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Interaction effect of primings and tobacco genotypes on some chemical constituents

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Abstract

The study was conducted to estimate the percentage of reducing sugar, nicotine content, total nitrogen, total ash and volatile oil, potassium, chloride content and also C/N ratio in the leaves of different priming of oriental tobacco (*Nicotiana tabacum* L.). The study included leaves from three priming of five oriental tobacco genotypes, namely; Gullsur, Gullspi, Madara, Pazarjik, and Bulgari which were grown in Zrguz Tobacco Station in Sulaimani, during the summer season of 2010. The product was stored in the warehouse for a long time to induce natural fermentation and aging. The factorial experiment was laid out according to Completely Randomized Design, with three replicates. Tobacco leaves from lower, middle and upper primings were prepared to study the difference of genotypes, primings, and their combinations in some chemical constituents of leaves.
Statistical analysis showed that there were highly significant differences among genotypes for all studied chemical constituents with the exception of total alkaloids as nicotine and chloride content. Also, the results indicated that there were highly significant differences between the primings regarding the studied chemical constituents except for the CN ratio and chloride content. The analysis also indicated that a combination between genotypes and primings were showed highly significant differences regarding all studied chemical constituents with the exception of total nitrogen and CN ratio, while the difference between the combination in chloride was significant.

Introduction

Tobacco ***Nicotiana tabacum*** L. belongs to the nightshade family Solanaceae. It is one of the most widely grown commercial nonfood crops in the world [1]. Tobacco is an economically important crop, cultivated all over the world especially in China, India, Brazil and USA. It is produced in at least 120 countries by approximately 33 million farmers [2], primarily between latitudes 45º N and 30º S [3]. Tobacco is one of the most important industrial crops that are used after leaves cured and fermented in making cigarettes, cigar, chewing tobacco, pipe tobacco, snuff and water pipe tobacco, and its leaves contain alkaline substances including nicotine and its related compounds. The genus *Nicotiana* has been divided into three subgenera (*Tabacum, Rustica and Petunioides*) containing more than 64 recognized species [4] and [5]. There are many tobacco varieties and landraces and the number of new genotypes in the world steadily increases. The cultivation of tobacco was introduced into Iraqi-Kurdistan Region during the early seventeenth century, which introduced from Europe through Empire Turkey [6].

The grading system of oriental tobacco leaf quality was used to consist of five grades, excellent (usually 1.0% of the total production). The first grade (22.0%), the second grade (53.0%), the third grade (21.0%), and finally the fourth grade of the lowest quality (3.0%). These grades are identified by certain visual physical properties such as leaf size, thickness, texture, and color conducted by tobacco experts who implemented their expertise in the tobacco monopoly official market grading [7]. Leaf qualities of tobacco for all tobacco types were known by their physical criteria and chemical constituents. These physical criteria include thickness, leaf area, shapes of the leaf, leaf position, color of the leaf, filling values, and the properties of burning. Chemical constituents include reducing sugars, carbohydrates, total nitrogen, total alkaloids, nicotine, C/N ratios, and other minor constituents. Nicotine is an important quality parameter for tobacco and it needs to be neither high nor low. In a study, researcher confirmed that high nicotine content causes hardness and bitterness to taste and low nicotine content causes weak taste and physiological nonsatisfaction [8]. Furthermore, of their physical criteria and chemical constituents of oriental tobacco, leaf qualities of oriental tobacco were recognized by their essential oil contents. The essential oils of the oriental tobacco leaves were recognized to be the most important constituent, whereas leaf quality and grades evaluated and priced in the world markets internationally depending on its essential oils [9]. There is an enormous amount of scientific information about the chemical composition of different types, varieties, and genotypes. To date, a vast number of bioactive compounds have been isolated from tobacco under different geographical coordinates [10] and [11]. On the other hand, the presence of tobacco alkaloids, together with the other biologically active compounds in the extracts creates grounds for their possible use in specific cosmeceuticals, in bio-pharmacy and in agriculture (insecticides, semi chemicals), etc. [12].

The aim of the study was to evaluate and investigate the chemical constituents of five oriental tobacco which were grown, cured and fermented in Zrguz, Sulaimani Governorate, and describe its constituents under the effect of primings and under the effect of the interaction between genotypes and primings.

Materials and Methods

Two local oriental tobacco genotypes "Gullsur, Gullspi, and three introduced genotype of oriental tobacco Madara, Pazarjik, and Bulgari" were implemented in this study. These genotypes of tobacco were cultivated at Zrguz Tobacco Station which belongs to Tobacco Cultivation and Development Center in Sulaimani- Ministry of Agriculture and Water Resources, during the summer season of 2010. After curing the product was stored in the warehouse for a long time to induce natural fermentation and aging. Sugars like glucose, fructose and sucrose, are natural components of tobacco after the curing process [13] and [14]. Curing process results in sugar levels of over 20% (flue-cured, like Virginia) and 10% (sun-cured, like Oriental) weight percent of dried tobacco, respectively, [13], [14] and [15]. An adequate amount of fermented tobacco leaves from lower, middle and upper primings was prepared for this study from those genotypes. To perform the chemical analysis for the samples of primings, the leaves from those primings were initially moistened and modified, for treating and conditioning tobacco leaves. The (Lower, Middle, and Upper) leaves of those oriental tobaccos were used as samples to determine chemical constituents [16]. And the different chemical analysis was performed according to their procedures. The chemical constituents of those different primings determine the special criteria and quality specifications of the tobacco genotypes. In addition, the evaluation in terms of their essential oil contents has occurred thus, the benchmark of the essential oil contents characterizes oriental tobacco quality. The studied characters included chemical constituents of the lower, middle, and upper leaves which were:

Percentage of reducing sugar was evaluated according to Joslyn method [17]. Percentage of total alkaloids as nicotine was determined according to Liquid Chromatography HPLC [18]. Percentage of total nitrogen was determined by using Micro-kijeldale method [19]. C/N ratio can be calculated by dividing total soluble carbohydrate by nitrogen content of tobacco leaf in percentage, accordingly, the high molecule weight carbohydrates such as starch should be degraded during the curing process into soluble reducing sugar and degradation of protein into simple amino acid to an account of C/N ratio [9]. The percentage of total ash was estimated by weighing 2 g of crushed leaf dry sample, put in a tarred crucible. Then transferred it in a muffle furnace for 6 hours at 600 ˚C until free from all carbonaceous materials and ash became white or grayish-white to determining the percentage of total ash [20]. And for determination of Volatile oil, weighed 20 g of the dry crushed sample, put in a beaker, and added (100 ml) of distilled water and placed it in the Clevenger device for 3 hours. 20 ml of hexane were added to the collected oil and separate the oil from the water droplets, this mean percentage of Volatile oil determined according to techniques for the extraction of essential oils by hydro distillation using the Clevenger apparatus [21]. Potassium was determined by weighing 1 g of dry, ground tobacco leaves in a 50 ml porcelain crucible, then place porcelain crucible in a cool muffle furnace, and increasing temperature gradually to 400 °C, for 6 hours, after the sample cooled, some drops of concentrated nitric acid (HNO3) were added into the crucible to moist the ashing sample and put it on the hotplate until the nitric acid evaporated. After several stages the percentage of potassium calculated as follows: potassium (ppm) = ppm micronutrients from calibration curve × (volume / weight of sample) % = ppm/10000, according to [22], and chloride content were also determined using %5 potassium chromate (K2CrO4) indicator and the solution was titrated by silver nitrate (AgNO3) method [23] and [24].

Statistical Analysis

The factorial experiment with two factors was laid out (the first factor was genotypes and the second factor were primings) according to Completely Randomized Design, with three replicates. The recorded data subjected to analysis of variance to determine significant differences among treatments and interactions, all data were statistically analyzed under 5% and 1% significant levels according to the methods of analysis of variance (ANOVA). Least significant differences (L.S.D.) under 5% and 1% significant levels were used to compare between mean treatments and treatment combinations [25]. All the references for the study were arranged and organized by Mendeley software.

Results and discussion

A. The effect of genotypes on some chemical constituents:

Percentage of reducing sugar: The content of reducing sugar is considered to be one of the positive quality characters of tobacco leaves, during smoking, which produces a smooth smoke sensation, desirable aroma, and smell without irritation. The sugar content of tobacco leaf related to the color of the leaf, as the moderate percentage of sugar content in oriental tobacco, gives it a bright red color as a physical characteristic. But the color of the leaf tobacco does not determine quality alone. Tobacco leaves with light color as a general rule are mild tobacco, and others with dark color are strong tobacco. Table 1 and 2, indicate the presence of highly significant differences among the genotypes for percentage of reducing sugar constituent. Among the genotypes, Gullsur gave the maximum value of percentage reducing sugar with 8.76%, followed by the values of 7.53% and 6.77% which recorded by Pazarjik and Gullspi respectively, while Bulgari exhibited the minimum value of percentage reducing sugar with 5.28%. Higher content of reducing sugars desirable as it imparts to the smoke an acidic character, lower content imparts alkalinity to smoke due to high nitrogenous constituents. In a study "Phytochemicals in leaves and extracts of the variety of Bulgarian oriental tobacco" the optimal range of the basic chemical content (reducing sugar) of cured leaves ranged between 10-14% [26]. Sugar compounds provide softness in smoking tobacco and accepted as affecting quality positively [8], [9] and [27]. It is recommended that high sugar content and low total ash as a general are required for tobacco quality [28].

Percentage of total alkaloid as nicotine: Nicotine, which is the predominant constituent of the bases contained in tobacco leaf. The percentage of total alkaloids is very important because it is an essential factor in leaf quality which affects and provides a physiological stimulus that makes the use of tobacco products pleasurable [29]. There were no significant differences among genotypes for the character total alkaloids as nicotine (Table 1 and 2). The percentage nicotine values for the genotypes restricted between (2.18- 2.44%). In a study on phytochemicals, the optimal range of the chemical constituent of total alkaloids as nicotine of the cured leaves ranged between 1.0-1.2% and reached 2.3% [26], this result agrees with the current study. Nicotine content of tobaccos that are produced in Zrguz is found to be higher than the normal levels, this may be due to their soil structure or it may be due to that, plant has to struggle to reach water and nutrients due to the village's non-productive soils, this means that plant has to develop a strong root structure, and nicotine are produced in roots [9] and [30].

Percentage of total nitrogen: Generally, high levels of the percentage of total nitrogen appear to affect the quality of tobacco leaf and contribute negatively to smoke and flavor [16]. Highly significant differences were found among the genotypes in percentage of total nitrogen (Table 1 and 2). The results of (Table 2) detected that the genotype Madara exceeded all other genotypes in the percentage of total nitrogen with 2.35%, followed by 2.25 and 1.95% recorded by Bulgari and Gullspi respectively. Moreover, the minimum value of percentage of total nitrogen was 1.83% exhibited by Pazarjik. According to the data obtained from the research in two years, total nitrogen values were determined between 0.88-2.72% [14]. In a study, the optimal value of the total nitrogen of cured leaves was 1.7% [26]. This result is similar to the current work.

C/N ratio: C/N is the ratio between positive compounds as reducing sugar to negative compounds as (nitrogen-containing compounds), it is generally used as a basis for evaluation of tobacco leaves and evaluation related to strength or smoothness of the tobacco products [9]. Data represented in (Table 1 and 2) confirmed the presence of highly significant differences among genotypes for the character C/N ratio. Among the genotypes, Gullsur recorded the maximum value of C/N ratio with 4.64, followed by the values of 4.06 and 3.60 which exhibited by Pazarjik and Gullspi respectively, while Bulgari recorded the minimum value of C/N ratio with 2.35. As a sensory trait, a high ratio of C/N may tend to indicate mildness and smoothness while a very low ratio may be indicative of harsh and strong smoke. During smoking, sugars react with amines to yield brown-colored Maillard reaction products that improve the taste of tobacco smoke [31], [32] and [33]. In tobacco, Maillard reactions result in 1.5‑2.0% w/w, amino-sugar compounds [14] and [33].

Table-1: Mean squares of variance analysis for some chemical constituents of five Oriental tobaccos

Genotypes	***Genotypes***	***Primings***	***Genotypes X Primings***	***Error***
D.f	***4***	***2***	***8***	***30***
%Reducing Sugar	*14.612***	*9.489***	*1.564***	*0.431*
%Total Alkaloids as Nicotine	*0.103N.S*	*7.597***	*1.202***	*0.177*
%Total Nitrogen	*0.474***	*0.237***	*0.071N.S*	*0.033*
C/N Ratio	*7.582***	*0.586N.S*	*0.317N.S*	*0.407*
%Total Ash	*6.403***	*36.145***	*5.639***	*1.302*
%Volatile Oil	*0.156***	*1.035***	*0.173***	*0.011*
%Potassium	*0.071***	*0.634***	*0.133***	*0.005*
%Chloride	*0.029N.S*	*0.044N.S*	*0.046**	*0.020*

N.S not Significant   ** Significant*   *** Highly Significant*

Percentage of total Ash: In general, the percentage of total ash is believed to have effects on tobacco leaf quality because total ash represents the number and amount of minerals in leaves and its effect on smoking, which causes a smooth smoke sensation or produces irritation. Data in (Table 1 and 2) have shown highly significant differences between genotypes for the character percentage of total ash. The results in Table 2, indicated that among genotypes, Bulgari recorded the maximum value of total ash with 22.20%, followed by the values of 21.94% and 20.85% which recorded by Gullspi and Madara respectively, while the minimum value of the percentage total ash was 20.20% exhibited by the genotype Pazarjik.

Table- 2: The effect of five tobacco varieties (genotypes) on some chemical constituents

Genotypes	***Some Chemical Constituents***							
	%Reducing Sugar	***%Total Alkaloids as Nicotine***	***%Total Nitrogen***	***C/N Ratio***	***%Total Ash***	***%Volatile Oil***	***% Potassium***	***% Chloride***
Gullspi	*6.77*	*2.22*	*1.95*	*3.60*	*21.94*	*2.26*	*2.28*	*0.803*
Gullsur	*8.76*	*2.44*	*1.91*	*4.64*	*20.78*	*2.19*	*2.18*	*0.925*
Madara	*6.70*	*2.32*	*2.35*	*2.84*	*20.85*	*2.20*	*2.18*	*0.813*
PazarjiK	*7.53*	*2.23*	*1.83*	*4.06*	*20.20*	*2.16*	*2.09*	*0.877*
Bulgari	*5.28*	*2.18*	*2.25*	*2.35*	*22.20*	*1.92*	*2.31*	*0.915*
$L.S.D_{0.05}$	***0.63***	***N.S***	***0.175***	***0.614***	***1.10***	***0.102***	***0.070***	***N.S***
$L.S.D_{0.01}$	***0.85***	***N.S***	***0.236***	***0.827***	***1.48***	***0.138***	***0.094***	***N.S***

In a study entitled "Influence of soil properties on yield and quality of tobacco plant in Akhisar region of Turkey". total alkaloid (nicotine), total reducing sugar, total nitrogen, and raw ash were determined as 0.126-

1.410%, 7.81-33.71%, 0.45-3.24 %, 8.49-30.01%, respectively [16] and [28], these results were in agreement with the current study.

Percentage of Volatile Oil: Volatile oils or Essential oils are highly concentrated oils extracted from various parts of plants. The aroma of tobacco essential oil is similar to that of the tobacco leaf and has an effect on the tobacco smoke. Oriental tobacco is known for its small leaf, as a physical property, with a distinct flavor and aroma. Data represented in (Table 1 and 2) confirmed the presence of highly significant differences among genotypes for the character percentage of volatile oils. Results in Table 2, showed that among genotypes, Gullspi with 2.26% volatile oil gave the maximum value, followed by the values of 2.20% and 2.19% which recorded by Madara and Gullsur respectively, while Bulgari exhibited the minimum value of percentage volatile oils with 1.92%. The results about the reducing sugar, total nicotine, total nitrogen, C/N ratio, total ash and volatile oil of the five oriental tobacco, comply with the conclusions of a number of researchers, that the particular individual compounds in tobacco, depend mainly on the tobacco type, environmental conditions, season, curing process, storage, and other factors[14], [34], [35] and [36].

***Potassium content*:** Potassium content is believed to have positive effects on tobacco leaf quality, especially during smoking, which affects the rate of burning. Data represented in (Table 1 and 2) confirmed the presence of highly significant differences among genotypes for the character percentage potassium content. Results in Table 2, showed that among genotypes, Bulgari with 2.31% of potassium content gave the maximum value, followed by values of 2.28 and 2.18% which recorded by Gullspi and Gullsur respectively, while Pazarjik exhibited the minimum value of percentage potassium content with 2.09%.

Combustibility or burning quality of tobacco is an important physical characteristic that involves several criteria like fire holding capacity, rate of burn, completeness of burn, and character of residual ash. Combustibility is varying with different types of tobacco and more factors affecting combustibility such as physical and chemical in nature. Among the chemical characteristics, the good burn is always exhibited by leaf containing high potassium and low chloride. The researchers reported that the constituents of chloride, reducing sugars, nitrogen, nicotine, and potassium contents of leaves increased with increasing KCl [37]. The preferred source of potassium is potassium sulfate but the cost of fertilizer attracts tobacco growers to use the cheaper muriate of potash source (potassium chloride, KCl used as a fertilizer- not in scientific use) [38].

***Chloride content*:** Chloride is one of an essential micronutrient, and there is evidence that the beneficial effects of chloride arise with tobacco from the presence of small amounts of this element in the fertilizer [39]. Data in (Table 1 and 2) showed that there were no significant differences among genotypes for the character percentage chloride content. These results of chemical constituents of the five oriental tobacco varieties have also concurred in comparison with data from a field experiment by [38] and [40]. In a scientific study, with the increase of chloride application rates, the Cl content in leaves from the first, second, and third priming was significantly increased [41]. In other study, the researcher found significant reductions in price, quality index, and leaf burning at above 0.53% of chloride in the cured Maryland tobacco [42].

B. The effect of primings on some chemical constituents:

As the physical properties of oriental tobacco, it had been known that length, width, and area of leaves become smaller as their position moves up the stalk [29]. Data in (Table 1 and 3) indicated that there were highly significant effects due to primings on all studied chemical constituents with the exception of CN ratio and percentage chloride content. Results in Table 3, showed that the lower leaves recorded the maximum values of percentage total alkaloids as nicotine, percentage total ash and potassium content with 2.94, 22.92 and 2.33% respectively, while the same leaves exhibited the minimum values of percentage reducing sugar, total nitrogen and percentage volatile oil with 6.22, 1.92 and 1.87% respectively. The upper leaves recorded the maximum values of percentage reducing sugar, percentage total nitrogen and percentage volatile oil with 7.81, 2.17 and 2.39% respectively, while the same leaves exhibited the minimum values of percentage total alkaloids as nicotine, percentage of total ash and potassium content with 1.53, 19.90 and 1.97% respectively.

In a study, the highest concentration of chloride is found in the lower leaves and it decreases progressively to the top of the plant [43]. The results of this study disagree with the result of the chloride content of current research. In other investigation, the variation in Cl contents in the second and third primings was not related to leaves' stalk position, which could be explained by the fact that Cl^- mobile within the plant [41].

Table- 3: The effect of primings on some chemical constituents

Primings	Some Chemical Constituents							
	%Reducing Sugar	%Total Alkaloids as Nicotine	%Total Nitrogen	C/N Ratio	%Total Ash	%Volatile Oil	% Potassium	% Chloride
Lower leaf	6.22	2.94	1.92	3.30	22.92	1.87	2.33	0.825
Middle leaf	7.00	2.37	2.07	3.51	20.78	2.18	2.32	0.846
Upper leaf	7.81	1.53	2.17	3.69	19.90	2.39	1.97	0.928
$L.S.D_{0.05}$	**0.49**	**0.314**	**0.136**	**N.S**	**0.85**	**0.079**	**0.054**	**N.S**
$L.S.D_{0.01}$	**0.66**	**0.423**	**0.183**	**N.S**	**1.15**	**0.107**	**0.073**	**N.S**

C. The effect of interaction between genotypes and primings on some chemical constituents:

Data in (Table 1) indicated that the effect of interaction between genotypes and primings was highly significant on all studied chemical constituents with the exception of total nitrogen and CN ratio, while the percentage chloride content showed only significant effect.

Percentage of reducing sugar: Results in Table 4, showed that the interaction between Gullsur and middle leaf revealed the maximum value of reducing sugar with 9.20% followed by the values of 8.91 and 8.61% which recorded by the interaction between Gullsur and upper leaf and Pazarjik with upper leaf respectively. While the interaction between Bulgari and lower leaf recorded the minimum value of reducing sugar with 4.71%. In a chemical study, some researchers suggested that sugars promote tobacco smoking, because they generate acids that neutralize the harsh taste and throat impact of tobacco smoke, moreover, the sweet taste and the agreeable smell of caramelized sugar flavors are appreciated in particular by smokers [44]. Sugar content less than % 8-10 in Virginia and Oriental tobaccos are considered inadequate in terms of quality [28].

Percentage of total alkaloid as nicotine: Data in Table 4, indicated that the interaction between Gullspi and lower leaf exhibited the maximum value of total alkaloid as nicotine with 3.67% followed by the values of 3.09 and 3.00% which showed by the interaction between Madara and lower leaf and Gullsur with middle leaf respectively. While the interaction between Gullspi and middle leaf recorded the minimum value of total alkaloid as nicotine with 1.16%. Nicotine is an important quality parameter for tobacco and it needs to be neither high nor low. The values of total alkaloid as nicotine for the genotypes in our study restricted between (1.16- 3.67%), these values adjust with a nicotine content of oriental tobaccos [3] and [30]. Nicotine as a chemical compound is one of the major representatives of the alkaloids contained in tobacco plant and an important indicator of quality of tobacco raw [42]. In the tested varieties from type Prilep in 2014, the content of nicotine was slightly lower and it is from 0.88% in the variety NS-72 to 0.94% in the control variety P-23 [45].

Percentage of total nitrogen: Data in Table 4, indicated that the interaction between Madara and upper leaf exhibited the maximum value of total nitrogen with 2.45%, while the interaction between Pazarjik and lower leaf recorded the minimum value of total nitrogen with 1.57%, although there were no significant differences between the interactions. According to Uzunoski, total nitrogen content increases from lower to the upper insertions and varies depending on the conditions of growing and the type of tobacco [46]. The results of our study concurred with the result of this study.

C/N ratio: Results in Table 4, showed that the interaction between Gullsur and upper leaf showed the maximum value of C/N ratio with 5.03%, while the interaction between Bulgari and lower leaf recorded the minimum value of C/N ratio with 2.24%, although, no significant differences were found between the interactions.

Percentage of total Ash: The results in Table 4, showed that the interaction between Bulgari and lower leaf recorded the maximum value of total ash with 24.56% followed by the values of 23.54 and 23.24% recorded by the interaction between Gullspi and lower leaf and the interaction between Pazarjik and lower leaf respectively. While the interaction between Pazarjik and upper leaf exhibited the minimum value of total ash with 17.15%.

Percentage of Volatile Oil: Results in Table 4, showed that the interaction between Gullsur and upper leaf exhibited the maximum value of percentage volatile oil with 2.43%, followed by the values of 2.41% and 2.35% which recorded by the interaction between Gullspi and upper leaf and Madara with upper leaf respectively. While the interaction between Bulgari and lower leaf recorded the minimum value of percentage volatile oil with 1.16%.

***Percentage of Potassium content*:** Data represented in (Table 4) confirmed that the interaction between Gullsur and lower leaf recorded the maximum value of potassium content with 2.53% followed by the values of 2.50 and 2.44% recorded by the interaction between Bulgari and lower leaf and the interaction between Pazarjik and middle leaf respectively. While the interaction between Madara and upper leaf and between Pazarjik and lower leaf recorded the same value of 1.84%, which exhibited the minimum value of percentage of potassium content. The researchers showed that potassium and chloride concentrations are much greater in stems than in lamina of the leaves, but the level of total alkaloids is much higher in lamina than in stems [47]. In the same study, it was assumed that during combustion the organic materials are burned and the inorganic materials such as calcium, potassium and magnesium salts, inorganic phosphates, chlorides, and sulfates, and silica remain as ash [47].

Table- 4: The effect of interactions between tobacco varieties and primings on some chemical constituents

Genotypes	*Primings*	*Some Chemical Constituents*							
		% Reducing Sugar	*% Total Alkaloids as Nicotine*	*% Total Nitrogen*	*C/N Ratio*	*% Total Ash*	*% Volatile Oil*	*% Potassium*	*% Chloride*
Gullspi	***Lower Leaf***	*6.21*	*3.67*	*1.85*	*3.36*	*23.54*	*2.19*	*2.38*	*0.805*
	Middle Leaf	*6.47*	*1.16*	*1.82*	*3.88*	*20.06*	*2.19*	*2.35*	*0.819*
	Upper Leaf	*7.63*	*1.83*	*2.17*	*3.56*	*22.22*	*2.41*	*2.11*	*0.786*
Gullsur	***Lower Leaf***	*8.18*	*2.95*	*1.85*	*4.41*	*20.97*	*1.95*	*2.53*	*0.994*
	Middle Leaf	*9.20*	*3.00*	*2.06*	*4.47*	*21.57*	*2.19*	*2.08*	*0.805*
	Upper Leaf	*8.91*	*1.38*	*1.82*	*5.03*	*19.81*	*2.43*	*1.93*	*0.975*
Madara	***Lower Leaf***	*6.21*	*3.09*	*2.24*	*2.78*	*22.27*	*2.07*	*2.41*	*0.738*
	Middle Leaf	*5.67*	*2.40*	*2.37*	*2.39*	*21.01*	*2.17*	*2.29*	*0.691*
	Upper Leaf	*8.22*	*1.48*	*2.45*	*3.36*	*19.29*	*2.35*	*1.84*	*1.008*
PazarjiK	***Lower Leaf***	*5.78*	*2.37*	*1.57*	*3.69*	*23.24*	*1.97*	*1.84*	*0.861*
	Middle Leaf	*8.20*	*2.96*	*1.75*	*4.51*	*20.21*	*2.15*	*2.44*	*0.828*
	Upper Leaf	*8.61*	*1.34*	*2.16*	*3.99*	*17.15*	*2.35*	*1.99*	*0.942*
Bulgari	***Lower Leaf***	*4.71*	*2.62*	*2.11*	*2.24*	*24.56*	*1.16*	*2.50*	*0.729*
	Middle Leaf	*5.45*	*2.31*	*2.37*	*2.30*	*21.03*	*2.19*	*2.44*	*1.089*
	Upper Leaf	*5.67*	*1.60*	*2.27*	*2.51*	*21.02*	*2.41*	*1.99*	*0.928*
L.S.D$_{0.05}$		***1.10***	***0.703***	***N.S***	***N.S***	***1.90***	***0.177***	***0.121***	***0.235***
L.S.D$_{0.01}$		***1.47***	***0.946***	***N.S***	***N.S***	***2.56***	***0.238***	***0.163***	***N.S***

***Percentage of Chloride content*:** As it has shown in (Table 1 and 4) only significant effects was found due to the interaction between genotypes and primings on percentage chloride content. Data in (Table 4) showed that the interaction between Madara and upper leaf recorded the maximum value of chloride content with

1.008% followed by the values of 0.994 and 0.975%, which recorded by the interaction between Gullsur and lower leaf and the interaction between Gullsur and upper leaf respectively. While the interaction between Madara and middle leaf recorded the minimum value of percentage of chloride content with the value of 0.691%. It has shown in Table. 4 that most values of chloride content were less than 1%. The researcher showed that chloride concentrations in excess of 1% can produce poor quality tobacco [43]. In another investigation, the researchers informed that average reducing sugar, nicotine, total nitrogen and chloride contents of Aegean Region tobaccos are 21.76 %, 0.66 %, 1.40 % and 0.48 %, respectively [48]. The result of the chemical content of reducing sugar and nicotine do not agree in comparison with the data of our research. In a scientific study, statistically significant linear dependence was noted between chloride treatments and the concentration of chloride in the leaf tissues [41].

Conclusion

In this study, some results were achieved as indicated above for reducing sugar, total nicotine, total nitrogen, total ash, volatile oil, and C/N ratio, with potassium, and chloride content. The study indicated that the local genotype Gullsur exhibited the maximum values of percentage reducing sugar and CN ratio, while the introduced genotype Bulgari recorded the maximum values of percentage total ash and Potassium content. The results showed that the minimum values of percentage reducing sugar, CN ratio and percentage volatile oil exhibited by the introduced genotype Bulgari. The results of the study indicated that the lower leaves recorded the maximum values of percentage total alkaloid as nicotine, total ash and percentage potassium, and also recorded the minimum values of percentage reducing sugar, total nitrogen and percentage volatile oil. The results of interactions revealed that the interactions between Gullsur and the upper leaves exhibited the maximum values of CN ratio and percentage volatile oil.

Recommendation

More study is needed to understand the chemical constituents of the local genotypes Gullsur and Gullspi as well as to study the introduced genotypes. However, further studies must be carried out in the future to determine the relationships between chemical constituents and physical properties and between soil properties, environment and yield and the quality of tobacco. It can be possible to improve the yield and quality of tobacco using these relationships. The studies on different genotypes of tobacco and its constituents could be important to its appropriate industrial use for different blends of tobacco and in manufacturing cigarettes.

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