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Original Article

Growth and Phytochemical Content of *Cynodon dactylon* (L.) Pers. as Affected by Trinexapac-ethyl and Paclobutrazol

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Abstract

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a perennial grass and has been long used as a medicinal herb in Iranian traditional medicine to treat varied ailments. Different parts of bermudagrass such as leaves, stolons, rhizomes and culms are rich sources of metabolites such as proteins, carbohydrates, mineral constituents, β-sitosterol, flavonoids, alkaloids, glycosides and triterpenoides. Growth regulators change plant physiological processes such as phytochemical accumulation. Paclobutrazol (PBZ) and Trinexapac-ethyl (TE) are two popular growth retardant that inhibit gibberellic acid (GA) biosynthesis. This greenhouse experiment was conducted to determine effects of TE and PBZ application on bermudagrass growth and phytochemical content. Paclobutrazol was applied twice at four weeks intervals at 0, 1, 2, 3 and 4 g a.i. (active ingredient) /100 m^2 and TE treatments (0, 0.25, 0.5, 0.75 and 1 g a.i. /100 m2) were applied biweekly over 8 weeks period. Shoot and root growth, were decreased with increasing PBZ and TE application rates except for $TE_{0.5}$ treatments that showed higher root growth than control plants. Chlorophyll, Total non-structural carbohydrates, soluble flavonoids and protein content were increased with increasing PBZ and TE application rates unless TE_1 treatment that exhibited lower total non-structural carbohydrates (TNC) content than untreated plants. The results suggest that TE and PBZ treatments were beneficial for bermudagrass phytochemical content enhancement. However, puclobutrazol application, because of higher phytotoxity than trinexapac ethyl (lower plant quality and sever shoot depression), seems to be less effective in raising medicinal properties.

Keywords: *Cynodon dactylon*, Trinexapac-ethyl, Paclobutrazol, Phytochemical content

Introduction

The interest on herbal medicines and their utilization have been increasing rapidly in recent years as they are claimed to be safe, economical and yet efficacious [1]. Bermudagrass *Cynodon dactylon* (L.) Pers.] is a perennial grass of the Poaceae family and is one of the most commonly occurring weeds in Iran. Bermudagrass has been long used as a medicinal herb in Iranian traditional medicine to treat varied ailments such as, hysteria, cough, headache, diarrhea, cramps, edema, dysentery, hemorrhage, hypertension and stones urogenital disorders [2]. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. [3]. Different part of bermudagrass such as leaves, stolons, rhizomes and culms is a rich source of metabolites such as proteins, carbohydrates, mineral constituents, β-sitosterol, flavonoids, alkaloids, glycosides and triterpenoides [4]. The extract of *C. dactylon* leaf has been reported to be anti-diabetic, antioxidant and hypolipidemic efficacy, healing of minor injuries, immunomo-dulatory and hepatic antioxidant activities. The aqueous fluid extract of *C. dactylon* rhizome is used for diuretic, anti-emetic, purifying agent and dysentery [5].
Growth regulators change plant physiological processes such as phytochemical accumulation [6].

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Paclobutrazol (PBZ) is used as a plant growth retardant for high maintenance in turfgrass management to suppress shoot growth and improving aesthetic [7]. Vadakkemuriyil *et al*. [8] reported that Enzymatic and Nonenzymatic antioxidants as well as α-tocopherol content, increased in the PBZ treated *Ocimum sanctum* plants. Similar result was observed in *Catharanthus roseus* [9]. Ratnadewi *et al*. [10] showed that C*inchona ledgeriana* plants treated by PBZ produced the highest level of quinine, major alkaloids in C*inchona* spp. Trinexapac-ethyl (TE) is a popular plant growth regulator in the turfgrass industry that inhibits gibberellic acid (GA) biosynthesis and effectively reduces leaf elongation and subsequent clipping production. Some studies indicate that TE application can enhance phytochemical properties of turfgrasses [11,12].

Objectives of the present study were to 1) Investigate whether TE and PBZ application can enhance phytochemical content of bermudagrass. 2) Compare morphological and physiological effects of different rates of TE and PBZ application on bermudagrass.

Materials and Methods

Plants Culture and Growth Condition

Bermudagrass [*Cynodon dactylon* (L.) Pers.] was sown (20 g.m^{-2}) in 15-cm-diameter × 30-cm-deep plastic pots filled with sandy loam soil in April. Plants were grown in a greenhouse with average 30 °C day/18 °C night temperatures under natural light (average: 800 μmol m^{-2} s^{-1} photosynthetically active radiation, 14-h photoperiod) at the University of Zanjan. Turf was hand-clipped weekly and pots were fertigated with a soluble 20N-20P-12K + micronutrients fertilizer, for 4 months prior to initiation of treatments.

Treatments, experimental design and data analysis

Paclobutrazol was applied twice at four weeks intervals at 0, 1, 2, 3 and 4 g a.i. /100 m^2 and TE treatments (0, 0.25, 0.5, 0.75 and 1 g a.i. /100 m2) were applied biweekly over 8 weeks period. The experiment was set out in a completely randomized design with four replications for each treatment. The data were statistically analyzed using the analysis of variance procedure [13]. Differences between treatment means were compared by Duncan's multiple range tests at 0.05 probability level.

Measurements

During treatments period, clipping yields were harvested weekly and dried at 70 °C for 48 h for dry weight determination. Following the final clipping harvest after 8 weeks of treatments, grass swards were harvested and divided into verdure and roots. Each fraction was dried at 70 °C for 48 h and then dry mass was measured. Shoot growth was calculated based on cumulative clipping and verdure dry weight [14].

Data on leaves total non-structural carbohydrates (TNC), chlorophyll, flavonoids, soluble protein and Turf quality were determined at the end of experiment.

Turf quality (TQ) was visually rated on a scale of 1 to 9 based on color, density, and uniformity [15]. Plants rated 1, were completely desiccated with a completely necrotic turf canopy. A rating of 9, represented healthy plants with dark green, turgid leaf blades, and a full turf canopy.

Total non-structural carbohydrates were measured according to the method of Lyons *et al*. [16] that was modified method of Ting [17]. Briefly, leaves were dried at 80 °C for 72 h. 40 mg of ground tissues were transferred to glass tubes containing 2.5 mL of 5.0% amylase and incubated at 37 °C for 24 h. After 24 h incubation period, 0.5 mL of 0.6 N HCl was added to the solution for an additional 18 h. The solution was then neutralized with 10 N NaOH and diluted to 50 mL with distilled water and filtered. Reducing sugars were measured by taking 1.0 mL of the solution and adding 1.5 mL of alkaline ferricyanide solution. The mixture was heated for 10 min in a 100 °C water bath and quickly cooled under running water. The pH of the solution was partially neutralized with 3.0 mL of 2 N H_2SO_4. Finally, 1.2 mL of arsenomollybdate solution was added and the total volume was adjusted to 25 mL with distilled water. Absorbance of the solution was measured at 515 nm and compared to a standard curve to determine TNC content.

Chlorophyll was extracted by soaking 0.1 g of fresh leaf sample in 20 mL of dimethyl sulfoxide in the dark for 72 h [18]. Absorbance of the extract at 663 and 645 nm was measured with a spectrophotometer and total Chl concentration was calculated using the formulas described by Arnon [19].

For protein extracts and assays, 0.1 g leaves were frozen in liquid nitrogen and then ground in 3 ml of buffer (62.5 mM Tris-base buffer pH 8.0). The homogenate was centrifuged at 15000 gn for 20 min at 4 °C, and the supernatant was collected for protein assays. Total soluble proteins were determined according to Bradford [20], spectrophotometrically at 595 nm, using bovine serum albumin as the standard.

Soluble flavonoids were measured as described by Nangle *et al*. [12]. Briefly, leaves were dried at 80 °C for 72 h. 30 mg of ground tissues were added to 10 mL of methanol. The homogenate was centrifuged at 10000 gn for 10 min. Extract was then placed into a tube with 1.5 mL CH3OH, 0.1 mL of 10% AlCl3, 0.1 mL potassium acetate and 2.8 mL distilled H2O. The absorbance was read at 415 nm. The amount of flavonoids was determined from a standard curve prepared by quercetin solutions.

Results

Shoot and Root Growth

Shoot growth of Bermudagrass were decreased with increasing PBZ and TE application rates. However, PBZ reduced shoot growth more vigorously than TE (Fig. 1). A similar pattern was observed for root growth related to PBZ application, whereas highest levels of root dry weight were recorded in TE-treated plants at 0.5 g·100 m^2, followed by $TE_{0.25}$, $TE_{0.75}$ and control treatments (Fig. 2).

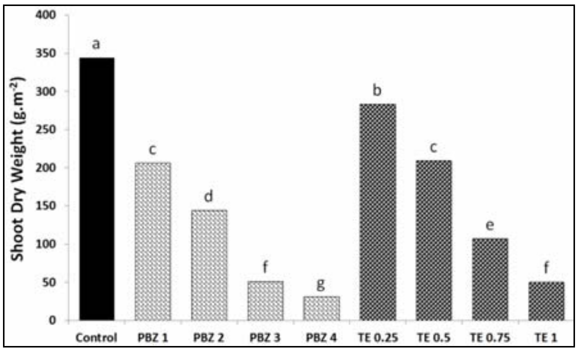

Fig. 1 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on shoot growth of Bermuda grass. Values followed by the same letter are not significantly different at 5% level (DMRT).

Turf Quality (TQ)

The best quality was observed in TE-treated plants at 0.5 g·100 m^{-2}. No significant difference existed in plant quality among PBZ_1, PBZ_2, $TE_{0.25}$ and control treatments. Other paclobutrazol and trinexapac ethyl treatments reduced visual quality and lowest quality was recorded in PBZ_4 treatment (Fig. 3).

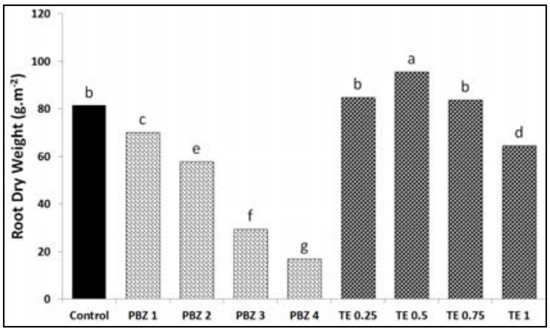

Fig. 2 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on root growth of Bermuda grass. Values followed by the same letter were not significantly different at 5% level (DMRT).

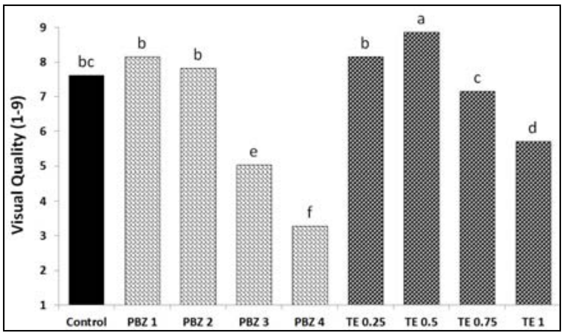

Fig. 3 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on quality of Bermuda grass. Values followed by the same letter were not significantly different at 5% level (DMRT).

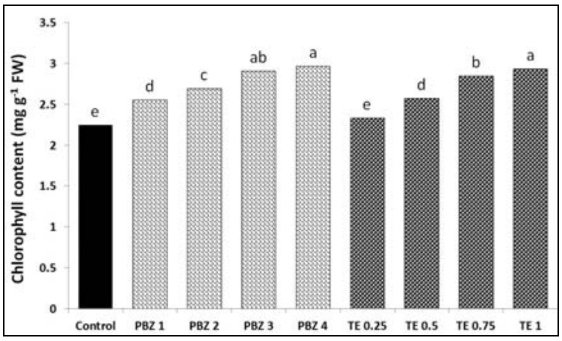

Fig. 4 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on chlorophyll content of Bermuda grass. Values followed by the same letter were not significantly different at 5% level (DMRT).

Chlorophyll content.

Chlorophyll content was increased with increasing PBZ and TE application rates and highest Chl content was observed in TE_1 and PBZ_4 treatments.

While no significant difference was observed between $TE_{0.25}$ and untreated plants (Fig. 4).

Total Non-structural Carbohydrates Content (TNC)

TNC content was increased in all PBZ and TE treated plants except for TE_1 treatment that exhibited lower TNC content than control plants. The highest TNC content was observed in TE-treated plants at 0.5 g·100 m^{-2} followed by $TE_{0.75}$ and PBZ_2 treatments (Fig. 5).

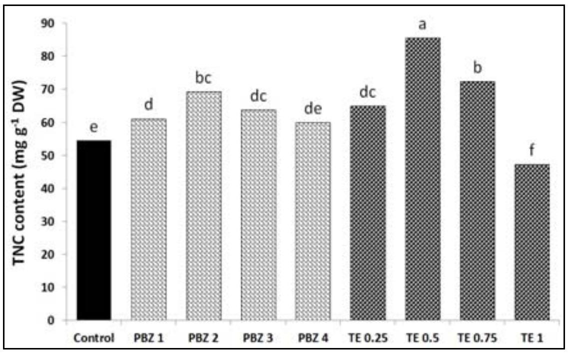

Fig. 5 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on total nonstructural carbohyrates (TNC) of Bermuda grass. Values followed by the same letter were not significantly different at 5% level (DMRT).

Soluble flavonoids content

Total flavonoids content was increased with increasing PBZ and TE application rates, except for PBZ_4 treatment that exhibited lower flavonoid content than PBZ_3 plants (Fig. 6).

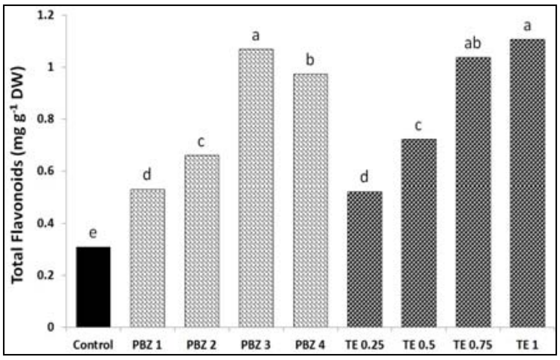

Fig. 6 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on total flavonoids content of Bermuda grass. Values followed by the same letter were not significantly different at 5% level (DMRT).

Total Soluble Proteins

Leaves soluble proteins was increased with increasing PBZ and TE application rates until PBZ_3 and $TE_{0.75}$ and then decreased in PBZ_4 and TE_1 treatments (Fig. 7).

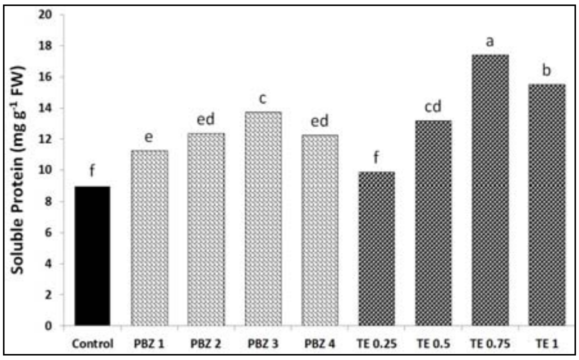

Fig. 7 Effect of different application rate (g a.i. /100 m2) of paclobutrazol (PBZ) and trinexapac-ethyl (TE) on soluble protein content of Bermuda grass. Values followed by the same letter were not significantly different at 5% level (DMRT).

Discussion

Morphological and physiological responses of Bermudagrass to trinexapacethyl and paclobutrazol were different in some aspect. Trinexapac-ethyl competitively inhibits the synthesis of gibberellin late in its biosynthetic pathway, 3ß-hydroxylase conversion of gibberellic acid-20 (GA_{20}) to gibberellic acid-1 (GA_1), resulting in reduced cell elongation, while Paclobutrazol inhibits gibberellin biosynthesis in the early stages of this pathway [21]. This early blockage prevents the synthesis of numerous gibberellins and could explaining the greater decline in shoot and root growth by PBZ. Effects of TE and PBZ on growth inhibition are well documented in bermudagrass and other turfgrass species [22-24]. On the other hand, relatively high TE and PBZ application rate may have resulted in phytotoxicity, leading to a decrease in overall visual quality [25,26].

Some studies have demonstrated that inhibition of GA_1 due to TE and PBZ action increases leaf cell density resulting in more chlorophyll content per unit area and darker green leaves [27-29]. Our results also showed that TE and PBZ application increased leaf Chlorophyll content. Chlorophyll content is an important factor in determining photosynthetic capacity. It has been shown that TE and PBZ application increases canopy photosynthetic rate and photochemical efficiency [30-32], while maintenance respiration may be decreased [33]. Photosynthate not used for leaf elongation must be stored or transported to other organs such as crowns, stems and roots and would explain increased tillering [34] and rooting [25] that has been reported in previous research. Parallel to these results we observed that, application of TE at 0.5 g·100 m^{-2} increased root growth, however increase in root mass was not observed in higher TE application rates or PBZ treatments may be due to sever growth retardation.

In the present study, application of TE and PBZ, in low or medium rates, increased leaf total nonstructural carbohydrates. The major TNC found in turfgrass shoots consist of the monosaccharides, glucose and fructose, the disaccharide sucrose, various oligosaccharides of the beta-2 - 6-linked polyfructosylsucrose type, starch, and long-chain fructans [35]. Total nonstructural carbohydrates in grasses provide a reservoir of energy in plants beyond the immediate requirements for growth and maintenance and have been described as a physiological measure of stress tolerance [36]. Increased TNC content by PBZ and TE, have also been observed in different plants [31, 37-40]. TNC improvement after PBZ and TE application may attributed to the enhanced leaf photosynthesis and accumulation of photosynthetic pigments (mainly chlorophyll) [30] or may be due to a direct effect of plant growth retardants on carbohydrate metabolism [39]. However, it appears that effect of plant growth retardants on TNC contents depends on the application rate and intervals. Han *et al.* [41] reported that trinexapac-ethyl, flurprimidol, and paclobutrazol significantly increased the TNC of creeping bentgrass 2 weeks after their initial application, but TNC levels began to decrease at week 4. Besides, a single trinexapac-ethyl application reduced TNC content more than did split applications, each at lower rates.

Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in Free State and as glycosides. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition etc. [42]. Flavonoids require larger amounts of carbon in their structure, so increasing in chlorophyll and leaf photosynthesis by PBZ and TE can conduces to enhanced flavonoids content. Our results agree with those of Nangle [12] and Srilatha and Reddy [43] who found that application of TE and PBZ increased total flavonoids content.

Proteins are the most abundant biological macromolecules occurring in all parts of the cells and it also occur in great variety. Moreover, proteins exhibit enormous diversity of biological function and are the most important final product of the pathways [44]. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [45]. Increase of protein content by PBZ in Common Bean [46] and wheat [47] have been reported in previous studies. Matysiak [48] has also shown protein enhancement due to TE application. Our results are in agreement with these reports.

In summary, the results reported here suggest that TE and PBZ treatments were beneficial for Bermudagrass phytochemical content enhancement, as manifested by improved chlorophyll, flavonoids and proteins. However, puclobutrazol application,because of higher phytotoxity than trinexapac ethyl, seems to be lees effective in raising medicinal properties. Therefore, additional studies are required to find proper TE and PBZ application rates.

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