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RESEARCH ARTICLE

Effect of organic fertilization on soil characteristics, yield and quality of Virginia tobacco in Mediterranean area

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ABSTRACT

As organic tobacco is a consumer choice, it is necessary to explore the proper fertilization program with environmentally friendly practices. A field experiment lasting 3 growing seasons established at the Agricultural University of Athens in order to examined the effect of comparison between organic and inorganic fertilization on (flue-cured) Virginia tobacco plants (*Nicotiana tabacum* L.) and soil. A randomized complete block design with four treatments (manure, compost, NPK and control) and three replications was set up. The results of analysis of variance for treatments showed that the organic matter of soil did not affected by the fertilization while $CaCO_3$ significantly affected by fertilization. The N concentration in soil does not significantly affected by fertilization. Plants height was higher under the inorganic fertilization in comparison to that of the organic fertilization treatments. The final yield was affected by year and it is higher in control treatment with highest value 7,053.42 kg ha^{-1} The fertilization does not significant affect the uptake N leaves was affected by fertilization and was higher under inorganic fertilizer, ranged from 2.73 to 0.88%. The nicotine and sugar on Virginia tobacco were not significant affected by fertilization. A strong correlation was observed between yield and reducing sugars (r = 0.66). The results of the current study revealed that the organic fertilization affected certain soil characteristics and not the quality characteristics of tobacco.

Keywords: Virginia; Organic fertilizers; N leaves uptake; Nicotine

INTRODUCTION

Although tobacco (*Nicotiana tabacum L.*) belongs to the category of industrial plants, it is directly related to human health, as it is used by inhaling the burning of leaves. This makes it necessary to produce a better quality product, free of toxic pesticide residues, in order to create a "clean" end product, reducing the harmful effects of smoking on consumer health. Virginia tobacco is one of the most important crops in Greece (Karaivazoglou at al., 2007). According to the cultivated area, the "oriental" type of tobacco is cultivated first and second type is Virginia with 30% of the Greek production (Bilalis et al. 2009). In Greece, Virginia leaves are collected by hand harvesting in 4–5 primings (Bilalis et al., 2009).

Tobacco yield is affected by climatic conditions and growing methods. Yield and root system of Virginia tobacco significant increased with green manure (Bilalis et al., 2009). Calcium supplementation is necessary for acidic soils where tobacco is grown (Karaivazoglou et al., 2007).

Crop productivity and quality are affected by the availability of macro and micronutrients in the soil. In terms of tobacco, its quality is a set of properties of the leaf, in terms of chemical composition it is the concentration of N, nicotine, reduction of sugar concentration, mineral components and ash content (Mendell et al., 1984). For high yield and smoke quality N is a required element (Kaiser et al. 2010). Regarding the application of N to tobacco, nitrate is recommended in contrast to ammonium which reduces yields and leaf quality (Cao et al., 1992). In addition, the nicotine composition depends directly on the form of nitrogen applied (Shang et al., 2017). Nitrogen must be available until flowering, otherwise the deficiency of nitrogen at this time is likely to lead to reduced yields. (Marchetti et al., 2006). Adequate amount of N

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in the soil can lead to premature yellowing of the leaves (Karaivazoglou at al., 2005). On the contrary Karaivazoglou at al. 2007 noted that tobacco growth does not depend on the form of nitrogen fertilizer. The reduction and accumulation of nicotine in tobacco is affected by applied N. Furthermore, the addition of K increased the N content in the leaves (Sabeti et al., 2013). But you need to pay attention to the amount of application of N, it must be done very specifically because the extravagant supply creates problems that prevent mechanical harvesting. (Hawks et al., 1983).

Organic agriculture is based on the soil and its quality. Soil quality is affected by both fertilization and agricultural practices such as tillage, crop residue management (Bilalis et al., 2005). The application of fertilizers, whether organic or inorganic, affects the physical properties of the soil, such as water retention and porocity, chemical and biological (Zhang et al., 2007 ;Singh Brar et al., 2015). The organic matter of the soil is directly affected by fertilization. Bilalis et al (2005) noticed there was more organic matter in the soil by adding organic fertilizer than inorganic fertilizer .,. Soil productivity increases significantly with increasing organic matter in the soil. Following the soil quality, one factor is $CaCO_3$, which dissolves with the addition of N (Gandois et al., 2011).

Organic tobacco cultivation is not particularly explored, and demand has been rising in recent years. Nicotine levels increase with green manure farming practice, which is a classic and important intervention for organic farming (Bilalis et al., 2009). Improving the quality of tobacco and reducing the nicotine content was restored by adding organic fertilizer containing *Phanerochaete chrysosporium* and *Bacillus thuringiensis* instead of pure rapeseed cake (Shang et al., 2017). Also, studies show that excessive application of nitrogen reduces the quality because the stem hardens, the color darkens and the strip yield decreases, therefore its commercial value decreases greatly. (Marchetti et al., 2006).

One of the main concerns in tobacco production, promotion and industry is the health of the smoker. Investigating this concern requires information that will set up new agricultural techniques that will allow for better quality tobacco production, free of any toxic agrochemical residue. Several aspects of traditional tobacco techniques need to be improved, and the traditional plant protection products used need to be replaced with more environmentally friendly and biodegradable substances to ensure the absence of toxic residues in the final product.

On the occasion of the effects of inorganic fertilization on the environmental level and the quality of tobacco, we decided to explore two forms of organic fertilization (compost, manure), inorganic (NPK) and control on Virginia type of tobacco which is an important crop for Greece and a significant incomefor producers. As an extensive crop, the first object of our study was yield and whether yield could be competitive with conventional cultivation. The second object of this study is the quality of the tobacco leaves in terms of the concentration of nicotine, sugar final and N content in leaves.

MATERIALS AND METHODS

Experimental Design

At the Agricultural University of Athens, in the Votanikos area, a three-year experiment was established with tobacco (*Nicotiana tabacum* L.), as the main crop and vetch (*Vicia sativa*) as green manure. The experiment was held on from 2016 to 2018. Tobacco type were studied was Virginia cultivar Nicki. The experiment was designed according to randomized complete block design (RCBD), with four different fertilizer treatments (control, 800 kg ha^{-1} inorganic fertilizer, 2,100 kg ha^{-1} manure and 1,500 kg ha^{-1}organic compost) and three replications. Green manure was applied to all plots, so it did not have major effect on nitrogen content. Each plot was 12.5 m^2, there were 12 plots (3x4), therefore the total experimental area was 150 m^2. The inorganic fertilizer was 12-12-17-2 ((N-P2O5-K2O-MgO, and trace elements), the manure came from caw farm of the Agricultural University of Athens facilities 3.5- 1.5- 3 and the organic compost was from livestock products 6-8-10+3.3+0.3B+1%$ZnSO_4$.

Soil properties and experimental site's preparation

The soil in the present field was Clay Loam. Its pH was 7.29 and it had 2.47% organic matter according to Wakley and Black (1934) method. As well, the calcium carbonate percentage was 15.99 and the soil's Nitrate Nitrogen was 12.4 mg per 1 kg soil.

The field preparation was the same for all the years. Every year the vetch sown, took place on November 24, 2015, on November 20, 2016 and on November 18, 2017. The vetch density was 150 kg seed ha^{-1}. One week later, fertilizer application and hoeing followed. Finally, before transplanting, a rotary hoe was performed.

Seedlings' production, transplanting and cultivation treatments

Seedling production was done by the float system method. Large water tanks were designed, in which the discs were placed, filled with plant substrate in order to carry out the germination of the seeds and then the development of the tobacco plants. In addition, liquid organic fertilizers and organic plant protection products Trianum P. (*Trichoderma harzianum* strain T22) were applied.

Tobacco seedlings were transplanted on 29 April, 24 April, 19 April, for the years 2016, 2017 and 2018 respectively. The transplant was done by hand and the density between them was 0.5 m (between rows) x 0.4 m (between the plants in each row). There were 8 rows of plants per plot. A drip irrigation system was used for irrigation and were carried out twelve irrigations for each growing season. The water used per irrigation was 3 m^3. Weed control was performed when and where needed by weeder.

Meteorological Data

During the years (2016-2018), in which the experiment took place, the annual meteorological data (mean air temperature (°C) and precipitation (mm)) are presented in the Fig. 1.

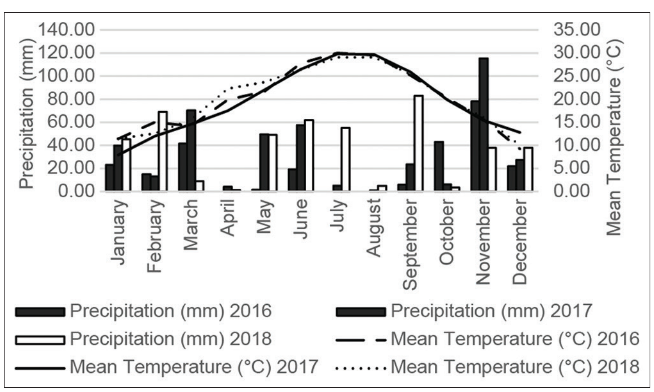

Fig 1. Annual meteorological data (mean air temperature (°C) and precipitation (mm)) during 2016, 2017 and 2018.

Measurements and Methods

Soil measurements

Soil measurements were made at the end of each growing season and the samples came from the first 30 cm of soil.

The organic matter of the soil was determined using the Wakley and Black method (1934). The percentage of calcium carbonate $CaCO_3$ (%) was measured by the Bernard device use. Water extracts were used to determine the pH, which were obtained in a 1: 1 ratio with soil and then shaken for sixty minutes. In addition as for the total nitrogen (mg g^{-1}) and assimilable phosphorus (ppm g^{-1}) determination there were used the Kjeldahl method (Bremner, 1960) and the Olsen (1965) method respectively.

Agronomic characteristics' measurements

Regarding the agronomic measurements, were used specific samples which came from of the middle lines of each plot. The measurements were held on the harvest date. The leaves harvest was made at one time. There were calculated the yield (kg ha^{-1}) and the total number of leaves, while the total plants height (cm) was also measured. Moreover, there was determined the leaf area with the following equation (eq. 1), which S symbolize the leaf area, L the leaf length, W the leaf width and k is an empirical constant, obtained by linear regression of a sample of measurement values of L, W and S (Moustakas and Ntzanis, 1998; Maw and Mullinix, 1992).

$$S = k\,(L * W) \qquad \text{eq. 1}$$

Quality characteristics' determination

There were determined the ash percentage, the total leaves' nitrogen content (mg g^{-1}) and the leaves' phosphorus content (ppm g^{-1}) with the methods mentioned above for soil measurements. As for the nicotine and sugars determination there were used the recommended methods from CORESTA (Bilalis et al., 2015), no.35 (ISO/DIS15152) (CORESTA, 1994a) and no.38 (ISO/DIS 15154) (CORESTA, 1994b) respectively.

Statistical analysis

Analysis of variance was set up on data using the Statistica (Stat Soft, 2011) logistic package as a randomized complete block design (RCBD). The significance of differences between treatments was estimated using Tukey's test and probabilities equal to or less than 0.05 considered significant. The tests of correlation coefficients and linear regression by Statistica software were set at two levels with significance (α = 0.05) and remarkable significance (α = 0.01).

RESULTS AND DISCUSSION

Soil properties

As it is presented to Table 1 the interaction between years with fertilizers was not statistically significant for all the characteristics studied. According to Kakabouki et al. (2019) organic matter plays an important role in the soil, as it is one of the components of the soil as well as a source of nutrients. More specifically, the organic matter of the soil did not show statistically significant differences between the years as well as between the different fertilizer treatments. However, several studies have shown that the use of synthetic nitrogen fertilizers has resulted in an increase in soil organic matter (Powlson et al., 2010; Ladha et al., 2011). The organic matter in the soil was ranged between 2.82 to 3.57%.

In addition, as for the calcium carbonate ($CaCO_3$) percentage according to Table 1, there were statistically significant differences between the fertilizer treatments, but the differences between the years were not statistically significant. In the first year between control and compost

Table 1: Soil characteristics as affected by fertilizer treatments (compost, manure, NPK, control)

	Organic matter (%)	$CaCO_3$ (%)	P soil (ppm g^{-1})	N soil (mg g^{-1})
2016				
control	3.24^{ns}	30.40^{ab}	19.66^{a}	2.04^{ns}
compost	3.12^{ns}	30.40^{ab}	18.14^{a}	2.05^{ns}
manure	3.18^{ns}	30^{a}	27.57^{ab}	2.10^{ns}
NPK	3.55^{ns}	31.60^{a}	34.42^{c}	2.17^{ns}
2017				
control	3.43^{ns}	29.13^{a}	23.65^{b}	1.71^{ns}
compost	2.96^{ns}	29.93^{ab}	20^{a}	1.90^{ns}
manure	2.94^{ns}	29.57^{a}	25.35^{b}	1.83^{ns}
NPK	3.04^{ns}	32.40^{b}	24.77^{b}	1.79^{ns}
2018				
control	3.38^{ns}	28.73^{a}	23.18^{b}	1.62^{ns}
compost	3.29^{ns}	29.83^{b}	20.68^{a}	2.11^{ns}
manure	3.57^{ns}	28.90^{a}	24.19^{b}	1.81^{ns}
NPK	2.82^{ns}	33.20^{c}	27.18^{b}	2.08^{ns}
F_{Fert}	*ns*	*10.107****	*7.616****	*ns*
F_{Year}	*ns*	*ns*	*ns*	*ns*
$F_{Fert\ x\ Year}$	*ns*	*ns*	*ns*	*ns*

F-test ratios are from ANOVA. Different letters within a column indicate significant differences according to Tukey's test (α = 0.05). Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$)

Table 2: Agronomic characteristics affected by fertilizer treatments (compost, manure, NPK, control)

	Plant height (cm)	Leaves (no $plant^{-1}$)	Leaf area (cm^2)	Yield (kg ha^{-1})
2016				
control	66.78^{a}	13.78^{a}	756.52^{ns}	5,705.23^{a}
compost	66.11^{a}	14.56^{a}	730.69^{ns}	4,182.59^{b}
manure	61.56^{b}	12^{b}	892.18^{ns}	4,001.81^{b}
NPK	72.67^{a}	14^{a}	655.93^{ns}	3,678.12^{b}
2017				
control	102.08^{a}	19.83^{a}	827.33^{ns}	7,053.42^{b}
compost	108.83^{b}	21.17^{b}	854.33^{ns}	5,329.56^{a}
manure	107.08^{b}	20.33^{a}	974.83^{ns}	6,218.44^{b}
NPK	104.17^{a}	21.67^{b}	786.17^{ns}	6,805.91^{b}
2018				
control	81.67^{c}	14.67^{a}	826.93^{ns}	6,686.93^{a}
compost	96.33^{ab}	16.33^{b}	827.51^{ns}	5,410.67^{b}
manure	90^{a}	17.33^{b}	968.51^{ns}	5,606.67^{b}
NPK	113^{b}	21.33^{b}	756.05^{ns}	6,716^{a}
F_{Fert}	*4.78***	*ns*	*ns*	*ns*
F_{Year}	*78.865****	*27.982****	*ns*	*4.147**
$F_{Fert\ x\ Year}$	*2.645**	*ns*	*ns*	*ns*

F-test ratios are from ANOVA. Different letters within a column indicate significant differences according to Tukey's test (α = 0.05). Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$)

treatments there were not statistically significant differences, however control and compost differ statistically significant with the inorganic fertilizer treatment. In the 2017 there were statistically significant differences between control and manure with compost and inorganic. The highest values for all the years were on the inorganic fertilizer application, while the lowest values differ between the years, for the 2016 was on the manure treatment and for the 2017 was on the application with inorganic fertilizer as well as for the 2018.

The phosphorus content in the soil showed statistically significant differences between fertilizer treatments, but there were no significant differences between the years. In 2016, there were statistically significant differences between control, compost and manure with NPK. In the 2017, compost was significantly difference with control, manure and NPK. In addition, the lowest value in compost treatment has been observed over the years 18.14 ppm g^{-1}, 20 ppm g^{-1} and 20.68 ppm g^{-1} for 2016, 2017 and 2018 respectively. However, the highest values for 2016 and 2018 was on the NPK treatments and for the 2017 on the manure treatment. Several studies have shown that organic fertilizers increase the available nutrients content in the soil, such as the phosphorus (Singh et al., 2019). On the other hand, according to the results of Arif et al. (2017), manure application increased soil organic carbon as well as available phosphorus.

With regard to soil nitrogen, there were no differences between fertilizer treatments and years (Table 1). For the first year, the lowest value was on control, 2.04 mg g^{-1}, and the highest on inorganic fertilizer treatment, 2.17 mg g^{-1}. Cui et al. (2008) reported that increased soil organic matter significantly affected soil nitrogen content.

Tobacco growth and yield

In the Table 2 presented the agronomic characteristics as like as plant height. The highest value was 113 cm in the NPK, in the 2018 and the lowest was 61.56 cm in the manure in the 2016. The manure had not statistically significant difference with the NPK, control and compost had not statistically significant difference with the control in the 2016.. In the 2018 the compost had not statistically significant difference with the manure. The manure had statistically significant difference with control and NPK.. It is observed that in organic fertilizers and especially in compost, there were high values in plant height as well as in inorganic fertilization (Table 2).

Karaivazoglou et al. (2007) states that the plant height of the tobacco was affected by nitrogen fertilization and mainly by the higher quantities applied. While Song et al. (2016) emphasized the fact that different concentrations of organic fertilizers affect the morphological characteristics of tobacco. The plant height had positive correlation with the number of leaves per plant (r= 0.43, p=0.001), (Table 3). Also, in the number of leaves per plant, manure had statistically significant difference with compost, NPK in the 2016 and the control, manure had statistically significant difference with the NPK, compost , in 2017. On the other hand, in the 2018, the control had statistically

Table 3 : Pearson's correlation coefficient (r) of soil, agronomic and quality characteristics. Significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant (p > 0.05)

	Organic matter (%)	$CaCO_3$ (%)	P soil (ppm g^{-1})	N soil (mg g^{-1})	Ash (%)	P (ppm g^{-1})	N leaves (%)	Nicotine (%)	Reducing sugars (%)	Yield (kg ha^{-1})	Plant Height (cm)	Leaves (no $plant^{-1}$)	Leaf area (cm^2)
Organic matter (%)	1.00	0.06^{ns}	0.15^{ns}	-0.13^{ns}	-0.01^{ns}	-0.03^{ns}	-0.06^{ns}	0.06^{ns}	-0.02^{ns}	0.00^{ns}	-0.23^{ns}	-0.05^{ns}	0.03^{ns}
$CaCO_3$ (%)	0.06^{ns}	1.00	0.14^{ns}	0.20^{*}	-0.17^{ns}	0.14^{ns}	0.06^{ns}	0.04^{ns}	-0.06^{ns}	-0.05^{ns}	-0.04^{ns}	0.28***	-0.13^{ns}
P soil (ppm g^{-1})	0.15^{ns}	0.14^{ns}	1.00	0.02^{ns}	-0.06^{ns}	0.09^{ns}	0.09^{ns}	-0.09^{ns}	0.13^{ns}	0.05^{ns}	0.00^{ns}	-0.06^{ns}	0.00^{ns}
N soil (mg g^{-1})	-0.13^{ns}	0.20^{*}	0.02^{ns}	1.00	0.13^{ns}	0.15^{ns}	0.14^{ns}	-0.19^{*}	0.14^{ns}	-0.05^{ns}	-0.18^{ns}	-0.15^{ns}	-0.08^{ns}
Ash (%)	-0.01^{ns}	-0.17^{ns}	-0.06^{ns}	0.13^{ns}	1.00	0.16^{ns}	0.04^{ns}	-0.08^{ns}	-0.01^{ns}	-0.04^{ns}	0.11^{ns}	-0.20^{*}	0.18^{*}
P (ppm g^{-1})	-0.03^{ns}	0.14^{ns}	0.09^{ns}	0.15^{ns}	0.16^{ns}	1.00	0.01^{ns}	-0.03^{ns}	-0.07^{ns}	-0.20^{*}	-0.16^{ns}	-0.09^{ns}	-0.03^{ns}
N leaves (%)	-0.06^{ns}	0.06^{ns}	0.09^{ns}	0.14^{ns}	0.04^{ns}	0.01^{ns}	1.00	-0.43***	0.58***	0.45***	-0.21^{*}	-0.31***	0.30***
Nicotine (%)	0.06^{ns}	0.04^{ns}	-0.09^{ns}	-0.19^{*}	-0.08^{ns}	-0.03^{ns}	-0.43***	1.00	-0.55***	-0.47***	0.10^{ns}	0.47***	-0.36***
Reducing sugars (%)	-0.02^{ns}	-0.06^{ns}	0.13^{ns}	0.14^{ns}	-0.01^{ns}	-0.07^{ns}	0.58***	-0.55***	1.00	0.66***	-0.36***	-0.61***	0.48***
Yield (kg ha^{-1})	0.00^{ns}	-0.05^{ns}	0.05^{ns}	-0.05^{ns}	-0.04^{ns}	-0.20^{*}	0.45***	-0.47***	0.66***	1.00	0.09^{ns}	-0.32***	0.49***
Plant Height(cm)	-0.23^{**}	-0.04^{ns}	0.00^{ns}	-0.18^{*}	0.11^{ns}	-0.16^{ns}	-0.21^{*}	0.10^{ns}	-0.36***	0.09^{ns}	1.00	0.43***	-0.09^{ns}
Leaves (no $plant^{-1}$)	-0.05^{ns}	0.28***	-0.06^{ns}	-0.15^{ns}	-0.20^{*}	-0.09^{ns}	-0.31***	0.47***	-0.61***	-0.32***	0.43***	1.00	-0.34***
Leaf area (cm^2)	0.03^{ns}	-0.13^{ns}	0.00^{ns}	-0.08^{ns}	0.18^{*}	-0.03^{ns}	0.30***	-0.36***	0.48***	0.4***	-0.09^{ns}	-0.34***	1.00

significant difference with the rest treatments (Table 2). The highest value was 21.67 per plant in the NPK, in the 2018 and the lowest was 12 per plant in the manure in the first year. Kurt and Ayan (2014) recorded higher leaves values per plant compared to our results. These differences are due to different climatic conditions as well as different fertilization. Otan (1983) reported that leaf length and width indicate different characteristics for each type of tobacco. Moreover, in the leaf area, none treatment was statistically significant in the three years. Kurt and Ayan (2014) emphasized that leaf surface was significantly raised with the increase of organic fertilization. In our study, manure was the fertilizer that had the highest leaf area values.

Furthermore, concerning yield, in the first year the three fertilizers were more efficient than control. In the second year, the compost shows the lowest yield and finally in the third year the control had a higher yield than the 2 organic fertilizers. The highest value was 7,053.42 kg ha^{-1} in the control and the lowest was 3,678.12 kg ha^{-1} in the NPK, in the second year (Table 2). In three years the highest yields among the three fertilizers occurred mainly in NPK fertilization. Similar results are presented with the study of Bilalis et al. (2010), where higher yields of Virginia tobacco in inorganic fertilization were recorded. Tobacco plants under fertilizers were higher with smaller leaves, which is why the accumulation of weight on the stem is suggested and not on the leaf. Fertilizers make the leaves thinner.

Tobacco quality

In Table 3 are presented the quality characteristics of tobacco. Concerning ash content none of treatment was statistically significant in the three years. The highest value was 20.64% in the manure, in the 2017 and the lowest was 15.76% in the control, in the third year. Karaivazoglou et al. (2007) stated that in the various forms of nitrogen fertilization that took place, none of them were statistically significant in terms of ash.

Moreover, in the content of P, during the three growing seasons the control had not statistically significant difference with the compost and manure with NPK. . The highest values were obtained in manure for all years(Table 4). In the percentage of nitrogen (N) in leaves, the control had not statistically significant difference with the NPK and the manure had not statistically significant difference with the compost, in the three years. The highest value was 2.73% in the control in 2017 and the lowest 0.88% in the manure in the 2018. Nitrogen content in leaves are higher in inorganic fertilization and in the three years of the experiment. Many studies reported that different forms of

Table 4: Quality characteristics affected by fertilizer treatments (compost, manure, NPK, control)

	Ash (%)	P (ppm g^{-1})	N leaves (%)	Nicotine (%)	Reducing sugars (%)
2016					
control	18.70^{ns}	440.38^{a}	1.88^{b}	2.15^{ns}	12.56^{ns}
compost	20^{ns}	444.98^{a}	1.41^{a}	1.90^{ns}	12.97^{ns}
manure	20.26^{ns}	784.83^{b}	1.32^{a}	1.96^{ns}	13.32^{ns}
NPK	18.11^{ns}	659.88^{b}	2.04^{b}	2.03^{ns}	12.93^{ns}
2017					
control	18.86^{ns}	364.11^{a}	2.73^{b}	1.74^{ns}	12.44^{ns}
compost	20.05^{ns}	405.57^{a}	1.91^{a}	1.79^{ns}	12.83^{ns}
manure	20.64^{ns}	841.35^{c}	1.75^{a}	1.46^{ns}	13.64^{ns}
NPK	19.07^{ns}	535.56^{b}	2.51^{b}	2.17^{ns}	12.51^{ns}
2018					
control	15.76^{ns}	528.05^{b}	1.02^{a}	2.55^{ns}	12.67^{ns}
compost	20.54^{ns}	397.63^{a}	0.91^{b}	2.02^{ns}	13.11^{ns}
manure	20.44^{ns}	631.26^{b}	0.88^{b}	2.46^{ns}	13^{ns}
NPK	18.61^{ns}	531.97^{b}	1.58^{a}	1.89^{ns}	13.35^{ns}
F_{Fert}	*3.008**	*6.373***	*5.273**	*ns*	*ns*
F_{Year}	*ns*	*ns*	*ns*	*ns*	*ns*
$F_{Fert\ x\ Year}$	*ns*	*ns*	*ns*	*ns*	*ns*

F-test ratios are from ANOVA. Different letters within a column indicate significant differences according to Tukey's test (α = 0.05). Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$)

nitrogen, either from inorganic or organic fertilizer, do not affect the concentrations of total nitrogen in the tobacco leaves (Ali et al., 1991; Fenn et al., 1994; Lo´pez-Lefebre et al., 2000). Morad-beigi et al. (2012) reported that higher concentrations of phosphorus and nitrogen in leaves were recorded in inorganic fertilization as opposed to organic fertilization.

In the percentage of nicotine none treatment was statistically significant affect in the three years. The highest value was 2.46% in the manure, in the third year and the lowest was 1.46% in the manure in the second year (Table 4). According to Leffingwell, (1999) the composition and content of nicotine is affected by various agronomic factors as well as environmental factors (rainfall and temperatures). As shown in Table 3 the yield had negative correlation with the percentage of nicotine, (r= -0.47, p= 0.001).

Tso, (1990) emphasized the fact that nitrogen concentrations in the leaves have a positive effect on nicotine concentrations. In contrast to Tso (1990), in our results the nitrogen content of leaves had a negative correlation with the nicotine (r= -0.43, p= 0.001), as shown in Table 3. In our study, the highest values appeared in inorganic fertilization the first two years and in the third it was in fertilization with manure. On the other hand, the reducing sugars had positive correlation with the nitrogen content in leaves (r= 0.58, p=0.001), (Table 3). An important indicator of the quality of smoking is reducing sugars concentrations (Weybrew et al., 1983). Furthermore, in reducing sugars , none treatment was statistically significant in the three years. The maximum value was 13.64% in the manure and the lowest was 12.44% in the control, in the second year (Table 4). According to Karaivazoglou et al. (2007), different amounts and forms of nitrogen did not affect in reducing sugars.

CONCLUSION

In conclusion the different fertilization had effect on the soil, agronomic and quality characteristics in Virginia tobacco. From organic fertilizers, fertilization with manure showed the highest values in several parameters as like as in the soil characteristics. Inorganic fertilization also affected on agronomic characteristics and especially yield. The quality characteristics were not affected by the different fertilization in any of the three years where the experiment was conducted.

Authors' contributions

All authors designed the study performed the experiment and the data collection, analysis and wrote the manuscript.

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