

## Allelopathic effects of *Festuca arundinacea* variety Millennium2 leaf aqueous extracts on seedlings growth of 10 fescue varieties

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### ABSTRACT

The allelopathic effects of fescue (*Festuca arundinacea*) variety 'Millennium' 2 leaf aqueous extracts on 10 fescue varieties were assayed. The 50 mg·ml<sup>-1</sup> treatment increased the biomass and physiological indices (soluble sugar content, malondialdehyde content and root activity), whereas the 100 mg·ml<sup>-1</sup> and 150 mg·ml<sup>-1</sup> treatments were inhibitory. There was variable response of fescue varieties to different treatments, the varieties (*Aries*, *Millennium2*, and *Free star*) were most sensitive to the leaf extracts, while *Greenlabel*, *Lenge maste*, and *Arid3* varieties were least sensitive. The treatments most affected the physiological factor, relative electrolyte leakage, followed by the soluble sugar content, malondialdehyde content, and root activity. These results suggested that sowing an appropriate combination and proportion of grass varieties may be better to improve the lawn quality than the continuous use of a single grass variety.

**Key words:** Allelopathy, biomass, fescue, *Festuca arundinacea*, leaf aqueous extracts, malondialdehyde, mix sowing, physiological indices, soluble sugar, root activity.

### INTRODUCTION

Plants release the allelochemicals in to the surrounding environment, which changes the soil micro-environment and affects the growth of nearby plants. For instance, the leaf extracts of *Festuca arundinacea* significantly inhibits the growth of *Trifolium* species and the extent of inhibition increases with the increasing extract concentrations (10). The pine needle extracts significantly decreases the seed germination and seedling growth of *Poa* and *Festuca* species, with greater harmful effects on *Poa* species (1). The sensitivity of grass roots to the allelochemicals is more than the grass shoots, and the grass seed germination is relatively less sensitive to pine needle extracts than seedling growth period. In the other reports, bermudagrass seeds treatment with the aqueous extracts from perennial ryegrass leaves and stems, decreased the germination rate, root weight, root length, and root density (14). The bermudagrass root extracts reduced the ryegrass germination (24), likewise, the root and stem extracts of switchgrass were allelopathic perennial to ryegrass and alfalfa (17).

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An (2) identified two highly allelopathic switchgrass varieties, their root exudates significantly inhibited the lettuce seed germination and plant growth, but the effects varied among ecotypes. Zhang (28) treated *Perennial ryegrass*, *Poa pratensis*, and *F. arundinacea* seeds with root extracts of *Imperata koenigii* and observed that the allelopathic effects were strongest on *Poa pratensis*, followed by *F. arundinacea* and *Perennial ryegrass*. Gao (6) reported that the *Conyza canadensis* leaf aqueous extract variably inhibited the seed germination and seedling growth of five crops (rape, radish, cucumber, sorghum and wheat), the rape and radish were more sensitive than sorghum, cucumber and wheat. Wang (21) studied the allelopathic effects of eight *P. pratensis* varieties on *Perennial ryegrass* seedlings, and found that *P. pratensis* variety *Kentucky* had the strongest effects, followed by *Platini* and *Park* varieties. Therefore, comparing the allelopathic effects among the species and varieties is important to improve the plant growth (8).



Figure 1. *Festuca arundinacea* variety *Millennium2*

The winter season fescue variety ‘*Millennium*’2 sown at high density, has excellent performance and shade tolerance, drought and disease resistance than other varieties (Figure1). It could be suitable in mixture of grasses. This study aimed to determine the allelopathic effects of fescue variety ‘*Millennium*’2 on 10- fescue varieties sown in lawns to improve the quality and ornamental value of lawns.

## MATERIALS AND METHODS

This study was conducted from September 2017 to September 2019 in Forestry University, Beijing (Longitude, 116° 23' 17" N; latitude, 39° 54' 27" E; Altitude, 60 m; Weather conditions: temperate monsoon climate; Annual precipitation: 448 mm).

### I. Pot culture

Ten fescue grass varieties (*Aries*, *Justice*, *Millennium 2*, *Free star*, *Regular*, *Lenge maste*, *BonSai3000*, *Greenlabel*, *Populars*, and *Arid 3*) seeds were purchased from Beijing Top Green Company Ltd. The germination of the grass seeds was >90 %. Two hundred

seeds of each cultivar were sown per plastic pot (10×10×12 cm) filled with 100 g vermiculite per pot and kept in growth chamber (25 °C, photoperiod: 16 h light/8 h dark). The seeds were soaked in water overnight before sowing, then were sown on the surface of substrate. The treatments were replicated thrice in complete randomised design. Plants were irrigated every other day and fertilized weekly with half-strength Hoagland's nutrient solution (9).

## II. Bioassays

### 1. Leaf aqueous extraction

Twenty g fresh leaves of *Millennium2* variety were harvested at 3-leaf stage and dried in vacuum at 80 °C, cut in pieces and soaked in 100 ml distilled water for 24 h. After filtering through cotton gauze, the leaf aqueous extract was obtained and diluted to concentration of 0 ml<sup>-1</sup>, 50 ml<sup>-1</sup>, 100 ml<sup>-1</sup> and 150 ml<sup>-1</sup>, respectively

### 2. Gas chromatography-mass spectrometry (GC-MS) analysis

The leaf aqueous extract of 100 mg·ml<sup>-1</sup> concentration was further purified by filtering through filter paper and millipore filter (0.45 µm). Five ml filtrate was mixed with 2 ml ethyl acetate. Then 1µl ethyl acetate extract was analyzed by GCMS-Qp2010 Ultra GC-MS (manufactured by Shimadzu Corporation). The GC was equipped with a quartz capillary column Rtx-5MS column (30 m×0.25 mm×0.25 µm). The GC conditions were set as under: inlet temperature 250 °C, helium (99.99 %) carrier gas flow at constant 1ml·min<sup>-1</sup>, injector split ratio 10:1. The temperature program for GC started at 50 °C and increased at the rate of 15 °C·min<sup>-1</sup> to 180 °C for 5 min, and then at the rate of 10 °C·min<sup>-1</sup> until it reached 250 °C for 15min. MS analysis was conducted in positive electron ionization mode. The source temperature was held at 230 °C, with an electron ionization potential of 70 eV and ions were scanned over the molecular weight range of 33-400 atomic mass units. Each metabolite was identified via the standard mass spectrum database of NIST 11.lib and NIST11s.lib and the relative content of each component was counted by area normalization (12).

### 3. Biomass and physiological index evaluation

Two-weeks-old *Festuca* seedlings were treated with the *Millennium2* leaf aqueous extracts, at 20 ml per pot on alternate days. After 7 d treatment, the fresh seedlings and roots were harvested, weighed and tested for physiological indices. The treatments were replicated thrice in complete randomised design.

(i). **Relative electrolyte leakage (REL):** One g fresh leaves were sampled, soaked in 30 ml deionized water and shaken overnight. The electrical conductivity was measured as R1. The solution with the leaves were further boiled for 15 min and shaken overnight. The electrical conductivity was measured as R2 (27). The relative electrolyte leakage was calculated as under:

$$REL = R1/R2.$$

Where, R1: Electrical conductivity of fresh leaves, R2: Electrical conductivity of boiled leaves

**(ii). Malondialdehyde content (MDA):** One g fresh leaves were ground in 1.5 ml trichloroacetic acid. After centrifugation, the supernatant was mixed with 0.6 % thiobarbituric acid in equal volume and kept in boiling water bath for 15 min. The absorbance value of the mixture was detected at wavelength of 450, 532, and 600 nm (27). The MDA content ( $\mu\text{mol}\cdot\text{g}^{-1}$ ) was calculated as under:

$$\text{MDA content} = [6.459 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}] \times V/W.$$

Where, OD: Optical density; V: Extraction volume (ml); W: Fresh sample weight (g).

**(iii). Soluble sugar content (SSC):** One ml deionized water with 0.1 g fresh leaves was boiled for 20 min. After centrifugation, 50  $\mu\text{l}$  supernatant was mixed with 450  $\mu\text{l}$  distilled water and 2.5 ml anthrone reagent, then boiled for 10 min again. The absorbance value of the mixture was measured at wavelength of 620 nm (27). The soluble sugar content ( $\mu\text{g}\cdot\text{g}^{-1}$ ) was calculated as under:

$$\text{SSC} = W1 \times V1 \times \text{dilution factor} / (V2 \times W2 \times 106).$$

Where, W1: SSC from standard curve ( $\mu\text{g}$ ); V1: extraction volume (ml), V2: Test sample volume (ml), W2: Fresh sample weight (g).

**(iv). Root activity:** The root samples were washed with distilled water, dried, and soaked in 5 ml 95 % ethanol at 60 °C water bath for 4 h. 0.1 g clean roots were harvested and mixed with 2.5 ml 0.4 % TTC (2,3,5-triphenyltetrazolium chloride) in dark for 24 h. Absorbance of the soaked solution was measured at 490 nm. The standard curve was made with the absorbance of gradient triphenyl formazan (TTF) solution (25  $\mu\text{g}$ , 50  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150  $\mu\text{g}$  and 200  $\mu\text{g}$ ) measured at 490 nm (23). The sample root vitality ( $\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) was then computed.

Root vitality = TTF content inferred with the standard curve /root dry weight/time.

#### 4. Response index (IR) calculation

Response index for each treatment was calculated as under:

$$\text{IR} = (\text{T1} - \text{T0}) / \text{T0}.$$

Where, T0: Physiological data in 0  $\text{mg}\cdot\text{ml}^{-1}$  leaf aqueous extract treatment; T1: Physiological data in 50 (or 100, 150)  $\text{mg}\cdot\text{ml}^{-1}$  leaf aqueous extract treatment. IR > 0: +ve Allelopathic effect, IR < 0: -ve Allelopathic effect.,

**Statistical analysis:** The data were analyzed statistically using Microsoft Excel 2013 for calculating mean and standard error, and the SPSS19.0 system for two-way ANOVA at 0.05 or 0.01 probability levels.

## RESULTS AND DISCUSSION

### I. Allelopathic compounds in the *Millennium2* leaf aqueous extract

On the basis of GC-MS analysis, 26 compounds were identified in the *Millennium2* leaf aqueous extract (Table 1). Their similarity indices to known compounds exceeded 80. This ensured that their allelopathic effects of *Millennium2* leaf aqueous extract could be significantly evaluated.

Table 1. The allelopathic compounds in leaf aqueous extract of *Millennium2* by GC-MS analysis

Retention Time (min)	Chemical Compound	Similarity Index	Area (%)
3.350	Cyclohexanol	93	1.02
3.817	4-Methylpentanoic acid	94	20.92
3.883	1,1-diethoxy-3-methylbutane	92	11.21
4.042	cis-4-Hepten-1-ol	95	19.36
4.158	Phenol	96	21.18
4.692	2-Ethylhexanol	93	1.00
4.817	Benzyl alcohol	93	0.96
4.975	3-ethyl-3-methylheptane	82	0.35
5.033	4,8-Dimethylundecane	80	0.09
5.175	p-Cresol	82	0.48
5.433	3,7-Dimethyldecane	84	0.63
5.492	2,3,5,8-tetramethyldecane	84	0.24
5.633	Phenylethyl alcohol	91	4.07
6.100	4-Ethylphenol	97	0.22
7.192	4,6-Dimethyldodecane	90	0.48
7.458	Indole	90	1.48
7.600	4-Hydroxy-3-methoxystyrene	85	2.89
9.158	1-Iodohexadecane	87	0.72
9.350	3,5-Di-tert-butylphenol	80	0.35
9.617	Heneicosane	84	0.54
11.242	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-butanone	83	5.34
11.442	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butan-2-ol	81	3.29
11.833	5,5-Diethyltridecane	83	0.61
11.933	4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)butan-2-one	82	1.20
12.633	Icosane	83	0.46
16.333	Methyl 2-benzoylbenzoate	89	0.91

Phenolic acids (21.18 %) were the main compounds in the *Millennium2* leaf aqueous extract, these secondary metabolites in plant tissues are closely associated with plant growth (3,22). Buta and Spaulding (4) also found phenolic inhibitors (p-coumaric and caffeic) in the leachates of tall fescue and their concentration was high enough to cause growth inhibition. The resistant cultivars of *Musa paradisiaca* contains very high contents of phenols and flavanoids and these were allelopathic to endophytic larvae (10). The exogenously applied p-coumaric acid (0.25 mM) stopped the premature soybean root

growth and at 0.5 mM decreased the cell viability and PAL activity (26). Fu (5) reported that three phenols (ferulic acid, chlorogenic acid, and protocatechuic acid) increased the chlorophyll and carotenoid contents in *Rhododendron delavayi* seedlings but inhibited the accumulation of biomass. Thus, phenolic acids might be crucial for the allelopathic effects of *Millennium2*. However, the other two main allelopathic components (4-methylpentanoic acid and cis-4-hepten-1-ol) of the leaf aqueous extract are not fully investigated.

## II. Effects on fescue biomass

The leaf aqueous extract treatment of 50 mg·ml<sup>-1</sup> *Millennium2*, increased the biomass (0.91 %) in all 10 tested fescue varieties. In contrast, the leaf aqueous extract at 100 mg·ml<sup>-1</sup> and 150 mg·ml<sup>-1</sup>, decreased the biomass by 5.61 % and 13.18 %, respectively (Table 2). The allelopathic index response (IR) was positive for all varieties at 50 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract (Figure 2). Whereas, the IRs were negative at 100 mg·ml<sup>-1</sup> and 150 mg·ml<sup>-1</sup> treatments. Thus the *Millennium2* leaf aqueous extract had significant allelopathic effects on the fescue biomass.

Table 2. The fresh weight (g) of fescue seedlings with *Millennium2* leaf aqueous extract treatments

Variety	Concentration of the <i>Millennium 2</i> leaf aqueous extracts			
	0 mg·ml <sup>-1</sup>	50 mg·ml <sup>-1</sup>	100 mg·ml <sup>-1</sup>	150 mg·ml <sup>-1</sup>
Arid3	1.08±0.11c	1.13±0.02c	1.04±0.07c	0.99±0.06b
Regular	1.30±0.15b	1.31±0.05b	1.25±0.12b	1.11±0.05b
BonSai3000	1.22±0.10b	1.29±0.08b	1.23±0.04b	1.13±0.08b
Greenlabel	1.27±0.11b	1.28±0.07b	1.24±0.11b	1.11±0.08b
Lenge master	1.22±0.14b	1.27±0.08b	1.20±0.05bc	1.11±0.10b
Justice	1.44±0.08b	1.45±0.05ab	1.39±0.05ab	1.29±0.17ab
Millennium2	1.73±0.13a	1.76±0.07a	1.57±0.06a	1.47±0.09a
Popular	1.19±0.10b	1.26±0.09b	1.16±0.09bc	1.07±0.08b
Aries	1.31±0.23b	1.32±0.12b	1.23±0.08b	1.13±0.08b
Free star	1.22±0.15b	1.25±0.09b	1.15±0.04bc	1.05±0.05b

Note: Data indicate means ± STDEV (n=3); Values labeled with different letters in the same column indicate significant difference among treatments ( $P<0.05$ ) according to LSD test (SPSS 19.0).

The tested fescue varieties showed variable responses to the treatments. Specifically, the growth of *BonSai3000* and *Popular* was more stimulated by 50 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract than other varieties. The leaf extract at higher concentrations stimulated the growth of *Millennium2*, *Free star*, *Aries*, and *Regular* than other varieties. The sum total of the absolute IRs for 3- concentrations (50, 100, and 150 mg·ml<sup>-1</sup>) revealed that *Millennium2* was the most affected variety (i.e., Auto-toxicity of leaf extracts), followed by *Free star*, *Aries*, *Regular*, *Popular*, *Arid3*, *Justice*, *BonSai3000*, *Greenlabel* and *Lenge maste*. The fescue plants are allelopathic to other plants growth. For example, Walters (20) reported that fescue sown into pots containing sweetgum seedlings, reduced the dry weight of sweetgum (29 to 95 %). Water extracts of tall fescue also reduced the germination and seedling growth of alfalfa, Italian ryegrass and clover (18,19).

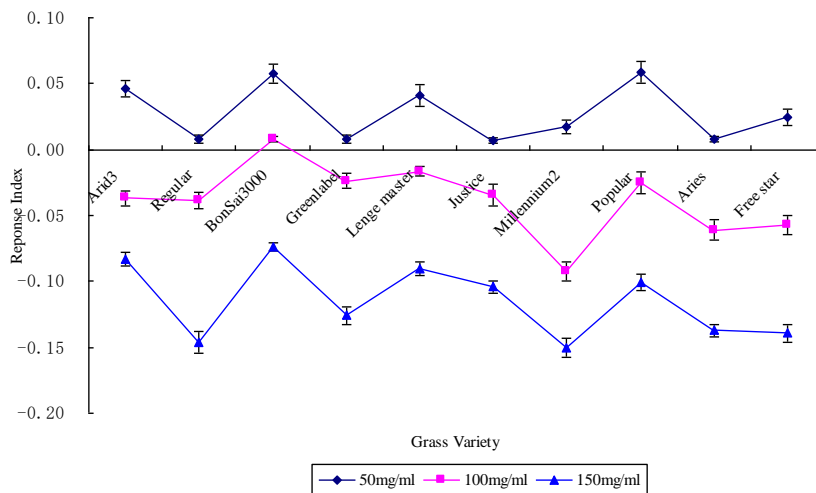


Figure 2. Response of seedling biomass to the leaf.

### III. Effects on selected physiological factors of fescue

*Millennium2* leaf aqueous extracts significantly affected the physiological performance of fescue plants. The 50 mg·ml<sup>-1</sup> treatment decreased the malondialdehyde (MDA) content and relative electrolyte leakage (REL), whereas, increased the soluble sugar content (SSC) and root activity than control (0 mg·ml<sup>-1</sup>) (Table 3). The IRs for the 50 mg·ml<sup>-1</sup> treatment were mostly negative for MDA and REL, but positive for the SSC and root activity (Table 4), thus indicating the positive effects of this concentration. In contrast, the 100 mg·ml<sup>-1</sup> and 150 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract treatments increased the MDA content and REL, whereas, decreased the SSC and root activity than control. The IRs were mostly positive for MDA and REL, but negative for the SSC and root activity, suggesting that the 100 mg·ml<sup>-1</sup> and 150 mg·ml<sup>-1</sup> treatments adversely influenced the fescue. Thus, the allelopathic effects of the *Millennium2* leaf aqueous extract on fescue depended on the concentration. This is consistent with the findings of several previous studies. For example, the *Caragana microphylla* leaf extract concentration below 20 mg·ml<sup>-1</sup> promotes the seed germination and seedling growth of *Agropyron* species, whereas, higher concentrations were inhibitory (25). The *Oxytropis glabra* leaf extract > 10 mg·ml<sup>-1</sup> concentrations adversely affects the seed germination rate and germination index of the 3-receptor plant species (*Melilotoides ruthenwa*, *Astragalus melilotoides* and *Agropyron mongolicum*), but concentrations < 5 mg·ml<sup>-1</sup> have no significant allelopathic effects (13). Thus, the threshold extract concentration for allelopathic effects differs among the test plant species, possibly because of the specificity of the allelochemicals. A previous study on *Pogostemon cablin* seedlings irrigated with salicylic acid, benzoic acid, malonic acid, vanillic acid, or p-hydroxybenzoic acid revealed that the various combinations of allelochemicals may have diverse allelopathic effects on the same organism (7).

Table 3. The effects of *Millennium2* leaf aqueous extracts on the physiological performance of 10- fescue varieties

Concentration	Index	Artd3	Regular	Bonsai3000	Greenibel	Langje master	Justice	Millennium2	Popular	Aries	Free star
0 mg ml <sup>-1</sup>	YDA content (μmol.g <sup>-1</sup> )	4.92±0.20 <sup>a</sup>	5.30±0.01 <sup>ab</sup>	4.31±0.14 <sup>a</sup>	4.03±0.14 <sup>a</sup>	5.20±0.13 <sup>ab</sup>	4.68±0.20 <sup>a</sup>	4.66±0.31 <sup>a</sup>	5.62±0.34 <sup>a</sup>	5.78±0.20 <sup>a</sup>	5.33±0.37 <sup>a</sup>
	SSC (μg.g <sup>-1</sup> )	0.39±0.06 <sup>b</sup>	0.59±0.07 <sup>a</sup>	0.43±0.09 <sup>ab</sup>	0.40±0.09 <sup>b</sup>	0.49±0.10 <sup>ab</sup>	0.54±0.11 <sup>a</sup>	0.40±0.07 <sup>b</sup>	0.48±0.07 <sup>ab</sup>	0.56±0.06 <sup>a</sup>	0.50±0.15 <sup>a</sup>
	Root activity (mg.g <sup>-1</sup> .h <sup>-1</sup> )	5.51±0.13 <sup>a</sup>	4.80±1.05 <sup>bc</sup>	5.20±0.48 <sup>a</sup>	5.25±0.62 <sup>a</sup>	5.05±0.07 <sup>bc</sup>	5.27±0.39 <sup>a</sup>	5.82±0.76 <sup>a</sup>	4.99±0.38 <sup>bc</sup>	4.45±0.10 <sup>b</sup>	5.30±0.07 <sup>ab</sup>
50 mg ml <sup>-1</sup>	YDA content (μmol.g <sup>-1</sup> )	0.15±0.01 <sup>b</sup>	0.14±0.02 <sup>b</sup>	0.12±0.02 <sup>b</sup>	0.16±0.02 <sup>b</sup>	0.13±0.01 <sup>b</sup>	0.14±0.01 <sup>b</sup>	0.14±0.01 <sup>b</sup>	0.19±0.01 <sup>a</sup>	0.14±0.01 <sup>b</sup>	0.14±0.01 <sup>b</sup>
	SSC (μg.g <sup>-1</sup> )	4.77±0.09 <sup>b</sup>	5.03±0.08 <sup>ab</sup>	4.17±0.09 <sup>bc</sup>	3.94±0.12 <sup>c</sup>	5.03±0.03 <sup>ab</sup>	4.34±0.10 <sup>bc</sup>	4.69±0.26 <sup>b</sup>	5.33±0.05 <sup>a</sup>	5.42±0.05 <sup>a</sup>	5.39±0.09 <sup>a</sup>
	Root activity (μg.g <sup>-1</sup> )	0.45±0.03 <sup>c</sup>	0.65±0.04 <sup>a</sup>	0.47±0.04 <sup>c</sup>	0.45±0.01 <sup>c</sup>	0.54±0.03 <sup>b</sup>	0.61±0.02 <sup>a</sup>	0.46±0.01 <sup>c</sup>	0.55±0.02 <sup>b</sup>	0.60±0.05 <sup>a</sup>	0.56±0.02 <sup>b</sup>
100 mg ml <sup>-1</sup>	YDA content (μmol.g <sup>-1</sup> )	5.81±0.39 <sup>a</sup>	5.14±0.41 <sup>ab</sup>	5.37±0.44 <sup>ab</sup>	5.35±0.39 <sup>ab</sup>	5.28±0.46 <sup>ab</sup>	5.32±0.51 <sup>ab</sup>	5.70±0.52 <sup>a</sup>	5.17±0.21 <sup>ab</sup>	4.75±0.24 <sup>b</sup>	5.41±0.11 <sup>ab</sup>
	SSC (μg.g <sup>-1</sup> )	0.13±0.01 <sup>bc</sup>	0.12±0.01 <sup>bc</sup>	0.10±0.01 <sup>c</sup>	0.14±0.01 <sup>b</sup>	0.12±0.01 <sup>bc</sup>	0.10±0.01 <sup>c</sup>	0.13±0.01 <sup>bc</sup>	0.17±0.01 <sup>a</sup>	0.11±0.01 <sup>bc</sup>	0.12±0.01 <sup>bc</sup>
	Root activity (mg.g <sup>-1</sup> .h <sup>-1</sup> )	4.90±0.46 <sup>bc</sup>	5.39±0.41 <sup>b</sup>	4.38±0.37 <sup>c</sup>	4.10±0.23 <sup>d</sup>	5.23±0.37 <sup>b</sup>	4.70±0.32 <sup>c</sup>	4.92±0.31 <sup>bc</sup>	5.66±0.56 <sup>ab</sup>	5.91±0.46 <sup>a</sup>	5.62±0.32 <sup>ab</sup>
150 mg ml <sup>-1</sup>	YDA content (μmol.g <sup>-1</sup> )	0.41±0.03 <sup>c</sup>	0.58±0.05 <sup>a</sup>	0.42±0.03 <sup>c</sup>	0.40±0.02 <sup>c</sup>	0.49±0.03 <sup>b</sup>	0.51±0.03 <sup>ab</sup>	0.39±0.02 <sup>c</sup>	0.47±0.04 <sup>b</sup>	0.54±0.09 <sup>ab</sup>	0.47±0.04 <sup>b</sup>
	SSC (μg.g <sup>-1</sup> )	5.52±0.28 <sup>a</sup>	4.81±0.22 <sup>b</sup>	5.14±0.24 <sup>b</sup>	5.22±0.16 <sup>ab</sup>	5.00±0.47 <sup>b</sup>	5.18±0.28 <sup>ab</sup>	5.54±0.56 <sup>a</sup>	4.93±0.40 <sup>b</sup>	4.42±0.34 <sup>c</sup>	5.25±0.39 <sup>ab</sup>
	Root activity (mg.g <sup>-1</sup> .h <sup>-1</sup> )	0.14±0.01 <sup>b</sup>	0.14±0.02 <sup>b</sup>	0.13±0.02 <sup>b</sup>	0.16±0.01 <sup>b</sup>	0.14±0.02 <sup>b</sup>	0.15±0.03 <sup>b</sup>	0.15±0.03 <sup>b</sup>	0.20±0.01 <sup>a</sup>	0.16±0.05 <sup>ab</sup>	0.15±0.03 <sup>b</sup>
150 mg ml <sup>-1</sup>	YDA content (μmol.g <sup>-1</sup> )	5.13±0.43 <sup>b</sup>	5.72±0.23 <sup>ab</sup>	4.74±0.46 <sup>bc</sup>	4.44±0.40 <sup>c</sup>	5.46±0.39 <sup>b</sup>	5.00±0.39 <sup>b</sup>	5.26±0.31 <sup>b</sup>	5.93±0.37 <sup>a</sup>	6.25±0.33 <sup>a</sup>	5.95±0.45 <sup>a</sup>
	SSC (μg.g <sup>-1</sup> )	0.37±0.02 <sup>bc</sup>	0.52±0.04 <sup>a</sup>	0.38±0.01 <sup>bc</sup>	0.36±0.04 <sup>c</sup>	0.44±0.02 <sup>b</sup>	0.47±0.03 <sup>ab</sup>	0.32±0.03 <sup>c</sup>	0.43±0.03 <sup>b</sup>	0.50±0.05 <sup>a</sup>	0.44±0.02 <sup>b</sup>
	Root activity (mg.g <sup>-1</sup> .h <sup>-1</sup> )	5.47±0.46 <sup>a</sup>	4.34±0.28 <sup>b</sup>	4.81±0.51 <sup>ab</sup>	4.89±0.19 <sup>ab</sup>	4.67±0.43 <sup>ab</sup>	4.85±0.31 <sup>ab</sup>	5.20±0.08 <sup>a</sup>	4.60±0.20 <sup>ab</sup>	4.25±0.30 <sup>b</sup>	4.81±0.36 <sup>ab</sup>
	REL	0.17±0.03 <sup>ab</sup>	0.17±0.01 <sup>ab</sup>	0.15±0.02 <sup>b</sup>	0.19±0.02 <sup>ab</sup>	0.18±0.02 <sup>ab</sup>	0.18±0.01 <sup>ab</sup>	0.19±0.01 <sup>ab</sup>	0.22±0.02 <sup>a</sup>	0.19±0.01 <sup>ab</sup>	0.19±0.01 <sup>ab</sup>

Data indicate means ± STDEV (n=5); Values labeled with different letters in the same line indicate significant difference among treatments ( $P < 0.05$ ) as per LSD test (/SPSS 10.0)

Table 4. Response index (IR) of *Millennium2* leaf aqueous extract on fescue plants

Concentration	Species	Response index value (IR)			
		MDA Content	SSC	Root activity	REL
50 mg·ml <sup>-1</sup>	Arid3	-0.03 <sup>b</sup>	0.08 <sup>a</sup>	0.05 <sup>b</sup>	-0.09 <sup>c</sup>
	Regular	-0.05 <sup>bc</sup>	0.05 <sup>b</sup>	0.07 <sup>a</sup>	-0.07 <sup>bc</sup>
	BonSai3000	-0.03 <sup>b</sup>	0.05 <sup>b</sup>	0.03 <sup>bc</sup>	-0.05 <sup>b</sup>
	Greenlabel	-0.02 <sup>b</sup>	0.06 <sup>ab</sup>	0.02 <sup>c</sup>	-0.07 <sup>bc</sup>
	Lenge master	-0.03 <sup>b</sup>	0.07 <sup>a</sup>	0.05 <sup>b</sup>	-0.04 <sup>ab</sup>
	Justice	-0.07 <sup>c</sup>	0.07 <sup>a</sup>	0.01 <sup>c</sup>	-0.16 <sup>d</sup>
	Millennium2	0.01 <sup>a</sup>	0.00 <sup>d</sup>	-0.02 <sup>d</sup>	-0.02 <sup>a</sup>
	Popular	-0.05 <sup>bc</sup>	0.06 <sup>ab</sup>	0.03 <sup>bc</sup>	-0.09 <sup>c</sup>
	Aries	-0.06 <sup>c</sup>	0.03 <sup>c</sup>	0.07 <sup>a</sup>	-0.08 <sup>c</sup>
	Free star	-0.02 <sup>b</sup>	0.04 <sup>bc</sup>	0.02 <sup>c</sup>	-0.07 <sup>bc</sup>
100 mg·ml <sup>-1</sup>	Arid3	0.00 <sup>c</sup>	0.05 <sup>a</sup>	0.00 <sup>a</sup>	-0.04 <sup>d</sup>
	Regular	0.02 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>a</sup>	0.05 <sup>c</sup>
	BonSai3000	0.02 <sup>b</sup>	-0.02 <sup>bc</sup>	-0.01 <sup>bc</sup>	0.08 <sup>bc</sup>
	Greenlabel	0.02 <sup>b</sup>	0.01 <sup>b</sup>	-0.01 <sup>bc</sup>	0.00 <sup>cd</sup>
	Lenge master	0.01 <sup>bc</sup>	0.00 <sup>b</sup>	-0.01 <sup>bc</sup>	0.06 <sup>c</sup>
	Justice	0.00 <sup>c</sup>	-0.05 <sup>c</sup>	-0.02 <sup>b</sup>	0.11 <sup>b</sup>
	Millennium2	0.06 <sup>a</sup>	-0.03 <sup>bc</sup>	-0.05 <sup>c</sup>	0.09 <sup>b</sup>
	Popular	0.01 <sup>bc</sup>	-0.04 <sup>c</sup>	-0.01 <sup>bc</sup>	0.05 <sup>c</sup>
	Aries	0.02 <sup>b</sup>	-0.04 <sup>c</sup>	-0.01 <sup>bc</sup>	0.20 <sup>a</sup>
	Free star	0.02 <sup>b</sup>	-0.06 <sup>c</sup>	-0.01 <sup>bc</sup>	0.10 <sup>b</sup>
150 mg·ml <sup>-1</sup>	Arid3	0.04 <sup>c</sup>	-0.04 <sup>a</sup>	-0.01 <sup>a</sup>	0.13 <sup>c</sup>
	Regular	0.08 <sup>b</sup>	-0.12 <sup>c</sup>	-0.10 <sup>d</sup>	0.24 <sup>b</sup>
	BonSai3000	0.10 <sup>ab</sup>	-0.10 <sup>b</sup>	-0.08 <sup>c</sup>	0.25 <sup>b</sup>
	Greenlabel	0.10 <sup>ab</sup>	-0.10 <sup>b</sup>	-0.07 <sup>c</sup>	0.23 <sup>b</sup>
	Lenge master	0.05 <sup>c</sup>	-0.10 <sup>b</sup>	-0.07 <sup>c</sup>	0.35 <sup>ab</sup>
	Justice	0.07 <sup>b</sup>	-0.13 <sup>c</sup>	-0.08 <sup>c</sup>	0.33 <sup>ab</sup>
	Millennium2	0.13 <sup>a</sup>	-0.17 <sup>d</sup>	-0.11 <sup>d</sup>	0.34 <sup>ab</sup>
	Popular	0.05 <sup>c</sup>	-0.11 <sup>bc</sup>	-0.08 <sup>c</sup>	0.12 <sup>c</sup>
	Aries	0.08 <sup>b</sup>	-0.10 <sup>b</sup>	-0.04 <sup>b</sup>	0.38 <sup>a</sup>
	Free star	0.08 <sup>b</sup>	-0.12 <sup>c</sup>	-0.09 <sup>cd</sup>	0.37 <sup>a</sup>

MDA: Malondialdehyde, SSC: Soluble sugar content, REL: Relative electrolyte leakage.

IR = (T1-T0)/ T0 [T0, the physiological data under the treatment of 0 mg·ml<sup>-1</sup> leaf aqueous extract; T1, the physiological data under the treatment of 50 (or 100, 150) mg·ml<sup>-1</sup> leaf aqueous extract. Data indicate means ± STDEV; Values labeled with different letters in the same column indicate significant difference among treatments ( $P < 0.05$ ) according to LSD test (SPSS 19.0).

Diverse physiological changes were observed in the tested fescue varieties in response to the *Millennium2* leaf aqueous extract. For example, at 50 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract treatment, *Greenland* had the lowest MDA content, which was 72.69 % of that of *Aries*. Additionally, *Regular* had the highest SSC, which was 1.44 times than that of *Arid3*. Moreover, *Arid3* showed the strongest root activity, which was 1.22 times that of *Aries*. Furthermore, *BonSai3000* and *Justice* had the lowest REL, which was 58.82 % of that of *Popular*. At 100 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract treatments

the total absolute IRs for the 4- analyzed physiological factors (MDA content, SSC, REL, and Root activity) were 5-31 for the tested varieties. Further, the summed absolute IRs in the 100 mg·ml<sup>-1</sup> and 150 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract treatments were 4-27 and 22-75, respectively. A combined analysis of the effects of the three extract concentrations on the four physiological factors based on the total absolute IRs indicated that *Aries* was the most affected variety, followed by *Justice*, *Millennium2*, *Free star*, *Regular*, *Lenge maste*, *BonSai3000*, *Greenlabel*, *Popular*, and *Arid3*. The differences in the allelopathic effects of leaf extract on fescue varieties were consistent with the results of other studies. For example, Wang (20) evaluated the allelopathic effects of stem extracts of 8- *Poa pratensis* L. varieties (21). The *Poa pratensis* L. extracts drastically inhibited the seed germination and seedling growth of *Lolium perenne* varieties. Zhang (28) reported that the root extracts of *Imperata cylindrica* had variable allelopathic effects on three lawn grass species and followed the order (weakest to strongest effect) of *Perennial ryegrass* > *Tall fescue* > *Poa pratensis*. Li (11) studied the effects of walnut leaf aqueous extracts on four herbal plants. The synthetic inhibitory effect was greatest in recipient *Salvia multiorrhiza*, followed by *Isatis tinctoria*, *Atractylodes macrocephala*, and *Polygala tenuifolia*. Thus, the interactions between the allelochemical source and receptor plants were variable. The tall fescue genotypes had variable inhibitory effects on other species (15,16).

The, physiological factors responded differently to various treatments. For example, the 50 mg·ml<sup>-1</sup> *Millennium2* leaf aqueous extract treatment induced greater changes in REL than in MDA content in *Justice* variety, with IRs of -0.16 and -0.07, respectively. The total absolute IRs for the 10-tested fescue varieties for the MDA content, SSC, root activity, and REL were 0.37, 0.5, 0.37 and 0.74, respectively. Similar results were also obtained for the applied two higher leaf extract concentrations. A comparison of 10-varieties treated with three leaf extract concentrations indicated that the REL was most affected physiological factor, followed by SSC, MDA content and root activity. Therefore, to evaluate the allelopathic effects, the analyses of various physiological factors should be integrated.

## CONCLUSIONS

Our results showed the Auto-toxicity of grass variety *Millennium2*, if this variety is continually grown year after year, it adversely affected the plant growth. The diverse allelopathic effects among the test varieties showed that growing of different combinations and proportions of grass varieties may improve and balance the turf quality and effective lawn management strategy.

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