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Research Article

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Inter-Relationships among Chemical Traits of Cured Tobacco Leaves under Various Levels of Water Stress in a Dry Year

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Abstract Paper discusses the results of the evaluations done on the basses of data obtained from filed experiment, carried out on the effects of various levels of water supply and water stress imposed at different growth stages, on chemical traits cured tobacco plant (*Nicotiana tabacum* L.), during a year with insufficient precipitation rate. Three known stages of the plant, were considered and a total of 14 irrigation levels were applied in the experiments with K-326 Virginia tobacco cultivar, grown soil and climatic conditions of Kirklareli region of Turkey. All the treatments of the study were irrigated at the same time as the control irrigated at each growth stage, with water amount enough to replenish the water deficit in 0-90 cm soil layer, and three levels of water amount reductions (0, 40 and 60 %) were applied at each development stage. Evaluating the results obtained during a dry year it was concluded that irrigation application or water stress imposed at various stages of growth, markedly influenced the chemical traits of the cured tobacco leaf. It was also determined that close linear relationships exist between the seasonal irrigation water amount or seasonal consumptive use, and the chemical traits contents as nicotine, chloride, nitrogen, sodium and potassium in the leaves of tobacco plant. Mutual relationships of high significance between nicotine and each of the traits i.e. nitrogen, chloride, potassium, and sodium, were also identified.

Keywords Tobacco, Water, Stress, Chemical Traits, Relationships

Introduction

Due to insufficiency of the precipitations during the critical stages of growth, irrigation appears a factor of vital importance for Virginia tobacco production grown in Northwest part of Turkey. Almost no commercial dryland production of tobacco could be considered in the region, since the high water requirements of the crop during the periods of rapid plant growth and leaf expansion could be hardly met by the precipitation events during the mentioned periods.The abiotic stress factors including water stress and drought, significantly limit the yield potential and chemical composition of crops in any agricultural system. Nathawat et al. [1] claimed that drought and salinity stresses are especially harmful to food production in arid and semi-arid regions. Recently researchers from all over the world have been concentrating on physiological and biochemical responses of stressed plants, as a tool to understand control mechanisms and imparting stress tolerance.

The importance of irrigation in tobacco production has been described by [2]. Water is involved in all plant growth processes of tobacco plant and continuous water supply is essential for leaf yields of high quality and quantity. Some researchers [3,4], and [5], reported that imposition of drought from 14 to 30 days after transplanting is beneficial in stimulating root development. Though, the availability of water in the soil profile during leaf formation and ripening stages of growth may permanently affect development of the plant either in leaf yield or leaf quality. The association of high alkaloids and low sugars with drought conditions and conversely, of low alkaloids/high sugars with wet weather crops is well known [6-7]. According to [8] water influences the chemical composition of curried tobacco leaf and ample water contributes to an increase in sugar content, alkalinity, ash content, and potassium content, and decreases nitrogen, nicotine, and chlorine content. While excess soil water by rainfall or irrigation, however, may change the leaf chemistry away from a desired level [9]. In Queensland Australia, [10] have studied the effect of water stress on yield and quality of flue-cured tobacco and determined that water stress during the period of rapid growth has detrimental effects on leaf yield and quality under conditions. Some authors [11] claimed that tobacco quality and chemical compound of cured leaf are as important, as the yield and in order to obtain tobacco production with good taste and flavor, special attention should be given to irrigation at right time with appropriate irrigation water amount.

Taking into account the harmful effects of nicotine on the heath of the smokers, nowadays great attention is given to the effect of the other agricultural practices on nicotine content of cured tobacco leaf. Karim et al. [12] have studied the effect of topping stages and levels, on chemical composition of flue-cured Virginia tobacco leaves and determined that topping at 16 leaves level provides most desirable chemical characteristics of the leaves. While other authors [13] reported that it is noteworthy that grafting in tobacco plants to tomato rootstocks essentially eliminates foliar nicotine levels (reduced to 1%) and this technique implies a rapid, efficient and natural alternative in increasing tobacco-leaf quality and thus reducing harmful effects of this alkaloid on the health of smokers.

There are several publications related to the effects of water supply on growth and dry matter accumulation dynamics [14] and on yield and yield response to water [15] of Virginia tobacco plant grown in the region of the study.

The purposes of this study are to evaluate the effects of water stress imposed during different growth stages on chemical composition of cured tobacco leaf, and to discuss the relationships among irrigation water and chemical traits at one side, and between nicotine and other chemical compounds of the leaf on the other.

Materials and Methods

The study was carried out on the fields of the Soil and Water Resources Research Institute in Kirklareli. Seedlings of K-326 flue-cured tobacco cv, were transplanted during the second half of May of the experimental year, on a 0.5 m X 1.0 m grid. Field experiments were carried out in a randomized complete block design, with three replications.

Three known growth stages of the plant, Vegetative (V), Yield formation (F) and Ripening (R), were considered and a total of 14 (including rain fed) irrigation treatments were applied. All the experimental treatments were irrigated at the same time as (VFR) irrigated at each growth stage with the amount of water required to fill the 0-90 cm soil depth to field capacity and three levels of water amount reductions (0, 40 and 60 %) were implied at each development stage. More details on the experimental site and experimental procedure, irrigation application and moisture monitoring are published in our previous publication related to similar study [15].

Undisturbed and disturbed soil samples collected from different (0-30, 30-60, 60-90 and 90-120 cm) layers of the experimental plots, and water samples collected at the initial, middle and final stage of the irrigation season were analyzed following procedures given by [16]. The soil properties of the experimental area and irrigation water characteristics are summarized in Table 1 and Table 2, respectively.

Table 1: Some principle properties of the experimental soil

Depth (cm)	Field Capacity (%)	Wilting Point (%)	Bulk Density gr/cm^3	Organic Matter (%)	Electrical Conductivity dS/m	pH	Soil Texture*
0-30	22.7	9.7	1.46	2.1	2.34	7.7	L
30-60	19.8	9.0	1.52	1.5	2.06	7.7	SCL
60-90	10.8	5.1	1.54	0.9	1.29	7.7	SL
90-120	13.2	6.7	1.63	0.9	1.58	7.8	SL

* L, loam; SL, sandy loam; SCL, sandy clay loam

Table 2: Chemical characteristics of irrigation water applied to experimental treatments

pH	EC dS/m	Cations , me/l			Anions, me/l				SAR	Class
		Na^+	$Ca^{2+}+Mg^{2+}$	K^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}		
7.0	1.12	2.72	9.14	0.25	-	6.70	3.08	2.33	1.26	S3A1
6.9	1.08	2.54	8.90	0.22	-	6.43	2.80	2.43	1.32	S3A1
7.0	1.11	2.38	8.72	0.18	-	6.20	2.77	2.31	1.35	S3A1

Daily weather climatic parameters as precipitation, relative humidity, evaporation and average temperature were measured at a weather station, located adjacent to the experimental site and the values during the dry experimental year, accompanied by long-term averages are presented in Table 3. Data included in the mentioned table show that the experimental year is much drier than normal. The experimental years were different also from the point of distribution of precipitation during the different parts of the growing season. The total monthly rainfall amounts during the periods of rapid vegetative growth and yield formation stages (June-August) of the experimental year was about 13 mm vs. 100.6 mm for long-term average rainfall amount.

Table 3: Some meteorological data for the months of the year of study and for long years period

Month and Meteor. Event	May	June	July	August	September	October	Seasonal	Yearly
Precipitation, mm-								
Long year avg.	51.7	45.4	30.8	24.4	29.8	51.7	233.8	594.7
Exp. Year	19.2	5.2	3.2	5.0	60.8	2.8	96.2	315.4
Rel. humidity, %								
Long year avg.	69	63	61	62	68	75	66.3	73
Exp.Year	67	59	57	59	66	73	63.5	74
Evaporation,mm								
Long year avg.	92.9	116.5	158.6	159.1	108.4	64.5	700	922.6
Exp. Year	137.5	141.8	164.0	154.1	105.7	57.0	760.1	983.3
Av. Air Temp.,°C								
Long Year avg.	17.0	21.2	23.2	22.5	18.8	13.7	19.2	13.0
Exp. Year	16.9	20.9	25.5	24.6	20.1	15.0	20.5	13.6

Harvesting proceeded up the stalk as more leaves mature and was completed generally in six harvests. Mature leaves of the plants on a central part of 20 m^2 area of each experimental plot was harvested at each harvesting date, the leaves were fresh weighed, placed into metal boxes and put into curing barns and cured in a typical oven for Virginia tobacco. Following the curing process, the cured leaves obtained from each individual experimental plot were weight and the dry leaf yield was determined. In order to avoid variations in chemical traits of the leaves with the position on a plant stalk, samplings from the lower leaves (1^{st} prime) and upper leaves (5^{th} and 6^{th} prime) were omitted. Composite samples from all commercial fractions of 2^{nd}, 3^{rd} and 4^{th} prime of the experimental year were prepared and used for chemical analyses. The samples were analyzed for nicotine, nitrogen, chloride, potassium and sodium. Nicotine analysis were performed following procedure published by [17],while total nitrogen was analyzed employing the Kjeldahl procedure [18]. All the other analyses were performed following procedures given by [19]. Evaluations of the experimental data were performed following procedures given by [20].

Results and Discussions

Evaluations on the effect of irrigation water amounts and the levels of water stress on nicotine, nitrogen, chloride, sodium and potassium content of cured tobacco leaf, under application of various irrigation programs during the experimental dry year, were supported from results of the regression statistical analysis between seasonal irrigation water amounts and content of chemical traits in tobacco leaves from certain prime of the experimental year (Fig 1,2,3,4, and 5). The summary of the results from regression analysis included in Table 4showed, that seasonal irrigation water amounts and seasonal consumptive water use had significant effect on nicotine, chloride, nitrogen, sodium and potassium content in cured leaves of 2^{nd}, 3^{rd}, 4^{th} and over all of the primes of the dry experimental year. Moreover, all the relationships related to seasonal irrigation water amount and nicotine and/or chloride percentage (Fig 1 and 2) on one hand, and relationships for seasonal water consumptive use and nicotine and/or chloride of all the primes on the other (Table 4), were statistically significant at (p<.01) level.

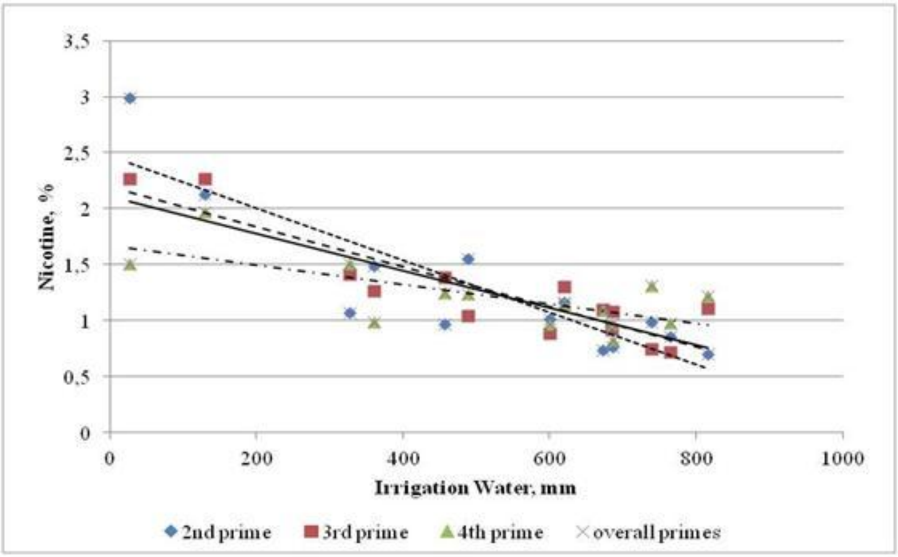

Figure 1: Seasonal irrigation water – nicotine content (%) relationship

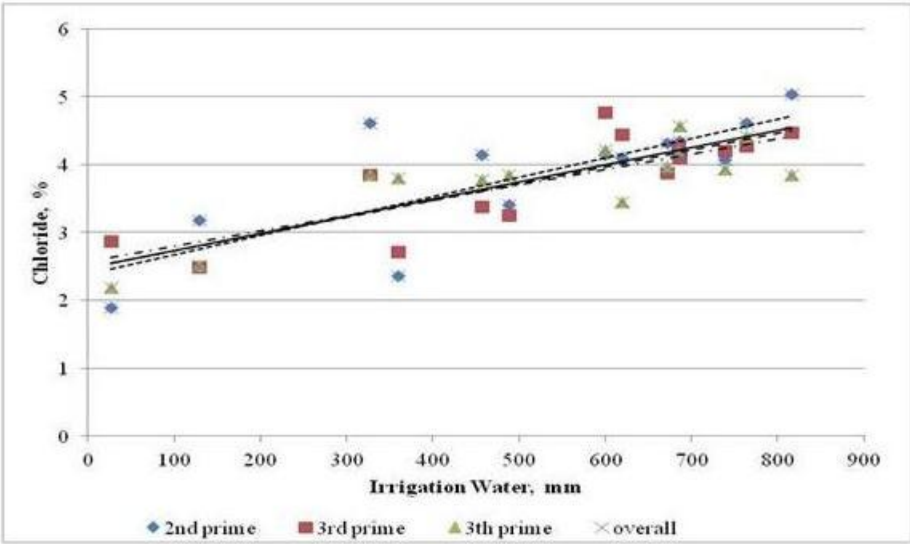

Figure 2: Relationships between seasonal water use and chloride percentage of tobacco leaves

On the other hand, the relationships between seasonal water use and irrigation water quantities and/or nitrogen, sodium and potassium percentage of leaves from 2nd prime, and nitrogen from 3rdprime were significant at p<0.05 level, and potassium in the leaves of 4th prime were not statistically significant.

As could be concluded from Fig 1, nicotine content of the leaves decreases linearly with increase of irrigation water amounts. In addition, slopes of the regression lines related to all primes of the experimental year are similar. Since, seasonal irrigation water is the major component of seasonal evapotranspiration, results of the regression analysis from terms of the relationship between seasonal evapotranspiration and nicotine percentage of tobacco leaf were also statistically significant (p<.01) for all primes of the dry experimental year (detailed data not presented). In addition, the rate of linear decrease is relatively similar in leaves of 2nd, 3rd and overall the primes (Table 4). The regression types and regression coefficients for overall primes were determined as, Y=-0.0017Irr+ 2.109 R= 0.81**, and Y=-0.0019ET+2.447 R= 0.81** for irrigation water and seasonal water consumption, respectively.

Based on the discussed findings it could be said, that irrigation water amount could be used as an effective tool in making efforts to decrease the harmful effect of tobacco products on the health of human being.

Different type of relationships was determined in the case of chloride content of the leaf, which dramatically increases with irrigation water amount increase (Fig 2). The figure shows also that lines of the regressions related to 2nd, 3rd, and 4th primes and that of overall primes almost overlap. The results of the analysis of regression were statistically significant at $p< 0.01$ level for all primes of the experimental year, and the types of the regression equation for overall primes were determined to be Y=0.0029ET+1.994, R=0.78** and Y=0.0025Irr+2.476, R= 0.80**, respectively for relationships of chloride with seasonal consumptive use and irrigation water applied (Table 4).

Our results for linear increase of chloride in tobacco leaves agree with those published earlier. Both chloride uptake and accumulation are often reported to increase linearly over a wide range of chloride concentration in the soil or in water used for irrigation. Some authors [21-22] reported that chloride concentration of dried tobacco leaves increases linearly with increasing level of chloride in the soil. Sifola [23] have studied the effect of saline waters on quality characteristics of Burley tobacco, and determined that leaf accumulates chloride linearly within the rate of application in the ranges of 40.3–5100 kg ha^{-1} and no further accumulation appears beyond those levels due to chloride saturation of the leaf. Other researchers [24] reported that chloride concentrations in the leaves of all primes, had a significant linear response to rates of chloride in irrigation water source. However [25] pointed out earlier, that the increase of chloride concentration of flue cured tobacco leaves is curvilinearly related to the amount of chloride added by irrigation water.

Results of regression analysis between seasonal irrigation water amount and nitrogen or sodium content and of the leaf, showed that linear relationships exist between pointed out parameters for all primes, though statistically significance is not as high as in the cases of nicotine and chloride (Fig 3 and 4).

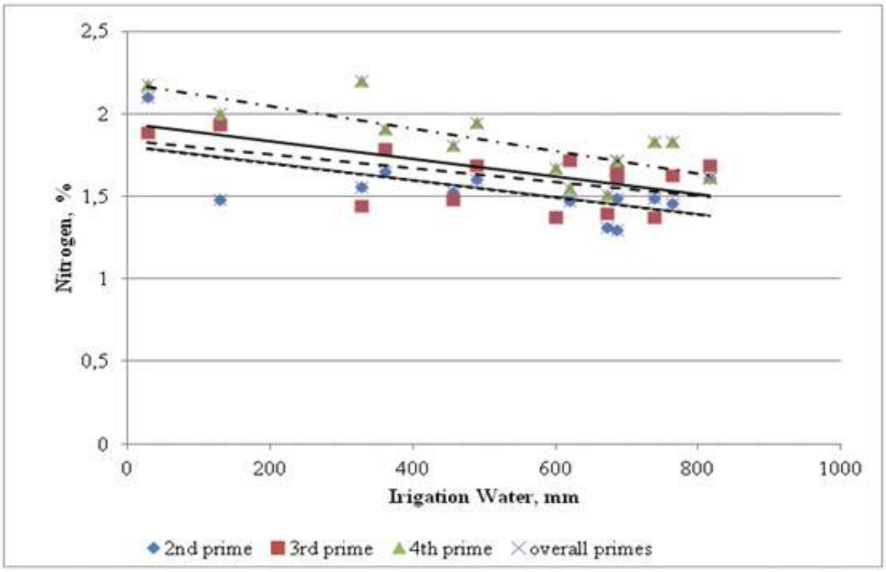

Figure 3: Relationship between seasonal irrigation water and nitrogen content of cured tobacco leaves

While all the relationships concerning the seasonal water amount and nicotine, and/or chloride percentage were statistically significant at ($p<0.01$) level, the relationships between seasonal irrigation water quantities and nitrogen content of leaves from 2nd and 3rd prime of the experimental year were significant at $p<0.05$ level. However, the major part of the relationships between irrigation water amount and nitrogen, and irrigation water and sodium were also statistically significant at ($p<0.01$) level.

Even less statistical evidence was determined in terms of relationships between irrigation water amount and potassium content of tobacco leaf (Fig 5 and Table 4).

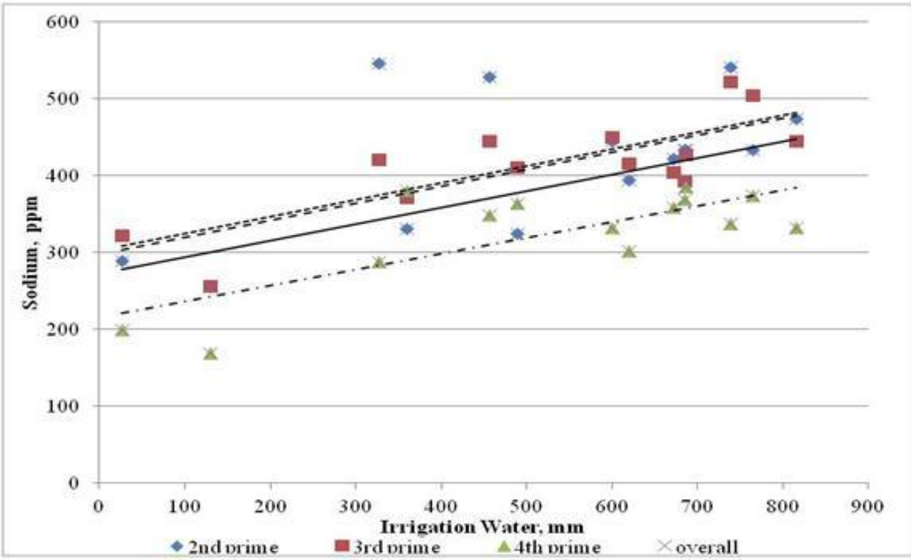

Figure 4: Seasonal irrigation water amount- sodium content relationship

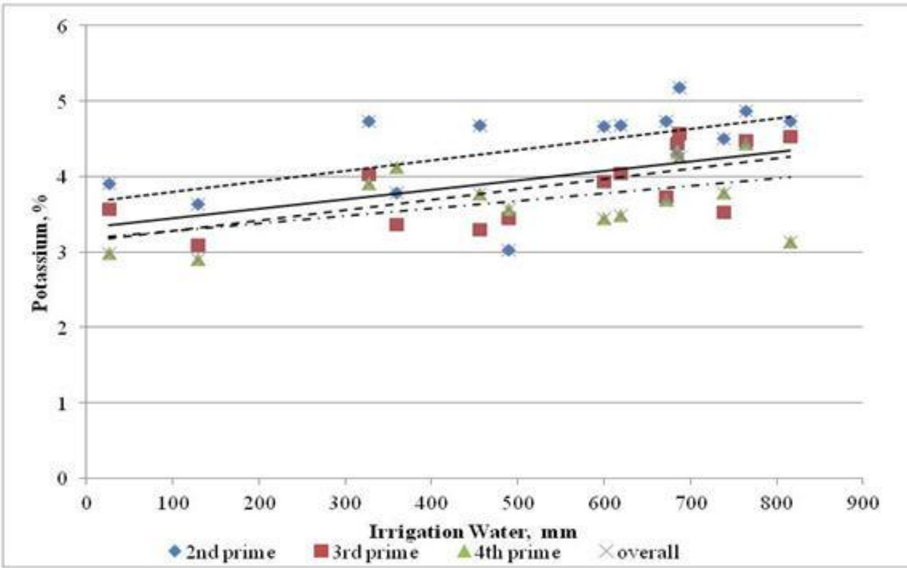

Figure 5: Relationships between irrigation water amount and potassium percentage of cured tobacco leaves

In this study the interactions between nicotine and any of the other chemical traits of cured tobacco leaves were also evaluated (figures not included). Results of the regression analysis (details not included) shows mutual dependency between nicotine and each of the other chemical parameters in cured leaves. Relationships of high significance ($p<0.01$) were determined from nicotine-nitrogen and nitrogen-nicotine regressions for all primes and overall for the year except for 4^{th} prime, where the statistically significance was proved at $p<0.05$ level. Close relationships at $p<0.01$ level for all primes were established between nicotine and chloride, though slopes in the regression graphs related these variables is negative. Negative relationships at $p<0.01$ or $p<0.05$ significance levels were estimated for the relationships between nicotine and potassium, and nicotine and sodium for all primes and overalls. Our results related to nitrogen- nicotine relationship supports the view of [9], that alkaloids interact with nitrogen and increase with nitrogen content. Parker [26]also pointed out recently that total alkaloids increase as nitrogen rate increases up to 112 kg N/ha. Karaivazoglou et al. [24], published previously that the nitrate nitrogen form favoured the leaf nicotine concentration comparably to ammonium nitrogen in both primes. Though other authors [23] reported that, alkaloids did not vary with salinity of the irrigation water and chloride did not influence synthesis of alkaloids in flue-cured tobacco leaves. The decrease of the nicotine content with increasing chloride content, observed in our study can be explained with the view of [27] that chloride affected the nicotine of Burley leaf by decreasing the amounts of nitrogen compounds.

Table 4: Regression Equations and correlation coefficients of the relationships among irrigation water amount and consumptive water use and chemical composition parameters of tobacco leaves under conditions of dry year

Chem. Composition Primes	2nd prime		3rd prime		4th prime		Overall primes	
	Regression equation	**R**	**Regression equation**	**R**	**Regression equation**	**R**	**Regression equation**	**R**
Nicotine	Y=-0.0027ET+2.96	0.92**	Y=-0.0021ET+2.55	0.90**	Y=-0.001ET+1.83	0.68**	Y=-0.0019ET+2.447	0.81**
	Y=-0.0023Irr+2.46	0.88**	Y=-0.0018Irr+ 2.20	0.89**	Y=-0.0009Irr+ 1.67	0.69**	Y=-0.0017Irr+ 2.109	0.81**
Chloride	Y=0.0033ET+ 1.81	0.78**	Y=0.0027ET+2.07	0.78**	Y=0.0026ET+2.10	0.83**	Y=0.0029ET+1.994	0.78**
	Y=0.0029Irr+ 2.38	0.78**	Y=0.0025Irr+ 2.48	0.83**	Y=0.0023Irr+2.57	0.82**	Y=0.0025Irr+2.476	0.80**
Nitrogen	Y=-0.0006ET+1.92	0.65**	Y=-0.0005ET+ 1.92	0.53*	Y=-0.0008ET+2.33	0.79**	Y=-0.0006ET+2.054	0.56**
	Y=-0.0005rr+ 1.80	0.64*	Y=-0.0004Irr+ 1.84	0.54*	Y=-0.0007Irr+2.18	0.77**	Y=-0.0005Irr+1.939	0.55**
Potassium	Y=0.0015ET+ 3.43	0.54*	Y=0.0015ET+ 2.94	0.60*	Y=0.0011ET+ 3.03	0.46ns	Y=0.0014ET+ 3.13	0.46**
	Y=0.0014Irr+ 3.65	0.57*	Y=0.0014Irr+ 3.14	0.66**	Y=0.0010Irr+ 3.18	0.49ns	Y=0.0013Irr+ 3.23	0.50**
Sodium	Y=0.0024ET+ 2.67	0.54*	Y=0.0025ET+ 2.57	0.76**	Y=0.0024ET+ 1.71	0.77**	Y=0.0024ET+ 2.32	0.57**
	Y=0.0022Irr+ 3.02	0.58*	Y=0.0022Irr+ 2.97	0.79**	Y=0.0021Irr+ 2.15	0.76**	Y=0.0022Irr+ 2.71	0.59**

Conclusions

Evaluating the results obtained from study carried out during a year with low precipitation rates, it could be concluded that irrigation application or water stress imposed at various stages of growth, markedly influenced the chemical traits of te curied tobacco leaf.

Close linear relationships were determined between the seasonal irrigation water amount or seasonal consumptive useand the chemical traits content as nicotine, chloride, nitrogen, sodium and potassium in the cured leaves of tobacco plant. Mutual relationships of high significance between nicotine and each of the traits i.e. nitrogen, chloride, potassium, and sodium, were also determined.

The results of the study related to values of the chemical traits under conditions of various soil moisture levels and water use by plant, could be succesfully used as an effective toolfor moderating leaf nicotine content, and may provide a more desirable tobacco leaf chemistryreducing harmful effects of this alkaloid on the health of smokers.

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