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Effect of nitrogen fertilizations, with and without inhibitors, on cotton growth and fiber quality

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Abstract. Considering cotton, one of the most non environmentally friendly crops, new types of fertilizers, such as the urease inhibitor, are now being used for fertilization. Furthermore, the need of increasing the nutrient use efficiency which is an important contributor to yield has arisen. The objective of this study was to assess the impacts of four different urea combinations (Urea, Urea+NI+UI, Urea+NI, Urea+UI) on cotton (*Gossypium hirsutum* L.) yield and fiber traits. For this purpose, different inhibitors used on urea fertilizer such as nitrification inhibitor (NI), dicyandiamide (DCD), urease inhibitor (UI), N-(n-butyl) thiophosphoric triamide, and a combination of urease (UI) and nitrification inhibitor (NI) (double inhibitor). Additionally, Nitrogen indicators were also used to evaluate the efficiency of these combinations. Two field experiments were conducted in Agrinio and Copaida region, Central Greece during 2019. The total dry weight ranged from 13,027 to 14,481 kg ha^{-1} in Agrinio area and from 12,567 to 14,136 kg ha^{-1} in Copaida area. The highest seed cotton yield was recorded under Urea+NI+UI fertilization at 5,145 kg ha^{-1} application in Copaida area and 5,318 kg ha^{-1} application in Agrinio area. Also, the total plant nitrogen uptake (kg N ha^{-1}) was affected by the inhibitors NI and UI. The range for Nitrogen Utilization Efficiency (NUtE) index was 9.27 to 23.06. Moreover, results indicated that NI and UI inhibitors have a marked effect on fiber quality such as strength (g Tex^{-1}). In the Mediterranean region of Greece, the combined use of inhibitors UI and NI resulted in higher yield and finest fiber quality.

Key words: cotton, fiber quality, nitrogen inhibitor, iNUE.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.), is considered one of the major industrial plants, for most countries, including Greece (Avgoulas et al., 2005; Bilalis et al., 2010).

Nitrogen is most often the major limiting nutrient to cotton cultivation. It is classified among the soil minerals that get absorbed by cotton, and influences the crop's height, fruiting, yield and fiber quality (Ma et al., 2008; Ducamp et al., 2012). N deficiency, affects the number of leaves per plant, thus reducing the photosynthetic capacity and accumulation of sugars in the boll set and ultimately affecting the plant's maturation and height (Wullschleger & Oosterhuis 1990; Emara & El-Gammaal 2012). Dong et al. (2012) report a 10% increase of the biological yield, the photosynthesis and protein concentration, when high nitrogen amounts are applied. Urea production rose to 174.3 million tons in 2016, down 0.6% to 2015.

Overall, nitrogen is an essential macronutrient for cotton cultivation, due to its pivotal role in cotton growth and yield. The right amount of nitrogen during the plant growth, affects the photosynthetic capacity of leaves, providing the growing of the productive components (Bondada & Oosterhuis, 2001; Wullschleger & Oosterhuis, 1990). Furthermore, nitrogen has an impact on boll and seed formation by increasing their size and weight (Bondada et al., 1996 and Saleem et al., 2010). Cotton yield showed a significant increase which followed the increase of N application rates and the mulching consistently exerted the additive effect of N fertilization on cotton growth and yield (Allanov et al., 2019).

Since cotton fibers are primarily composed of cellulose, any influence on the plant's photosynthetic rate and production of carbohydrates will cause similar influence on fiber growth. Micronaire (MIC) measures the rate of airflow, under pressure, of a plug of lint cotton (of known weight) compressed into a chamber of fixed volume. Micronaire, is often treated as the fiber maturity measurement in classing-office (Bradow & Davidonis, 2000). Micronaire is considered more important in spinning and fiber maturity and seems to impact more on dye-uptake rate. Maintaining fiber quality standards is essential for growers in order to avoid price reduction, however the expression of fiber properties genetic potential depends on complex interaction among crop management and growth environment (Darawsheh, 2010). Micronaire (fineness-maturity), length, strength and color grade are very important parameters for spinning, while maturity, elongation, and short fiber index are also important fiber quality characteristics (Christidis, 1965; Deussen, 1986). Fiber quality parameters is a genetic characteristic (Bauer et al., 2000; Davidonis et al., 2004; Bednarz et al., 2006), however these fiber parameters are significantly affected by crop management and environmental conditions (Subhan et al., 2001; Darawsheh et al., 2009).

The use of urea based fertilizers leads to high Nitrogen losses due to ammonia volatilization. During the volatilization, ammonium is converted to ammonia and is lost in the atmosphere. Through the years 2006 to 2016, the tendency of urea production was to annually increase by 2.8%. The biggest producing countries are at the same time the largest consumers, referring to China and India. China is self-sufficient for nitrogen fertilizers, but India's demand for imports is significant. Most of the new nitrogen capacity in the world is in the form of urea, so naturally the production/consumption growth rates are higher for urea than for ammonia/total nitrogen. Nowadays, the difference has been quite large, as urea has a market share. Compared to other products, urea has high nitrogen content (46%), a fact that makes its transport relatively cheap. The most commonly used N fertilizer is urea (46-0-0), due to high N content, low cost and easy transport storage and application (Glibert et al., 2006).

One of the most useful Nitrogen Indicators is the Internal crop Nitrogen Use Efficiency (iNUE). This indicator refers to the ratio between the applied nitrogen and the nitrogen that is removed by the crop. In addition, iNUE calculates the nitrogen loss to the environment (Brentrup & Lammel, 2016). Other indicators are Nitrates (NO_3^-) and ammonium (NH^+_4), which are the major forms of organic N in agricultural soils. Nitrate is water soluble and is commonly used to calculate the availability of N in soils. Ammonium is often used for the same purpose as well (Maynard et al., 2016).

The most widely used inhibitors are the urease inhibitor, thiophosphorictriamide (NBPT) and the nitrification inhibitor, dicyandiamide (DCD) (Li et al., 2020). Urease inhibitors delay urea hydrolysis in soil, by reducing the formation of NO_3^- and NH^+_4. In that way, the toxic effect of high ammonia concentration on seed germination is narrowed. The existence of the inhibitor in the soil, affects the effectiveness of controlling NH_3 losses. According to Krol et al. (2020), urease inhibitors, when added to urea, reduced ammonia loss and thus increased cotton yield and N uptake, compared to single urea application. Due to the fact that N is a component of the chlorophyll structure, the addition of NBPT causes an increase of the chlorophyll content in the leaves (Makino & Osmond, 1991). Liu et al. (2017) reported that the nitrification inhibitor did not alter yield; however the N use efficiency of cotton increased, under a drip-fertigation system. Double inhibitor NBPT and DCD, are unknown to slow down the N conversion to meet the crop's needs (Li et al., 2020). As for crops an increase on 5–12% to iNUE is reported, while urease inhibitor increase the yield of cotton crop Cantarella et al., 2018). On the other hand, Li et al. (2020), demonstrated that cotton boll yield, lint percentage, lint yield and fiber quality, were not affected by fertilizer treatments, including polymer-coated urea (ESN) and urease inhibitors.

The scope of this study is to determine the improvement of the fertilizer yield by adding nitrification (DCD) and urease (NBPT) inhibitors in urea.

MATERIAL AND METHODS

Location and soil classification of the experimental site

The experiments were conducted as an open-field experiment at two areas in Greece, during 2019. The first site was located in Agrinio region, West Greece (Latitude: 38°35′ N, Longitude: 21°25′ E, Altitude: 80 m above sea level). The type soil is characterized as Clay Loam (40.9% clay, 26.5% silt and 32.6% sand), organic matter content in the topsoil of approximately 1.46% (Wakley & Black, 1934) and pH 7.44. The second experimental field was located in the drained Copaida basin, Veotia prefecture, Central Greece (Latitude: 38° 24' N, Longitude: 22° 59' E, Altitude 110 m above sea level) in an alluvial plain of lake deposits, intensively cultivated with maize, wheat and cotton. The experimental soil of Copaida area was (43.7% clay, 25.6% silt and 30.7% sand), pH 7.32 and organic matter 2.29.

The meteorological data, collected from a nearby weather station, regarding temperature and rainfall during the crop growing season is given in Fig. 1.

Experimental design and treatments

A randomized complete block design (RCBD) with factorial arrangement (taking urea combinations and application methods as factors with equal importance) was followed with 4 replications and plot size of 5.0 m × 6.0 m. The total experimental area was 600 m^2, which was devised in 4 replicates with 5 plots. The experiment consisted of four Urea combinations (Urea, Urea+NI+UI, Urea+NI, Urea+UI) and control. In all experiments, the following treatments and doses applied as followed:

1) Control (0 kg N ha^{-1}) 2) Urea (46-0-0): at a rate of 160 kg N ha^{-1}, 3) Urea + Nitrification Inhibitor (NI) + Urease Inhibitor (UI): at the same rate as urea. 4) Urea+NI:

at the same rate as urea. 5) Urea+UI: at the same rate as urea. Half dose was applied before sowing and the remaining half was side-dressed applied 4 weeks after sowing. The nitrification inhibitor was dicyandiamide (DCD) and urease inhibitor was N-(n-butyl) thiophosphoric triamide (NBPT).

For the purpose of this experiment, we used the very early maturity cotton variety ST 402. The planting of cotton (*Gossypium hirsutum* L.) took place on April 22 & 24, 2019 (at Agrinio & Copaida region, respectively) by using 20 kg seed ha^{-1}. The plant density was evaluated over row spacing 95 cm and intra- row spacing 4 cm. Soil tillage encompasses 35 cm deep agronomic chisel plough, followed by rotary hoeing. Drip lines irrigation system was applied over the soil surface and water was being distributed every 10 days. Two manual hoeings were carried out to achieve weed control. The final hand picking took place in October 3rd.

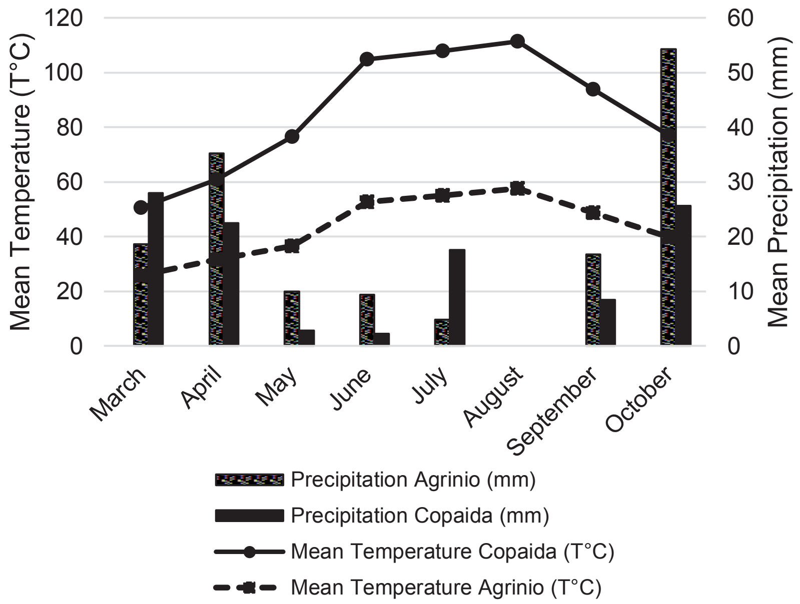

Figure 1. Meteorological data, mean month temperature and precipitation for experimental site during the growing periods in Agrinio and Copaida regions (April-October, 2019).

Samplings, measurements and methods

Agronomic traits

The height of plants was measured from the base of the plant to the tip of the main stem. Leaf Area Index (LAI) was measured by using SunScan ΔT devices. Total weight of opened bolls (g) was counted per plant.

Yields

The *Total Dry Weight (kg ha^{-1})* was measured during the harvest period. A number of 10 plants was selected from the middle rows and was dried at 64°C for 48 h. Then the Seed Cotton Yield (kg ha^{-1}) was calculated according to Eq. (1).

$$\textit{Seed Cotton Yield} = \textit{Density} \times \textit{Number of Bolls} \times \textit{Bolls Yield} \qquad (1)$$

The Above-Ground N Content (%) & Seed Cotton N Content (%) were determined by the Kjeldahl method (Bremner, 1960) using a Buchi 316 device.

Seed Cotton N Yield (kg N ha^{-1})

To estimate the N yield in seed cotton, N concentration (%) is multiplied by the dry weight of the seed cotton (kg ha^{-1}) resulting the yield in N (kg ha^{-1}).

Total Plant Nitrogen Uptake (kg N ha^{-1})

Once the seed cotton was calculated less than 13%, it was ginned on a 10_saw, and after ginning the lint yield (kg ha^{-1}) determination followed.

Fiber quality

To estimate fiber quality parameters and lint proportion, 500 g. seed cotton was selected for each plot; subsequently a laboratory gin machine with saw ginning system was used to separate the fibers from the seeds. Fiber quality characteristics, micronaire, length, strength, uniformity and **spinning consistency index (SCI)** were determined under standard ambient laboratory conditions (21 ± 1°C and 65% ± 2% relative humidity) by High Volume Instrument (HVI-100), USTER Technologies AG., according to the international standards, ASTM D586 (standard test methods of measurements of physical properties of raw cotton classification instruments). Cotton samples before measurement were air conditioned for 12 hour according to ASTM D1776 (standard practice for conditioning and testing textiles).

The Uniformity Ratio expresses the ratio of the Mean Length to the Upper Half Mean Length, expressed as a percentage according to Eq. (2).

$$\textit{Uniformity Ratio} = \textit{Mean Length} / \textit{Upper Half Mean Length} \qquad (2)$$

Spinning consistency index (SCI) was calculated based on a regression equation (Eq. 3) which considers the measured indexes.

$$SCI = -414.67 + 2.9 \times \text{strength} - 9.32 \text{ x micronaire} + 49.17 \times \text{length ('')} + 4.74 \times \text{uniformity} + 0.65 \times RD + 0.36 \times +b \qquad (3)$$

Nitrogen indicators

Nitrogen Utilization Efficiency (NUtE) and internal crop Nitrogen Use Efficiency (iNUE) indicators were used to evaluate the efficiency of nitrogen in cotton cultivation (Gerloff & Gabelman, 1983). The NUtE is calculated according to Eq. (3).

$$\textit{NUtE} = \textit{seed yield (kg ha}^{-1}\textit{)/ total plant N uptake (kg ha}^{-1}\textit{)} \qquad (4)$$

This ratio shows the seed yield (kg ha^{-1}) to the N concentration (kg ha^{-1}) in the above-ground part of the plant per crop.

Crop iNUE was determined in field experiments by Eq. (4) and it indicates how efficiently cotton produces lint in relation to the amount of N, accumulated by the crop. Crop iNUE measurements have been reported for cotton (Bronson 2008; Zhang et al., 2008a; Rochester, 2011).

$$\textit{iNUE} = \textit{kg lint kg}^{-1} \textit{ crop N uptake} \qquad (5)$$

Soil Nitrate N_NO_3 and extractable Ammonium N_NH_4 estimated by flow injection Analyzer Method (Kenney & Nelson, 1982) at 3 different stages, 60, 100 and 140 DAS (days after sowing).

Statistical analysis

Analysis of variance was carried out on data using the STATISTICA (Stat Soft, 2011) logistic package as a Completely Randomized Design. The significance of differences between treatments was estimated using the *LSD* test and probabilities equal to or less than 0.05 were considered significant.

RESULTS

All different fertilizers had an effect on total dry weight and in seed cotton yield, in both areas, as shown in Table 1. The values in total dry weight ranged from 7,941 to 14,136 kg ha^{-1} in Copaida area and from 8,206 to 14,881 kg ha^{-1} in Agrinio area. The total dry weight resulting from fertilization with Urea+NI+UI containing inhibitors, Nitrification (NI) and Urease (UI) and fertilization with Urea+UI containing inhibitor Urease showed statistically significant difference comparing to all other treatments. Urea+NI+UI treatment marked the highest value in total dry weight, in both areas. It is worth emphasizing that $F_{Copaida * Agrinio}$ was not statistically significant (Table 1,2,3) due to the fact that Copaida and Agrinio areas are characterized by similar type of soil and climatic conditions. For all the above reasons there is no differentiation.

Table 1. Agronomic characteristics as affected by fertilizer treatments in Copaida and Agrinio regions

	Plant Height (cm)	LAI	Total Dry Weight (kg ha^{-1})	Seed Cotton Yield (kg ha^{-1})	Weight of open bolls per plant (g)	Lint yield (kg ha^{-1})
Copaida						
Urea + NI + UI	131.20^{a}	4.75 ^{a}	14,136 ^{a}	5,145 ^{a}	80.29 ^{a}	2,287.20 ^{a}
Urea + UI	119.70^{ab}	4.58 ^{ab}	14,028 ^{a}	5,105 ^{b}	79.27 ^{ab}	2,255.30 ^{a}
Urea + NI	112.50^{ab}	4.43 ^{ab}	13,564 ^{b}	4,932 ^{b}	73.74 ^{ab}	2,188.30 ^{a}
Urea	101.10^{bc}	3.70 ^{b}	12,567 ^{b}	4,568 ^{b}	62.75 ^{b}	1,939.80 ^{b}
Control(0 kg)	81.30^{c}	2.28 ^{c}	7,941 ^{c}	1,634 ^{c}	25.22 ^{c}	687 ^{c}
Agrinio						
Urea + NI + UI	139.80^{a}	4.94 ^{a}	14,881 ^{a}	5,318 ^{a}	85.11 ^{a}	2,442.50 ^{a}
Urea + UI	127.60^{ab}	4.87 ^{a}	14,794 ^{a}	5,201 ^{ab}	84.09 ^{ab}	2,406.50 ^{a}
Urea + NI	117.40^{ab}	4.57 ^{a}	14,106 ^{b}	4,953 ^{ab}	76.09 ^{ab}	2,256 ^{b}
Urea	105.40^{bc}	3.88 ^{ab}	13,027 ^{b}	4,572 ^{b}	66.06 ^{b}	1,986 ^{b}
Control	*84.80* ^{c}	2.34 ^{b}	8,206.50 ^{c}	1,650.25 ^{c}	26.14 ^{c}	707.30 ^{c}
$F_{Copaida\ Value}$	*8.46**	10.91**	24.92** → 20.85**	51.26***	25.34**	18.22**
$F_{Agrinio\ value}$	*6.14**	8.27*	24.92**	42.34**	16.47**	40.53**
$F_{Copaida*Agrinio}$	*ns*	*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 ($p > 0.05$).

The values of the seed cotton yield ranged from 1,634 to 5,145 kg ha^{-1} in Copaida area and from 1,650 to 5,318 kg ha^{-1} in Agrinio area, respectively. Urea+NI+UI treatment showed statistically significant difference comparing to all other treatments in Copaida area, whereas in Agrinio area, Urea showed statistically significant difference comparing to Urea+NI+UI treatment and Control. As for the plant height factor, in both regions, Urea+NI+UI treatment showed statistically significant difference comparing to Urea. The highest value was reported in Agrinio area at 39.80 cm, when using Urea with inhibitors Nitrification (NI) and Urease (UI) treatment, while the lowest value was reported in Copaida area at 81.30 cm, in Control (Table 1). LAI values (Leaf Area Index), in Agrinio area, ranged from 2.34 to 4.94 (highest value, in Urea+NI+UI fertilization). Urea with inhibitors Nitrification (NI) and Urease (UI), Urea+UI and Urea+NI treatments showed statistically significant difference with Control. On the contrary, in Copaida area, Urea+NI+UI treatment showed statistically significant difference comparing to Urea and Control, with LAI values ranging from 2.28 to 4.75 (Table 1). The weight of open bolls per plant values ranged from 25.22 to 80.29 g in Copaida area whereas in Agrinio area the range value was from 26.14 to 85.11 g, respectively. Concerning, the Lint yield factor, Urea showed statistically significant difference comparing to other treatments, in Copaida region. Urea+NI+UI and Urea when containing inhibitor Urease (UI) treatments showed statistically significant difference comparing to Urea+NI and Urea treatments, in Agrinio region (Table 1). The lint yield values ranged from 687 to 2,287.20 kg ha^{-1} in Copaida region and from 707.30 to 2,442.50 kg ha^{-1}in Agrinio region. Moreover, in Agrinio area, Urea containing inhibitors Nitrification (NI) and Urease (UI) treatment marked a higher value from the corresponding treatment in Copaida area (Table 1). In the current study, statistically significant differences were found among the fertilizer treatments, concerning the agronomic characteristics of cotton such as total dry weight, seed cotton yield, lint yield, LAI and plant height. The Inhibitors used in this study, were NBPT (Urease inhibitor), DCD (Nitrification inhibitor) and double inhibitors NBPT+DCD.

Our results indicated that the different treatments in both regions (Agrinio & Copaida) showed statistically significant difference, in both Nitrogen Utilization Efficiency (NUtE) and Internal crop N use efficiency (iNUE) sectors. The values of iNUE, ranged from 6.82 to 9.81 in Copaida area and from 6.10 to 9.16 in Agrinio area. Respectively, the values of NUtE ranged from 16.20 to 23.06 in Copaida area and from 9.27 to 13.74 in Agrinio area. Urea with double inhibitors showed statistically significant difference with Urea+NI, Urea+UI and with Urea, in Copaida area. Also, Urea’s value with inhibitor Urease, in Copaida area, was 21.53, higher from the corresponding treatment in Agrinio area, which were 12.94. It is worth pointing out that, the treatments showed statistically significant difference comparing to control (Table 2).

Furthermore, the Seed Cotton N Content, Seed Cotton N Yield and Total Plant Nitrogen Uptake showed statistically significant difference between treatments in Copaida and Agrinio regions. The fertilizations that Urea showed statistically significant differences were Urea+NI+UI, Urea+UI, Urea with inhibitor Nitrification (NI) treatments and Control in Copaida area (Table 2). The seed cotton N yield values ranged from 43 to 179.25 kg N ha^{-1} in Copaida area and from 48.50 to 211.75 kg N ha^{-1} in Agrinio area. The seed cotton N content values ranged from 2.64 to 3.48% in Copaida area and from 2.71 to 3.66% in Agrinio area. In regard to total plant nitrogen uptake, the values ranged from 103.50 to 250.50 kg N ha^{-1} in Copaida area and from 117.75 to 286.75 kg N ha^{-1} in Agrinio area. In total, plant nitrogen uptake value in Urea was 198.50 kg N ha^{-1} in Copaida area, and it was lower than the value of Urea in Agrinio area (218 kg N ha^{-1}). In the above ground N content, the value ranged from 1.29 to 1.77% in Copaida area and from 1.42 to 1.94% in Agrinio area. Urea+NI+UI and Urea with inhibitor Urease (UI) showed statistically significant difference with Urea in Copaida area, and Urea+NI+UI and Urea+UI showed statistically significant difference with Urea+NI and Urea in Agrinio area.

Also, in Agrinio area, all treatments marked higher values when compared to the ones in Copaida area. The highest values in all parameters concerning nitrogen content are given by Urea with inhibitors Nitrification (NI) and Urease (UI) in both areas (Table 2).

Table 2. Content nitrogen in seed cotton, cotton yield, in plant, NUtE, iNUE as affected by fertilizer treatments in Copaida and Agrinio regions

	Above Ground N Content (%)	Seed Cotton N Content (%)	Seed Cotton N Yield (kg N ha^{-1})	Total Plant Nitrogen Uptake (kg N ha^{-1})	Nitrogen Utilization Efficiency (NUtE)	Internal crop N use efficiency (iNUE)
Copaida						
Urea + NI + UI	1.77 ^{a}	3.48 ^{a}	179.25 ^{a}	250.50 ^{a}	23.06 ^{a}	9.81 ^{a}
Urea + UI	1.74 ^{a}	3.45 ^{a}	176 ^{a}	245.50 ^{a}	21.53 ^{b}	9.56 ^{a}
Urea + NI	1.69 ^{ab}	3.34 ^{a}	164.75 ^{a}	229.25 ^{a}	20.79 ^{b}	9.17 ^{b}
Urea	1.57 ^{b}	3.08 ^{b}	140.50 ^{b}	198.50 ^{b}	20.52 ^{b}	9.12 ^{b}
Control (0 kg)	1.29 ^{c}	2.64 ^{c}	43 ^{c}	103.50 ^{c}	16.20 ^{c}	6.82 ^{c}
Agrinio						
Urea + NI + UI	1.94 ^{a}	3.66 ^{a}	211.75 ^{a}	286.75 ^{a}	13.74 ^{a}	9.16 ^{a}
Urea + UI	1.89 ^{a}	3.62 ^{a}	204.75 ^{ab}	282.25 ^{ab}	12.94 ^{a}	9.07 ^{a}
Urea + NI	1.76 ^{b}	3.42 ^{ab}	183.75 ^{b}	249 ^{bc}	12.05 ^{b}	8.51 ^{b}
Urea	1.66 ^{b}	3.16 ^{b}	157.50 ^{c}	218 ^{c}	11.97 ^{b}	8.50 ^{b}
Control (0 kg)	1.42 ^{c}	2.71 ^{c}	48.50 ^{d}	117.75 ^{d}	9.27 ^{c}	6.10 ^{c}
$F_{Copaida\ Value}$	*25.34***	*26.30***	*73.31****	*37.38***	*5.56**	*6.21**
$F_{Agrinio\ value}$	*30.73***	*22.31***	*69.23****	*35.01****	*6.44**	*9.56**
$F_{Copaida\ *\ Agrinio}$	*ns*	*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 ($p > 0.05$).

Generally, micronaire was higher in Copaida than in Agrinio region (Table 3). However, in both regions it was significant higher (4.04–4.30) when Urea+NI+UI treatment was applied and lower in Control and Urea treatments (3.25–3.39). Micronaire in all other treatments (Urea+UI and Urea+NI) marked intermediate values of about 3.80 and 4.07 in Copaida and Agrinio regions, respectively.

SCI (Spinning Consistency Index) generally was higher in Agrinio than in Copaida region. In Copaida area, SCI marked significant lower values in Urea and Control (137.52 and 125.31 respectively) whereas the values were the same in the rest three treatments (Urea+NI+UI, Urea+UI and Urea+NI) (Table 3). In Agrinio area, SCI showed significant differences between all treatments, marking the higher value in Urea+NI+UI (158.16) treatment, the lower value in Control (129.33), and intermediate values in all the rest treatments (Urea+UI, Urea+N & Urea, 149.66, 140.31 and 140.06, respectively). As for the other fiber parameters, fiber length in both two regions (Copaida and Agrinio), was significantly higher in Urea+NI+UI treatment (28.50, 28.35 mm in both two regions respectively) and lower in Control (26.94 and 26.33 mm in both two regions respectively).

Table 3. Fiber quality as affected by fertilizer treatments in Copaida and Agrinio regions

	Upper Half Mean Length (mm)	Strenght g Tex^{-1}	Elongation (%)	Micronaire	Spinning Consistency Index	Lint Propotion (%)
Copaida						
Urea + NI + UI	28.50 ^{a}	30.47 ^{a}	9.18 ^{a}	4.04 ^{a}	140.38 ^{a}	43.87 ^{a}
Urea + UI	28.14 ^{b}	30.07 ^{a}	9.06 ^{a}	3.83 ^{a}	139.17 ^{a}	43.82 ^{a}
Urea + NI	28.02 ^{b}	29.95 ^{a}	9.02 ^{b}	3.81 ^{a}	138.52 ^{a}	43.55 ^{b}
Urea	27.75 ^{c}	28.71 ^{b}	8.94 ^{b}	3.39 ^{b}	137.78 ^{b}	42.52 ^{b}
Control (0 kg)	26.94 ^{d}	27.62 ^{b}	8.69 ^{c}	3.25^{b}	125.31 ^{c}	41.49 ^{c}
Agrinio						
Urea + NI + UI	28.35 ^{a}	32.34 ^{a}	9.76 ^{a}	4.3 ^{a}	158.16 ^{a}	46.20 ^{a}
Urea + UI	28.32 ^{a}	31.82 ^{a}	9.66 ^{a}	4.07 ^{ab}	149.66 ^{ab}	45.91 ^{a}
Urea + NI	27.64 ^{b}	31.06 ^{ab}	9.41 ^{b}	3.99 ^{ab}	140.31 ^{bc}	45.57 ^{ab}
Urea	27.17 ^{b}	29.85 ^{bc}	9.20 ^{b}	3.54 ^{bc}	140.06 ^{c}	43.94 ^{bc}
Control (0 kg)	26.33 ^{c}	28.61 ^{c}	9.06 ^{c}	3.39 ^{c}	129.33 c	42.86 ^{c}
$F_{Copaida\ Value}$	*62.23****	*8.71***	*21.97***	*19.83***	*19.28***	*5.34**
$F_{Agrinio\ value}$	*16.12***	*11.01***	*22.67***	*ns*	*6.67**	*5.11**
$F_{Copaida\ *\ Agrinio}$	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

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

Fiber strength (Table 3) data demonstrates the same alterations with SCI, marking significant higher values in Urea+NI+UI (30.47 g tex^{-1}), in Urea+UI (30.07 g tex^{-1}) and in Urea+NI (29.95 g tex^{-1}) treatments in Copaida region and lower values in Urea and Control treatments (28.71 and 25.62 g tex^{-1}, respectively). Additionally, in Agrinio region significant differences between all treatments were marked but the values were significantly higher in Urea+NI+UI treatment (32.34 g tex^{-1}) and significantly lower in Control (28.61 g tex^{-1}). Also, fiber strength was significantly higher in Agrinio than in Copaida region.

Fiber elongation, showed similar variations for both two regions. It was significantly higher in Urea+NI+UI and Urea+UI treatments (9.18% and 9.76% in Copaida and Agrinio regions, respectively) and it was lower in the rest of the treatments (Urea+NI, Urea and Control). Finally, it was higher in all treatments, in Agrinio region.

Lint proportion was generally higher in Copaida than in Agrinio region, marking significant higher values in Urea+NI+UI and Urea+UI treatments rather than in all other treatments.

It is emphasized that the application of Urea containing Nitrification (NI) and Urease (UI) inhibitors as fertilizer, resulted all fiber quality parameters to mark the highest values in both regions (Copaida&Agrinio), as shown in the Table 3.

Fig. 2 depicts the N NH_4 concentrations in different DAS (Days After Sowing). Observing the case of Copaida area, it is demonstrated that at 40 DAS, all treatments marked high values, ranged from 23 ppm to 37 ppm. Urea+NI treatment marked the highest value and Control the lowest. At 100 DAS a value decrease was noticed, with Urea+NI, Urea treatments and Control demonstrating 26 ppm, 24 ppm and 16 ppm N NH_4 concentrations, respectively. Although at 140 DAS, Control showed an increased value compared to 100 DAS, still, it marked the lowest values along with Urea+NI treatment (23 ppm for both treatments). The highest values were marked by Urea+UI and Urea treatments. It is worth pointing out that while Urea+NI treatment showed originally the highest value at 60 DAS, it shows the lowest at 140 DAS.

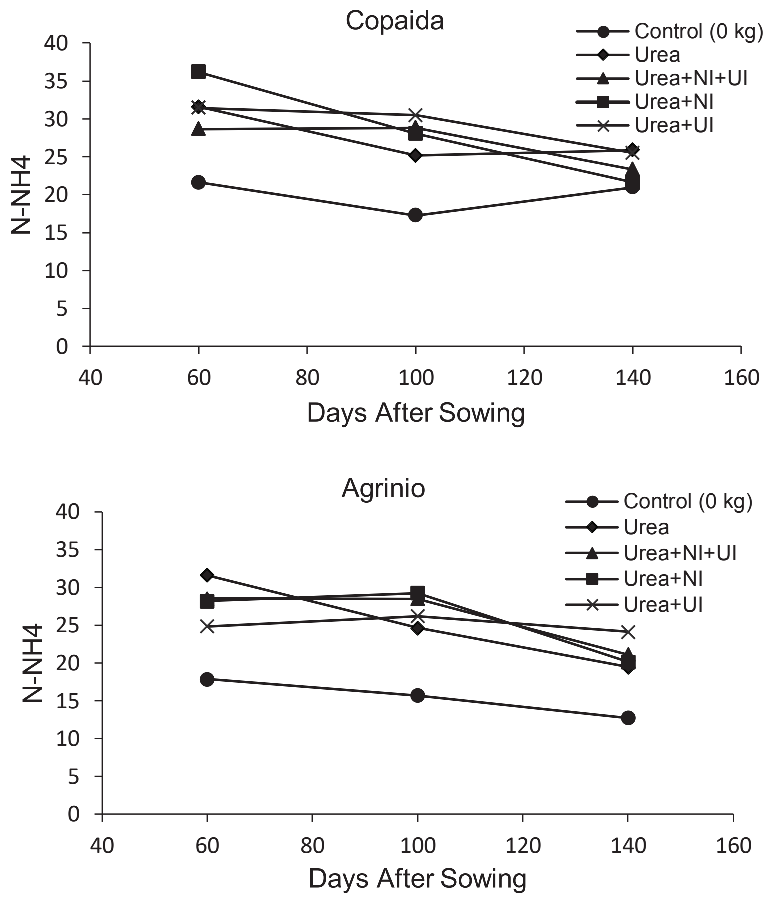

Figure 2. Ammonium concentrations (N_NH_4) in Copaida and Agrinio regions at 60, 100 and 140 DAS.

Observing Agrinio area, at 60 DAS Urea marked the highest value and Control the lowest. At 100 DAS Urea value decreased. At 140 DAS the lowest value is given by Control and the highest by Urea+UI treatment, (14 ppm and 25 ppm, respectively). The remarkable fact is that we noticed a decrease in Urea, at 140 DAS in Agrinio area, while the highest value marked at 60 DAS. On the contrary, in Copaida area, Urea+NI treatment showed the highest value at 60 DAS, and then it decreased at 140 DAS. Additionally, we observed that Control in Copaida area demonstrates an increase at

140 DAS compared to 100 DAS while in Agrinio area it continuously declines until140 DAS.

Fig. 3 depicts the N NO$_3$ concentrations at different DAS (Days After Sowing). While noticing Copaida area, it is demonstrated that Urea marks the highest value at 60 DAS but at the same time Urea marks low values at 140 DAS (35 ppm and 15 ppm, respectively). The values of Control are the lowest at 60, 100 and 140 DAS (17 ppm, 14 ppm and 12 ppm respectively). Urea marked the highest decrease in comparison with all other treatments. From 60 DAS to 140 DAS the decrease in all treatments was impressive.

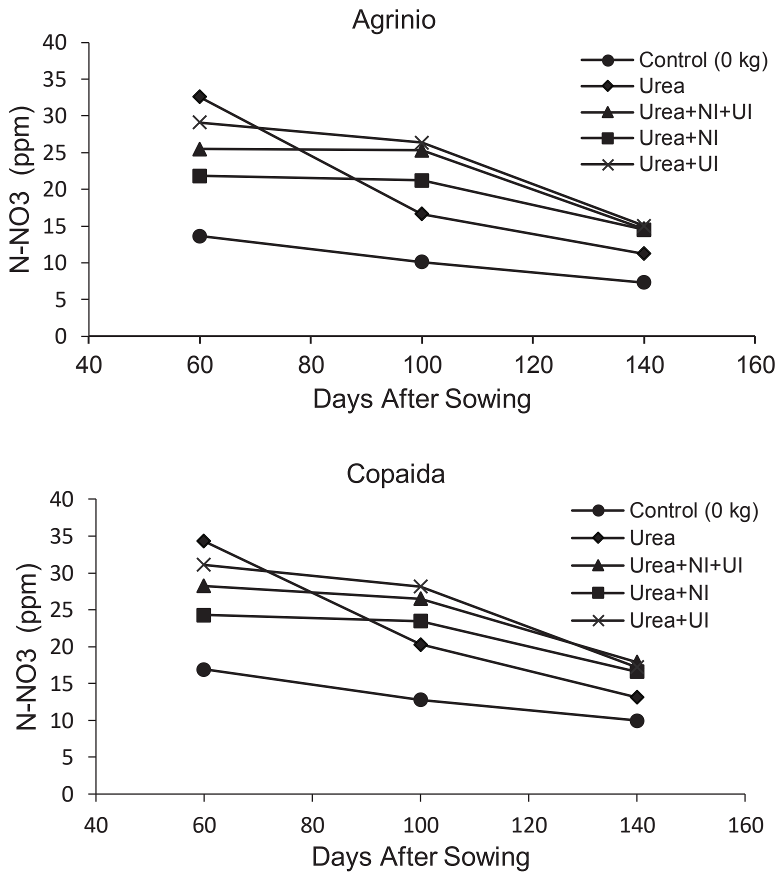

Figure 3. Nitrate concentrations (N_NO$_3$) in Copaida and Agrinio regions at 60, 100 and 140 DAS.

In Agrinio area, Control values at 60 DAS and 140 DAS were 14 ppm and 10 ppm respectively. Urea value at 60 DAS was 35 ppm but at 140 DAS was 15 ppm. Urea+NI+UI, Urea+NI and Urea+UI treatments marked the same value at 140 DAS (20 ppm). At 60 DAS all three fertilizations marked different values (27 ppm, 30 ppm and 24 ppm respectively). Control's values are significantly lower than the corresponding in Copaida area. The values of all treatments decreased from 60 DAS to 140 DAS.

DISCUSSION

Agronomic characteristics

Urea with inhibitors Urease and Nitrification treatment demonstrated significant differences in the total dry weight. Similar studies have also demonstrated a positive effect of NBPT on Urea especially in total dry matter (Oosterhuis et al., 1983; Bondada & Oosterhuis, 2001).

In the present research, during treatment with double inhibitors, differences in LAI were recorded. Similar results presented by Makino & Osmond (1991), who demonstrated that NBPT increases the leaf chlorophyll concentration. This is due to the positive correlation between N and leaf chlorophyll concentration in cotton (Buscaglia & Vacro, 2002).

Seed cotton yield and lint yield were higher when Urea was applied along with NBPT and DCD. Also, seed cotton yield and lint yield marked a significant positive correlation with above ground N, total plant nitrogen Uptake and with seed cotton N (Table 4). Meaning that, these forms of N enhances the seed cotton yield and lint yield. Kawakami et al. (2012) in his study, also demonstrated, that N uptake, in Urea and NBPT treatment, results in higher cotton lint and seed yields.

Table 4. Correlation matrix between nitrogen index, plant growth parameters and yields

	Total Dry Weight (kg ha^{-1})	Seed Cotton Yield (kg ha^{-1})	Lint yield (kg ha^{-1})	LAI	Plant Height (cm)
Above-Ground N (%)	.83***	.80***	.82***	.76***	.76***
Seed Cotton N (%)	.84***	.81***	.83***	.78***	.74***
Seed Cotton N Yield (kg N ha^{-1})	.97**	.93**	.98**	.92**	.83***
Total Plant Nitrogen Uptake (kg N ha^{-1})	.98**	.94**	.96**	.92***	.87***
Nitrogen Utilization Efficiency (NUtE)	.18^{ns}	.32*	.26^{ns}	.18^{ns}	.04^{ns}
Internal crop N use efficiency (iNUE)	.60***	.74***	.72***	.56***	.39*

F-test ratios are from ANOVA. Different letters within a column indicate significant differences according to Tukey's test ($\alpha = 0.05$).

Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Quality characteristics

Fiber growth and development is affected by most factors that also influence the plant's growth. Since the fiber is primarily cellulose, any influence on the plant's photosynthetic capacity and production of carbohydrate will cause similar influence on fiber growth (Bange et al., 2009). Variations in fiber maturity were linked with source-sink modulations related to flowering date (Bradow et al., 1997).

In this study, while comparing Control and Urea treatments, micronaire was higher when Urea+NI+UI, Urea+NI and Urea+UI treatments were applied. Additionally, micronaire marked significant positive correlation with plant N uptake (Table 5) and with the N content in the above the ground part of plants. Therefore, these forms of N enhances the micronaire, and this may by related with the higher LAI values in Urea+NI+UI, Urea+NI and UI treatments.

Table 5. Correlation matrix between nitrogen index and cotton parameters quality

	Micronaire	Upper Half Mean Length (mm)	Strength (g Tex^{-1})	Elongatio n (%)	Spinning Consistency Index	Lint Propotion (%)
Above-Ground N Content (%)	.68***	.73***	.72***	.72***	.67***	.72***
Seed Cotton N Content (%)	.67***	.81***	.72***	.64***	.62***	.66***
Seed Cotton N Yield (kg N ha^{-1})	.71***	.80***	.74***	.71***	.71***	.70***
Total Plant Nitrogen Uptake (kg N ha^{-1})	.71***	.81***	.73***	.71***	.68***	.72***
Nitrogen Utilization Efficiency (NUtE)	$-.07^{ns}$	.34*	$-.22^{ns}$	-.44**	$-.10^{ns}$	-.34*
Internal crop N use efficiency (iNUE)/ Nitrogen Physiological Use Efficiency (NPUE)	$.29^{ns}$	.49**	$.29^{ns}$	$.22^{ns}$	.42**	$.28^{ns}$

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

N is a component of both proteins and chlorophyll. For instance, Bondada et al. (1996) found a strong relationship among lint yield, canopy photosynthesis, and soil N. From a fiber development perspective, changes in the relationship between canopy leaf area and boll number affect the maturity (thickness of the secondary cell wall) of developing fibers leading to differences in micronaire (Bange et al., 2009).

The N effect on canopy photosynthesis is probably predominately caused by the effect N has on leaf area production and light interception. N deficiency also impacts photosynthesis through effects on both the dark and light reaction components of photosynthesis, something that isn't unusual, considering that N is a component of both proteins and chlorophyll Reddy et al. (1996), Pettigrew (2016) demonstrated a close relationship between CER, Rubisco activity, and leaf N concentration. Based on previous reports regarding the effect of N on cotton fiber micronaire, the results of the present research, indicated that the same can be assumed regarding the other fiber parameters (length, strength, SCI and lint proportion) that marked significant higher values, as in the case of micronaire, when comparing Control and Urea treatment with Urea+NI+UI, Urea+NI and Urea+UI treatments.

Also, all the fiber quality parameters demonstrated significant positive correlation, as in micronaire, with N content in cotton seed, with above the ground part of plants and with total plant N uptake. The degree of deposition of cellulose in the fiber cell is significantly affected by factors that affect photosynthesis (Bange et al., 2009). The present research findings showed this factor may be LAI since it marked significant higher values when Urea and Control treatments compared to Urea with inhibitor nitrification (NI), Urea with inhibitor Urease (UI) treatments.

Few agronomic or climatic conditions indicated a consistent effect on fiber bundle strength, as the loss of leaf area can reduce photosynthesis. The strength of cotton fibers is related to the degree of wall thickening. Important, however, substantial differences in strength of fibers will depend on the chemical structure of the cellulose being laid down in the secondary wall. The longer the cellulose molecule chains that are laid down, the stronger the fiber becomes. The length of fiber is analogous to the yarn's strength (the longer the fiber is, the yarn is made stronger). The different fiber strength among cotton varieties is related to the composition of the cellulose. Nitrogen and potassium nutrition can have a significant effect on fiber quality (Pettigrew, 2016).

According to Boquet et al. (1993), the nitrogen fertilization demonstrated significant impacts on plant growth, lint yields and fiber quality.

Higher values in treatments with inhibitors, were observed mainly because Urease inhibitors delay Urea hydrolysis in soil and, this way, decreases the intensity of the soil pH while NH_3/NH_4^+ concentration is increased in the surrounding area of the fertilizer granule, thus reducing the toxic effect of high ammonia concentration on seed germination (Xiaobin et al., 1995; Grant & Bailey, 1999). The benefit of using Urea with inhibitor Urease (UI) fertilization in crops is well documented (Norman et al., 2006; Mozaffari et al., 2007). The nitrification inhibitor, by blocking nitrification, caused the soil NH_3/NH_4^+ concentration to remain high for a longer period, allowing volatilization losses to continue (Soares et al., 2012). The use of Urea with inhibitor nitrification has been reported to positively affect N fertilization and yield of crops (Di & Cameron, 2002).

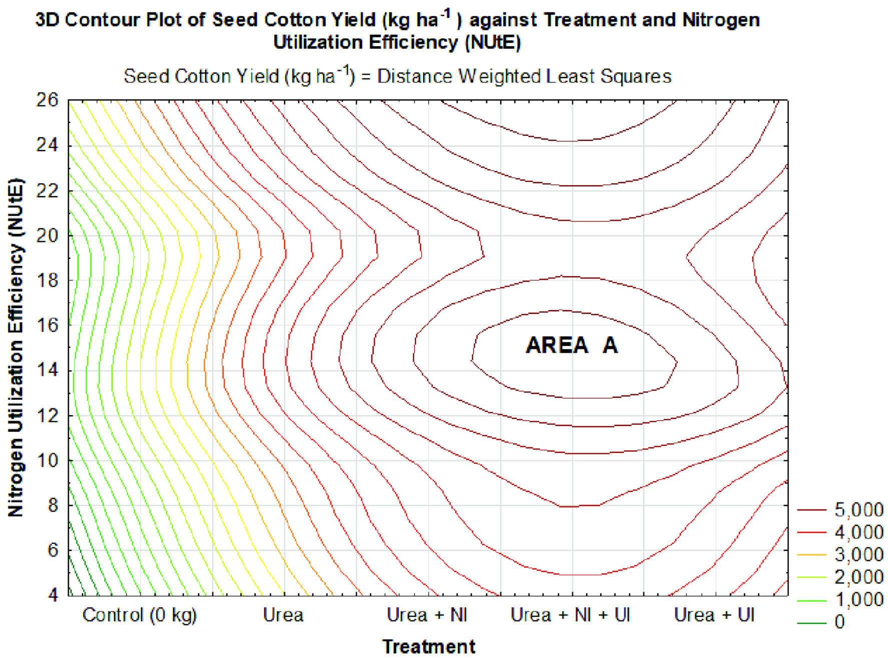

Figure 4. 3D-plot of seed yield against treatments and NUtE.

Each year larger amounts of N fertilizers are applied to croplands and cost billions of money (Nour, 2015). The estimated efficiency of applied N ranges from about 30% to about 70% (John D., 2007). Concerning to Nitrogen Utilization Efficiency (NUtE), we created Fig. 4, which shows the optimal area. According to Table 1 and Fig. 4, Urea with double inhibitors marked higher seed yield (12.63%), Urea+UI (11.76%) and Urea+NI (7.97%) than Urea. It should be noted that the same quantities of fertilizers were used in all treatments. In general, the efficiency of the inhibitors can reduce from 12.63% to 7.96% the quantities of Urea and therefore the losses arising from its use.

Corresponding to Fig. 5, the optimal area is depicted, when using Urea+NI+UI treatment. Under the same quantities of fertilizers, Urea+NI+UI treatment increases micronaire by 20.34%, Urea+UI treatment by 13.99% and Urea+NI treatment by 12.55% compared to Urea. This results from improved Nitrogen Use Efficiency (NUE), which leads to better fiber quality.

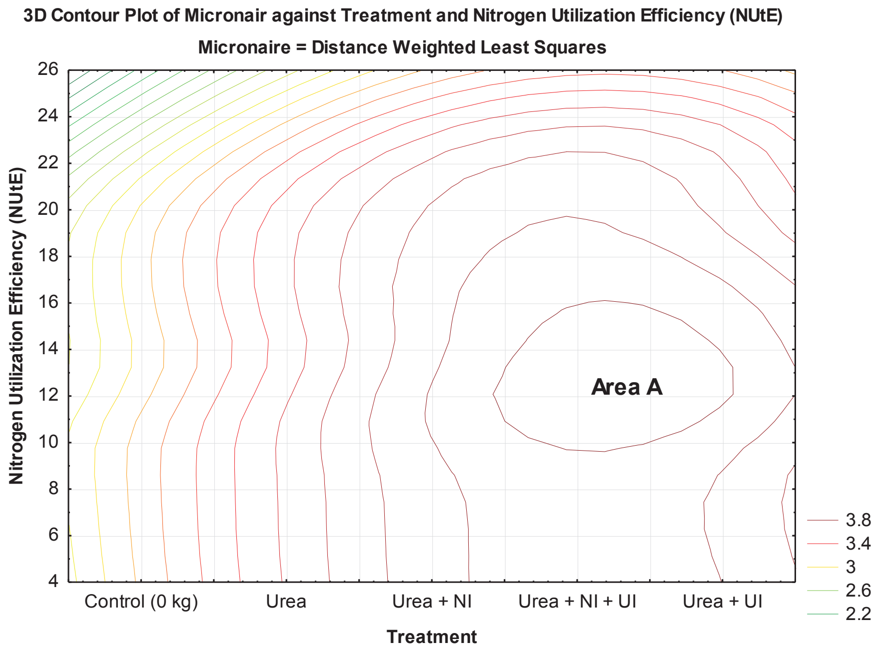

Figure 5. 3D-plot of micronaire value against treatments and NUtE.

CONCLUSION

The findings of the present study clearly indicate that, Urea with inhibitors Nitrification (NI) and Urease (UI) results in better plant growth, Nitrogen Indices as NUE, and better fiber quality, compared to Urea. Urea with Urease (UI) inhibitor and Urea with Nitrification (NI) inhibitor showed the immediate best results in cotton cultivation. It is emphasized that the above apply in both experimental regions (Agrinio and Copaida). According to the above, when using the same amount of fertilizer in all treatments, Urea with double inhibitors increases the seed yield by 12.63% and micronaire by 20.34% compared to Urea. Fertilizers that contain inhibitors, have the potential to increase yields, as well as quality characteristics with less losses to the environment.

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