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The Allelopathic Effects of Scots Pine (*Pinus sylvestris* L.) Leaf Extracts on Turf grass Seed Germination and Seedling Growth

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Allelopathic effects of aquous extracts of 10, 20, 30, 40 and 50 g/L dry Scot pine (*Pinus sylvestris*) leaves on seed germination and seedling growth of 4 turf grass varieties were investigated. All the concentration extracts tested had significantly inhibitory effects on seed germination percentage of *Lolium perenne* var. *Ovation*, *Festuca rubra rubra* var. *Franklin*, *Festuca rubra commutata* var. *Koket* and *Festuca ovina* var. crystal compared with the control. This inhibition effect increased with increasing Scot pine extract concentration. The treatments of 20, 30, 40 and 50 g/L concentration on hard fescue showed no germination. However, 10 g/L treatment of leaf extracts promoted root elongation on perennial ryegrass and shoot elongation on strong creeping red fescue, compared with the control. The average time of germination (ATG) and the time to 50 % of the final germination (GT_{50}) were significantly reduced by the extracts in comparison with the distilled water control.

Key Words: Allelopathy, Leaf Extract, *Pinus sylvestris*, Seed Germination, Scots Pine.

INTRODUCTION

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the release of chemical compounds the environment. These chemicals (also called allelo chemicals) often have inhibitory or contributory effects on seed germination and plant growth[1]. Allelo chemicals are present in stem, leaves, roots, flowers, inflorescence, fruit and seeds of the plant[2]. These compounds are usually synthesized in the leaves[3]. Allelopathic chemicals can be released or escape from a plant by several means *e.g.*, evaporating in to the air or from the soil surface, erosion or leaching from plant surfaces, exudation from roots and release by decaying dead organic materials. The released chemicals can be both water-soluble and oil soluble. They can be absorbed to soil surfaces, coat plant residues and be carried away in the wind[4].

Chemicals with allelopathic activity are present in many woody plants. Previous researches showed that plant extracts from shrubs and trees can cause increase or reduced germination or growth of neighboring plants[5-10].

Scots pine (*Pinus sylvestris* L) is the most widely distributed pine in the world. The naturally habitat ranges across Europe and Asia, from the Iberian peninsular and Turkey in the south to the edge of the Siberian tundra. It has also been introduced to other countries such as Canada and northern United States[11,12]. Besides timber uses are grown for forestry plantations, shelterbelts, Christmas tree, erosion control are grown in the garden and urban parks as a ornamental tree due to visual impact.

Turf grasses are of importance worldwide in enhancing and maintaining the function and beauty of natural and man-made landscapes. They are widely used for beautifying all types of surroundings, because of the diversity of species and varieties, turf grasses can be used in various applications and conditions[13].

In cool season, the most commonly planted herbaceous groundcover plants in residential landscapes are cool season turf grasses, due to their quality of appearance and its adaptability to environment and culture. Establishment of turf grass areas is most commonly accomplished with mix of turf grass seed species. Direct seeding is the least expensive and most efficient method for revegetating large parcels of residential landscapes with herbaceous species such as turf grasses[14].

There are numerous studies between woody plants and natural grasses related to allelochemicals[15-17]. However, up to know there are no studies on cultural turf grass related to allelochemicals.

Turf grasses are planted for aesthetic and functional reasons beneath or near the ornamental tress especially with Scot pine and others such as *Acer* sp., *Betula* sp., *Fraxinus* sp., *Querqus* sp., *Picea* sp., *Abies* sp., *etc.* in urban green areas. It is reported that Scots pine inhibited seed germination of various herbaceous under storey plants[18-20]. On the other hand, turf grasses under Scot pine trees shows low seed germination and seedling growth which could be resulted in economical losts. Therefore, this research was conducted to investigate the allelopathic potential of Scots pine leaf extract on seed germination and seedling growth of various varieties of turf grasses.

EXPERIMENTAL

Erzurum is located in the east of Turkey at 1850 m asl. At this altitude the most used cool-season turf grass for establishing green areas are perennial ryegrass, strong creeping red fescue, chewings fescue and hard fescue. The best season for the establishing of turf grass area in Erzurum is spring or autumn. Therefore, the Scots pine leaves was collected during in autumn season.

Mature green leaves were picked from Scots pine trees grown at the green areas of Atatürk Üniversity Erzurum, Turkey on September 5, 2005. Seeds of perennial ryegrass (*Lolium perenne* var. *Ovation*), strong creeping red fescue (*Festuca rubra rubra* var. *Franklin*), chewings fescue (*Festuca rubra commutata* var. *Koket*) and hard fescue (*Festuca ovina* var. *Crystal*) were obtained from Mommersteeg BV Grass Seed Company, the Netherlands.

Collected leaves of scots pine were washed several times with distilled water, oven dried at 45ºC for 72 h. After drying the leaves were stored in polyethylene bags at room temperature until use. Sample from these leaves were soaked in distilled water at 10, 20, 30, 40 and 50 g L^{-1} at 27 ± 1ºC for 24 h. The mixture were than filtered through Whatman No. 2 filter papers.

To determine germination percentage of four turf grass varieties, 4 mL of each extract and distilled water control were dispensed in 90 × 12 mm plastic petri dishes lined with Whatman No. 1 filter papers. Thirty seeds (three replicates of each experiment) were placed on petri dishes. The petri dishes were put in-to the culture chamber with controlled lighting and temperature (light phase: 16 h at 25ºC; dark phase 8 h at 25ºC) and relative humidity of about 60-65 %. The dishes where supplied extracts and distilled water when the seeds need.

Germinated seeds in each dish were counted daily. Emergence of 1 mm of the radicle was used as the criterion for germination[21]. An average time of germination (ATG) was calculated as follows: (nº germinated seeds × days)/total number of seeds germinating[22]. The experiment was continued for 40 d. Germination percentage, root and shoot length were recorded at the end of the experiment. In addition, the time (days) to 50 % of the final germination percentage were arcsin and square root $(X + 0.5)^{½}$ transformed for analysis[16]. The results were quantified as percentages of germination, shoot and root length (cm), average time of germination and the time to 50 % of the final germination. Data were subjected to analysis of variance (Anova) and means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

The present results showed that the seed germination percentage, ATG, GT_{50}, root and shoot length of 4 turf grass varieties were significantly affected by scots pine leaf extracts (Tables 1-4). The final seed germination percentage in all 4 turf grass varieties exhibited lower germination percentage than control. Germination percentage decreased progressively with the increase of extract concentration (Table-1).

The most effective germination inhibition in all turf grass varieties was recorded on 50 g/L extract treatment. This treatment inhibited sharply

the germination percentage of varieties hard fescue, strong creeping red fescue, chewings fescue and perennial ryegrass (0, 1.11, 11.11, 28.89 %), respectively. The results of 50 g/L extract treatment showed that, germination of perennial ryegrass seeds was the least inhibited variety compared to the other varieties.

The lowest concentration of Scots pine extract (10 g/L) caused the lowest inhibition on germination percentage of all turf grass varieties as compared to the other treatments. Germination of perennial ryegrass and chewings fescue seeds was slightly affected by 10 g/L Scots pine leaf extract. There was no significant difference between the control and 10 g/L application. Among all varieties, 10, 20, 30, 40 and 50 g/L extract treatments had the least inhibition percentage effect on perennial ryegrass (Table-1).

Root elongations were decreased by all concentration of the leaf extracts in all the varieties. However, root length decreased with the increase of extract concentrations. It was interesting that 10 g/L concentration of leaf extracts promoted the root elongation on perennial rye grass (10.18 cm) compared with the control (4.88 cm) (Table-2).

All scots pine leaf extracts treatment significantly decreased average shoot elongation in all species except 10 g/L concentration by strong creeping red fescue (4,07 cm) and chewings fescue (3.25 cm) without significant differences compared with the control (3.71 cm).

In comparing the effectiveness of treatments on shoot elongation of chewings fescue, hard fescue, strong creeping red fescue and perennial rye grass seedlings, 50 g/L concentration of extract was found to be the most inhibitory treatment (Table-2). The effect of extract concentrations of Scot pine on the average time of germination (ATG) of the selected turf grass seeds is shown in Table-3. Of the four turf grass varieties studied, hard fescue and strong creeping red fescue were not significantly affected ($p < 0.01$) any of the five concentrations.

In chewings fescue and perennial ryegrass, however, the ATG was significantly ($p < 0.01$) affected by the extract concentrations. At the high concentrations of the treatments (40 and 50 g/L), germination was slower than those of from the other treatments and the control. The highest ATG was obtained from perennial ryegrass by 10 g/L extract concentration.

Time to reach 50 % of the final germination (GT_{50}) results showed statistically significant differences (at the % 0.01 level) among the control and concentrated extracts (Table-4). Generally, GT_{50} values increased with the increase of extract concentrations, except 10 g/L extract concentration on perennial ryegrass. This application increased GT_{50} germination percentage but chewings fescue and hard fescue did not show significant differences as compared to the control. The decrease in GT_{50} germination

percentage was recorded on 40 and 50 g/L extracts in all turf grass varieties. The GT_{50} for strong creeping red fescue, treatments of 30, 40 and 50 g/L extracts could not be reached because of limited germination, therefore the GT_{50} was calculated as zero (Table-4).

The term allelopathy has traditionally been considered as only chemical ware fare of one organism upon another to inhibit of the plant growth. Modern research suggest that allelopathic chemical could be both stimulation and inhibition effects on the growth of economically important plants[4].

Previous works suggested that extracts from different higher plant such as trees and shrubs, caused increase or reduce seed germination, ATG, GT_{50}, root and seedling growth of herbaceous plants[1,9,23-25].

In present study, in general seed germination, root and shoot elongation of 4 turf grass varieties were strongly inhibited by the treatment of Scots pine leaf extracts. Similar results[18] were indicated with *Pinus sylvestris* in earlier studies in *Festuca becheri* Hack. Trautu and *Festuca rupicola* (Heull). In other study, Al-Humait *et al.*[16] reported that *Cynodon dactylon* seed germination were strongly inhibited by *Prosopis julifora* leaf extract treatment.

This study showed that all concentrations of Scots pine leaf extracts decreased seed germination of all turf grass varieties. Seed germination of turf grass varieties progressively decreased as the extract concentration increased. The most effective germination inhibition was seen on hard fescue seeds while the least germination inhibition was seen in perennial rye grass. Similar findings were obtained by Chaves and Escudero[6] who studied the effects of *Cistus ladanifer* leaf extract on *Lolium rigidum* seed germination. Watson[9] and Kocaçaliskan *et al.*[25] reported that smaller seeds are more sensitive to allelochemicals than greater seeds. In this study, seeds of hard fescue have the smaller seeds than the other varieties. And the greatest seeds have perennial rye grass. Therefore, the present findings are in agreement with above mentioned literatures.

Stimulation or inhibition effect of allelochemicals on the growth of plants depending on treatment dose of chemicals and plant species[4]. In the present study 10 g/L of Scots pine extract had stimulant effect on root elongation of perennial rye grass and shoot elongation of strong creeping red fescue and all the other concentrations had inhibitory effects. The ATG results showed that the Scots pine extract concentrations delayed germination of chewings fescue and perennial ryegrass seeds but not in hard fescue and strong creeping red fescue seeds.

Al-Humaid and Warrag[16], the GT_{50}, an index which has been used to evaluate seed germination percentage in meaningful biological units[26,27] showed statistically significant differences among the varieties, and concentrations. Generally GT_{50} values increase with the increase of extract

TABLE-1
EFFECTS OF SCOTS PINE LEAF EXTRACTS ON % GERMINATION OF SEEDS OF FOUR TURF GRASS VARIETIES

	Scots pine leaf extracts (g/L)						
Species	Control	10	20	30	40	50	LSD
Festuca rub. com. Koket	71.11 a	57.78 ab	51.11 b	45.55 b	24.45 c	11.11 c	0.01:16.28
Festuca ovina Crystal	83.33 a	10.00 b	0 c	0 c	0 c	0 c	0.01:9.59
Festuca rub.rub.Franklin	78.89 a	60.00 b	44.44 b	18.89 d	2.22 d	1.11 d	0.01:11.08
Lolium perenne Ovation	95.56 a	93.33 a	71.11 b	62.22 b	40.00 c	28.89 d	0.01:9.19

Means in rows followed by different letter differ significantly

TABLE-2
EFFECTS OF SCOT PINE LEAF EXTRACTS ON ROOT AND SHOOT ELONGATION OF SEEDLINGS OF FOUR TURF GRASS VARIETIES

	Scots pine leaf extracts (g/L)						
Species	Control	10	20	30	40	50	LSD
Root length (cm)							
Festuca rub. com. Koket	8.90 a	4.78 b	1.02 c	0.42 c	0.19 c	0.11 c	0.01:11.28
Festuca ovina Crystal	8.37 a	4.01 b	0.00 c	0.00 c	0.00 c	0.00 c	0.01:4.21
Festuca rub.rub.Franklin	9.76 a	6.38 b	0.87 c	0.60 cd	0.20 de	0.03 e	0.01:4.67
Lolium perenne Ovation	4.88 b	10.18 a	3.98 c	3.78 c	0.61 d	0.42 d	0.01:8.11
Shoot length (cm)							
Festuca rub. com. Koket	3.44a	3.25 a	2.48 b	1.32 c	0.69 d	0.55 d	0.01:5.98
Festuca ovina Crystal	4.03a	1.37 b	0.00 c	0.00 c	0.00 c	0.00 c	0.01:1.35
Festuca rub.rub.Franklin	3.71a	4.07 a	2.21 b	1.96 b	0.96 c	0.16 c	0.01:9.60
Lolium perenne Ovation	8.01a	4.22 b	4.21 b	4.11b	1.65 c	1.23 c	0.01:8.33

Means in rows followed by different letter differ significantly.

TABLE-3
EFFECTS OF SCOTS PINE LEAF EXTRACTS ON ATG OF SEEDS OF FOUR TURF GRASS VARIETIES

	Scots pine leaf extracts (g/L)						
Species	Control	10	20	30	40	50	LSD
Festuca rub. com. Koket	9,63 b	10,63 b	11,12 b	12,72 b	17,74 a	19,94 a	0,01:2.99
Festuca ovina Crystal	20,26 a	20,33 a	0 b	0 b	0 b	0 b	0,01:3.37
*Festuca rub.rub.*Franklin	NS	NS	NS	NS	NS	NS	NS
Lolium perenne Ovation	6,36 c	6,33 c	8,54 b	8,65 b	12,62 a	13,54 a	0,01:1.93

Means in rows followed by different letter differ significantly.

TABLE-4
EFFECTS OF SCOTS PINE LEAF EXTRACTS ON GT_{50} OF SEEDS OF FOUR TURF GRASS VARIETIES

	Scots pine leaf extracts (g/L)						
Species	Control	10	20	30	40	50	LSD
Festuca rub. com. Koket	7,3 e	8,55 de	10,03 cd	12,26 c	16,20 b	19,13 a	0,01:2,33
Festuca ovina Crystal	17,30 a	18,63 a	0 b	0 b	0 b	0 b	0,01:1,63
*Festuca rub.rub.*Franklin	7,37 c	11,07 b	13,17 a	0 d	0 d	0 d	0,01:1,78
Lolium perenne Ovation	4,33 c	4,1 c	7,30 b	7,48 b	11,60 a	13,00 a	0,01:2,34

Means in rows followed by different letter differ significantly.

concentrations, except for 10 g/L extract concentration on perennial ryegrass. The most significant reduction in GT_{50} germination percentage was recorded on 40 and 50 g/L extracts in all turf grass varieties.

The different results of ATG and GT_{50} for the four turf grasses clearly indicated that effectiveness of scots pine leaf extracts on inhibition was varieties, dependent

Allelochemicals are synthesized in plants as secondary metabolites and located in certain specialized organs of donor plants. Nerg *et al.*[28] reported that terpenes, resin acids and phenolics main allelochemicals in parts of Scots pine especially with a higher concentration in the leaves. We hypothesize that from these allelochemicals one or more substances that soluble in water may inhibit the germination of seeds and low concentrations stimulated root and shoot elongation of certain turf grass varieties. In present study it is showed that among turf grasses, the most sensitive variety to allelochemicals were hard fescue.Therefore in new turf grass plantations under Scots pine, peoples should avoid to add hard fescue seeds to mixture.

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