Foster, (2003); Kacar and Azkan, (2005); and Kacar and Azkan, (2007). As mentioned above, it was concluded that hypericin content was variable and influenced by environmental factors.

CONCLUSION

In the developing countries, the demand for natural products is increasing. In continuation to its use in the past, the method reports its variations among different plant parts such that the maximum fresh & dry herbage and dry leaf yield were obtained from top part of the plant. Yields were affected by development stages and growing years. Furthermore, the different developmental stages were effective on herbage yield and hypericin content. But, St. John's Wort plant showed morphogenetic variability showed the highest hypericin content from top part of the plant. Additionally, agronomic studies for Diyarbakir ecological conditions on St. John's Wort were determine the high herbage yield and hypericin content lines. Moreover, previous studies indicate that the maximum rate of hypericin reached at full flowering stage followd by rapid decrease, usually the flowering period of plant takes 2-3 weeks (Poutaraud and Girardin, 2005). In conclusion, this study was helpful to investigate and determine the best harvest time and the post harvest drying processes in understanding the effects of semi arid climatic conditions on high quality herbage yield of St. John's Wort.

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