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Effects of organic and inorganic fertilization on growth, yield and nicotine content of flue-cured and oriental tobacco (*Nicotiana tabacum* L.) seedlings grown in organic and conventional float system

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Abstract

Organic tobacco consists of a new industrial crop product. Also, organic transplant production is the major problem for organic agriculture in Greece. Thus, field experiments were conducted to determine (a) the effects of organic and conventional float systems on tobacco seedlings quality and (b) the effect of organic and inorganic fertilization on the growth, yield and nicotine content of flue-cured and oriental type of tobacco (*Nicotiana tabacum* L.). Experiment (a) was conducted as complete randomized block design with three replicates and two treatments (organic and conventional float system). Experiment (b) was conducted as split plot design with three replicates, two main plots (flue-cured and oriental tobacco) and two sub-plots (organic and inorganic fertilizers). Our results (Experiment a) clearly indicate that there were no significant differences between the organic and conventional float systems for root biomass of tobacco seedlings. Conventional fertilization treatment is responsible for the production of seedlings with high water content (82-83%). Moreover, in both tobacco types (Experiment b), there were no significant differences between the organic and inorganic fertilization concerning the height and yield of tobacco crop. The yield in the oriental tobacco was less than in the Virginia tobacco type. In both tobacco types the use of organic fertilizer increased the nicotine content and N% of leaves. High correlation coefficient ($r = 0.88$, $p<0.001$) between N% and nicotine content of leaves was found. The nicotine content of leaves in oriental tobacco type was always higher than that in Virginia type. Controlled release of nitrogen in organic fertilizers may be a useful practice for increasing nicotine content in tobacco crop.

Key words: Float system, organic fertilization, tobacco, yield, nicotine content, *Trichoderma* spp.

Introduction

Organic transplant production is the major problem for organic agriculture. Tobacco (*Nicotiana tabacum* L.) seeds need special environment during emergence because of small size. For this reason, seeds are not sown directly in the field. Instead, seedbed for seedling production is prepared, seeds are sown on the bed and seedlings are transplanted to the field. In conventional tobacco production, the cold seedbeds have been used for seedling production. A direct-seeded float system has been developed as a possible alternative to the conventional methods for seedling production [1].

The float system is an efficient method of seedling production. Float system technology is used widely to produce tobacco transplants in greenhouse [1-3] but is scarcely used for horticulture and other crops [4-8].

Agricultural production has become heavily reliant, over the past 50 years, on the use of synthetic fertilizers. Management methods that decrease requirement for agricultural chemicals are needed to reduce adverse environmental impacts. The organic fertilization is one of the basic cultivation techniques of organic agriculture. The results obtained by other researchers [9-11] showed that organic fertilization not only improved the soil physicochemical properties but also increased the yield of crops significantly. A niche market for organic tobacco appears to already exist and constantly grow. There are not many references on organic tobacco in science community [9, 12].

The aim of this study was to determine (a) the effects of organic and conventional float systems on tobacco seedlings quality and (b) the effect of organic and inorganic fertilization on the growth, yield and nicotine content of flue-cured and oriental type of tobacco (*Nicotiana tabacum* L.).

Materials and Methods

Experiment 1:

Experimental design: Experiment was conducted at the greenhouse of the Laboratory of Crop Production, Agricultural University of Athens (23.43°E and 34.58°N). The experiment was carried out according to a randomized complete block design. There were two basins (dimensions 68 cm x 55 cm x 10 cm) for each of the three replications (2 systems x 2 tobacco types x 3 replicates). The volume of each basin was 3.9 lt. The conventional basin was managed by means of the conventional (common) technique (CON) with water-soluble fertilization, 16 g of fertilizer (20-10-20) and two fungicides, 2 ml Previcur N 72.2 SL (i.e. propamocarb) by Bayer Crop Science and 2 g Derosal 50 WP (i.e. carbendazim) by Syngenta. The organic basin followed organic treatments (organic ORG) with organic water-soluble fertilizer, 24 ml Fish–Fert (2-4-0.5 and trace elements) by Humofert Co., 27 g Bioilsa (12-0-0) and 2 ml Trichomic (*Trichoderma* sp.) by Trichodex-Spain Co.

Two polystyrene floating trays with 198 cells (17 cm^3 per cell) per tray were used for each basin. Each cell was filled with substrate mixture (peat:perlite 1:1). Either oriental type of tobacco (*Nicotiana tabacum* L. var. S53) or flue-cured tobacco (*Nicotiana tabacum* L. var. VE9) was used in the experiment. Tobacco was hand-sown, one seed per cell, on 12th March 2008. Each seed was placed on the surface of the substrate without additional covering.

Measurements: Root samples were taken on 27th May 2008 based on 10 plant samples per treatment. Root sample was cleaned of peat/perlite media by washing the samples over 5 mm mesh-sieve. The roots retained on sieves were transferred to a 0.1% trypan blue FAA staining solution (mixture of 10% formalin, 50% ethanol and 5% acetic acid solutions). For the determination of root length (mm) and diameter (mm), the stained root samples were placed on a high-resolution scanner using DT-software (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burwell, Cambridge, UK).

Furthermore, the seedlings were weighed and then oven-dried at 70°C for 3 days, in order to measure dry weight (DW) and fresh weight (FW) in grams per plant. For each basin, electrical conductivity (EC) (Zonder by DOCH Inc.), pH (pH212 by Hanna Inc.) and the concentration of the dissolved oxygen (HI 9142 by Hanna Inc) of the solution were measured. Chlorophyll content was determined non-destructively using a CCM-200 chlorophyll content meter (Opti-Sciences, Inc). The CCM-200 uses absorbance to estimate the chlorophyll content in leaf tissue. SPAD measurements were made at two areas of the leaves. The ability to predict chlorophyll content on a leaf area basis from SPAD readings was demonstrated in tobacco crop [13].

Experiment 2:
Experimental design: A tobacco crop (*Nicotiana tabacum* L.) was established in the experimental "organic" field of the Agricultural University of Athens (23.43°E, 34.58°N). The soil was clay loam (29.8% clay, 34.3% silt and 35.9% sand) with pH 7.24, 1.17% organic matter and 0.54 mS cm^{-1} of EC. The oriental type of tobacco (*Nicotiana tabacum* L. var. S53) and flue-cured tobacco (*Nicotiana tabacum* L. var. VE9) were used in the experiment. The experiment was set up on an area of 300 m^2 according to split plot design with 2 fertilization treatments (organic and inorganic), 2 types of tobacco (oriental and flue-cured) and 3 replicates. The fertilization treatments were organic fertilizer (Bioilsa 12-0-0, 600 kg ha^{-1}) and inorganic fertilizer (20-10-20, 780 kg ha^{-1}). The plot size of fertilization treatment was 8 m x 8 m. The plot size of tobacco type treatment was 4 m x 4 m. Fertilizers were applied on 3rd June 2008, 7 days after transplanting.

Seedlings were transplanted on the 27th May 2008. Flue-cured tobacco seedlings were transplanted by hand in rows of 90 cm apart. Transplants were set at 40 cm between each other. Also, oriental tobacco seedlings were transplanted by hand in rows of 60 cm apart. Transplants were set at 20 cm between each other. Finally, weeds were controlled by hand.

Measurements: The samplings were taken 113 days after the transplanting date of tobacco. To define biomass 3 plants per plot were cut. Dry matter was determined after being placed in paper bags at 50°C in an oven for 72 hours. Plant height was determined by measurement of 5 plants per plot. Root samples were taken 113 days after the transplanting (DAT) date of tobacco. Samples were collected from each plot at soil depth of 0-30 cm with a 30 cm x 30 cm sampler. In each sample roots were separated from soil after standing for 24 h in water + $(NaPO_3)_6$ + Na_2CO_3. The %N from biomass was measured with the Kjeldahl method [14]. Nicotine content of roots and leaves was measured according to AOAC [15]. Proper quantity of sample (containing preferably 0.1-1.0 g nicotine) was placed into a 500 ml spherical flask of a steam distillation apparatus and moistened with 20 ml of 3.75 N NaOH solution. The mixture was steam distilled, and the distillate was collected in a 250 ml receiving flask, containing 5 ml of 2 N HCl solution. After complete distillation, 10 ml of 12% w/v SiO_2 $12WO_3.26H_2O$ (Aldrich) aqueous solution was added slowly under stirring to the distillate. The precipitate was filtered off through a tared Gooch funnel, previously dried at 105°C, and washed with 0.005 N HCl solution. The funnel+precipitate was dried at 105°C for 3 h and weighed. The % nicotine was calculated on the dry weight of sample by equation (1):

$$\% \text{ nicotine} = (w \times 1012) / (W \times (100-m)) \quad (1)$$

where w = weight of precipitate, W = weight of sample taken and m = % moisture content of sample.

Statistical analysis: The data of two experiments were subjected to statistical analysis according to the experiment (randomized complete block or split plot) design. Differences between treatment means were compared at $p = 0.05$ with ANOVA to find significant differences and LSD to compare the plots. The statistical analysis of the data was performed with the STATGRAPHICS Plus 5.1 logistic package.

Results

Tobacco seedlings production (Experiment 1):
Water solution measurements: There was a remarkable reduction in pH values (Fig. 1) of the solution after the organic float system. Also, the lowest electrical conductivity was found in organic float system. There were significant differences between the organic and conventional float system. On the other hand, there were no significant differences between the organic and conventional float system concerning the dissolved oxygen concentration.

Seedlings growth: Concerning the germination percentage (range 85-90%) there were no significant differences between organic and conventional float system. Also, both organic and inorganic pesticides inhibited the growth of *Rhizoctonia solani* and *Pythium* spp. The lowest fresh weight was found in organic float system. There were significant differences between the organic and conventional float system. On the other hand, there were no significant differences between the organic and conventional float system concerning the fresh weight of roots, dry weight of biomass, root diameter, root length and chlorophyll content (SPAD value).

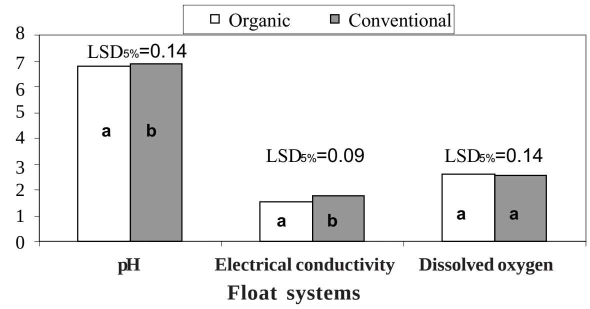

Figure 1. pH, electrical conductivity (EC mS cm^{-1}) and dissolved oxygen (ppm lt^{-1}) values for the two float systems (CON Conventional and ORG organic).

Tobacco crop growth (Experiment 2):

Crop growth and yield characteristics: In both tobacco types, there were no significant differences between the organic and inorganic fertilization concerning the dry weight of leaves, height and yield.

The dry weight of leaves and height in the oriental tobacco was less than in the Virginia tobacco type. Concerning the tobacco type statistically significant differences in the yield of tobacco crop were observed. The tobacco yield in Virginia type was always higher than that in oriental tobacco type.

Industrial characteristics: In both tobacco types the use of organic fertilizer led to increase N% (Table 1) and the nicotine content (Table 2) of leaves. On the other hand, there were no significant differences between the organic and inorganic fertilization concerning the nicotine content of roots and the total nicotine content of leaves (Table 2). The highest N% of leaves was recorded in the oriental tobacco type (Table 3).

The nicotine content of roots in the Virginia tobacco was less than in the oriental tobacco type. Moreover, the tobacco yield in oriental tobacco type was always higher (28.38-30.61%) than that in Virginia type. Concerning the tobacco type no statistically significant differences in the total nicotine content of leaves were observed.

Discussion

Tobacco seedlings production: Organic transplant production is the major problem for organic agriculture in Greece [5]. Float system technology is used to produce tobacco seedlings [2, 16]. Our results clearly indicate that seedling vigor (fresh weight) tended to be higher in the conventional floating systems compared to organic treatment. Conventional fertilization (mainly the nitrogen) treatment is probably responsible for the production of watery plants. Rideout and Overstreet [17] indicated that greater water content of seedling affects negatively the quality of tobacco transplants.

Significantly lower values of pH and EC in the solution from the organic treatment can be attributed to the properties of the organic fertilizer applied. Similar results have also been reported by Bilalis *et al.* [5]. Also, both organic (use of Trichomic (*Trichoderma* sp.)) and inorganic (Previcur N 72.2 SL (i.e. propamocarb) and Derosal 50 WP (i.e. carbendazim)) pesticides inhibited the growth of *Rhizoctonia solani* and *Pythium* spp. Tarantino *et al*. [3] also found the same level of disease control in plots with a high rate of iprodione and in plots with a low rate of fungicide, applied together with *Gliocladium roseum* (GNLr1 and GNLr2) iprodione-resistant strains. Less-disease control was obtained by a reduced dose of iprodione, when applied alone. Moreover, *Trichoderma* spp.

Table 1. Effects of two float systems (CON conventional and ORG organic) on tobacco (Virginia and oriental type) seedlings growth parameters: fresh weight of roots and above ground biomass (g $plant^{-1}$), dry weight of above ground biomass (g $plant^{-1}$), root diameter (mm), root length (mm) and chlorophyll content (SPAD value).

	Virginia			Oriental		
	ORG	CON	*$LSD_{5\%}$*	ORG	CON	*$LSD_{5\%}$*
Fresh weight of roots	0.98a	3.13a	*2.54*	0.92a	2.48a	*2.13*
Fresh weight of biomass	2.40a	12.64b	*3.18*	2.61a	8.56b	*2.21*
Dry weight of biomass	0.58a	2.26a	*1.78*	0.72a	1.44a	*1.32*
Root diameter	0.298a	0.313a	*0.22*	0.324a	0.329a	*0.12*
Root length	3443a	3135a	*455*	4055a	3547a	*655*
Chlorophyll (SPAD value)	16.97a	16.53a	*2.32*	19.40a	14.53a	*7.86*

Table 2. Effects of organic (ORG) and inorganic fertilization (CON) on tobacco (Virginia and oriental type) plants parameters: dry weight of plants (g $plant^{-1}$), height (m), yield (kg ha^{-1}) and total nitrogen (%).

Tobacco type	Fertilization			
	ORG	CON	ORG	CON
	Dry weight of leaves		Height	
Virginia	29.08	33.55	1.03	1.16
Oriental	22.11	21.32	0.84	0.91
LSDfert (p=0.05)	*4.55*		*0.21*	
LSDtype(p=0.05)	*3.21*		*0.10*	
	Yield		N%	
Virginia	2930	3390	1.843	1.620
Oriental	2300	2360	2.273	2.177
LSDfert (p=0.05)	*470.87*		*0.34*	
LSDtype(p=0.05)	*340.54*		*0.17*	

The LSD (p = 0.05) for fertilization and tobacco type are shown.

Table 3. Effects of organic (ORG) and inorganic fertilization (CON) on tobacco (Virginia and oriental type) plants parameters: nicotine content of roots and leaves (%) and total nicotine of leaves (kg ha^{-1}).

Tobacco type	Fertilization					
	ORG	CON	ORG	CON	ORG	CON
	Nicotine of roots		Nicotine of leaves		Total nicotine of leaves	
Virginia	0.490	0.483	0.893	0.739	26.23	25.00
Oriental	0.637	0.710	1.247	1.065	28.70	25.13
LSDfert (p=0.05)	*0.083*		*0.072*		*3.56*	
LSDtype(p=0.05)	*0.051*		*0.123*		*2.21*	

The LSD (p = 0.05) for fertilization and tobacco type are shown.

isolates were found effective to control Phytophthora foot rot disease of black pepper [18].

Tobacco crop growth - industrial characteristics: In both tobacco types, there were no significant differences between the organic and inorganic fertilization concerning the dry weight of leaves and height. The yield of Virginia tobacco crop under inorganic fertilization was higher (14%) than that with organic fertilization, although the yield difference was not statistically significant. In addition, Butorac *et al*. [12] reported that organic fertilizers had an obvious advantage over mineral fertilization. Organic fertilizers had a positive effect on the yield and quality of the flue-cured tobacco leaves. Moreover, Bilalis *et al*. [9] have also reported high yield (4080-5050 kg ha^{-1}) under vetch green manure, in tobacco crop irrigated by drip irrigation system.

The dry weight of leaves and height in the oriental tobacco was less than in the Virginia tobacco type. Concerning the tobacco type, statistically significant differences in the yield of tobacco crop were observed. The tobacco yield in Virginia type was always higher (21.50-30.38%) than that in oriental tobacco type. High positive correlation coefficient ($r = 0.84$, $p<0.01$, $n = 12$) between height and tobacco yield was found.

Nitrogen (N) supply is the most important factor affecting quality of tobacco [9, 19]. High negative correlation coefficient ($r = -0.83$, $p<0.01$, $n = 12$) between yield and nicotine content was found. In both tobacco types the use of organic fertilizer, led to increase N% (Table 1) and the nicotine of leaves (Table 2). Controlled release of nitrogen in organic fertilizers may be a useful practice for increasing nicotine content in tobacco crop. Nitrogen availability at late growth stages is critical factor affecting the nicotine content of tobacco leaves. Ju *et al*. [19] reported that soil N mineralization at late growth stages of tobacco was an important factor affecting N accumulation and therefore the nicotine content in tobacco leaves. Moreover, Wang *et al*. [20] reported that nicotine concentration in leaves of flue-cured tobacco plants increases at the late growth stage, especially after removing the shoot apex.

Moreover, the highest N% of leaves was recorded in the oriental tobacco type. High correlation coefficient ($r = 0.88$, $p<0.001$, Fig. 2) between N% and nicotine content of leaves was found. Thus, the nicotine of leaves in Oriental tobacco type was always higher than those in Virginia type. The nicotine content of roots in the Virginia tobacco was less than in the Oriental tobacco type. Concerning the tobacco type no statistically significant differences in the total nicotine of leaves were observed. Thus, both tobacco types, especially oriental type with high nicotine concentration, can be used for the production of bio-insecticides. The demand for natural plant based insecticides is increasing day by day [21]. Aqueous extracts of tobacco could be used for the control of insect's field infestation [22].

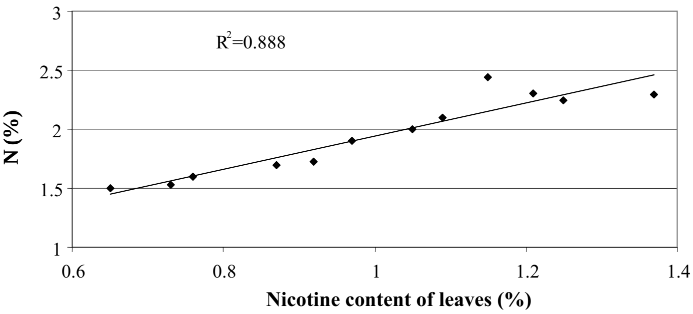

Figure 2. Correlation between the nitrogen concentration of leaves N(%) and nicotine content of leaves (n = 12).

Conclusions

Our results clearly indicate that seedling vigor (fresh weight) tended to be higher in the conventional floating systems compared to organic treatment. Conventional fertilization (mainly the nitrogen) treatment is probably responsible for the production of plants with high water content. Also, there were no significant differences between the organic and conventional float systems for root biomass of tobacco seedlings.

Moreover, in both tobacco types, there were no significant differences between the organic and inorganic fertilization concerning the dry weight of leaves, height and yield. The yield in the oriental tobacco was less than in the Virginia tobacco type. In both tobacco types the use of organic fertilizer led to increase the nicotine and N% of leaves. High correlation coefficient ($r = 0.88$, $p<0.001$) between N% and nicotine content of leaves was found. The nicotine of leaves in oriental tobacco type was always higher than that in Virginia type.

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