DOI: 10.1515/cerce-2016-0004
Available online: www.uaiasi.ro/CERCET_AGROMOLD/
Print ISSN 0379-5837; Electronic ISSN 2067-1865

Cercetări Agronomice în Moldova
Vol. XLIX , No. 1 (165) / 2016: 41-50

EFFECTS OF SOWING DATE ON SOME AGRONOMIC CHARACTERISTICS AND ALKALOID CONTENT OF DATURA STRAMONIUM IN SEMI-ARID CONDITIONS

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Received October 01, 2015. Accepted: January 26, 2016. Published online: April 08, 2016

ABSTRACT. Alkaloids of diferent plant, including many *Solanaceae* species, constitute important natural sources for variety of pharmaceutical products. The amounts of various secondary plant products are strongly dependent on the growing conditions and agronomical applications such as planting date, fertilization, irrigation etc. The aim of this was to study the effects of sowing date on some agronomic characteristics and total alkaloid content of thorn apple (*Datura stramonium*), investigated in the environmental conditions from Dicle University, Agricultural Faculty, Field Crops Department experimental area, during 2010 and 2011 growing years. In the research, plant height, stem diameter, number of branches per plant, number of capsule per plant, capsule width, capsule length, 1000-seed weight, fresh herb yield, herba yield, seed yield and total alkaloid content were examined. At the end of the study, in the trial of thorn apple with sowing dates, according to two years mean, seed yield, fresh herb yield, dry herb yield and total alkaloid yield were changed between 335 kg ha^{-1} and 704 kg ha^{-1}, 5933 and 20537 kg ha^{-1}, 1613 kg ha^{-1} and 4800 kg ha^{-1}, and 0.270% and 0.391%, respectively. The effect of sowing date was found significant on the investigated characteristics, when sowing delayed, agronomic characteristics were also decreased. The highest values related with seed yield, fresh and dry herbage of thorn apple were obtained sowing of 01 Apr. Moreover, thorn apple plant showed morphogenetic variation when compared leaf and seed alkaloid contents.

Key words: Thorn apple; Seed yield; Herb yield; Secondary metabolite.

INTRODUCTION

Datura stramonium L. (thorn apple) belongs to the family of *Solanaceae*, which includes species with valuable contents of tropane alkaloids. The plant is an herbaceous annual plant distributing throughout most parts of temperate regions of the world (Soni *et al*., 2102).

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Tropane alkaloids were found in all parts of thorn apple plant as well as in deadly. All the plant parts contain tropane alkaloids: L-hyoscyamine, atropine and scopolamine (Ricard *et al.,* 2012). They are extracted and used for the production of pharmaceutical preparations with anticholinergic properties (Temerdashev *et al*., 2012). The phytochemical analysis on the plant showed that thorn apple also contained saponins, tannins and glycosides. Some medicinal uses of the plant are its anti-inflammatory property of all parts of the plant, stimulation of the central nervous system, respiratory decongestion, treatment of dental and skin infections, alopecia and in the treatment of toothache (Soni *et al*., 2012).

In the South-eastern Anatolia of Turkey, thorn apple found in fields as a weed in many summer crops, particularly maize, cotton and vegetable crops, is generally attributed to its ability to compete with crops for moisture and nutrients. An important growing factor in the production of all crops is the sowing date and it is the most significant factor limited crop yield and quality, especially in arid and semi-arid regions (Turan *et al.*, 2011).

Limited information is available about the effect of cultivation practices of medicinally important thorn apple in Diyarbakir province, Turkey. The present study was undertaken to study the effect of sowing date on plant characteristics, yield and total alkaloid content of thorn apple growing in the semi-arid conditions of Turkey.

MATERIALS AND METHODS

Plant materials and field experiment

The research was conducted in the field trial area of Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, (latitude 37°53´N and longitude 40°16´E, 680 m above sea level), Turkey, during the growing seasons of 2010-2011. Trials were designed in randomized complete block design (RCBD) with three replications. Experimental factor was sowing dates as: first sowing date (01 Apr.), second sowing date (15 Apr.) and third sowing date (01 May). Seeds was sown by hand, fertilization was done two dates; first was applied at sowing date, second was done at initial of irrigation. Form of nitrogen is ammonium nitrate (33%). Normal cultural practices were followed throughout out the growing season.

The properties of soil sampled were taken in a depth of 30 cm. The experimental soil was salt with 0.16% content, pH of 7.46, available phosphorus (P_2O_5) with 1.45 kg ha^{-1}, potassium (K_2O) (8.2 kg ha^{-1}), available lime ($CaCO_3$) in 11.02% and organic matter (0.48%), respectively. According to the results, the trial area medium saline soil, slightly alkaline, medium calcareous and organic matter content is low.

General climatic characteristics of South-Eastern Anatolia region, summers are hot and dry, with temperatures above 30°C. Spring and autumn are generally mild, but during both seasons sudden hot and cold spells frequently occur in the region (Anonymous, 2012). The experimental area has a longterm (70 years) average precipitation of 469.9 mm,

mean temperature of 15.8 °C and relative humidity of 54.3%. Meteorological data for 2010 and 2011 growing period, indicated an average temperature of 17.7°C and 15.5°C, precipitation of 398 mm and 574 mm, respectively (Bulletin of Monthly Publication of Meteorology, Diyarbakir).

The seeds were sown on per the sowing date at 60x30 cm spacing. Plot sizes were 7.2 m^2 (3 m×2.4 m) each treatment. At harvest, five plants were randomly selected in each treatment for recording growth and yield parameters. The crop was harvested manually on flowering stage for leaves yield, on full ripening for seed yield. Statistical analysis (Analysis of Variance (ANOVA)) of the data generated was done using MSTAT-C package programme. The differences between means were compared by the LSD test at 5% level.

Determination of total alkaloid content

Determinations of total alkaloids were performed by Switzerland Codex (1971). According to the method, dried plant material was ground into a blender small pieces, were macerated in 50 ml 0.1 N HCL. Then kept at room temperature for a night and the solution was transferred to tube percolator. After maceration, the mixture was transferred to tube percolator, percolation process was continued with distilled water; a few drops of the percolation dropped in Dragendorff reagent. The process was continued until the mixture was orange. Perchlorate transferred to a 250 ml separatory funnel, after transferring media made alkaline with 10% ammonia solution. Then the solution was dissolved each date 2 min with 4×25 ml of chloroform ($CHCl_3$) by shaking, the extracts are collected, $CHCl_3$ completely were eluted from the media. The residue dried at 105 °C for 30 minutes, remaining material was dissolved in 25 ml of boiling water and 3 ml of 95º ethanol added into using methyl red as an indicator, was titrated with 0.01 N HCL. Titration, which is already transparent-yellowish color returns to pink, are given at the end.

1 ml 0.01 N HCL = 0.00289 g alkaloid. This value is multiplied by the acid used and amount of alkaloids from 3 g of samples has determined. This can then be converted to a percentage.

Preparation of methyl red solution

An amount of 0.05 N NaOH is added to 0.025 g of Methyl Red solution gently heated. Then, 5 ml of ethanol is added. Volume is completed to 250 ml with of 50% ethanol.

RESULTS AND DISCUSSION

According to variance analysis (*Table 1*) plant height, plant stem diameter, number of capsules, capsule width, number of seed per capsule, seed yield, fresh herb yield, dry herb yield and total alkaloid content of leaf were significant except number of branches, capsule length, 1000-seed weight and total alkaloid content of seeds. The highest plant height (110.4 cm) was obtained from the second sowing date and the lowest one (71.3 cm) from the third sowing date for 2010 year (*Table 2*). In 2011 growing season, plant height decreased with delay of sowing date while the highest plant height (75.1 cm) were recorded in the first sowing date (01 April), the lowest value (46.10 cm) was recorded in third sowing date (01 May) (*Table 2*). Declines were observed in plant

height related to delayed sowing date, differences between years were thought to be the result of changes in the amount of annual rainfall. Cakmak and Ozguven (1987) were found the highest plant height in 21 Mar. sowing with two different species. Decreasing plant height in late sowing may depend short growing period, early sown crop will have a longer vegetative period so plant height will be higher than the others.

Table 1 - Variance analysis of investigated characters for different sowing date on 2010 and 2011 growing seasons

Variation sources	Variance (%)		Mean square		F value	
	2010	2011	2010	2011	2010	2011
Plant height (cm)	9.82	13.29	1200.404	657.568	15.9294*	10.7702*
Plant stem diameter (mm)	8.01	10.95	13.510	12.214	10.6378*	11.3282*
Number of branch	12.57	15.12	1.404	0.324	7.90*	4.1714
Number of capsules	6.85	5.36	0.108	0.538	7.4615*	121.00**
Capsule width (cm)	3.21	1.73	2.963	1.343	8.0818*	13.00*
Capsule length (cm)	3.75	3.49	0.092	0.009	10.7456*	1.5048
Number of seed per capsule	14.33	7.38	18047.418	6907.765	10.0672*	31.9644**
1000-seed weight (g)	2.77	7.41	0.037	0.185	2.5363	1.0611
Seed yield (kg ha-1)	103.6	76.0	5089.35	3916.88	149.450*	346.184**
Fresh herb yield (kg ha-1)	212.2	215.1	8859281	7102484.4	84.3*	154.4*
Dry herb yield (kg ha-1)	153.9	158.3	479501.11	742893.33	146.2*	300.0**
Alkaloid content of seed (%)	6.77	11.61	0.006	0.007	11.4743*	4.9572
Alkaloid content of leaf (%)	4.56	9.25	0.000	0.004	1.3965	8.7711*

*p< 0.05, **p< 0.01

Different sowing date affected the plant stem diameter significantly for 2010 and 2011 seasons. Stem diameter was reduced with delay in planting. The plant stem diameter ranged from 12.60 mm to 16.5 mm. Plant stem diameter was influenced by delaying sowing date and the first sowing date (01 Apr.) had the highest plant stem diameter (11.4 mm), the lowest value was recorded in third sowing date (01 May) as 7.38 mm (*Table 2*). These differences between the years can be related to annual distribution of precipitation in growing season and the development and generative pattern of plant cause decreases in plant stem diameter.

Fetri *et al*. (2013) in their studies reported the stem diameter reduced with delay in planting for sunflower (*Helianthus annuus* L.).

Number of branches per plant, there was significant differences within sowing dates for 2010 but no differences for 2011. The number of branches decreased down from 3.93 to 2.60 branches per plant in 2010. First sowing date produced the highest number of branches (3.93), the lowest number of branches were recorded from third sowing date as 2.60 (*Table 2*). In 2011, delaying sowing date reduced the number of branches. The highest number of branches (2.07) was obtained when the crop was sown in 01 Apr., the lowest one (1.47) was obtained when the crop sown in 01 May. This may be due to changes in the amount of annual rainfall. Gurbuz (1994) reported number of branches changed between 7.48 and 14.13.

Table 2 - Effects of different sowing date on plant height, plant stem diameter, number of branch per plant, capsule width, capsule length and number of seed per capsule of *D. stramonium* during the two experimental seasons (2010 and 2011)

Sowing dates	Plant height (cm)	Plant stem diameter (mm)	Number of branch	Number of capsules	Capsule width (cm)	Capsule length (cm)	Number of seed per capsule
2010							
01 Apr.	110.4 a	16.50 a	3.93 a	1.97 a	1.99 a	2.61 a	378.4 a
15 Apr.	83.4 b	13.10 b	3.53 ab	1.70 ab	1.87 ab	2.53 a	283.2 ab
01 May	71.3 b	12.60 b	2.60 b	1.60 b	1.79 b	2.27 b	224.7 b
Mean	88.4	14.06	3.36	1.76	1.89	2.47	295.4
LSD (0.05)	19.7	2.55	0.95	0.26	1.373	0.22	95.98
2011							
01 Apr.	75.1 a	11.40 a	2.07	1.73 a	1.86 a	2.21	252.60 a
15 Apr.	55.3 b	9.67 ab	2.00	1.00 b	1.80 a	2.22	185.20 b
01 May	46.1 b	7.38 b	1.47	1.00 b	1.73 b	2.12	159.73 b
Mean	58.8	9.48	1.84	1.24	1.79	2.19	199.18
LSD (0.05)	17.7	2.31	NS	0.14	0.72	NS	33.33

*Means fallowed by same letters are not significantly different at 0.05 level of significance.

Statistical analysis revealed that different sowing dates had significantly effected on number of capsules per plant (0.05%). The average number of capsules varied from 1.60 to 1.97 with all sowing dates for 2010 growing season. The highest value was 1.97 in first sowing date and the lowest value was 1.60 in third sowing date (*Table 2*). Due to the changes in the amount of annual rainfall, number of capsules per plant

decreased depend on sowing dates, the highest number of capsules were obtained from first sowing date with 1.3, the lowest value was obtained from second and third sowing date with 1.0.

The highest capsule width was observed as 1.99 cm on 01 Apr., the lowest value was observed as 1.79 cm on third sowing date (01 May) for 2010. The first sowing date produced the highest capsule width (1.86 cm) and the third sowing date gave the lowest one (1.73 cm). Decreases and changes of capsule width may be due to annual rainfull regime.

Analysis of variance showed that variation within sowing dates was statistically significant for capsule length in 2010 but, the differences of 2011 were not significant. Maximum capsule length (2.61 cm) was for first sowing date and the lower one (2.27 cm) were recorded in third sowing date in 2010 growing season. In 2011, delaying in sowing date decreased the capsule length, the highest value was obtained in 21.2 cm and 01 Apr. sowing, sowing from May 01 with the lowest value was 2.12 cm. The maximum number of seeds per capsule showed at first sowing date (01 Apr.) with 378.37 and minimum number of seeds per capsule showed in third sowing date (01 May) with 224.70 (*Table 2*). The decreases in number of seeds per capsule due to delayed sowing dates took place in 2011 growing season. The highest number of seeds per capsule (252.60 /pieces) was obtained when the crop was sown in first sowing date, third sowing date (30 Apr.) with 159.73/pieces recorded as minimum seed per capsule in 2011 year. It is considered that differences arising between the two trial years may result from changes in the amount of annual rainfall as well as some of the other characters. Taher Sola *et al*. (2012) examine three sowing date and stated that maximum seed number in capsule were seen on first sowing date (21 Apr.).

Sowing date had no significant effect on 1000-seed weight for differences between the years (*Table 1*). According to the results, 01 Apr. with 4.46 g had highest, the lowest 1000-seed weight belonged to 15 Apr. with 4.24 g, respectively (*Table 3*). Thousand seed weight decreased with delaying sowing date, the highest 1000-seed weight was obtained from 01 Apr. (5.91 g), the lowest value was obtained from 01 May (5.42 g) in 2011. Differences arising between two of the trial for number of seeds in capsule may be provide the formation of seed in suitable environment for development. Siddique *et al*. (2002) reported that late sowings had fewer capsules per plant and per unit area, fewer seeds per capsule and lower 1000-seed weight. Taher Sola *et al*. (2012) similarly determined one seed weight for datura as 0.009 g from first planting date (21 Apr.).

The differences between the years were significant ($p<0.05$) in terms of seed yield. Seed yield were highest as 704 kg ha^{-1} on 01 Apr. sowing and lowest as 447 kg ha^{-1} for 01 May sowings (*Table 3*). The first

sowing date (01 Apr.) had highest seed yield (562 kg ha^{-1}), the last sowing date (01 May) had a significantly lower seed yield (335 kg ha^{-1}) for 2011 growing season. Differences between the experiment years for seed yield were probably due to differences in seed per capsule. Furthermore, the possible reason for low yield in delayed sowing might be insufficient date for vegetative growth as the plants entered the reproductive phase more rapidly. Hornok (1976) reported that earlier sowing produced higher seed yield in coriander. Taher Sola *et. al.* (2012) reported that first planting time (21 Apr.) caused highest seed yield in bush (38.38), that had significant differences with other treatments. Gurbuz stated that plant yield was lowest with latest sowing (01 May).

Table 3 - Effects of different sowing date on thousand seed weight, seed yield, fresh herbage yield, dry herbage yield, alkaloid content of seed and alkaloid content of leaves of *D. stramonium* during the two experimental seasons (2010 and 2011)

Sowing dates	1000-seed weight (g)	Seed yield (kg ha^{-1})	Fresh herbage yield (kg ha^{-1})	Dry herbage yield (kg ha^{-1})	Alkaloid content of seed (%)	Alkaloid content of leaves (%)
2010						
01 Apr.	4.46	704 a	20537 a	4800 a	0.391 a	0.291
15 Apr.	4.24	539 b	15607 ab	4033 a	0.328 ab	0.285
01 May	4.31	447 b	9683 b	2330 b	0.303 b	0.273
Mean	4.34	563	15276	3721	0.341	0.283
LSD (0.05)	NS	132	7349	1298	0.071	NS
2011						
01 Apr.	5.91	562 a	15373 a	4580 a	0.360	0.270 a
15 Apr.	5.59	431 b	8607 b	2187 b	0.347	0.230 b
01 May	5.42	335 c	5933 b	1613 b	0.270	0.197 c
Mean	5.64	443	9971	2793	0.326	0.232
LSD (0.05)	NS	7.63	4863	1002	NS	0.022

*Means fallowed by same letters are not significantly different at 0.05 level of significance.

Fresh herbage yield is one of the latest product for alkaloid production of thorn apple as with seed yield. High fresh herbage yield depends on several factors. The number of plants per unit area, good agricultural practices and irrigation are at the beginning among these factors. Data presented in *Table 3* show that sowing dates significantly affected fresh herbage yield between the years. Highest fresh herbage yield resulted by the first sowing date (20537 kg ha^{-1}) and the lowest ones resulted by the third sowing date (9683 kg ha^{-1}) (*Table 3*). In 2011, fresh herbage yield decreased with delaying sowing date. Highest fresh herbage yield (15373 kg ha^{-1}) was achieved 01 Apr. sowing, the lowest fresh herbage yield

(5933 kg/ha^{-1}) was recorded in 01 May sowing (*Table 3*). Sowing dates in 2010 and 2011 were statistically significant (0.05) for dry herbage yield. 01 Apr. sowing had greatest (4800 kg ha^{-1}) dry herbage yield. 01 May sowing produced the lowest dry herbage yield (2330 kg ha^{-1}). Sowing dates declining affected dry herbage yield in 2011 growing season. The highest dry herbage yield was obtained from 01 Apr. with 4580 kg ha^{-1}, the lowest valued was obtained from 01 M ay with 1613 kg ha^{-1} (*Table 3*). Cakmak and Ozguven (1987) were found the highest fresh and dry herbage yield in 21 Mar. sowing with two different *Datura* species.

Significant differences between the sowing dates were determined in 2010 year for alkaloid content of seeds but, not determined in 2011. In 2010, the highest value (0.391%) was obtained from 01 Apr., while the lowest one (0.303%) was obtained from 01 May sowing (*Table 3*). While the differences for 2011 were not important, the highest seed alkaloid content was obtained from 01 Apr., The lowest result was obtained from 15 Apr. sowing. Our results are in agreement with the results obtained by Berkov *et al*. (2005), who reported that alkaloid content and composition of crops changes depending on the development stage of plants. Kan and Arslan (2003) reported that *Datura* species differ in terms of total alkaloid content between the years. Akin and Ceylan (1986) observed that alkaloid content of plant varied 0.177% from 0.285%, Baytop (1971) also found 0.30% and 0.50%, and Sarin (1982) reported that alkaloid content of *Datura* plants was 0.25% and 0.40%. Henry (1949) observed that alkaloid content of thorn apple varied from 0.2% to 0.45% in leaves, from 0.2% to 0.5% in seeds, from 0.21% to 0.25% in roots and from 0.6% to 0.7% aerial parts. Our results are in agreement with findings of Henry (1949), Akin and Ceylan (1986), Baytop (1971), Sarin (1982).

The alkaloid content of the leaves was not significantly influenced by different sowing dates in 2010, but statistically significant in 2011. Maximum alkaloids content (0.29%) was found in the leaves harvested in 15 Apr. sowing. While minimum alkaloids content was 0.273% in the leaves of crop harvested in 01 May (*Table 3*). Differences between sowing dates was statistically important for alkaloid content of leaves, the highest value was 0.270% in 01 Apr., the lowest value was 0.197% in 01 May sowings. It was determined that the means of 2010 was higher than average value of 2011 for alkaloid content of leaves. This situation confirms that ecological conditions ecological factors have an effect on secondary metabolites. Kan and Arslan (2003) showed that there are morphogenetic variations in thorn apple plants and they reported alkaloid content of crop as 0.54-0.62% in herbage, 0.43-0.71% in leaves, 0.77-0.86% in flower, 0.48% in seed. Our results are lower than findings of Kan and Arslan (2003).

Iranbakhsh *et al*. (2006) reported that alkaloid ratio of thorn apple were identified as 0.127% in the vegetative period, 0.098% in capsule, 0.093% in vegetative and generative stage at the same rate, 0.069% and 0.050%, root and leaf generative period, respectively, and 0.020% in the seed. These variations might be due to different genotypic structure, ecological conditions and cultivation techniques.

CONCLUSION

Alkaloid analysis of the different parts of thorn apple (*Datura stramonium*) as leaf and seed showed that the alkaloid content was 0.391% in seed and 0.291% in leaf. The content of alkaloid was noticed to be highest in the seed, as compared to leaf content. First sowing date (01 Apr.) is the best date suggested for fresh and dry herbage yield and seed yield in this study.

Acknowledgements. This work was supported by a grant from Dicle University Research Funding (DUBAP; Project No. 08-ZF-39).

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