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EFFECT OF DIFFERENT NITROGEN DOSES ON SOME AGRICULTURAL CHARACTERISTICS AND ALKALOID CONTENT OF *HYOSCYAMUS RETICULATUS* L. AND *HYOSCYAMUS NIGER* L.

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ABSTRACT. Alkaloids, nitrogen containing basic substance, have a complex structure. They are one of the most important groups of secondary metabolites, which is synthesized in roots and transported to other organs. Since alkaloids are nitrogenous compounds, the availability of nitrogen is expected to play an important role in the biosynthesis and accumulation of alkaloids in plants. Nitrogen affects yield and quality of medicinal plants, therefore, growers usually apply large amount of nitrogen to obtain high yields. The objective of the present study was to determine yield, yield components and alkaloid content of two henbane species (*Hyoscyamus reticulatus* L. and *Hyoscyamus niger* L.), collected from wild flora of South-eastern Anatolia, grown under four nitrogen applications (0, 50, 100 and 200 kg ha^{-1}), in 2010-2011 growing seasons. In the field trial, plant height, stem diameter, number of branches per plant, number of capsule per plant, capsule width, capsule length, number of seed per capsule, 1000 seed weight, seed yield per plant and total alkaloid content were investigated. The results of study showed that nitrogen doses were found important for investigated characters but not important for *Hyoscyamus* species. Seed yield per plant varied from 8.4 to 11.6 g per plant, their alkaloid contents were found between 0.14% and 0.21%.

Keywords: henbane; herb yield; seed yield; total alkaloid content.

INTRODUCTION

The genus *Hyoscyamus* L. belongs to the tribe *Hyoscyameae* Miers of *Solanaceae* family with 18 species all over the world (Yousaf *et al.,* 2008). There are six species belonging to this species: *Hyoscyamus pusillus* L., *Hyoscyamus niger* L., *Hyoscyamus reticulatus* L., *Hyoscyamus albus* L., *Hyoscyamus aureus* L. and *Hyoscyamus leptocalyx* Stapf., found in Turkey (Baytop, 1978; Guner *et al.,* 2000).

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Hyoscyamus species are mainly rich in hyoscyamine and scopolamine, which are generally used in medicine as anticholinergic, antispasmodic, mildly pain relieving, hypnotic, hallucinogenic, pupil-dilating, and sedative compounds (Ghorbanpour *et al.,* 2013). Seeds and roots of *Hyoscyamus* species have been used by the Babylonians for treatment of different disorders, especially in relieving toothaches (Ceylan, 1994).

Since alkaloids are nitrogenous compounds, the availability of nitrogen is expected to play an important role in the biosynthesis and accumulation of alkaloids in plants (Ghorbanpour *et al.,* 20013). So, nitrogen is one the most important reqired nutrient in plants, which affects for growth and development. Al-Humaid (2005) reported that the availability of essential nutrient elements necessary in *Datura* growth and metabolism cause vigorous vegetation and high chemical production. Moreover, Lata (2007) stated that the fertilization of medicinal plants could increased in the yield of bioactive compounds.

Hyoscyamus species are also grown naturally in Southeast Anatolia Region, Turkey. But, comparatively very little work has been done relation with these species's alkaloid content or their agronomic traits. The secondary substances containing *Hyoscyamus* have an importance in medicine. These substances are met by exporting in Turkey, so a significant amount of foreign exchange is loss. However, not much information is available on the cultivation of *Hyoscyamus* in Turkey. Therefore, purpose of the present study was to determine the effects of different nitrogen doses on some agronomic characteristics and cultivation techniques of these species.

MATERIALS AND METHODS

Plant materials and experiments

Henbane species seeds were collected from Diyarbakır-Silvan at 10th km and Mardin-Mazidag at 15th km for *Hyoscyamus reticulatus,* and from Dicle and Egil districts of Diyarbakir province for *Hyoscyamus niger*. Both *Hyoscyamus* species are biennial.

The present study was carried out during 2009-10 and 2010-11 growing seasons, at Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey, at 37°N 53'latitude, 40°16' longitude and at an altitude of 680 meters above the mean sea level. Soil characteristics of the research area were clay-loam with 7.46 pH, 14.5 kg ha^{-1} P_2O_5, 0.43% organic matter and 11.02% lime.

Southeastern Anatolia Region has a continental climate features. The data, taken from the State Meteorology Institute, Diyarbakir (Turkey), showed that Diyarbakir had mean temperatures of January to December period during 2009-10 and 2010-11 was 17.6 and 15.4°C, respectively, with long term mean temperature of 15.8°C. It had mean relative humidity during 2009-10 and 2010-11 was of 49.7 and 52.4%, respectively, with long term mean relative humidity of 53.8%, and mean precipitation of 33.2 and 47.8 mm, respectively, with long term mean precipitation of 40.7 mm (*Table 1*).

Table 1 - Mean temperature, precipitation and humidity at the site of experimentation for long years, 2010 and 2011 years

Months	Mean temperature (ºC)			Precipitation (mm)			Humidity (%)		
	2010	2011	Long years	2010	2011	Long years	2010	2011	Long years
January	5.4	3.5	1.7	113.4	40.0	73.6	80.9	73.1	77
February	6.6	4.7	3.5	40.2	49.9	67.0	79.7	69.1	73
March	11.1	9.0	8.2	68.7	46.6	67.9	66.6	56.1	66
April	14.2	12.9	13.8	22.4	209.0	70.5	60.4	75.6	63
May	20.4	17.6	19.2	31.6	80.1	42.1	49.3	67.8	56
June	27.2	25.4	26.0	11.2	13.6	6.9	29.1	38.3	31
July	32.3	31.3	31.0	0.0	0.6	0.6	19.6	22.7	27
August	32.0	30.7	30.3	0.0	0.0	0.4	17.5	21.7	28
September	27.0	25.0	24.8	0.4	9.2	2.7	27.4	30.4	32
October	18.1	16.4	17.1	63.0	11.8	31.1	55.9	41.5	48
November	11.1	6.4	9.6	0.0	73.0	54.1	41.1	58.5	68
December	6.5	2.3	4.1	48.0	40.2	71.5	68.9	73.9	77

Source: State Meteorology Institute, Diyarbakir, Turkey

The experimental design was a split-plot design, with nitrogen rates (0, 50, 100, 200 kg ha^{-1}) as sub-plots and *Hyoscyamus* species (*H. niger* and *H. reticulatus*) as main plots and replicated with three times. The seeds were sown by hand at a spacing 45×20 cm, at the date of 01 April 2010. *Hyoscyamus* species seeds were not germinated until September 2010, because of the seed of *Hyoscyamus* species are very hard and germination occur at certain environmental conditions (Ceylan, 1994). The nitrogen fertilizer, amonium nitrate form (33% N) was applied in two parts, at sowing time and after germination, with first irrigation. Field practices (weed control and irrigation) had been done as needed. Plants were harvested in the full maturity stages, at July 2011.

Plant height, stem diameter, number of branch per plant, number of capsules per plant, capsule length, capsule width, number seed per capsule, seed yield per plant, thousand seed weight and alkaloid content of seed were measured from randomly selected 10 plants at harvest. Data were subjected to analysis of variance in the split plot design and LSD test was applied to compare the means at frequency level of 5%.

Determination of total alkaloids content

Determinations of total alkaloids were done modified method of *Pharmacopoea Helvetica* (Swiss Pharmacopoeia) by Baytop and Guner (1983). After harvesting, seed samples were air dried, grinded into fine powder. Then, 3 g of the sample of seeds was homogenized with 50 ml of 0.1 N HCl. Solution was kept at room temperature (22±2ºC) for maceration during a night. The macerated material was transferred to tube percolator, percolation process was continued with pure water. A few drops of the percolation dropped in Dragendorff reagent. The process was continued until the orange precipitate. Perchlorate transferred to a 250 ml separating funnel, after transferring media made alkaline with 10% ammonia solution. Then, the solution was extracted each time 2 min

with 4×25 ml of chloroform ($CHCl_3$) by shaking, the extracts were collected, $CHCl_3$ was evaporated from the media. The residue dried at 105°C for 30 min, remaining material was dissolved in 25 ml of boiling water and 3 ml of 95°C ethanol added into using methyl red as an indicator, was titrated with 0.01 N HCL. Titration, which was already transparent-yellowish color, returns to pink by methyl red solution; 1 ml 0.01 N HCL = 0.00289 g alkaloid, this value was multiplied by the acid used than total amount of alkaloid of 3 g of samples was determined and converted to percentage.

RESULTS AND DISCUSSION

The influence of nitrogen doses and species on the yield and yield component of henbane *(Hyoscyamus* species*)* are presented in this paper. The analysis of variance revealed that differences between nitrogen doses were significant for all investigated characters, except 1000 seed weight (g). Interaction between *Hyoscyamus* species and nitrogen doses were not found significant (*Tables 2 and 3*).

H. reticulatus are grown naturally in roadside slopes and edges of fields so it grows arid areas interm of water. Plant height of *H. reticulatus* (69.6 cm) is small, compared to *H. niger* (132.1 cm). Means of different nitrogen doses in *H. reticulatus* were not significant. Whereas there were significant differences due to increasing nitrogen doses in plant height of *H. niger*. There was significant difference between *Hyoscyamus* species for plant height. Plant height also varied depending on fertilization doses (*Table 2*).

Table 2 - Means of some agronomical characters of Hyoscyamus species

Species	Nitrogen doses (kg ha^{-1})	Plant height (cm)	Stem diameter (mm)	Number of branches	Number of capsules	Capsule width (mm)
H. reticulatus	0	63.7	9.8	6.9	110.5	0.57
	50	68.8	10.0	8.5	117.5	0.67
	100	72.3	11.0	10.4	158.9	0.71
	200	73.6	10.2	10.3	137.4	0.71
Mean		69.6 B*	10.2 B	9.14	129.3	0.66 B
H. niger	0	114.2	16.3	6.9	125.2	0.84
	50	130.6	18.8	8.7	168.6	1.00
	100	144.5	24.3	10.9	183.9	0.98
	200	139.3	23.0	10.4	184.7	0.97
Mean		132.1 A	20.6 A	9.26	165.6	0.95 A
Nitrogen doses	0	88.9 c	13.0 c	6.9 c	114.3 c	0.70 b
	50	99.7 b	14.4 bc	8.8 b	143.1 b	0.83 a
	100	108.5 a	17.7 a	10.7 a	171.4 a	0.85 a
	200	106.4 b	16.6 ab	10.3 a	161.0 ab	0.84 a
LSD (0.05)		S:18.0 ND: 6.7	S:9.4 ND:2.4	S: ns ND: 1.24	S: ns ND: 19.5	S:0.28 ND:0.07

S: Species; ND: Nitrogen doses; ns: no significant

*Means in each column followed by the same letter are not significantly different (P< 0.05).

Table 3 - Means of some agronomical characters of Hyoscyamus species

Species	Nitrogen doses (kg ha^{-1})	Capsule length (mm)	Number of seed per capsule	1000-seed weight (g)	Seed yield per plant (g)	Alkaloid content (%)
H. reticulatus	0	1.71	76.3	0.78	5.1	0.14
	50	1.75	90.4	0.83	5.9	0.17
	100	1.75	93.5	0.71	7.6	0.19
	200	1.62	89.9	0.77	7.1	0.18
Mean		1.71 B*	87.5 B	0.77 A	6.4	0.17
H. niger	0	2.10	229.6	0.60	11.7	0.14
	50	2.22	294.5	0.56	13.4	0.17
	100	2.53	320.4	0.54	15.5	0.24
	200	2.40	293.1	0.62	14.4	0.18
Mean		2.31 A	284.4 A	0.58 B	13.78	0.18
Nitrogen doses	0	1.91 b	153.0 c	0.68	8.4 c	0.14 c
	50	1.99 b	192.4 b	0.69	9.7 b	0.17 bc
	100	2.14 a	207.0 a	0.63	11.6 a	0.21 a
	200	2.01 b	191.5 b	0.69	10.7 a	0.18 ab
LSD (0.05)		S: 0.08 ND:0.11	S: 18.8 ND:12.9	S: 0.02 ND: ns	S: ns ND: 0.88	S: ns ND: 0.03

S: Species; ND: Nitrogen doses; ns: no significant
*Means in each column followed by the same letter are not significantly different (P< 0.05).

Plants height of *H. reticulatus* ranged from 63.7 to 73.60 cm. Nitrogen applied at N_{200} resulted in maximum plant height, compared to the minimum control. *H. niger* had a higher plant height. The highest plant height was 144.5 cm for N_{100}, the lowest was 114.2 cm for N_0 nitrogen doses in *H. niger*. Increasing N fertilization increased plant height (*Table 3*). Similarly, in *Datura innoxia*, plant height was found to increase linearly with increasing levels of N application then, all parameters tended to decline at certain level (Al-Humaid, 2005).

Hyoscyamus species have high amount of herbage yield because of wide leaves. Stem diameter of *H. niger* (20.6 cm) was larger from *H. reticulatus* (10.2 cm). Nitrogen treatments enhanced plant development and, as a result, the stem diameter values were increased at increasing nitrogen doses. The maximum stem diameter (17.7 mm) was produced at N_{100} dose, compared to the minimum stem diameter at control which produced 13.0 mm (*Table 2*).

Hyoscyamus species have high the number of branches and capsules. Number of branches per plant, the parameter related to seed yield of the crop, especially positively. Significant differences were not observed for number of branch per plant. Nitrogen doses significantly affected number of branch per plant. The highest value was observed (10.7 per plant) from N_{100}, and the lowest result (6.9 per plant) was found in control treatment.

Number of branch per plant was increased with increasing nitrogen applications, particularly N_{100} and N_{200}. Gupta *et al* (2011) found that maximum number of leaves and branches were recorded with the application of 75% recommended NPK along with 2.5 t vermicompost and biofertilizers as 16.53 per plant. The stimulating effect of nitrogen in increasing number of branches per plant was reported by Omar (2013) on *Datura*.

Varying nitrogen doses had significant effect on number of capsules per plant, but not species. Application of N_{100} produced the highest number of capsules per plant (171.4), as compared with the lowest number of capsules per plant (114.3), obtained from N_0 (*Table 2*). Although not statistically significant, fertilization of two *Hyoscyamus* species with higher levels of nitrogen increased number of capsules per plant. According to Losak and Richter (2004), with the increasing dose of nitrogen the number of capsules per a plant during the harvest and their volume increased irregularly in poppy plants (*Papaver somniferum* L.).

The more capsule volume affects number of seed positively. The capsule of *H. niger* are bigger than the capsule of *H. reticulatus*, so the differences are observed due to capsule size. Width of *H. reticulatus* capsules ranged between 0.5 cm and 0.7 cm. The results revealed that maximum width of capsule was obtained by applying N_{100} and minimum width of capsule by applying N_0 doses (*Table 2*). For *H. niger*, the maximum value (1.00 cm) was obtained at N_{50} treatment, the minimum (0.8 cm) at N_0 dose (*Table 2*).

As capsule width, capsule length has also positive effect on number of seed per capsule. The maximum capsule length (1.7 cm) was recorded on N_{50} dose, whereas minimum capsule length (1.6 cm) was recorded from N_{200} dose for *H. reticulatus* (*Table 2*). Fertilization doses had important effect on capsule length of *H. niger*. The maximum capsule length was found in N_{100} dose as 2.5 cm, the minimum was 2.1 cm, in control (*Table 2*). This may be due to botanical properties of studied species.

Hyoscyamus species have a lot of stem and capsules. Therefore, this situation positively affect seed yield. The dramatic differentiation was observed between species for of number of seed per capsule. The highest number of seeds per capsule varied between 76.3 and 93.5. Increasing N doses slightly increased number of seeds per capsule in *H. niger* and the differences were significant. The highest number of seeds per capsule was obtained from N_{100} dose as 320.4, the lowest one was 229.6 at control. N_{100} fertilization dose gave the highest values among nitrogen treatments (*Table 2*).

There were significant differences between species for 1000 seed weight. Seed of *H. reticulatus* has larger structure than *H. niger*. Thousand seed weight of

H. reticulatus changed from 0.7 g to 0.8 g. Increasing nitrogen doses have no significant effect on 1000 seed weight of *H. niger*. The highest 1000 seed weight was obtained (0.6 g) at N_{200}, whereas the lowest 1000 seed weight (0.5 g) was recorded at N_{100} treatment. Nitrogen doses did not affect 1000 seed weight of two species (*Table 3*).

Seed yield per plant is closely associated with number of capsules per plant and number of seeds per capsule. There were marked differences in means of seed yield per plant. Seed yield per plant of *H. reticulatus* ranged from 5.1 g to 7.6 g. Seed yield per plant increased in increasing nitrogen doses for *H. niger*. The highest seed yield per plant of 15.5 g was produced from N_{100} treatment, the lowest seed yield per plant was recorded from N_0 treatment as 11.7 g (*Table 3*).

Alkaloids are one of the most important groups of secondary metabolites, which is synthesized in roots and transported to other plant organs. Quantity and quality of alkaloids are different in various plant organ in the same plant. The distribution of alkaloids in the plant reveals that is the organ (roots, stems, leaves, flowers, seeds and fruit) important in terms of the active substance. Alkaloid content may also vary in different species of the same genus and taxonomic and genetic variations in the same species (Kan and Arslan, 2001). *Hyoscyamus* species are important plants for alkaloid production. They are containing in seed, leaf, flower and roots. Economically, leaves and seeds are used in the production of alkaloids. The alkaloid content was ranging from 0.148% to 0.191% for *H. reticulatus* (*Table 3*). On the other hand, total alkaloid content of *H. niger* varied from 0.140% to 0.240%, the maximum rate obtained from N_{100} dose, compared to control (N_0). Ceylan (1994) reported that alkaloid content of *H. niger* varied from 0.117% to 0.173%. There were no differences between species. Kan and Arslan (2001) showed that the difference between varieties was not found significant in terms of alkaloid content in *Datura* plants. Henry (1949) reported that total alkaloid content of *H. niger* ranged from 0.045% to 0.08% in leaves, 0.16% in roots, from 0.06% to 0.1% in seeds, from 0.07% to 0.1% in flowers, total alkaloid content of *H. reticulatus* was 0.08% in seed, varied from 0.12% to 0.24% in whole plant. Zeybek and Zeybek (1994) stated that alkaloid content of *H. niger* has 0.04% – 0.17% in leaves. Our results for alkaloid content are in agree with results obtained by Henry (1949), Ceylan (1994), Zeybek and Zeybek (1994). Alkaloid content of species was affected by nitrogen applications. Gholamhosseinpour *et al.* (2011) reported that nitrogen is a constituent of the alkaloids, which play an important role in the synthesis of alkaloids. Therefore, increasing nitrogen nutrient leads to an increase in alkaloid content: 0.04% – 0.17%

CONCLUSION

The results of present study showed that the highest seed yield per plant of *H. niger* was higher than *H. reticulatus* in connection with number of seed per capsule. The highest alkaloid content was obtained from N_{100} nitrogen doses, the lowest one was obtained from control. No difference was observed between species for alkaloid content.

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