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The effect of plant density and quantity of nitrogen fertilizer on vegetative function of *Lawsonia inermis* L. in Jiroft

Seyed Mohammad Ali Vakili Shahrbabaki

Department of Biology, Jiroft Branch, Islamic Azad University, Jiroft, Iran.

Abstract

In order to examine the effect of the quantity of Nitrogen fertilizer and bush density on vegetative and productive function of henna medicinal plant (*Lawsonia inermis)* factorial experimentation in the form of block plan was carried in three stages in agriculture research center in south of kerman province in corp of 2012. The first factor containing Nitrogen fertilizer in 4 levels including 50,100,150,200 kg net Nitrogen fertilizer and the second one as bush density in three level including 110000-160000-210000, bushes were examined. The measured traits included weight of fresh stem, stem, height weight of dried stem and leave, and total dried and fresh weight and all of productive traits including weight of fresh and dried flower, number fruits in bush, number of seeds in a fruit, number of seeds in frui weight of 1000 seeds and function of seeds. The results indicate that that effect of bush density, nitrogen fertilizer qualities and contrast effect between two factor on weight of fresh and dried leave Statistically was in to concern up to 1%, but nitrogen fertilizer shows no difference in statistical respect on this quality, nitrogen fertilizer effect on all of productive qualities except number of seeds in fruit Was up to 5% fruitless as the main consumption of henna is its leaves. Finally with density of the bush 160000 to 210000 bushes in 1 hectare and consuming 50kg nitrogen fertilizer with function of dried leaves weight up to 3.54, 3.34 tons in one hectare is recommended. Even though consumption of 100kg nitrogen fertilizer in hektar resulting of maximum effect on dried weight.

Key words: *Lawsonia inermis* - dried leave-vegetative organ- productive organ-seed function.

Introduction

Medicinal plants have an important role for human communities that have been used to prevent different diseases. There is a lot of useful information about medicinal plant (omidbigy2005) According to the World Health Organization report in 2013, 80 percent of developed countries cannot provide needed drugs. Since the plants are the major source of medical supply, it needs to be more attention. Due to biological and healing properties of medicinal plants in developed countries request is very high for these plants. Efforts to update the information about medicinal plants especially henna are increasing now (Orwa et *al*., 2009).

Corresponding Author E-mail: mohammadvakili72@yahoo.com

Sowing date and N fertilizer are the two most important factors that affect directly on the yield, and yield components (Marisol et al., 2003).

Lawsonia inermis have a wide distribution and exist in Iran, Pakistan, Syria, Egypt and etc.

The most common name for it is "Henna". Infusion of Henna flowers is useful for contusion remedy. The seeds of the plant have Deodorant property and contain especial oil for bloody diarrhea. Bark decoction is used for treatment of Jaundice, Leprosy, spleen and dermal problems. Due to medicinal properties of plant roots, these are used to therapy of Herpes simplex, burn, inflation and Eyesore (Chaudhary *et al.*, 2010).Roa et al, 2003 showed that the effect of five ton of organic fertilizer increased leaf dry weight to 1.4 % in comparison to control. Using of organic fertilizer including of 80kg Nitrogen and 40kg. Phosphor (p2o5) increased leaf dry weight 19.3 % compared with the control. Dry leaf yield of this treatment was 1302.6 kg ha. Dry leaf yield increased significantly with increasing distance from 30 cm to 45 cm in wide rows. Askari *et al*., 2012 in research on amounts of nitrogen fertilizer and plant density on the growth of Hanna showed that highest number of leaves per plant was obtained in density of 7 plants/m^2 and consumed 100 kg of pure nitrogen, respectively (90 leaves in plant) While in the density of 11 plants/m^2 and consumption of 50 kg N per hectare, maximum number of leaves, was about 85 leaves per plant. The maximum dry weight (17g) obtained in density of 11 plants/m^2 and consumption of 100 kg of nitrogen. The maximum plant dry weight (25g) was achieved in density of 7 plants/m^2 and consumption of 100 kg of nitrogen. Consumption of 50 kg of nitrogen in this condition was resulted to 23 g plant dry weight. Maximum plant height (85 Cm) was observed in the density of 7 plants/m^2 and 100 kg of nitrogen. Khusk and Hisbani, 2004 reported that 120 kg nitrogen and 60 kg phosphorus increases the henna plant height up to 225 cm, number of branches to 27.84, fresh weight of leaf to 175.9 and leaf dry weight to 52.7. The maximum of dye content is obtained from this treatment. According to Orwa *et al*. (2009) to get 1000 kg of dried henna leaves 180Kg N, 150 kg of potassium and 10-30 kg of phosphorus was used. Also plant density was 200000 plants per hectare and the distance between plants was 15 cm. Distance of 30-20 cm between plants was reported in transplant cultivation of henna. A study on the other species from different family confirmed these results (Jamshidi, 2000; Alizadeh and etal ,2006; Cooper ,1974).

Materials and Methods

This study was conducted in Jiroft that is situated at latitude 57°48" and longitude 28°35"with the highest peak of 625.6 m (above sea level). According to Meteorology Organization the lowest and highest extreme temperatures are –14° C and 40° C. Average annual rainfall is 130 mm. Precipitation in years of low rainfall is 70 mm and in high rainfall is 250 mm. The average relative humidity is 55 to 65%.

Table1. The results of soil analysis

Soil texture	pH	EC (ds/m)	K (ppm)	P (ppm)	N (%)	C (%)	depth cm
Loam - sandy	7.6	1.64	330.63	4.2	0.023	0.115	0-30

According to information on soil analysis and fertilizer, the following procedure was carried out. 200 kg ha triple super phosphate equivalent to 60 kg P was used. Phosphorus, nitrogen and organic matter of soil are poor but K in soil is rich.

This case study was performed in agricultural experimental farm located in 2011-2012 in Jiroft and Kahnooj city. This study has been done in a randomized complete block design in three replicate.

The first factor is the amount of nitrogen (Urea Fertilizer, 46% N). Four levels of nitrogen fertilizer were applied.a_1) 50 Kg/ha, a_2) 100 Kg/ha, a_3)150 Kg/ha a_4)200 Kg/ha.

The second factor is plant density included three levels: 210,000 plants per hectare, 160,000 plants per hectare, and 110,000 plants per hectare. A total of 12 treatments, 36 plots in an area of 561m2 were examined.

To study the physical and chemical properties of soil, a composite sample prepared from the depths of 0-30 cm soil profile and it was sent to a lab to analyses the soil .In order to transplant cultivation, the seeds planted in small pots and were transferred to the field when they reach a length of 20 to 30 cm in April. After field preparation, including the plan was performed on the field. The distance between rows was 30 cm and plant spacing on the line according to density treatments was 10, 20 and 30 cm, respectively. Each plot had 6 lines Planting 5 m in length.

Based on the analysis of soil, fertilized with phosphorus and potassium with half the nitrogen fertilizer along with planting and the remaining half was used for about a month after the first application. Each plot had 6 lines planting that half was used for vegetative characteristics and half for reproductive traits. In the beginning plots were irrigated every three days and in the middle of growing were irrigated once a week. The field was cleared from the weeds in the 25 days and 50 days after planting respectively.

Measurements of the tested parameters

In this stage half-meter of both sides of the border was eliminated then all of present plants harvested and the related parameters were investigated. From each plot 10 plants were selected randomly and average plant height, number of primary branches per plant and dry weight of stem and branch was recorded.

Plant height: measured by a ruler
Stem diameter: Third and fourth internodes diameter of each plant was measured by caliper.
Wet weight of inflorescences: was measured by digital precision balance.
Dry weight of stems, leaves and inflorescences: put each sample in oven at 75°C for 48h and weight it again.

Statistical analyses:

Data for each parameter were subjected to ANOVA and significant differences between treatment means were determined by Duncan Multiple Range test using the SAS software. Data are shown as means with three replicates and significance was determined at the 95% confidence ($p \leq 0.05$) limits.

Results

As shown in table 1-4, all of the parameters were affected at least by one or interaction between two factors.

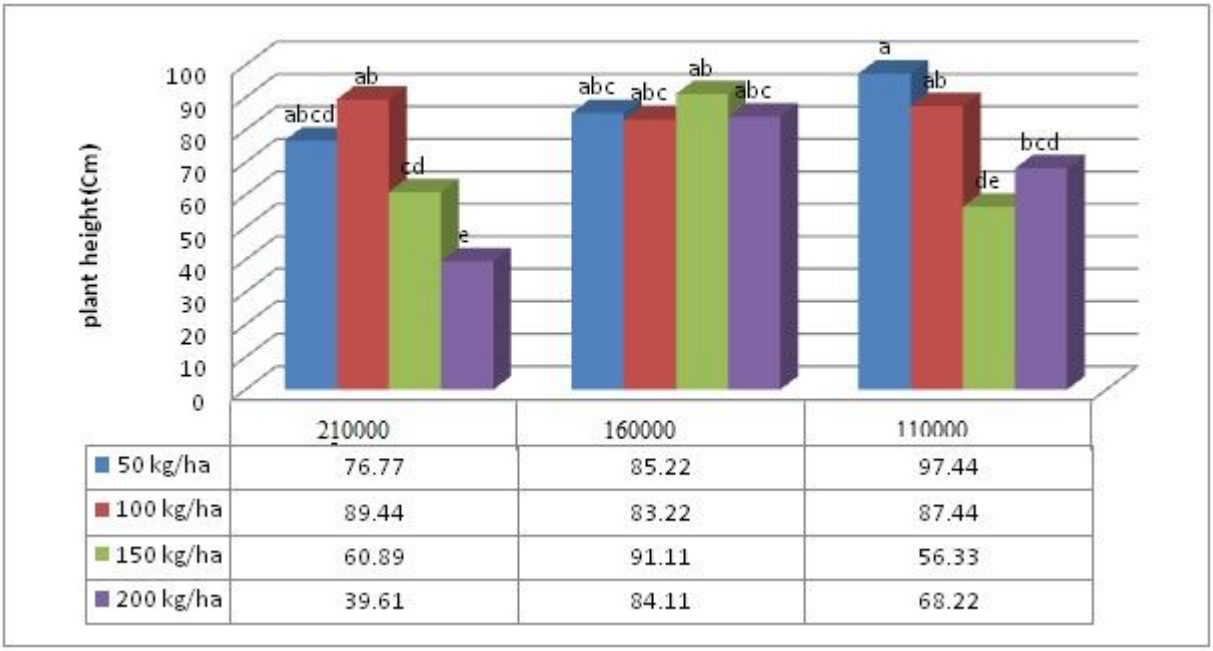
Vegetative parameters:

Plant height:

Results of analysis of variance showed that plant height is affected by different levels of plant density, nitrogen quantity and interaction between them at 0.01level (Table1).Comparing the average of interaction between plant density and nitrogen amount showed that

However the maximum height of plants (44- 97cm) is reported in treatment of 110,000 plant per ha with 50kg nitrogen per ha but there is no significant difference between plant height in this treatment and other treatments. The minimum height of plants is reported in treatments of 210,000 plant per ha with 200kg nitrogen per ha (39.61cm) and 110,000 plant per ha with 150kg nitrogen per ha (56.33cm).

With increasing the plant density to 160,000 plant per ha, the plant height increased but in higher density the plant height reduced because of severe competition for nutrient absorption.With increasing nitrogen from 50 kg to 100 kg per ha the height increased but this increasing was not significant. With more than 100 kg of nitrogen, plant height was reduced (Fig 1).



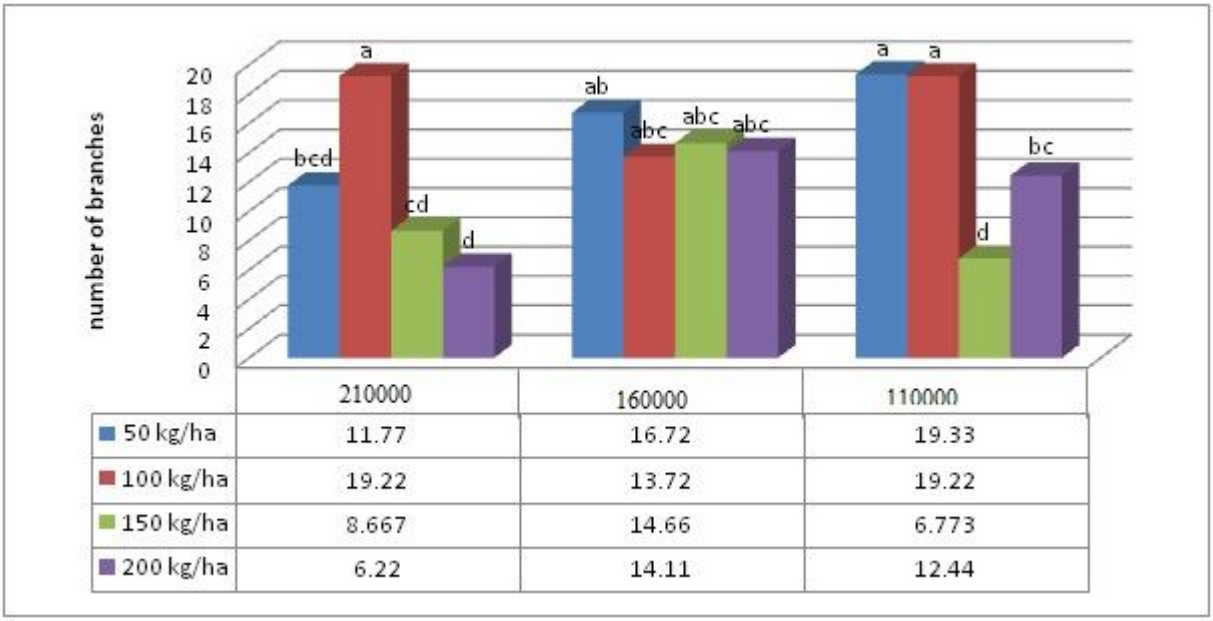
	210000	160000	110000
50 kg/ha	76.77	85.22	97.44
100 kg/ha	89.44	83.22	87.44
150 kg/ha	60.89	91.11	56.33
200 kg/ha	39.61	84.11	68.22

Fig. 1. Effect of plant density and nitrogen fertilizer on plant height of *Lawsonia inermis* L.

Number of branches

Different levels of density, different amounts of nitrogen and the interaction between them affected on the number of branches ($p \leq 0.05$, $p \leq 0.01$) (Table 1-4). Maximum number of branches belonging to the density of 110,000 plant per ha Combined with the use of 50 and 100 kg N (Respectively 33/19 and 22/19 branches) and the density of 210,000 plant per ha with 100 kg of nitrogen (22/19 branches). No significant differences were observed in the density of 160,000 plant per ha in all levels of plant nitrogen. It could be concluded that the plant produce lateral branches to compensate the reduced density. Nitrogen

increased vegetative growth of plant and increased number of branches to compensate the low density (Fig. 2).

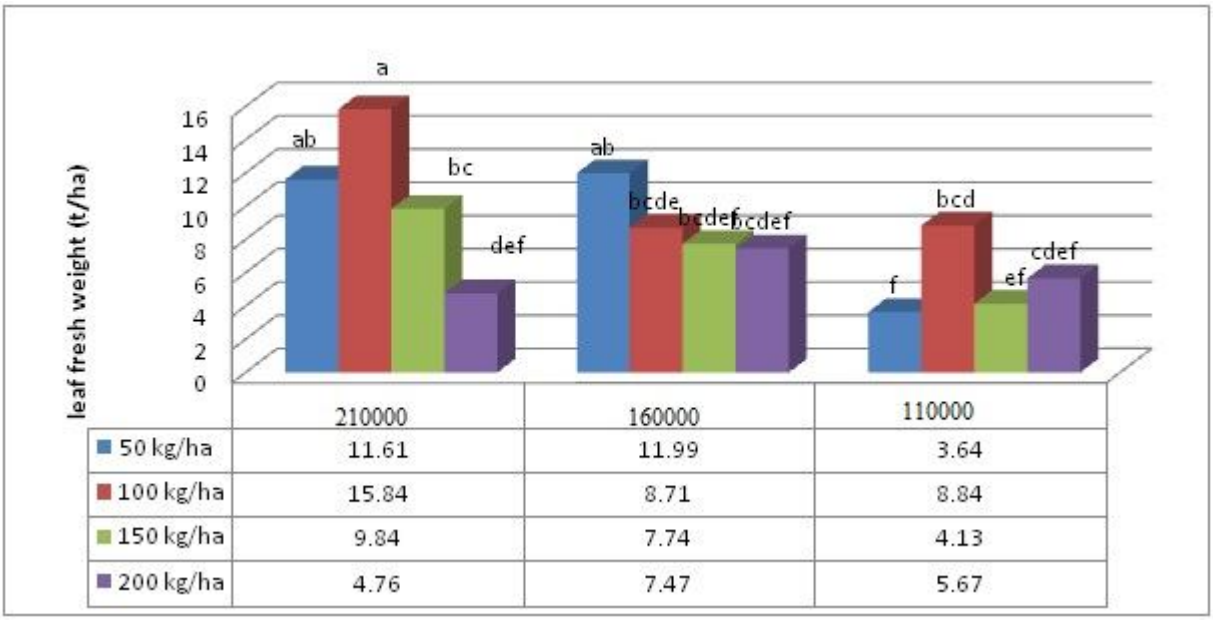


	210000	160000	110000
50 kg/ha	11.77	16.72	19.33
100 kg/ha	19.22	13.72	19.22
150 kg/ha	8.667	14.66	6.773
200 kg/ha	6.22	14.11	12.44

Fig 2.. Effect of plant density and nitrogen fertilizer on number of branches of *Lawsonia inermis* L.

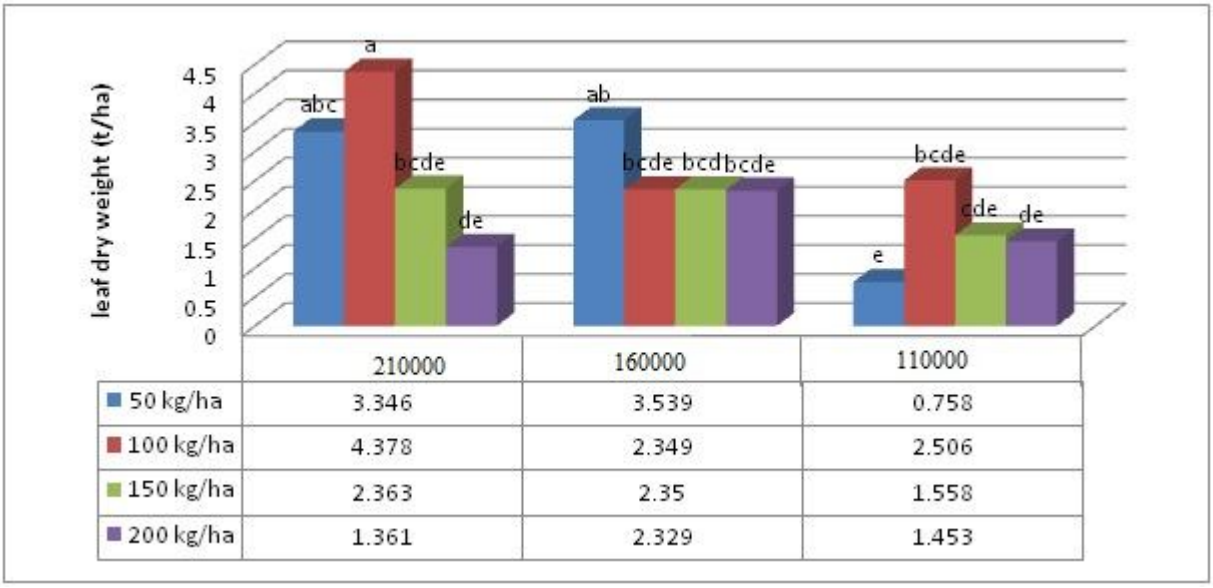
Fresh and dry weight of leaves

Different levels of density, different amounts of nitrogen and the interaction between them affected on fresh and dry weight of leaves at 99% level. Increasing plant density led to an increase in leaf fresh weight. So that the maximum of leaf fresh weight was reported in the density of 210,000 plant per ha with 100 kg of nitrogen (15.84 ton in ha). There was no significant difference between leaf fresh weigh in noted treatment and 50 kg of nitrogen and also in the density of 210000 and 160000 plant per ha (Respectively 99/11 and 61/11 t ha). Fertilizer consumption levels in the density of 110,000 plant per ha, had no significant effect on leaf fresh weigh. The lowest leaves fresh weight was also observed in this treatment. It seems that increasing plant density along with the availability of sufficient nutrition (through the use of nitrogen fertilizers) led to an increase in branches and leaves(Abbaszadeh, 2006) With the reducing density from 210000 to 110000 plants per hectare Henna Leaf fresh weight was reduced to about 5 tons. Also with increasing nitrogen fertilizer, leaf fresh weight increased (from 9.08 ha to 11.13 ha) but this increase was statistically insignificant. With further increase of nitrogen, henna leaf fresh weight was reduced (Fig. 3 and 4).



	210000	160000	110000
50 kg/ha	11.61	11.99	3.64
100 kg/ha	15.84	8.71	8.84
150 kg/ha	9.84	7.74	4.13
200 kg/ha	4.76	7.47	5.67

Fig. 3. Effect of plant density and nitrogen fertilizer on leaf fresh weight of *Lawsonia inermis* L.



	210000	160000	110000
50 kg/ha	3.346	3.539	0.758
100 kg/ha	4.378	2.349	2.506
150 kg/ha	2.363	2.35	1.558
200 kg/ha	1.361	2.329	1.453

Fig. 4. Effect of plant density and nitrogen fertilizer on leaf dry weight of *Lawsonia inermis* L.

Comparison of the interaction between the different levels of nitrogen and plant density showed similar results with the fresh weight. The maximum dry weight was achieved from 100 kg of nitrogen in the density of 110,000 plant per ha (378/4 t ha). There was no significant difference between this treatment and consumption of 50 kg of nitrogen in the density of 160,000 and 210000 plant per ha.

Roa *et al*., 2007 reported that consumption of 80 kg nitrogen increased maximum dry weight by about 19% compared with the control and the maximum amount of the dried leaves of this treatment were

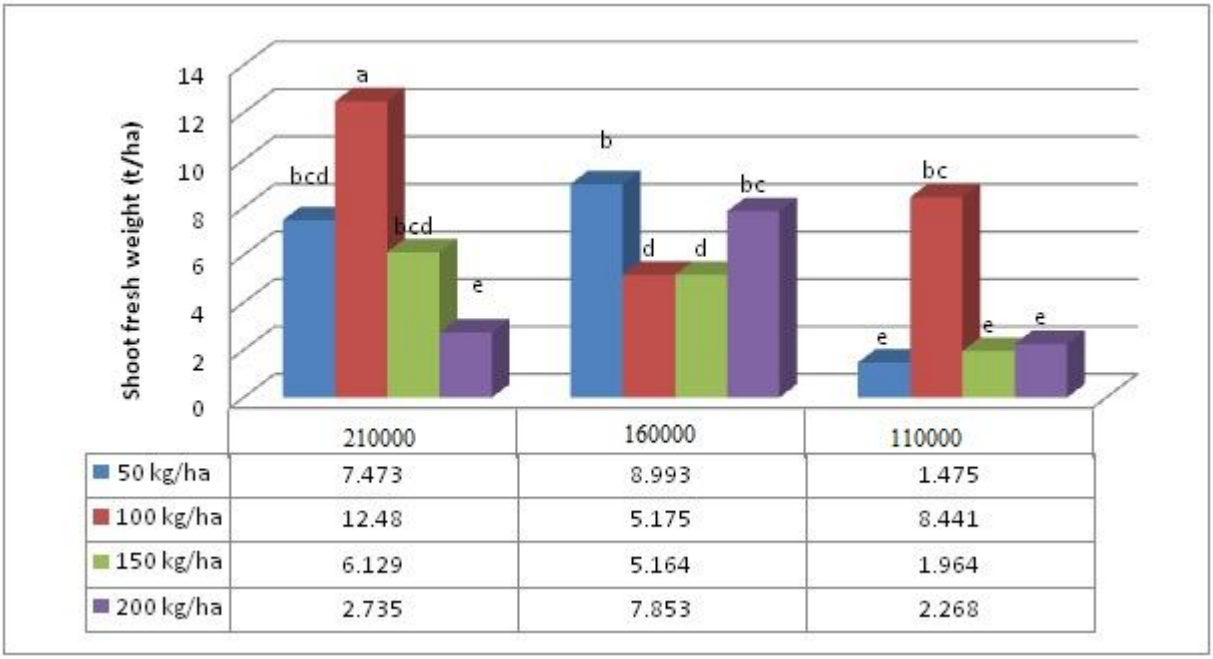
reported 1302.6 kg ha. In another study Roa *et al*., 2003 in India reported the highest yield of dry leaves (603.8 kg per ha) from the application of 60 kg N.

Askari *et al*., 2012 that had examined effect of nitrogen fertilizer and plant density on Henna,

repobrted the highest leaf dry weight per plant in density of 7 plant per m^2 with 100 kg nitrogen (25g) , in density of 13 plant per m^2 with 100 kg nitrogen (20g) and in density of 11 and 15 plant per m2 with 100 kg nitrogen fertilizer (17g) (Fig4).

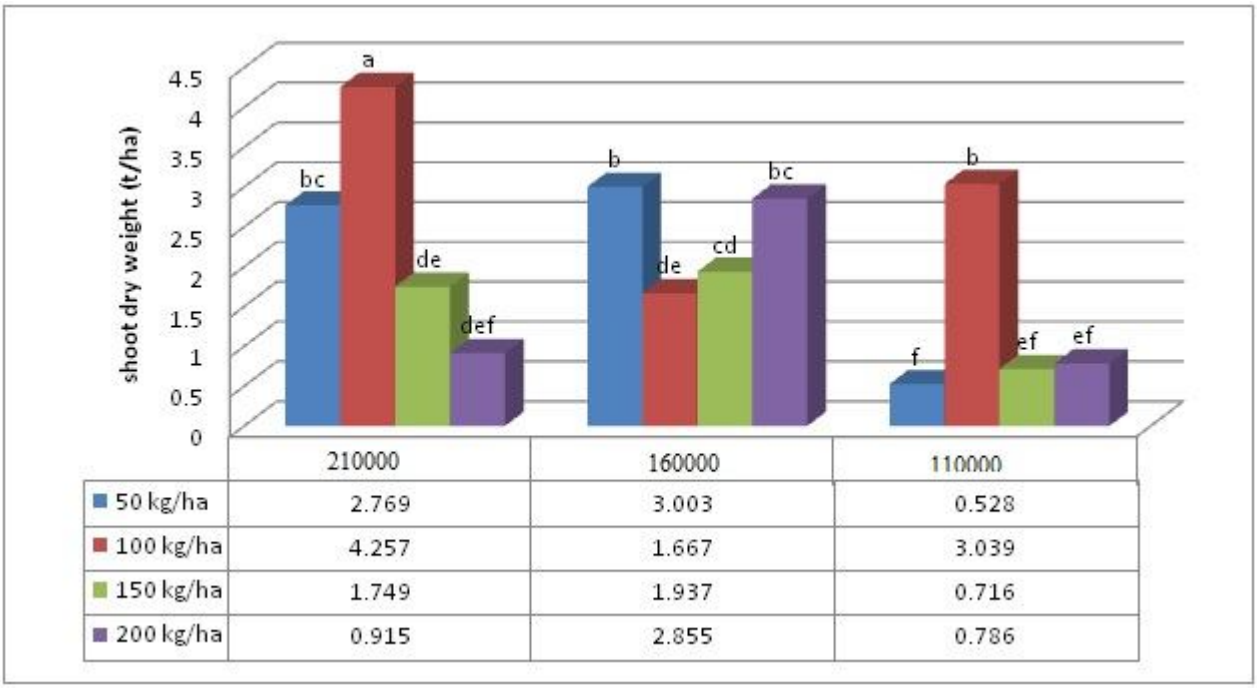
Fresh and dry weight of stem

Different levels of plant density, different amounts of nitrogen and the interaction between them affected on fresh and dry weight of stem at 99% level (Table1). Comparison of average interaction plant density and different amounts of nitrogen fertilizer showed similar results on fresh and dry weight of stem. As the highest fresh and dry weight (12.48 and 4.257 ton per ha) found in density of 210,000 plant per ha with 100 kg nitrogen. However, in this density, consumption of 50, 150 and 200 kg nitrogen has led to a reduction in shoot dry weight that indicated deficiency and overload of the element. In density of 110,000 plant per ha with consumption of 50, 150 and 200 kg nitrogen led to a sever reduction in shoot fresh and dry weight but in this density, consumption of 100 kg nitrogen has increased shoot dry weight considerably. At high densities, concentrations of auxin and gibberellin increased due to loss of light in the vegetation and thus the plant height will increase (Abbaszadeh, 2006) .With suitable plant nutrition increase in plant height could lead to an increase in shoot dry weight (Fig 5, 6).



	210000	160000	110000
50 kg/ha	7.473	8.993	1.475
100 kg/ha	12.48	5.175	8.441
150 kg/ha	6.129	5.164	1.964
200 kg/ha	2.735	7.853	2.268

Fig. 5. Effect of plant density and nitrogen fertilizer on shoot fresh weight of *Lawsonia inermis* L.



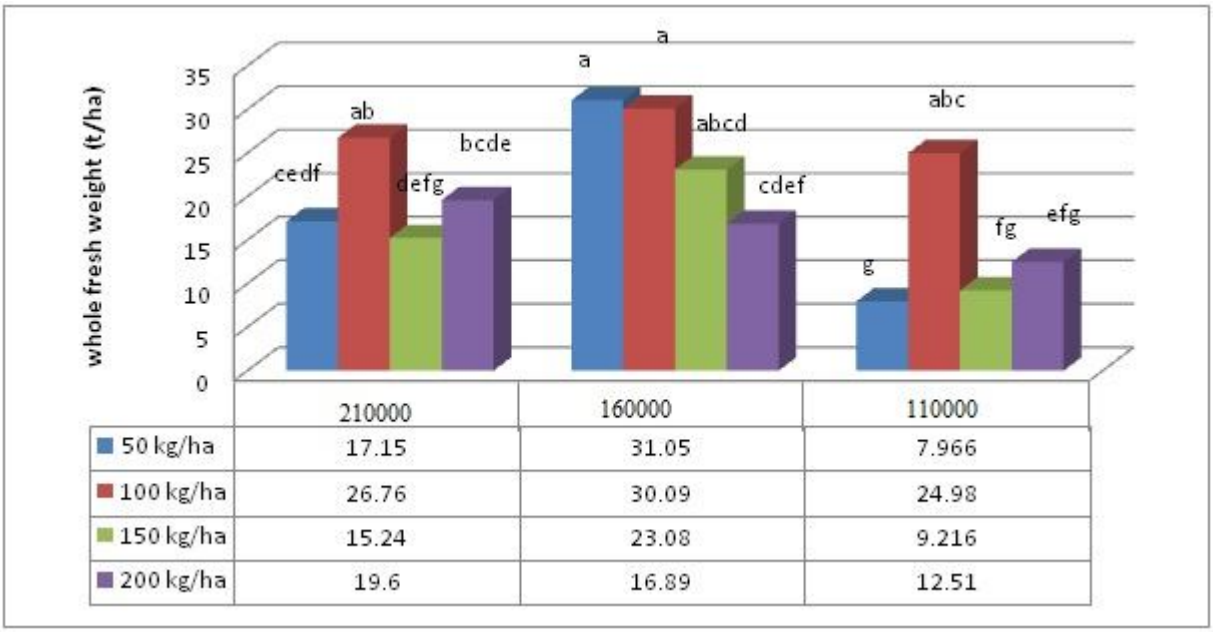
	210000	160000	110000
50 kg/ha	2.769	3.003	0.528
100 kg/ha	4.257	1.667	3.039
150 kg/ha	1.749	1.937	0.716
200 kg/ha	0.915	2.855	0.786

Fig. 6. Effect of plant density and nitrogen fertilizer on shoot fresh weight of *Lawsonia inermis* L.

Plant fresh and dry weigh:

Different levels of plant density, different amounts of nitrogen and the interaction between them affected on plant fresh and dry weigh at 99% level (Table1).

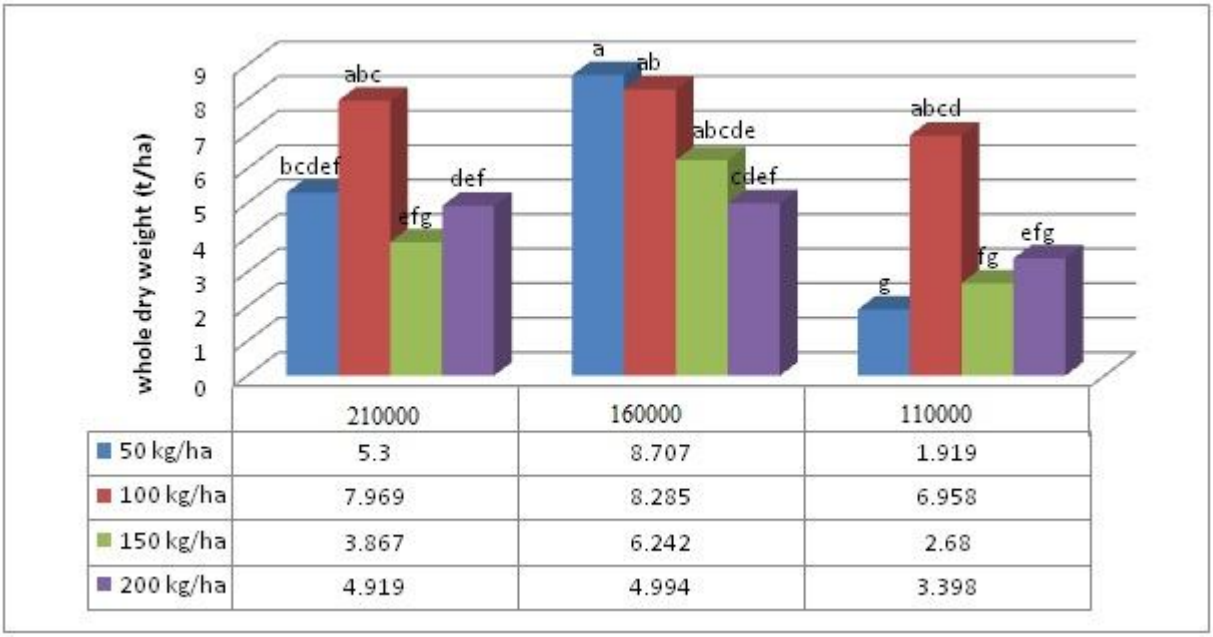
Comparison of average interaction plant density and different amounts of nitrogen fertilizer on whole plant fresh weight showed that the highest fresh weight of plant is recorded in 160,000 plant per ha density with 50 kg and 100kg nitrogen (31.05 and 30.090 ton per ha). Although between this treatment and the others was not observed the significant difference. The minimum weight of the whole plant belongs to 110,000 plant per ha density with consuming of 50, 150 and 200 kg nitrogen. This result shows that in high (210,000) and low (110,000) plant densities with consumption of 100 kg nitrogen and in the intermediate density with consumption of 50 kg nitrogen can help to increase vegetative growth. These results have also been obtained in other vegetative parameters Fig7).



	210000	160000	110000
50 kg/ha	17.15	31.05	7.966
100 kg/ha	26.76	30.09	24.98
150 kg/ha	15.24	23.08	9.216
200 kg/ha	19.6	16.89	12.51

Fig. 7. Effect of plant density and nitrogen fertilizer on whole fresh weight of *Lawsonia inermis* L.

Comparison of average interaction plant density and different amounts of nitrogen fertilizer on whole plant dry weight was similar to the effect on the whole plant fresh weight. As the highest dry weight of whole plant is related to 160,000 plant per ha density with 50 and 100kg nitrogen (8.80 and 80.28 ton per ha), 210,000 plant per ha density and 100 kg nitrogen (7.969 ton per ha), 110,000 plant per ha density and 100 kg nitrogen per ha (6.958 ton per ha) and 160,000 plant per ha and 150 kg nitrogen per ha respectively. Balak and *etal* 1999 showed that the highest growth indices and yield of *Anthemis* recorded with consumption of 120 kg nitrogen per ha Fig8).



	210000	160000	110000
50 kg/ha	5.3	8.707	1.919
100 kg/ha	7.969	8.285	6.958
150 kg/ha	3.867	6.242	2.68
200 kg/ha	4.919	4.994	3.398

Fig. 8. Effect of plant density and nitrogen fertilizer on whole dry weight of *Lawsonia inermis* L.

Conclusion

The results this study, showed that in treatments of 110,000 plant per ha density with 100 kg nitrogen , 160,000 plant per ha with 50 and 100 kg nitrogen and 210,000 plant per ha with 100 kg nitrogen have resulted in increasing of vegetative growth and majority of studied parameters..other Researcher showed similur resulets too,(Roa *et al*., 2007) reported that consumption of 80 kg nitrogen increased maximum dry weight by about 19% compared with the control and the maximum amount of the dried leaves of this treatment were reported 1302.6 kg ha. In another study Roa *et al*., 2003 in India reported the highest yield of dry leaves (603.8 kg per ha) from the application of 60 kg N. (Askari *et al*., 2012) that had examined effect of nitrogen fertilizer and plant density on Henna, reported the highest leaf dry weight per plant in density of 7 plant per m^2 with 100 kg nitrogen (25g) , in density of 13 plant per m^2 with 100 kg nitrogen (20g) and in density of 11 and 15 plant per m2 with 100 kg nitrogen fertilizer (17g) (Balak and *etal* 1999) showed that the highest growth indicate and yield of *Anthemis* recorded with consumption of 120 kg nitrogen per ha. (Abbaszadeh,2006) reported that At high densities, concentrations of auxin and gibberellin increased due to loss of light in the vegetation and thus the plant height will increase .It seems that increasing plant density along with the availability of sufficient nutrition (through the use of nitrogen fertilizers) led to an increase in branches and leaves. Roa et al, 2003 showed that the effect of five ton of organic fertilizer increased leaf dry weight to 1.4 % in comparison to control. Using of organic fertilizer including of 80kg Nitrogen and 40kg(Khusk and Hisbani, 2004) reported that 120 kg nitrogen and 60 kg phosphorus increases the henna plant height up to 225 cm, number of branches to 27.84, fresh weight of leaf to 175.9 and leaf dry weight to 52.7. The maximum of dye content is obtained from this treatment. According to (Orwa *et al*. (2009) to get 1000 kg of dried henna leaves 180Kg N, 150 kg of potassium and 10-30 kg of phosphorus was used. Also plant density was 200000 plants per hectare and the distance between plants was 15 cm..Distance of 30-20 cm between plants was reported in transplant cultivation of henna. A study on the other species from different family confirmed these results (Jamshidi, 2000; Alizadeh et al., 2006; Cooper ,1974).

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Table 1- Mean square of plant density and nitrogen on growth parameters in henna

Mean-square								freedom	Sources of variation
Whole dry weight	Whole fresh weight	Shoot dry weight	Shoot Fresh weight	Leaf dry weight	Leaves fresh weight	Number of branches	Plant height		
^{ns} 0.04	^{ns} 0.6	^{ns} 0.05	^{ns} 0.71	^{ns} 0.63	^{ns} 1.75	^{ns} 19.09	**801.29	2	repeat
**33.08	**417.09	**5.08	**48.47	**6.68	**76.73	*40.13	**1144.83	2	Plant density (A)
**11.66	**133.75	**4.49	**37.88	**2.84	**45.51	**62.08	**1210.95	3	Nitrogen Content (B)
**10.77	**126.39	**3.62	**27.38	**2.44	**23.51	**74.18	**612.26	6	Plant density × Nitrogen Content (AB)
1.4	11.74	0.17	0.99	0.52	3.32	7.12	99.21	22	Error
21.84	17.47	20.88	17.07	29.84	21.82	19.67	13.01	-	% Coefficient of Variation (cv)

ns, *.** : The non-significant and significant at 5 and 1% probability level