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The Effect of Leaf Extracts in Different Growth Periods of *Bituminaria bituminosa* (L.) C.H. Stirt. on Some Germination and Seedling Development Parameters of Wheat

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ABSTRACT
The aim of this study was to determine the effect of *Bituminaria bituminosa* (L.) C.H. Stirt. leaf extracts obtained at beginning of growth, budding and beginning of flowering on germination and seedling growth characteristics of wheat (*Triticum aestivum* L.). In the study, 12 *B. bituminosa* genotypes leaf extracts (G) were used, and the control group was consisted of distilled water. In the study, germination percentage, root and shoot length, root and shoot fresh-dry weight, root and shoot biomass and seedling vigor index were investigated. The highest germination percentage was found in G1 as 99.55%, and the lowest was found in G2 with 93.93%. The root and shoot length ranged from 6.49-16.28 and 7.58-12.67 cm, respectively. The highest seedling vigor index was found in G5 (2813.50), G9 (2619.74), G11 (2657.05) and G12 (2657.71), while it was lowest control group (1343.74). As a result, the G9, G10, and G11 showed higher performance in terms of investigated traits. Besides, the *B. bituminosa* genotypes leaf extracts obtained at the budding stage were higher and had positive allelopathy effect compared to the other growth stages.

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Keywords
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Bituminaria bituminosa (L.) C.H. Stirt.'in Farklı Gelişme Dönemlerindeki Yaprak Ekstraktlarının Buğdayın Bazı Çimlenme ve Fide Gelişimi Parametreleri Üzerine Etkisi

ÖZET
Bu çalışmada, büyüme başlangıcı, tomurcuklanma ve çiçeklenme başlangıcı dönemlerinde elde edilen *Bituminaria bituminosa* (L.) C.H. Stirt yaprak ekstraktlarının buğdayın (*Triticum aestivum* L.) çimlenme ve fide gelişimi üzerine etkilerinin belirlenmesi amaçlanmıştır. Çalışmada 12 adet *B. bituminosa* genotipinin yaprak ekstraktı kullanılmış, kontrol grubunu ise saf su oluşturmuştur. Çalışmada çimlenme oranı, kök-gövde uzunlukları, kök-gövde yaş ve kuru ağırlıkları, kök ve gövde biyokütlesi ve fide canlılık indeksi incelenmiştir. En yüksek çimlenme oranı 1 numaralı (% 99.55), en düşük ise 2 numaralı (% 93.93) genotipten elde edilmiştir. Çalışmada kök ve gövde uzunlukları sırasıyla 6.49-16.28 cm ve 7.58-12.67 cm arasında değişmiştir. En yüksek fide canlılık indeksi G5 (2813.50), G9 (2619.74), G11 (2657.05) ve G12 (2657.71), en düşük ise kontrol uygulamasından (1343.74). elde edilmiştir. Sonuç olarak çalışmada 9, 10 ve 11 numaralı genotipler incelenen özellikler bakımından daha üstün performans göstermiştir. Ayrıca tomurcuklanma döneminde alınan *B. bituminosa* yaprak ekstraktları diğer gelişme dönemlerine göre buğdayın gelişimi üzerinde daha yüksek ve olumlu allelopatik özellik göstermiştir.

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INTRODUCTION

Bituminaria bituminosa (L) C.H. Stirt. a perennial herb of the Leguminosae family was widely distributed in Turkey, Southern Europe, Crimea, West Syria, Cyprus, Caucasus, Israel, North Africa, Portugal, Spain and has a long history (Hooker and Jackson, 1960; Gulumser and Acar, 2012). *B. bituminosa* is known as a forest trefoil, iron hay or bitumen alfalfa and it is cultivated in the Canary Islands and Morocco. In recent years, its varieties were developed in southern regions such as; Australia, Israel, Italy and Spain. Also, it grows on roadsides, in woodland, forests and marginal lands (Davis, 1965-1988).

In nature, allelopathy plays an important role. Many of the compounds claimed to be allelochemicals have a biological activity on plants in soil, due to their instability, rapid degradation by microbes, or other interactions with soil. Indeed, these compounds are defence mechanism in plants, therefore removing these compounds from their content will affect the growth of others (Duke, 1991). *B. bituminosa* contain some compounds such as tannins, furanokumarin (psoralen and angelisin), isoflavone (daidzein and genistein) and pterocarbon groups (Bouque et al., 1998; Pecetti et al., 2007; Rio et al., 2010) and they are called allelochemicals. These allelochemicals have an allelopathic characteristics and, they spread allelochemicals to the environment. Therefore, the growth of other plants in the environment exposed to these compounds are affected.

In the agricultural fields, the determination of the allelopathic effect is very difficult since it is necessary to reveal the differences in the development of the plants, due to competition or other reasons. Besides, the allelochemicals of plants may have antibacterial, antifungal and antiviral effects. The allelochemicals can be used as natural herbicides and natural insecticides (Gurevitch et al., 2006).

The aim of this study was to determine the effect of *B. bituminosa* leaf extracts obtained at beginning of growth, budding and beginning of flowering on germination and seedling growth characteristics of wheat.

MATERIAL and METHODS

Plant materials

In this study, 12 *B. bituminosa* genotypes were used. The genotypes were collected from 3 provinces and 11 different locations in Turkey and, also the one genotype is Spanish origin (Table and Figure 1). The genotypes were collected and recognized by Prof. Dr. Zeki Acar.

The collected seeds were sown in peat media, and then seedling were transplanted to the field with 70x70 cm apart. Fifteen plants were used for each population and, the experiment was conducted in May, 2016 in Samsun, Turkey. Plants were harvested at 3 different growth stages (beginning of growth, budding and beginning of flowering) and, the leaves were separated. In the germination assay, the wheat (*Triticum aestivum* L.) var. "Flamura-85" were tested.

Plant extraction procedure

The harvested leaves were thoroughly washed with distilled water to avoid contamination and laid in distilled water for 7 hours. About 500 g of plant leaves were macerated in 3000 ml distilled water to be used in germination of wheat seeds. The obtained extracts were filtered with filter paper stored at +4°C in refrigerator.

Petri experiment

Germination and seedling growth experiments were performed under fully controlled climate room conditions in Biotechnology Application and Research Centre of Bilecik Şeyh Edebali University in 2018. The experiment was arranged in split plot design with four replications, main plots were growth stage and sub plots were genotypes..

Table 1. *B. bituminosa* genotypes

Genotype number	Collection site	Coordinates	
G1	Spain	-	-
G2	Kastamonu İnebolu	41° 58' 32.8"	33° 46' 10.4'
G3	Samsun-Çatalzeytin	41° 57' 48.4"	34° 09' 07.8'
G4	Sinop Kanlıçay	41° 40' 40.3"	35° 22' 22.8'
G5	Samsun-Kozağzı	41° 28' 05.1"	35° 49' 56.8'
G6	Samsun-Çarşamba	41° 04' 35.1"	36° 40' 09.0'
G7	Samsun-Bağkur	41° 18' 39.0"	36° 20' 02.5'
G8	Samsun- Baruthane	41° 19' 08.5"	36° 19' 13.6'
G9	Samsun-Nebyan	41° 23' 35.9"	35° 59' 06.2'
G10	Samsun-Kurupelit	41° 22' 16.0"	36° 11' 46.7'
G11	Sinop-Tıngıroğlu	41° 47' 41"	35° 00' 23"
G12	Samsun-Kavak	41° 03' 14.35"	35° 56' 59.84"

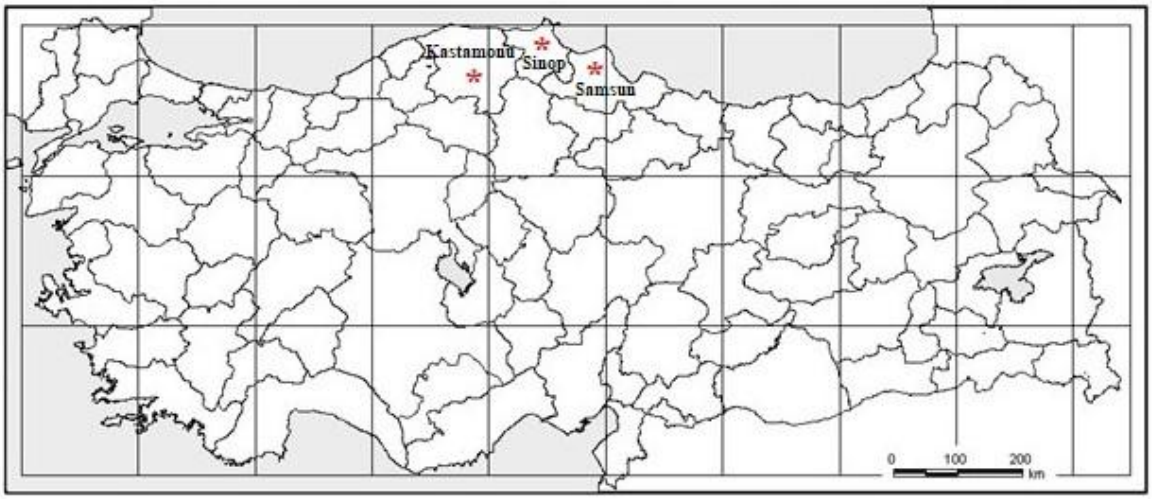


Figure 1. *B. bituminosa* genotypes collecting locations of (Davis, 1965-1988)

For the germination assay, twenty seeds of the wheat species were placed between filter paper in 12.0 cm diameter Petri dishes. The wheat seeds were irrigated with 6 ml of *B. bituminosa* leaves extract for 21 days and the touch of radicula to the paper was accepted as the beginning of the germination. The experiment lasted 21 days and distilled water was used as control group. In the study, germination percentage (GP), root and shoot length, root and shoot fresh-dry weight, root (RB) and shoot (SB) biomass and seedling vigor index (SVI) were analysed.

GP = 100 × (Total number of germinated seeds/total number of seeds tested) (Kayacetin et al., 2018).

RB: (Root dry weight* petri dish area)/10^{-8} (Caliseki et al., 2016)

SB: (Shoot dry weight* petri dish area)/10^{-8} (Caliseki et al., 2016)

SVI: (Average radicula length+average plumula length) x Germination percentage (Böhm, 1979).

Statistical analyses

The obtained data was analysed according to split plot design and the differences between the means were determined by the DUNCAN multiple comparison test.

RESULT and DISCUSSION

Germination percentage and seedling vigor index

The effect of B. *bituminosa* leaves extracts on seed germination percentage of wheat was not significant in the genotypes, growing stage and genotype x growing stage interactions. The highest germination percentage was in G1 (99.55%), while the lowest was in G2 (93.93%). The germination percentage of genotypes excepted in G2, G7 and G12 were greater than the control group (Figure 2). Besides, the average growing stage germination percentages were determined as 97.84% (beginning of growth stage), 96.41% (budding stage) and 96.41% (beginning of flowering stage), respectively.

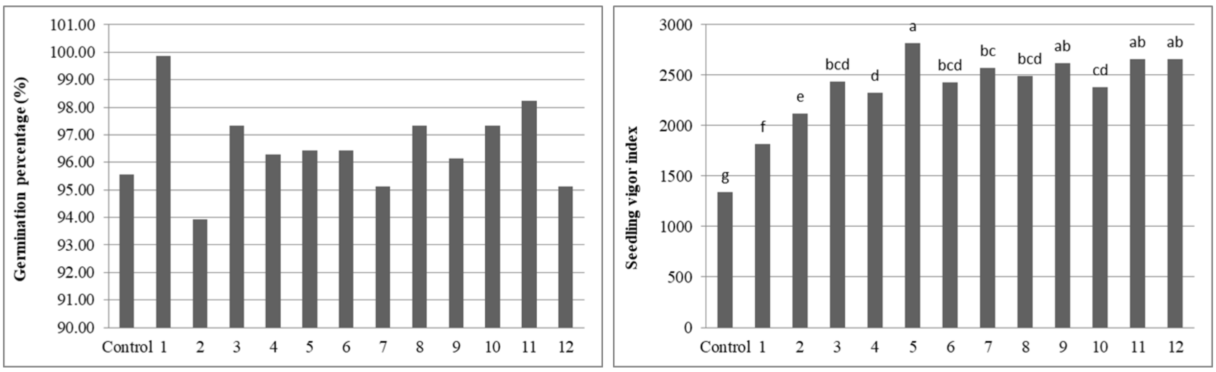


Figure 2. The average germination percentage and seedling vigor index of wheat treated with distilled water and *B. bituminosa* leaves extracts

The seedling vigor index was given Figure 2. The genotypes, growing stage and genotype x growing stage interactions was significant ($p<0.001$). The highest seedling vigor index was 2813.50, 2619.74, 2657.05 and 2657.71 for G5, G9, G11 and G12, respectively. On the other hand, the lowest seedling vigor index was observed in the control group as 1343.74. The average seedling vigor index in the growing stage was listed from the highest to lowest; budding > beginning of growth = beginning of flowering. Abbas *et al.* (2014) reported that the positive effect of distilled water compared to some weed extracts of wheat seed germination and root-shoot development.

Root and shoot length

The root and shoot length was significantly ($p \leq 0.01$) different in genotypes, growing stage and genotype x growing stage interaction. The highest root length was determined in G5 (16.28 cm), G9 (15.53 cm), G11 (15.52 cm) and G12 (16.14 cm), while it was the lowest in control group (distilled water and 6.49 cm). The shoot length ranged from 7.58 cm (distilled water) to 12.67 cm (G5). The root and shoot length of wheat that obtained of all the genotypes were higher than control group (distilled water) (Figure 3). This indicated that *B. bituminosa* leaf extracts have a growth-promoting effect on the root and shoot development of wheat. Akin *et al*. (2017) reported that the *Lythrum salicaria* L. extracts had a negative effect on the germination of lettuce. Their result was not similarly compatible with ours and, the difference between the two studies resulted from the secondary metabolites of the plants and the positive or negative effects of these metabolites depending on the plant species. Regarding to the growing stage, the highest root and shoot length were determined in budding stage (17.24 cm and 12.77 cm, respectively), and then it was followed by beginning of flowering stage (11.99 cm and 10.29 cm, respectively). The beginning of growth root and shoot length was as 11.29 cm and 9.60 cm, respectively.

Root and shoot fresh-dry weight

The effect of *B. bituminosa* leaves extracts on root fresh and dry weight of wheat was significant ($p \leq 0.01$) in the genotypes, growing stage and genotype x growing stage interactions. The root fresh and dry weight ranged from 0.498 g (control) to 1.274 g (G12) and 0.0718 g (control) – 0.171 g (G5), respectively (Figure 4).

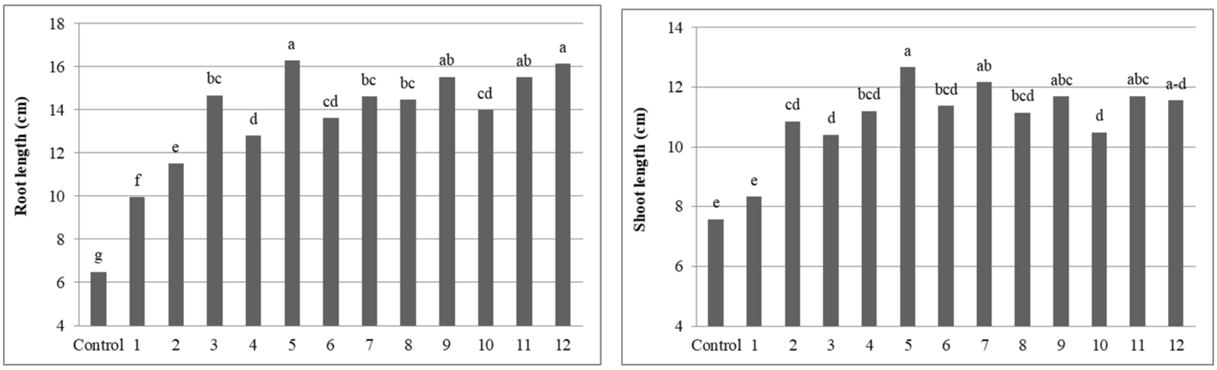


Figure 3. The average root and shoot length of wheat treated with distilled water and *B. bituminosa* genotype leaves extracts

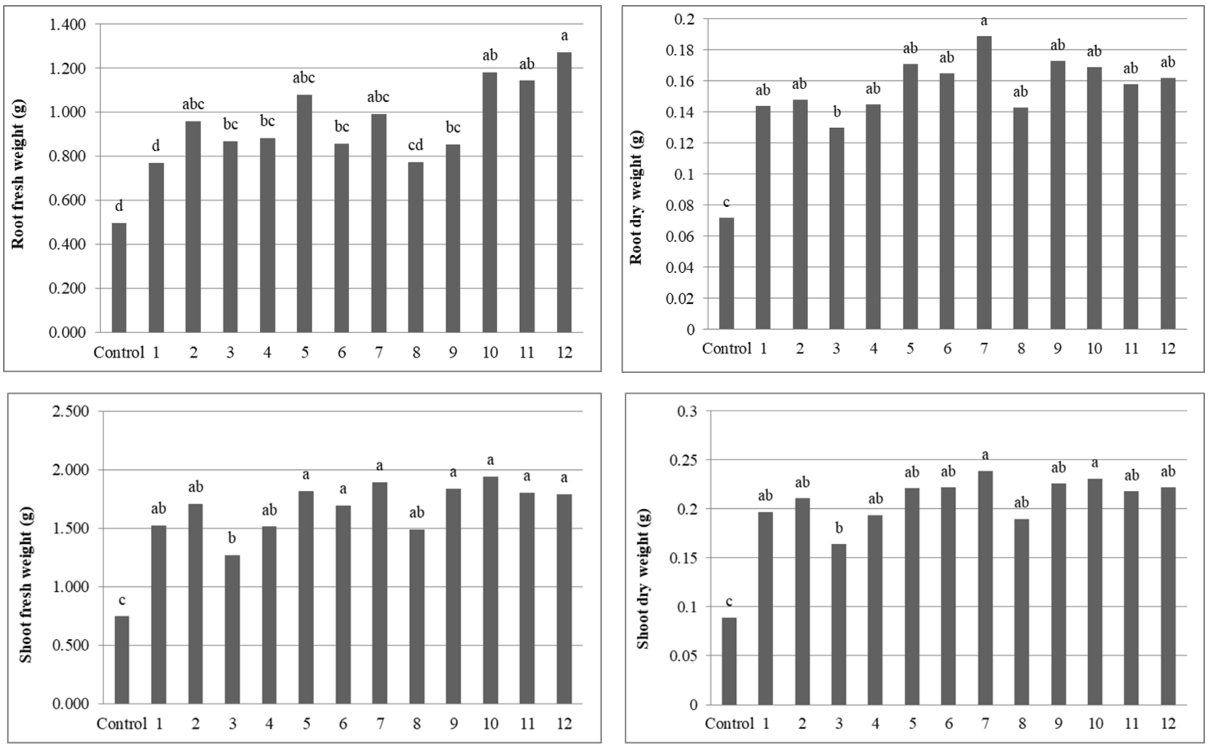


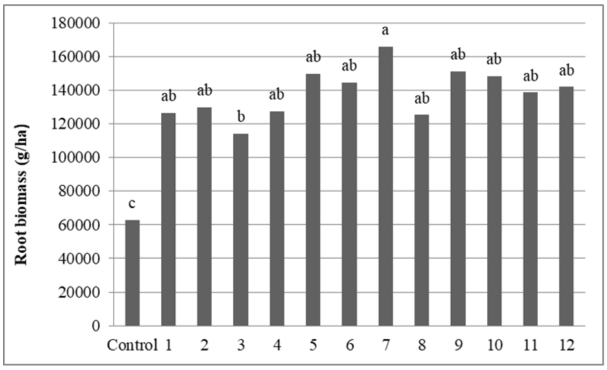
Figure 4. The average root and shoot fresh-dry weight of wheat treated with distilled water and *B. bituminosa* genotype leaves extracts

The shoot fresh and dry weight were significantly ($p \leq 0.01$) different in genotypes and growing stage, while they did not have significant genotype x growing stage interaction. According to root and shoot fresh-dry weight, there are significant differences between genotypes and growth stage. This indicated that there was a difference between genotypes in terms of secondary metabolites and development period of plants. Murad et al. (2016) reported that the *Silene villosa* extracts had lower effect than compared to distilled water on the germination of *T. aestivum.*

Root and Shoot Biomass

The average root and shoot biomass were given Figure 5. The root and shoot biomass had significantly ($p \leq 0.01$) different depending on genotypes, growing stage and genotype x growing stage interaction. The wheat seeds root and shoot biomass ranged from 62891.24 g/ha to 166379.47 g/ha and 78078.60 g/ha to 209897.10 g/ha, respectively (Figure 5). The highest root and shoot biomass were determined in G7, while the lowest were in control group. The average root biomass as its growing stage was listed here in order of high to low; beginning of growth = budding > beginning of flowering, while shoot biomass growing stage; budding > beginning of growth = beginning of flowering.

Gella et al. (2013) indicated that the *Argemone mexicana* and *Amaranthus hybridus* L. were found to be effective on the root and shoot biomass of wheat compared to the distilled water. In the same study, the root and shoot biomass of wheat ranged from 2.75 to 311 mg and 2.85 to 3.32 mg.



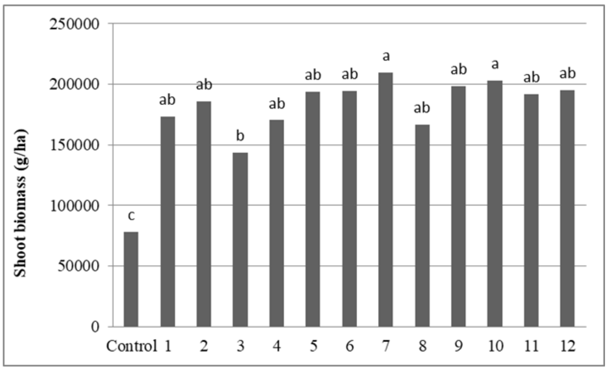


Figure 5. The average root and shoot biomass of wheat treated with distilled water and *B. bituminosa* genotype leaves extracts

CONCLUSION

In the study, *B. bituminosa* genotypes leaves extracts obtained at different growth stages were determined a positive effect on germination and seedling development of wheat. According to studied traits, G9, G10 and G11 showed higher performances. Besides, the *B. bituminosa* genotypes leaves extracts obtained at budding stage had higher and positive allelopathy effect compared to the other growth stage.

As a result of the study, the total secondary metabolites obtained from the leaves of *B. bituminosa* found to have a positive allelopathic effect. Therefore, furanocoumarin (psoralen and angelisin), isoflavone (daidzein and genistein) and pterocarbon groups have to be isolated from *B. bituminosa*, and allelopathic effects of these allelochemicals must be determined separately.

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