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Relations between growth, N level, NH_4-N ratio of fertilizer, climatic variables, harvest time and tipburn of cos lettuce grown under the cold glasshouse

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Abstract

A study was conducted to analyze relations between growth, N level, NH_4-N ratio, climatic variables, harvest time and tipburn, and also the effects of N level, NH_4-N ratio of fertilizer and climatic variables on plant growth, yield and tipburn in cos lettuce grown in an unheated glasshouse in 2008-2009 winter-early spring growing period. Results showed that effects of applied N and NH_4-N ratios on plant growth, yield and tipburn were not statistically significant, but data suggested that lettuce yield tended to decline with the increased N and increased NH_4-N ratio of fertilizer. Moreover, of all the variables evaluated for internal tipburn incidence, the night relative humidity had the highest correlation coefficient ($r = 0.910$, $p<0.01$) while for the external tipburn incidence, soil K/Ca ratio had the highest correlation coefficient ($r = 0.738$, $p<0.01$), but these two variables were not the only variables correlated with the tipburn in cos lettuce. Our results suggest that, by statistical means, complex contributions of many variables had effect on tipburn of cos lettuce.

Key words: Tipburn, lettuce, nitrogen, ammonium, harvest time, relative humidity, K/Ca ratio.

Introduction

Despite the advantages in comparison to open field production, the undercover production is subjected to some certain physiological disorders caused by stress situations. Tipburn is considered a calcium-related disorder affected by these stress situations. Tipburn symptoms become apparent as margin rot in the outer leaves, another form of disorder occurs as an internal browning and blackening of the inner leaves within enclosed growing points [25].

Previous findings have revealed that complex contributions of factors influence, directly or indirectly, availability and uptake of calcium and its distribution into the plant tissues are main cause of this disorder. These factors include high temperature, relative humidity, insufficient water and Ca in root zone, weak root growth, pH, salinity of root solution, low rates of transpiration, slow xylem tissue development, low root pressure, insufficient amount of carbohydrates, fast plant growth, auxin, gibberellins, enzyme activities, cultivar and fertilization [1, 2, 4, 6, 7, 9, 11-13, 16, 17, 19-22, 20, 21, 24-27].

Calcium-related disorders are of considerable concern to certain species of plants [25]. The translocation of Ca, mainly through the xylem, depends on transpiration. Thus, Ca deficiency in these species such as head lettuce is more evident in young inner leaves, where transpiration is reduced [10].

Due to relatively shorter growing period than in open field, plants frequently are subjected to the excessive nitrogen fertilizer in greenhouse cultivation of lettuce. Nitrogenous fertilizer, mainly in form of ammonium, stimulates tipburn disorder [8] and should be avoided especially when the soil Ca concentration is higher and soil pH is higher than 7.

Although Barta and Tibbits [3] have documented the critical low levels of Ca (0.2 to 0.4 mg g^{-1} DW) that can occur in enclosed leaves of plants, and, which apparently leads to the marginal apex necrosis of developing leaves, Taylor and Locascio [24] have pointed out that Ca deficiency is not the cause since a critical Ca concentration has not been found. Additionally, Hartz *et al.* [9] have suggested that Ca fertigation (fertilization with drip irrigation) does not improve lettuce Ca uptake or reduce tipburn. The disorder appears to be primarily affected by environmental conditions during greenhouse growth [4].

The objective of this study was to analyze relations between growth, N level, NH_4-N ratio, climatic variables, harvest time and tipburn and also the effects of N level, NH_4-N ratio of fertilizer and climatic variables on plant growth, yield and tipburn in cos lettuce grown in an unheated glasshouse in 2008-2009 winter-early spring growing period.

Materials and Methods

Plant material, location and cultivation method: The planting experiment was run in an unheated glasshouse associated with the Horticulture Department of Agricultural Faculty, of the University of Namık Kemal, during winter-early spring growing season, in Tekirdağ, Turkey (40°59'N, 27°29' E).

Seeds of Yedikule (MayAgro Co.), a cos type of lettuce, were sown in a multicellular tray filled with peat (Klasmann Potgrond H, Germany) on 15th December 2007. Soil transplantation was carried out at the three to four true-leaf stage with 40 cm x 40 cm distances between the rows and in the rows, and with border plants on their sides on the 22nd January 2008 into the unheated greenhouse.

Climatic data were collected using by data collector (HOBO) placed above 50 cm of greenhouse surface, every 2 hours. The environmental condition of glasshouse during the period of experiment is presented in Fig. 1.

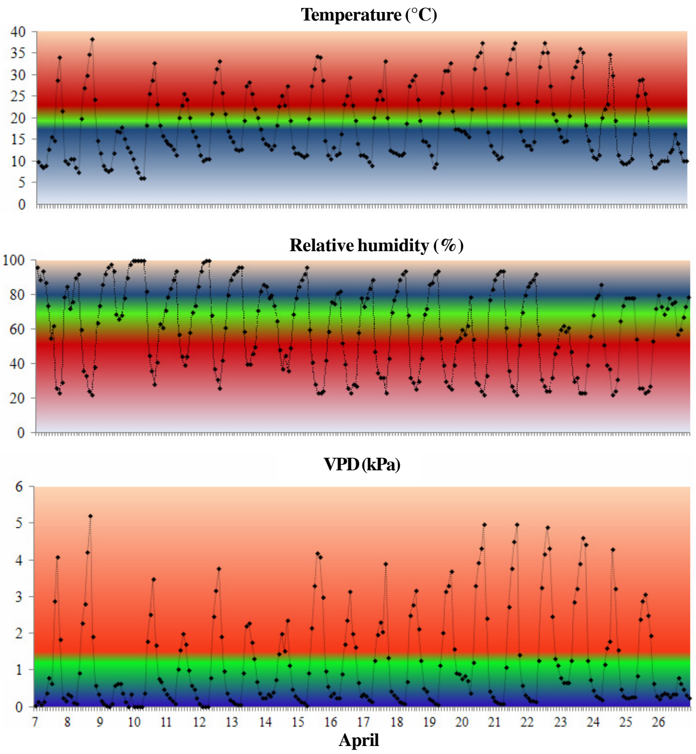

Figure 1. Environmental conditions of glasshouse during the period of experiment. Green lines represent the interval of optimum conditions.

The seedlings were watered and liquid-fed regularly until they were planted in the glasshouse border. The content of the fertilizer, applied once every fourth watering, was N 104, P_2O_5 60 and K_2O 340 mg l^{-1}. Seedlings were divided into 3 groups containing 156 plants in each group. Following planting, plants were liquid-fed according to fertilizing schedule. Three different fertilizer regimes conducted were: Fertilizer 1: (F1) No fertilizer, control; Fertilizer 2: (F2) N 6, P 0.8, K 13 and Ca 2.2 kg da^{-1} (N application schedule: 2 kg da^{-1} at planting, 2 kg da^{-1} 1 month after planting and 2 kg da^{-1} 1 month after 2nd application), NH_4-N ratio 20%; Fertilizer 3: (F3) N 10, P 0.8, K 13 and Ca 2.2 kg da^{-1} (N application schedule: 4 kg da^{-1} at planting, 3 kg da^{-1} 1 month after planting and 3 kg da^{-1} 1 month after 2nd application), NH_4-N ratio 30%.

Criteria considered, data evaluation and statistical analysis: Starting from 20 days prior to harvest, from each parcel of different fertilizer regimes, 6 plants (∑18 plants) were harvested in every day during 20 day-harvest period.

The data collected from this period were as follows: marketable weight (g), plant height (cm), head diameter (cm), number of leaves, head firmness (%), internal tipburn severity (ITS), external tipburn severity (ETS), internal tipburn rate (%) and external tipburn rate (%). Firmness of lettuce heads were evaluated by hand and the heads were given marks of no head, ≤50, 50, 75 and 100, the firmest being the 100. In every second day during the 20-day harvesting period, root fresh and dry weight were measured and head/root ratio was calculated. Also, once a week from each parcel of fertilizer treatment, soil and plant samples were collected to analyze for macro and micro element compositions.

Tipburn was evaluated individually on longitudinally cut-open heads by giving scores [27]: 1= no tipburn, 2 = 0.5 cm, 3 = 1.0 cm, 4 = 1.5 cm, 5 = 2.0 cm, 6 = 2.5 cm, 7 = 3.0 cm, 8 = 3.5 cm, 9 = 4.0 cm, 10 = ≥4.5 cm.

The experiment was laid out in a completely randomized block design with each treatment comprising six replications (∑468 plants). Treatment effects were compared by Duncan's multiple range test and regression analyses were performed to evaluate variables for tipburn incidence. Statistical analysis was performed with the aid of the SPSS statistical package (17.0 for Windows).

Results and Discussion

At the end of the experiment, all data from each harvest during the 20-day harvesting period were evaluated. Results showed that different N levels and percentage of NH_4-N did not have significant effect on plant growth, yield, plant and soil chemical composition and tipburn (Table 1, Figs 2- 4). Yield was not affected by different N levels or NH_4-N level significantly, but averages of yield from F2 and F3 plots were lower than those of the control plots (F1). Averages of yield obtained from F1, F2 and F3 plots were 428, 383 and 359 g $plant^{-1}$, respectively. Control plants also provided higher head diameter, plant height, head firmness and head/root ratio.

In contrast with this finding, Magnusson [16] has reported that increasing N level affects the yield. Simonne *et al.* [23] on the other hand report that nitrogen source affects crunchiness but not lettuce yield. In addition to this, Broadley *et al.* [5] suggested that periods of N deprivation at any early stage of crop growth will affect yields, although this effect is likely to be smaller as crops approach maturity, or if N is resupplied.

The reason for increasing N levels did not affect yield might be the decreasing dry matter production caused by low VPD [14] or the disturbed balance between absorption an assimilation at the late stage of the growth due to the higher irridation [15, 18]. Plants grown

Table 1. Effect of N level and NH_4-N ratio on plant growth, yield and tipburn incidence of cos lettuce (cv. Yedikule) (at the conclusion of the experiment) (n = 120).

Fertilizer/N level (da)	Marketable yield (g)	Number of leaves	Head diameter (cm)	Plant height (cm)	Firmness (%)	Head/Root ratio (g)	Internal tipburn rate (%)	External tipburn rate (%)
F1/0kg	428±46.1	54.49±2.66	10.35±0.68	30.37±0.57	64.83±6.9	20.36±0.7	19.17±0.41	22.50±0.49
F2/6 kg	383±46.1	54.97±2.66	9.99±0.68	29.58±0.57	62.97±6.9	17.97±0.7	16.67±0.41	24.17±0.49
F3/10 kg	359±46.1	50.96±2.66	9.32±0.68	29.26±0.57	56.73±6.9	18.72±0.7	13.33±0.41	22.50±0.49
LSD % 5	ns	ns	ns	ns	ns	ns	ns	ns

Fertilizer 1: No fertilizer; Fertilizer 2: N 6, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 20% (N application schedule: 2 kg da^{-1} at planting, 2 kg da^{-1} 1 month after planting and 2 kg da^{-1} 1 month after 2nd application); Fertilizer 3: N 10, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 30% (N application schedule: 4 kg da^{-1} at planting, 3 kg da^{-1} 1 month after planting and 3 kg da^{-1} 1 month after 2nd application).

under high VPD (low air humidity) show a higher photosynthetic capacity than plants grown under a low VPD (high air humidity)[28].

Internal and external tipburn incidence (%), although statistically significant, were slightly lower with increasing N level, while control plots produced the highest internal tipburn incidence of 19.17% and highest external tipburn incidence of 22.50%. Additionally control plots gave the highest internal and external tipburn severity (Table 1 and Fig. 2).

A negative correlation between the internal tipburn incidence and marketable yield (r = -0.203, p<0.01), plant height (r = -0.220, p<0.01) and number of leaves (r = -0.117, p<0.05) were observed,

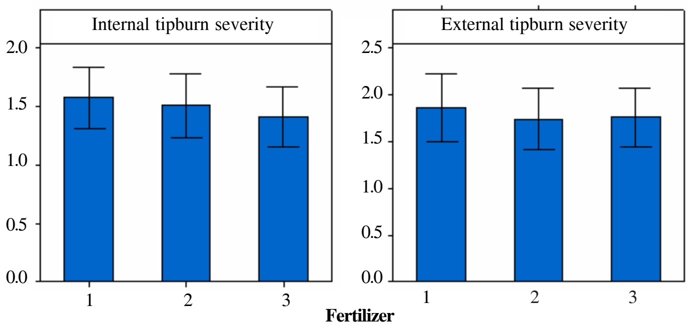

Figure 2. Effect of N level on tipburn severity of cos lettuce (cv. Yedikule)*, 95% CI for the Mean. Fertilizer 1: No fertilizer; Fertilizer 2: N 6, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 20% (N application schedule: 2 kg da^{-1} at planting, 2 kg da^{-1} 1 month after planting and 2 kg da^{-1} 1 month after 2nd application); Fertilizer 3: N 10, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 30% (N application schedule: 4 kg da^{-1} at planting, 3 kg da^{-1} 1 month after planting and 3 kg da^{-1} 1 month after 2nd application).* Mean separation by Duncan's multiple range test (P<0.05, n=120).

while external tipburn severity was effected by only head/root ratio (r = 0.306, p<0.05) (Table 2).

High external tipburn severity together with the high head/root ratio might suggest an impact of plant maturity as suggested by the Wissemeier and Zühlke [27] or root grow. Susceptibility of plant to tipburn might be attributed to altered head/root ratio, altered hormonal balance or effects of morphological and metabolic changes on Ca uptake, translocation or utilization of Ca at the tissue level [21, 27]. Another reason for this result, considering head firmness and head diameter had no significant effect while the number of leaves had increasing effects on tipburn, may be that, rather than the weight, the proportion of the Ca affected internal tipburn.

Harvest time (plant age) showed positive significant correlation with the internal tipburn severity (r = 0.79, p<0.00) (Table 2). While occurrence of external tipburn was more pronounced between 9th and 20th day of 20-day harvesting period, although fluctuating, internal tipburn occurrence was observed at the last week of harvest period (Figs. 5 and 6).

Significant positive correlations between internal tipburn severity and the sum of night temperature, night relative humidity, the sum of day temperature and the sum of daily temperature, significant negative correlations between internal tipburn severity day relative humidity and daily relative humidity were obtained. In contrast, these parameters had no effect on external tipburn severity (Table 3).

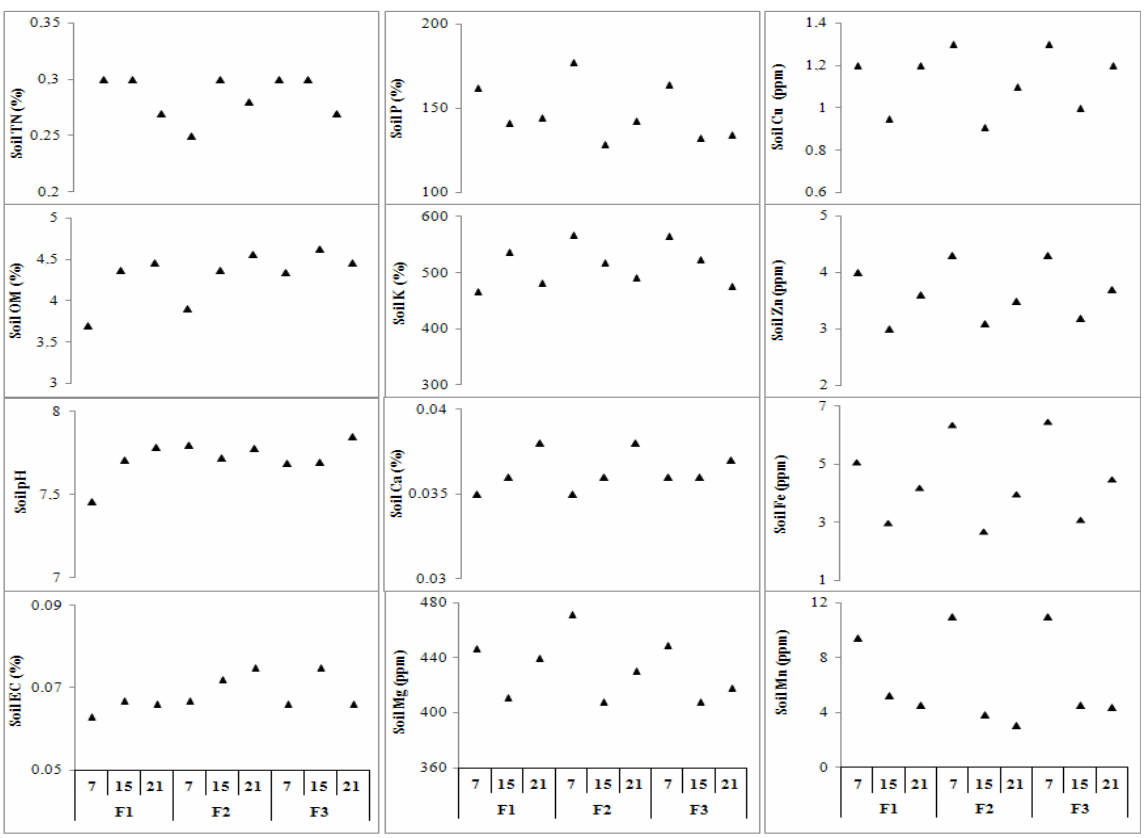

Figure 3. Effect of N level and NH_4-N ratio on soil composition.
F1: No fertilizer; F2: N 6, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 20% (N application schedule: 2 kg da^{-1} at planting, 2 kg da^{-1} 1 month after planting and 2 kg da^{-1} 1 month after 2nd application); F3: N 10, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 30% (N application schedule: 4 kg da^{-1} at planting, 3 kg da^{-1} 1 month after planting and 3 kg da^{-1} 1 month after 2nd application); 7: 7th day of harvest period; 15: 15th day of harvest period; 21: 21st day of harvest period (n=27).

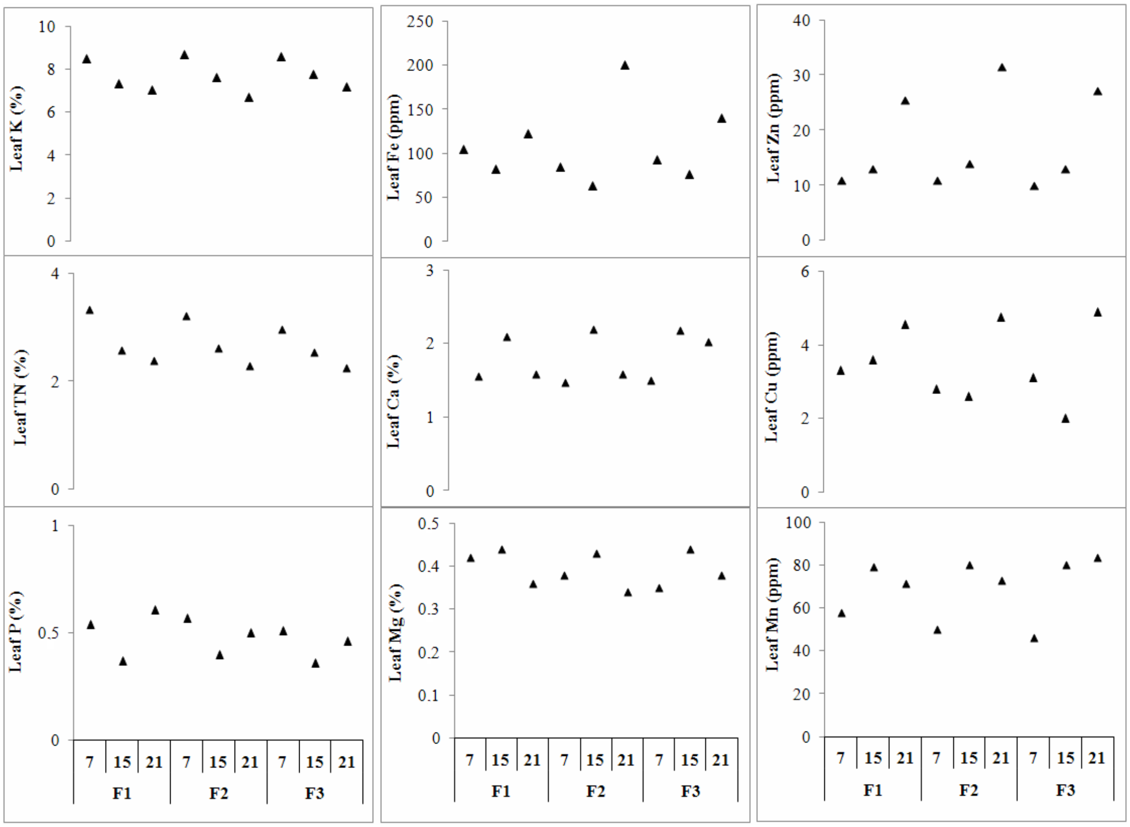

Figure 4. Effect of N level and NH_4-N ratio on leaf composition of cos lettuce (cv. Yedikule).
F1: No fertilizer; F2: N 6, P 0.8, K 13 and Ca 2.2 kg da^{-1}, NH_4-N ratio 20% (N application schedule: 2 kg da^{-1} at planting, 2 kg da^{-1} 1 month after planting and 2 kg da^{-1} 1 month after 2nd application); F3: N 10, P 0.8, K 13 and Ca 2.2 kg Ca da^{-1}, NH_4-N ratio 30% (N application schedule: 4 kg da^{-1} at planting, 3 kg da^{-1} 1 month after planting and 3 kg da^{-1} 1 month after 2nd application); 7: 7th day of harvest period; 15: 15th day of harvest period; 21: 21st day of harvest period (*n*=27).

Table 2. Correlation coefficients between tipburn and yield and growth of cos lettuce (cv. Yedikule) (*n* = 360).

Variable	Fertilizer	Harvest Time	Marketable Weight	Plant Height	Head Diameter	Number of Leaves	Head/Root Ratio
Internal tipburn severity	0.003	0.794**	-0.203**	-0.220**	-0.079	0.117*	-0.045
External tipburn severity	0.015	-0.254	0.147	0.002	0.123	0.024	0.306*

* $p<0.05$, ** $p<0.01$

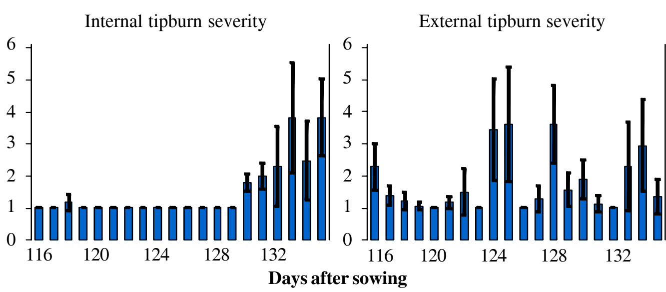

Figure 5. Effect of harvest time on tipburn severity of cos lettuce (cv. Yedikule). 95% CI for the Mean. Means and SE bars, (*n*=18).

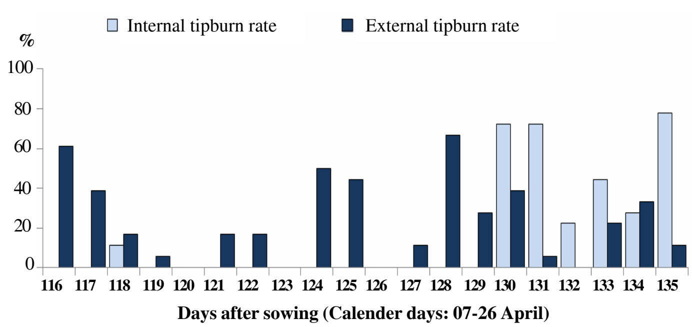

Figure 6. Effect of harvest time (plant age) on tipburn level of cos lettuce (cv. Yedikule) (*n*=18).

Table 3. Correlation coefficients between climatic and variables tipburn of cos lettuce (cv. Yedikule) (*n* = 240).

Variable	T_{night}	RH_{night}	T_{day}	RH_{day}	T_{sum}	RH_{daily}
Internal tipburn severity	0.790**	0.910**	0.765**	-0.614**	0.773**	-0.419**
External tipburn severity	-0.250	-0.519**	-0.222	0.081	-0.231	-0.070

* p<0.05, ** p<0.01

The significant negative correlation between RH_{day} and internal tipburn indicates that effect of transpiration was more pronounced than root pressure as suggested by Wien and Villias [26]. Authors have reported that the disorder was most severe in ponds whose atmosphere was constantly humid. Treatments which provided dry conditions, either during the day, or continuously, were least affected. Hence root pressure is less important than transpiration in distributing Ca to the edges of young leaves of lettuce. This is also correlated with the positive correlation between tipburn and temperature in our study.

According to Tibbits [25], high relative humidity at night improves the Ca transport, even to the non-transpiring tissues, by improving root pressure. However, improving effect of night relative humidity on Ca transport is less pronounced at lower soil temperature. Author also reported that plants grown at root temperature of 23.5°C had marginally higher Ca concentration in inner leaves. In order to increase Ca uptake through root pressure, root temperature should be between 15 and 25°C as reported by author. During the experimental period, average greenhouse temperature was 9-10°C at night and root temperature was between 4 and 23°C. This suggestion is further supported by the work of Hernandez *et al.* [10], in which authors have pointed out that row cover reduced the incidence of tipburn by raising temperatures under cover and altering transport in the inner leaves.

Different N levels and percentage of NH_4-N did not have significant effect on soil and leaf composition. While total N and Ca content of leaf were low, Fe content of leaf was high for cos lettuce. The highest Total N and P content of leaf were obtained from control treatment and these two elements were decreased with the increasing NH_4-N ratio and N. On the other hand, the highest K, Ca, Mg, Cu and Mn content of leaf were increased with the decrease in N level and NH_4-N ratio (Figs 3 and 4).

There were significant negative correlations between internal tipburn severity and soil N, soil K, soil EC, leaf Mg and leaf K/Ca ratio, while the reverse being true for external tipburn severity (Tables 4 and 5). On the other hand, significant positive correlations between internal tipburn severity and soil Ca, leaf P, leaf Fe, leaf Cu, leaf Zn and leaf Mn were observed, again, as these parameters showed negative correlation with external tipburn severity. Moreover, with the increase in soil Ca, while external tipburn severity decreased, internal severity increased. In reverse, with the increase in leaf Ca external tipburn increased. Also with the increase in soil pH and organic matter internal tipburn severity was increased, but correlation between soil EC and internal tipburn severity was negative. Additionally, correlation between leaf K/Ca ratio and both internal and external tipburn severity were significant. It was negative for internal tipburn severity as it was positive for external tipburn severity. Interestingly, however, of all minerals, the best predictor for internal tipburn severity was leaf Zn, while it was leaf K/Ca ratio for external tipburn severity.

The inhibitory effect of increasing soil Ca in external tipburn might be considered the effect of transpiration; however, a positive significant correlation coefficient between leaf Ca and external tipburn indicates that increased Ca of leaves was not the main factor for reducing tipburn. An increase in disorder at the distant part of the leaves might be resulted from an increase in leaf K/Ca ratio. In contrast with this relation, however, an increase in K/Ca content of leaves decreased internal tipburn. Ho *et al.* [11] have pointed out that in relation to the increased plant age, K/Ca content of plant tend to increase, especially at night, but this is not in line with our result since the internal tipburn was also increased by the plant age. Moreover internal tipburn was increased with the increased Zn, Cu, Fe and Mn content of leaves, while it was decreased with the decreased N, K, K/N and K/Ca ratios. Interestingly, however, external tipburn decreased with decreased Zn, Cu and Fe content of leaves.

The assumption of high relative humidity encourage root pressure flow during night time was not proven by this study. On the contrary, internal tipburn increased by increasing night relative humidity. This may be prove rather the concentration of mineral constituents in the root pressure exudates (K/Ca) seemed to be related to reduced tipburn incidence as suggested by Holtschulze [12]. Additionally, highest K concentrations toward the leaf apex and upper margin where injury symptoms occurred indicate a relation between disorder and K[3] .

Table 4. Correlation coefficients between tipburn and soil mineral composition (*n* = 27).

Variable	Soil									
	TN	P	K	Ca	Cu	Zn	Mn	pH	EC	OM
Internal tipburn severity	-0.42**	-0.29*	-0.57**	0.80**	0.18	-0.04	-0.50**	0.52**	-0.43**	0.40**
External tipburn severity	0.32*	-0.05	0.47**	-0.33*	-0.34*	-0.27*	0.06	-0.05	0.36**	0.09

* p<0.05, ** p<0.01

Table 5. Correlation coefficients between tipburn and leaf mineral composition of cos lettuce (cv. Yedikule) (*n* = 27).

Variable	Leaf										
	TN	P	K	Ca	Mg	FE	Cu	Zn	Mn	K/N ratio	K/Ca ratio
Internal tipburn severity	-0.65**	0.33**	-0.69**	-0.13	-0.56**	0.746	0.82**	0.88**	0.29*	-0.69**	-0.82**
External tipburn severity	0.08	-0.46**	0.14	0.33*	0.40**	-0.46**	-0.40**	-0.43**	0.06	0.14	0.73**

* p<0.05, ** p<0.01

Conclusions

According to the results, it might be pointed out that plant yield was not statistically affected by different N levels or NH_4-N level, but was reduced with the increasing N levels and NH_4-N. As for the tipburn incidence, positive or negative correlations between some variables were found, and of all the variables evaluated for internal tipburn incidence the night relative humidity had the highest correlation coefficient (r= 0.910, p<0.01) while for the external tipburn incidence leaf K/Ca ratio had the highest correlation coefficient (r = 0.738, p<0.01).

From the view point of the results of this experiment, however, it can be concluded that, although there were higher correlation coefficients between some of the variables than the others, tipburn in cos lettuce was affected by a complex contributions of variables evaluated in this study. It may be considered that statistics employed did not account for the full complexity with which variables may induce tipburn under fluctuating greenhouse conditions [27].

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