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# PIGMENT CHANGES OF RUBINIA TINCTORUM ROOT UNDER DIFFERENT LEVELS OF SALINITY AND THEIR RELATIONSHIP WITH SOME CHEMICAL CHARACTERISTICS OF WATER

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# **Abstract:-**

To achieve sustainable development in water, soil, and medicinal and industrial plant issues, and also, according to different uses of Rubinia tinctorum, especially in dye industry and the importance of salinity in it, this study was carried out to investigate the effect of water salinity stress on Pigments changes of Rubinia tinctorum Root and their relationship with some chemical characteristics of water in 6 regions under cultivation of this plant. This experiment was done on the basis of a compllyete randomized design with three replications in 6 regions under cultivation of it. After sampling and determining the electrical conductivity of irrigation water, the water samples were classified into three groups of 4, 4-8, and 8-16 ds/m and the effect of salinity on desire parameters was investigated. Data analysis was done using SPSS and Excel software. The results showed that with increasing salinity, the wool brightness ( $L^*$ ) was decreased (p<0.01). The amount of  $A^*$  (redness and greenness) (p<0.05) and  $B^*$  (yellowness and blueness) (P<0.01) parameters was increased with increasing water salinity. Investigation of the correlation between some chemical characteristics of water and pigments parameters, showed that there is a negative correlation between B\* parameter and water acidity. There is also a negative correlation between  $L^*$  parameter and all chemical characteristics of water except acidity (p<0.01). However, there is a positive correlation between A\* parameter and electrical conductivity and SAR, so that A\* parameter increases after increasing these parameters (p<0.01). In general, the quality of Rubinia tinctorum color was increased with increasing water salinity. So, progressing toward economic production of this industrial and medicinal plant is possible by using appropriate salinity treatments.

Keywords:- Pigment, Rubinia tinctorum, Salinity, Water chemical characteristics

## INTRODUCTION

Salinity is one of the main and common stresses in today's world which reduces the agricultural production and cause the loss of vast areas of the earth's natural vegetations. According to the estimates by Postini (1995) and Kaafi and Stewart (1998) 7% and 3% of the world lands are salty and very salty, respectively.

Salinity is one of the most important stresses in our country. About 2.1 percent of the land of Iran is faced with the problem of salinity. Therefore, plants that have a high tolerance to salinity, especially in desert areas are very important. Madder (Rubia tinctorum) is one of those plants that have multiple uses such as economic, medical, industrial and forage uses (Tavakkoli Saberi, 1989; Niebuhr, 1970; Chiej, 1984; Grieve, 1984; Bown, 1995). Rubia tinctorum belongs to the herbaceous and perennial madder family, which grows as a self-pollinated plant in the Mediterranean from Spain to Asia Minor and also, North Africa and some other parts of Asia. It is extremely salt resistant plant by tolerance method and the soil salinity is well tolerated up to 0.3 percent (Dashtakian and Bahrani, 2007). Abbasi et al., (2009) showed that by increasing salinity the resistance of madder was increased and specific leaf weight and the number of branching stems, leaf area, and root to shoot ratio, the central cylinder diameter to the root diameter, leaf weight of each plant, stem and root weight were decreased. Sepaskhah and Beirouti (2009) studied the effect of irrigation intervals and water salinity on growth of madder and concluded that the growth reduction per unit increase in soil salinity and irrigation water salinity for top growth are 2.0,3.7 % per dS m1, respectively. These values are 1.9, 3.1 % per dS m-1, respectively for root growth. Baghalian and Maghsodi (2010) have studied the Genetic diversity of Iranian madder (Rubia tinctorum) population based on agro- morphological traits, phytochemical content and RAPD markers. They have stated that the population had a significant effect on all evaluated traits. Also, correlation analysis showed that shoot weight had a significant positive correlation with root weight and dye content. Banakar and Khorsandi (2012) have stated that the madder plant is tolerant to salt in the vegetative stage and irrigation water with minimum salinity of 10 ds / m can be used for its cultivation. Madder plant is adapted to arid and desert climates and it is able to tolerate salinity. It is cultivated in some areas such as Yazd province using saline waters (Zargari, 1988).

In order to achieve sustainable development in the water, soil and Medicinal plant sectors, and with regard to different uses of the madder plant, especially in the dye industry and the importance of salinity, the objective of this study was set to investigate Pigments changes of Rubinia tinctorum Root under different levels of salinity and their Relationship with Some Chemical Characteristics of water in areas under cultivation of this plant.

# **Materials and Methods**

In order to investigate the effect of salinity on Rubinia tinctorum pigments, the experiment was conducted in the form of complete randomized block design with three replicates in 6 areas under cultivation of this plant in Ardakan, Yazd. It should be noted that the soil characteristics are identical in these areas and they differ only in terms of their irrigation water. After sampling and determining the electrical conductivity of irrigation water, the water samples were classified into three groups of 4, 4-8, and 8-16 ds/m. After transferring of soil and water samples to the laboratory, parameters such as electrical conductivity (EC), acidity (pH), sodium adsorption ratio (SAR) and ions of calcium, magnesium, sodium was measured in saturated extract. Also, to determine the pigment components of madder, the roots of the plants were harvested in autumn and after washing they have been dried and made into powder. 10 grams of powder was poured into 100 cc of distilled water and after boiling for an hour it was filtered. At this stage, 1 gr of wool in 30 cc of the solution was boiled at 70° C within an hour and it was washed and dried to remove excess dye. After preparation of the samples, the values of dye components including, L \* (indicates the brightness so that the greater amount, the brighter and less color absorption of wool), A \* (indicated the redness and the greenness of wool so that in the more positive value, the wool is more red, and in the more negative value, the wool is more green) and B \* (indicates the yellowness and the blueness of wool (it is not very effective in this test because the color is red)) were determined in the laboratory using spectrophotometer (model X-RITE).

Finally, statistical analysis of data was performed using SPSS and the graphs was drawn in EXCEL. The ANOVA method was used to analyze the data, and Duncan test was used to compare means.

# Results

# Effect of different salinity treatments on wool color components

The results of the variance analysis of data related to the color component of wool dyed with madder root color showed that the salinity treatments had a significant effect on all three color components (p<0.01) (table 1).

Table 1: Variance analysis of the effect of salinity treatment on the color component of wool dyed with madder root color

Sources of changes	Degrees of freedom	mean squares			
		L*	A*	B*	
salinity	2	53/15**	15/71*	18/10**	
error	6	10/1	0/811	1/67	

Significance at the level of 0.05:\* significance at the level of 0.01:\*\*

Mean comparison of data using Duncan's test showed that with increasing soil salinity, wool brightness (L \*) has decreased. The most amount of the L\* value was equal to 40 and it was observed in the salinity of less than 4 ds/m. There was no significant difference between it and the measured amount of 34.1 in the salinity of 4-8 ds/m. While there was a significant difference between it and the measured amount of 32.38 in the salinity of 8-16 ds/m.

In the case of the redness and the greenness of wool, with increasing soil salinity, the component (A \*) was increased and there was a significant difference between treatments. The component in the salinity of less than ds/m is equal to 24.33 and it has its maximum value of 39 in the salinity of 8-16 ds/m. In other words, the red and green color components by increasing soil salinities to over 8 ds/m will be increased to 62.5 percent.

The results obtained by mean comparison also showed that with increasing soil salinity in the 8-16 ds/m treatments, the yellowness and the blueness of wool (B \*) has increased significantly compared to treatments with electrical conductivity of less than 4 ds/m and 4-8 ds/m. The component in the salinity of 8-16 ds/m is equal to 26.17 and it shows an increase of 23% compared to the other two treatments.

# The correlation between some of the chemical properties of the irrigation water and dye components of wool

By examining correlations between some of the chemical properties of the irrigation water with each other and with dye components of wool such as  $L^*$ ,  $A^*$  and  $B^*$ , it has been found that there is a correlation between  $B^*$  and the acidity of water (p <0.01),  $A^*$  is correlated with EC and SAR (p <0.01) and with the rest of the parameters (p <0.05). Also,  $L^*$  has correlation with all the examined chemical characteristics in this experiment except for acidity (p <0.01). In Table 2, the correlation between these factors has been shown.

Table 2. Correlations between some of the chemical properties of the water used in the cultivation of madder with each other and with dye components of wool

	* B	**	*1	EC	Hd	Ca+Mg	S	SAR	Total hardness
B*	1	0/825*	-0/303	0/562	-0/786	0/464	0/494	0/519	0/464
A*		1	-0/707	0/800	0/768*	0/676*	0/786*	0/850	0/676*
L*			1	-0/920	0/664	-0/864	/945** -0	-0/948	-0/864
EČ				1	-0/877	0/971**	0/993	0/958	0/971**
рН					1	-0/863	0/817	0/754*	-0/863
Ca+Mg						1	0/960	0/878*	1/00**
Na							1	0/976	0/960**
SAR								1	0/878**
Total hardness									1

The yellowness and the blueness of wool (B \*) has a negative correlation with water acidity and it has been decreased as pH value increases (p <0.01). But the redness and the greenness of wool (A \*) has a positive correlation with electrical conductivity and sodium adsorption ratio and with the increase in these parameters, the component A \* also increased (p <0.01). By increasing the electrical conductivity, sodium adsorption ratio, calcium, magnesium, sodium and total water hardness, the wool brightness is reduced, it means that there is a negative correlation between these parameters and components L \* (p <0.01).

## Discussion

Study results showed that with increasing salinity, the wool brightness ( $L^*$ ) was decreased, in other words, it causes the increase in the pigments of the madder roots. The components have the maximum value in the EC of less than 4 ds/m. It had no significant difference with the measured amount in the EC of 4-8 ds/m while it had a significant difference with the measured amount in the EC of 8-16 ds/m. Decrease in the brightness level of wool indicates that the absorbed amount is more and the dyed wool seems to be darker. High negative correlation of  $L^*$  and chemical properties of the water is a clear proof of this claim.

Changes in the compounds which added to the dye, can cause the changes in the molecular structure of madder pigments. Montazer and Parvinzade (2002) have investigated the effect of ammonia on color change in wool dyed with natural colored materials such as madder and pointed to the effects of ammonia on the molecular structure of madder pigments. Also, the results of the present study about the rate of the redness and the greenness of wool (A \*) showed that with increase in salinity to over 8 ds/m, A \* will be increased to 62.5 percent. The rate of the yellowness and the blueness of wool (B \*) also increased with increasing in soil salinity. Moreover, the results showed a positive correlation between soil characteristics and A \* and B \* parameters.

Against all agricultural and horticultural crops which damaged by environmental stress, the active ingredients of medicinal and industrial plants will increase greatly in stress conditions (Rashidi, 2011). The general effect of salinity on the plants is reduction in the amount of their pigments, but depending on the species and the additive effects can be seen (Parida and Das, 2005). In some plants, the pigment amount increases with increasing in salinity. Destruction of the fine structure of chloroplasts and instability of pigment-protein complexes, chlorophyll degradation and changes in the content and composition of pigments such as carotenoids are the results of salinity. In general, according to the results, the presence of the correlation between irrigation water salinity and color components of the examined dyed wool and also due to the high resistancy of madder to salinity, increase in water salinity can lead to improve the color quality of madder. Therefore, the use of appropriate salinity treatment could be a step towards economic production of industrial and medicinal plant.

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