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Original Paper

The changes of the assimilation pigments content of turf *Festuca* spp. leaves after application of different nutrition forms

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The aim of this experiment was to compare find out of the changes of assimilation pigments content of turf *Festuca* spp. leaves after application of different nutrition forms under non-irrigated conditions. In period April 2012 – May 2015 (without June 2014 and February 2015) experiment was carried out in warm and dry conditions in area of Nitra (Slovak Republic). Concentration of assimilation pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) was determined spectrophotometrically. The experiment was included 10 treatments: 1. Without fertilization; 2. Saltpetre with dolomite, superphosphate, potassium salt; 3. Turf fertilizer NPK 15-3-8 (+ 3 MgO + 0.8 Fe + 18S); 4. Slow release fertilizer NPK 14-5-14 (+ 4CaO + 4MgO + 7S); 5. Controlled release fertilizer NPK (S) 13-9-18 (+ 6S); 6. Organic fertilizer NPK 5-1-1,7. Organic fertilizer NPK 3-2-1 and 3 mycorrhizal preparations. The use of inorganic and organic fertilizers resulted in an increase chlorophyll *a*, *b* content and total chlorophyll in leaves *Festuca* spp. More pronounced increase in chlorophyll content was found by the application of the Turf fertilizer. Application of this fertilizer has a statistically significant effect on content of chlorophyll *a* + *b* than in the other evaluated treatments without turfs fertilized by Controlled release fertilizer and Organic fertilizer NPK 5-1-1. A statistically significant increase in the total carotenoids concentration was observed after the use of Saltpetre with dolomite, superphosphate, potassium salt and Turf fertilizer as compared to the non-fertilized control.

Keywords: turf, *Festuca* spp., fertilizers application, chlorophyll, total carotenoids

1 Introduction

Colour, as one of the qualitative indicators of turfs, is closely related to the concentration of green leaf dye-chlorophyll. The most important pigments are chlorophyll *a* (blue-green) and chlorophyll *b* (yellow-green). Carotenoids, together with chlorophyll, are part of photosynthetic organisms and form a reciprocal process (Beard, 1973; Xu et al., 1995; Kovár and Gregorová, 2009). Biosynthesis of assimilation pigments in plants is affected by variety of external and internal factors (Masarovičová et al., 2000). The color of leaves, as the main component for assessing the aesthetic quality of turfs, is often evaluated in field experiments (Karcher and Richardson, 2003; Gregorová and Kovár, 2010; Hric et al., 2016a).

Nitrogen (N) is a component of chlorophyll. N is involved in the conversion of kinetic solar energy into chemical energy. In the absence of nitrogen of plants change their appearance. If the nitrogen content in the leaves of plants is low, then the leaves turn yellow. With a large nitrogen deficiency, the leaf dies (Hrabě et al., 2009; Aldous, 2011).

Nitrogen application significantly improves turf grass color and other turf qualitative parameters (Bell et al., 2004; Bilgili and Acikgoz, 2007; Pessarakli, 2007; Altissimo and Peserico, 2008; Hejduk, 2012). Several studies have been conducted to determine the effects of N application time and rates on turf quality and plant growth (Wehner et al., 1988; Moore et al., 1996; Bilgili and Acikgoz, 2007; Hric et al., 2016b).

The aim of this experiment was to compare the changes of the assimilation pigments content of turf *Festuca* spp. leaves after application of different nutrition forms under non-irrigated conditions. In the contribution compares the effect of fertilization on inorganic, organic and mycorrhizal preparations on the content of chlorophyll *a*, *b* and total carotenoids.

2 Material and methods

In period April 2012 – May 2015 (without June 2014 and February 2015) turf experiment was located in moderate climatic zone of warm and dry area in Nitra (Slovak Republic). In June 2014 and February 2015 samples were not analyzed (decimated turf).

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The experiment was established in early October 2011. We used turf mixture designed for low slowly growing turfs with following composition: *Lolium perenne* L. (30%), *Festuca rubra* L. (50%) and *Festuca ovina* L. (20%). Gradually dominated *Festuca* spp. leaves in turf.

Experimental plots area was 2.4 m^2 and each treatment was in 3 random replications. Experiment was established in the form of a Latin square.

There were used 10 treatments:

1. Without fertilizing (Control).
2. LAD (27% N), P_2O_5 (19% P), K_2O (60% K) (N + P + K).
3. Turf fertilizer NPK 15-3-8 (+ 3 MgO + 0.8 Fe + 18 S) (TF).
4. Slow release fertilizer NPK 14-5-14 (+4 CaO + 4 MgO + 7 S) (SRF).
5. Controlled release fertilizer NPK (S) 13-9-18 (+6S), (CRF).
6. Organic fertilizer NPK 5-1-1 (OF1).
7. Organic fertilizer NPK 3-2-1 (OF2).
8. Mycorrhizal preparation (MP1).
9. Mycorrhizal preparation (MP2).
10. Mycorrhizal preparation (MP3).

For the recommended dose of fertilizer the value 18 g m^{-2} N was taken, which meets the requirements for intensively used turfs (Cagaš et al., 2011). System of fertilizing is presented in Table 1.

LAD contains 27% nitrogen with dolomite. Nitrogen is in the ammonium and nitrate form. Superphosphate is 19% P_2O_5 and potassium salt is 60% K_2O.

Turf fertilizer 15-3-8 (+ 3 MgO + 0.8 Fe + 18 S) is the granulated fertilizer intended for use for turfs throughout the year in the form of multiple fertilizer applications (3–5×) during the growing season. Nitrogen is in the ammonium form.

SRF NPK 14-5-14 (+ 4 CaO + 4 MgO +7 S) is a complex NPK fertilizer containing urea formaldehyde component as a source of nitrogen enriched with micronutrients. Part of major NPK nutrients is founded in fast-dissolving form.

CRF NPK (S) 13-9-18 (+ 6S) is coated controlled release fertilizer nutrient (for 5–6 months).

OF 5-1-1 content is comprising C, H, O, N, P, K, Ca, Mg, S, Fe etc., in the form of organic components of the starch material from the milled cereals (30%), enriched hydrolysate of whey (30%), lignocelluloses raw material from wood processing (30%), by hydrolysis of whey enriched (30%) and in 10% mineral constituent zeolite of sodium aluminium silicate. Philosophy of this fertilizer is unlike mineral fertilizers aimed at improving the carbon balance.

OF 3-2-1 is produced by modern technology from natural materials without the use of chemicals and preservatives. Production procedure at high temperature leads to inactivation of pathogens and weed seeds. This fertilizer is characterized as high-quality organic fertilizer with gradual release of the main nutrients and essential trace elements. Its high biological value is increased due to harmless processing, content balance, easy handling and hygiene applications in practice. Compared with manure it constitutes a modern compensation for of manure.

Mycorrhizal preparation 1 is grass conditioner consisting of natural argillaceous media, 6 kinds of mycorrhizal fungi, natural ingredients that support mycorrhiza (humates, ground minerals, extracts of marine organisms), biodegradable polyacrylamide granules, sapropel (biological sediment). It contains 1.3% N, 0.6% P, 3% K, 1% Mg, 2.8% Ca and 0.8% S.

Mycorrhizal preparation 2 is based on the basis of endomycorrhizal fungi. It contains natural argillaceous media, 6 kinds of mycorrhizal fungi, natural ingredients

Table 1 System of fertilizing of individual treatments (2.4 m^2)

Type of fertilizer (number of applications per year)	Yearly dose (g)	Beginning of vegetation (g)	App.* 5. June (g)	App. 20. July (g)	Half of July (g)	Half of August (g)	Half of September (g)
LAD (4×)	160.00	40.00	40.00		40.00		40.00
P_2O_5 (1×)	130.43	130.43					
K_2O (2×)	69.40	34.70			34.70		
TF (3×)	288.00	96.00		96.00		96.00	
SRF (2×)	288.00	144.00			144.00		
CRF (2×)	332.32	166.16			166.16		
OF1(1×)	864.00	864.00					
OF2 (1×)	1440.00	1440.00					

* app. – approximatelly

that support mycorrhiza (humates, ground minerals, extracts of marine organisms) and biodegradable polyacrylamide granules.

Mycorrhizal preparation 3 contains mycorrhizal fungi, keratin, natural humates, ground minerals (zeolite, serpentine, apatite) a 5% N, 6% P, 4% K, 2% Mg, 2% S and 4% Ca.

Mycorrhizal preparations were applied before sowing turfgrass mixture in dose 360 g per variant.

Concentration of assimilation pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) was determined spectrophotometrically by Spectrofotometer CAMSPEC M108 UV/VIS. Samples of plant material were taken each time in the last decade of the respective month and were analyzed immediately after collection. Four *Festuca* spp. leaves were analyzed in each replication 4 times. After sawing leaves on underlying glass we removed the protruding parts and measured the width of leaves. The leaf segments thus prepared were homogenized in mortar in 80% acetone-containing blend. The impurities were separated by centrifugation at 2500 rpm for 2 minutes. Absorbance of leaf extract supernatant was measured by spectrophotometer at wavelengths (λ) 663 nm (chl *a*), 647 nm (chl *b*) and 470 nm (total carotenoids). The absorbance of impurities (proteins, tannins) in the supernatant was measured at wavelength of 750 nm. The absorbance value of the solution at λ 750 nm was deducted from absorbancies at λ 663, 647 and 470 nm. The concentration of the individual assimilation pigments (mg l^{-1} extract) was calculated according to the following equations (Lichtentaler, 1987):

$$\text{Chl } a = (12.25A_{663} - 2.79A_{647})D$$
$$\text{Chl } b = (21.50A_{647} - 5.10A_{663})D$$
$$\text{Total carotenoids} = [(1000A_{470} - 1.82\text{chl } a - 85.02\text{chl } b)/198]D$$

that, A_{663}, A_{647} a A_{470} is the absorbance of the extract at individual wavelengths (663, 647 a 470 nm) and D is the thickness of the spectrophotometrically cuvette (cm).

Total concentration of chl *a*, chl *b* and total carotenoids in leaves of used grasses was calculated on mg m^{-2} leaf area by relationship:

$$\text{mg m}^{-2} = \text{mg l}^{-1} \times K ===> K = x$$

where:

K – coefficient of conversion
V – sample volume (ml)
A – leaf area of segments (m^2)

Results were statistically evaluated by the Analysis of Variance (One-way ANOVA, Method: 95.0 percent LSD) using statistical software STATISTICA 7.1 (Stat Soft. Inc. 2007).

3 Results and discussion

The average values of chlorophyll *a* and *b* are given in Table 2. The content of chlorophyll *a* in turfgrass leafs was higher (p = 0.000302) on fertilized treatments (467.77–496.69 mg m^{-2}) compared to non-fertilized control (404.05 mg m^{-2}) and mycorrhizal preparation 2 (422.99 mg m^{-2}). The lowest content of chlorophyll in leaves we measured on control (404.05 mg m^{-2}). Similar results were obtained by Larimi et al. (2014) and Mahmoud et al. (2017). Conversely, the highest content of chlorophyll *a* (496.69 mg m^{-2}) in the leaves of grasses was recorded on the treatment fertilized by turf fertilizer. The highest connect values of chlorophyll *a* were on turfs fertilized by inorganic fertilizers (475.62–496.69 mg m^{-2}) and organic fertilizer 1 (492.16 mg m^{-2}). Treatment fertilized by TF was also characterized by the highest variability in chlorophyll *a* content (δ = 146.5).

The concentration of chlorophyll *b* in grass tissues was in the reference period in from 167.85 mg m^{-2} (control) to 218.67 mg m^{-2} (TF). Also, with this assimilation pigment, its content on treatment with application TF (218.67 mg.m^{-2}) was higher (p = 0.000029) than on control and on turfs with mycorrhizal preparations 1 and 2 (202.92 and 183.17 mg m^{-2}). Similar results were obtained by Larimi et al. (2014). The most variability content of chlorophyll *b* over the whole experiment period was treatment fertilized by turf fertilizer (δ = 59.31).

Total chlorophyll content (chl *a* + chl *b*) is presented in Table 3. Values were found in a range from 571.91 mg m^{-2} (TF) to 757.02 mg m^{-2} (control). Application of TF resulted in the highest (p = 000006) concentration of the monitored parameter (757.02 mg m^{-2}) compared to fertilized treatments N + P + K (680.65 mg m^{-2}), SRF (675.84 mg m^{-2}), OF2 (671.36 mg m^{-2}) and mycorrhizal preparations (606.166–675.29 mg m^{-2}). Control treatment has lowest total chlorophyll content. The use of the mycorrhizal preparation resulted in an increase in the total chlorophyll content as compared to the control. Equally, Vafadar et al. (2013) in their experiment after application of mycorrhizal fungi found an increase in chlorophyll content compared to control. Kuo (2015) found in his experiment with using SRF and fast release fertilizers 3× higher chlorophyll content than the control. Once again, the highest imbalance in chl *a* + chl *b* concentration was represented by TF (δ = 271.72). The most stable connect of chl *a* + chl *b* reached treatments with application mycorrhizal preparations (δ = 31.07–30.67).

Table 2 The average values of chlorophyll *a* and *b* in leaves

Treatment	Chl *a* (mg m^{-2})	δ	Chl *b* (mg m^{-2})	δ
Control	404.05^{b}	119.01	167.85^{d}	53.87
N + P + K	478.32^{a}	125.60	202.34^{abc}	55.89
TF	496.69^{a}	146.50	218.67^{c}	59.31
SRF	475.62^{a}	90.05	200.22^{abc}	40.74
CRF	483.90^{a}	98.85	209.24^{ab}	40.99
OF1	492.16^{a}	91.81	206.86^{ab}	45.12
OF2	467.77^{a}	93.45	203.59^{ab}	46.52
MP1	472.37^{a}	89.23	202.92^{ab}	46.02
MP2	422.99^{b}	88.56	183.17^{cd}	45.33
MP3	468.94^{a}	85.49	196.79^{ac}	42.66

$^{a, b, c, d}$ – statistically significant differences (Fisher LSD test, = 0.05), δ = standard deviation, *n* = 6

Table 3 The average values of chlorophyll *a* + *b* and total carotenoids in leaves

Treatment	Chl *a* + *b* (mg m^{-2})	δ	Total carotenoids (mg m^{-2})	δ
Control	571.91^{bc}	167.97	133.19^{a}	41.11
N + P + K	680.65^{a}	195.33	152.34^{b}	44.57
Turf fertilizer	757.02^{b}	271.72	152.26^{b}	46.55
SRF	675,84^{a}	121.88	141.44^{ab}	36.88
CRF	693.15^{ab}	137.51	148.37^{ab}	40.81
OF1	699.02^{ab}	128.81	147.52^{ab}	34.06
OF2	671.36^{a}	123.74	140.69^{ab}	31.25
MP1	675.29^{a}	126.19	144.80^{ab}	31.07
MP2	606.16^{cd}	128.12	135.89^{a}	31.41
MP3	665.73^{ad}	123.63	144.60^{ab}	30.67

$^{a, b, c, d}$ – statistically significant differences (Fisher LSD test, = 0.05), δ = standard deviation, *n* = 6

The concentration of total carotenoids was higher in fertilized treatments by N + P + K (152.34 mg m^{-2}) and TF (152.26 mg m^{-2}). In previous experiment (Hric et al., 2016a), the effect of use fertilizers on the content of total carotenoids did not notice a positive effect. Statistically significantly highest content of total carotenoids was found on the treatment fertilized with N + P + K and turf fertilizer compared with control and mycorrhizal preparation 2. At the same time, turf fertilized by turf fertilizer was characterized by the highest imbalance of this assimilation pigment (δ = 46.55). Again the most stable content of total carotenoids reached treatments with application mycorrhizal preparations (δ = 30.67–31.41).

4 Conclusions

On the basis of the results, it can be stated that the use of inorganic and organic fertilizers as well as the mycorrhizal preparation resulted in an increase of the assimilation pigments content of *Festuca* spp. leaves. The highest values of assimilation pigments were observed on treatments fertilized by inorganic fertilizers (N + P + K, TF, SRF, CRF and OF1). These fertilizers are likely to provide the greenest colour of turf. The highest increase in assimilation pigments occurred after application of TF. At same, this treatment was characterized by the greatest imbalance in assimilation pigment values. In the next period, is planning to continue in the experiment and spread it on other turfgrass species and fertilizers.

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