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# Determining the tolerance of various Turkish hazelnut cultivars (*Coryllus avellane* L.) against *Xanthomonas arboricola pv. corylina*

## Türk fındık çeşitlerinin (*Corylus avellana* L.) bakteriyel yanıklık hastalığına (*Xanthomonas arboricola* pv. *corylina*) karşı toleranslarının belirlenmesi

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## ABSTRACT

**Objective**: This study was conducted to define the tolerance of various hazelnut cultivars against *Xanthomonas arboricola* pv. *corylina* causing agent of bacterial blight.

**Material and Methods**: The Turkish hazelnut cultivars (Acı, Çakıldak, Foşa, İncekara, Kalınkara, Kan, Kargalak, Kuş, Mincane, Palaz, Sivri, Tombul, Uzunmusa, Yassı Badem and Yuvarlak Badem) were used as plant material. AT8/Z strain of *Xanthomonas arboricola* pv. *corylina* isolated from the field was tested. Parameters measured to detect bacterial blight tolerance were infected leaf percentage and leaf lesion rate.

**Results:** İncekara showed the minimum percentage of infected leaves (%17.57) and Yassı Badem reflected the highest (%77.51). Yassı Badem had the highest leaf lesion degree (3.00) and İncekara the lowest (1.34). The results suggested that Yassı Badem cultivar was the most susceptible and İncekara the most resistant to the pathogen. The highest antimicrobial activity was represented by Mincane with leaf diameter of 20 mm.

**Conclusion:** In this study, resistance to *Xanthomonas arboricola pv. corylina* pathogen tolerance of Turkish hezelnut cultivars grown in our country was determined. It is thought that the results obtained from the study will shed light on the breeding studies of resistant varieties against this pathogen in the future.

**Keywords**: Bacterial blight, *Corylus avellane,* hazelnut, *Xanthomonas arboricola pv. corylina*

## ÖZ

**Amaç**: Türk fındık çeşitlerinin bakteriyel yanıklığa sebep olan *Xanthomonas arboricola* pv. *corylina* patojenine karşı toleranslarının saptanması amaçlanmıştır.

**Materyal ve Metot**: Materyal olarak Acı, Çakıldak, Foşa, İncekara, Kalınkara, Kan, Kargalak, Kuş, Mincane, Palaz, Sivri, Tombul, Uzunmusa, Yassı Badem ve Yuvarlak Badem Türk fındık çeşitleri kullanılmıştır. *Xanthomonas arboricola* pv. *corylina* bakterisinin araziden izole edilen AT8/Z ırkı test edilmiştir. Bakteriyel yanıklık toleransını tespit etmek için enefekte olmuş yaprak yüzdesi ve yaprak lezyon derecesi parametreleri ölçülmüştür.

**Araştırma Bulguları**: Bu doğrultuda toplam lezyonlu yaprak oranı esas alındığında %17.57 ile İncekara çeşidi en az lezyonlu yaprak oranına, %77.51 ile Yassı Badem çeşidi ise en fazla lezyonlu yaprak oranına sahip olmuştur. Yaprak lezyon derecesi incelendiğinde Yassı Badem en yüksek (3.00), İncekara is en düşük (1.34) puanı almıştır. Bu sonuçlar doğrultusunda, Yassı Badem bakteriyel yanıklığa en duyarlı, İncekara ise en toleranslı fındık çeşidi olarak tespit edilmiştir. Fındık yaprak ekstraktlarının patojene karşı göstermiş olduğu antimikrobiyal etki bakımından 20 mm çap ile en yüksek Mincane çeşidinde belirlenmiştir.

**Sonuç:** Bu çalışma ile ülkemizde yetiştiriciliği yapılan Türk fındık çeşitlerinin *Xanthomonas arboricola pv. corylina* patojenine karşı toleransları saptanmıştır. Çalışmadan elde edilen sonuçlar, bu patojen karşı dayanıklı çeşit ıslahı çalışmalarına ışık tutacağı düşünülmektedir.

**Anahtar kelimeler:** Bakteriyel yanıklık *Corylus avellana,* fındık, , *Xanthomonas arboricola* pv. *corylina*,

## INTRODUCTION

Turkey, together with many other fruit species, is also homeland of hazelnut (*Corylus avellana* L.). Hazelnut belongs to genus *Corylus*, sub-family *Coryleae*, family *Betulacea*, order *Fagales*. Especially *C. avellana* specie, including domesticated hazelnut, is native to the Black Sea Region of Turkey and is known to be cultivated in this area for 2500 years (Ayfer et al., 1986; Köksal, 2002). Turkey is World leader in hazelnut production and cultivation with 500.000-700.000 tons production per year (Anonymous, 2019a). Thus, hazelnut is of great economic importance to Turkey. Together with high production amounts, there are some problems regarding hazelnut cultivation, viz: many hazelnut orchards especially in the Black Sea Region are very old and spring frost damages are seen, along with rainfalls in the harvest period causing aflatoxin formation in the nuts. Nut weevil, gall mite, green shield bug, European shot hole borer and hazel aphid, as well as European canker and armillaria are among the most important pests and diseases of hazelnut (Köksal, 2002; Anonymous, 2019b). *Xanthomonas arboricola* pv. *corylina*, agent of bacterial blight, causes severe yield loses in hazelnut. *X. arboricola* pv. *corylina,* which is a gram-negative aerobic bacterium with a single polar flagellum, mainly attacks *C. avellan*a (Anonymous, 2019c). The pathogen was reported in the USA, Italy, France, Spain, Turkey, Serbia, United Kingdom, Southern Russia, Switzerland, Chile, Canada, Australia, New Zealand, Iran, and Poland (Anonymous, 2019d; Pisetta et al., 2016). *X. arboricola* pv. *corylina* was found to prevail in hazelnut orchards of Samsun, Ordu, Giresun, Trabzon, Rize and Artvin provinces of Turkey, and all of the investigated hazelnut cultivars in these regions were susceptible to the disease (Alay et al., 1973). However, the distribution rate of the pathogen in Düzce, Sakarya and Zonguldak provinces of Turkey was %7.3, %10.4 and %10, respectively (Karahan et al., 2011). Bacterial blight tolerance of Allahverdi, Çakıldak, Foşa, Mincane, Palaz, Sivri and Tombul, important hazelnut cultivars grown in Black Sea Region, was investigated during 1994-1995 years. Allahverdi was found to be the most susceptible cultivar with regard to leaf lesions occurring after infection with *X. arboricola* pv. *corylina*. The infection rate in this cultivar was %37.57 and %21.43 in 1994 and 1995 years, respectively. The highest tolerance level was shown by Mincane cultivar (Ecevit et al., 1996).

Ecological conditions, applied cultural methods, plant age and plant variety are among the factors affecting the spread of *X. arboricola* pv. *corylina*. The pathogen mainly enter to the plant through stomata and 1-4 year old orchards are most susceptible (Anonymous, 2019e). When the mother plant is infected the vegetatively propagated plants from the suckers are also contaminated. Due to the lack of certified hazelnut saplings in Turkey, suckers taken from the existing orchards are used in creation and regeneration of orchards. For this reason, the spreading level of *X. arboricola* pv. *corylina* is relatively high. The susceptibility of hazelnut cultivars to bacterial blight differs and the tolerance level of the cultivars grown in Turkey is unknown. Therefore, the current study was conducted to determine the tolerance level of various cultivated Turkish hazelnut cultivars to *X. arboricola* pv. *corylina*, causal agent of bacterial blight.

## MATERIAL and METHODS

### Materials

Rooted hazelnut suckers of Acı, Çakıldak, Foşa, İncekara, Kalınkara, Kan, Kargalak, Kuş, Mincane, Palaz, Sivri, Tombul, Uzunmusa, Yassı Badem and Yuvarlak Badem hazelnut cultivars were provided from the 'Hazelnut Research Center' in Giresun, Turkey during October, 2011. The suckers were placed in plastic tubes (25x30 cm) containing 3:1:1 proportion of forest soil, peat and manure, respectively. Cultural practices were applied until inoculation with the bacteria. The condition of saplings before infection is presented in Figure 1. AT8/Z strain of *Xanthomonas arboricola* pv. *corylina* isolated from the field was tested on the hazelnut cultivars.

**Figure 1.** Appearance of hazelnut saplings before bacterial infection.
***Şekil 1.*** *Bakteriyel enfeksiyondan önce fındık fidanlarının görünümü.*

## Methods

### Inoculum production

The bacteria were inoculated on 21 g/l Mueller-Hinton Broth (Oxoid CMO405) medium using cotton swaps, followed by incubation for 24 hours at 25 $^{o}$C under dark conditions. 1 ml of these bacterial culture medium was added to each falcon tube containing 35 ml of 21 g/l Mueller-Hinton Broth medium, after which left to incubate at 30 $^{o}$C, 30 rpm water bath for 24 hours. Following this, the falcon tubes were centrifuged at 4000 rpm for 10 minutes. After discarding the supernatant from the falcon tubes, 25 ml sterile distilled water was added over the bacteria within the falcons and vortexed to homogeneity. The cultures were diluted with sterile distilled water to an optical density of $10^6$ bacteria/ml, and the dilutions were used to inoculate the hazelnut saplings.

### Disease reaction tests

Pressure sprayer (Gardener) filled with $10^6$ bacteria/ml optical density solution of *X. arboricola* pv. *corylina* was used to completely spray the hazelnut sapling leaves in the morning, when stomatal opening was maximum (26 May 2012). Bacterial blight symptom observations were recorded after 20 days following inoculation. Total number of leaves per sapling were counted including recording of unaffected and lesion formed leaves. Thus, the percent (%) of affected leaves per plant was determined. The degree of lesion formation per leaf was also measured. Leaf lesion degree was measured using 1-4 scale (with 1 least affected leaf area, 2 half of the leaf area was affected, 3 about 0.75 of the leaf area was affected, and 4 completely affected leaf area).

### Antimicrobial activity assay

Antimicrobial activity was assessed through disc diffusion and agar dilution methods using Muller Hinton Agar (Oxoid) medium. Bacteria were propagated on Muller Hinton Broth (Oxoid) medium, and ¼ Tris Buffer (Amresco) was used as minimum inhibition concentration. The leaf extracts were prepared

according to the method of Holopainen et al. (1988). The collected leaves were dried and smashed. 10 gr leaf samples of each hazelnut cultivar were blended with 50 ml ethanol in a separate sterile tube and kept for two days at 4 $^0$C, following extraction with membrane filter and vacuumed. The leaf solutions were kept at -20 $^0$C until used. The antimicrobial activity was detected according to the method of Ronald (1990). The agar medium was autoclaved prior to use and 20 ml medium was poured homogenously into each sterile petri dish (10 cm in diameter) and left to cool, following inoculation with bacteria through streaking with sterile cotton swaps onto the solidified agar surface. After this, discs were placed on each petri dish and 15 µl of the aforementioned leaf solution was dripped on each disc surface, following keeping at 37 $^o$C overnight. Measurements were recorded as mm on the formed inhibition zones.

**Statistical analysis**

The data were subjected to Analysis of Variance (ANOVA) followed by Duncan test using significance level at P≤0.05. Significant differences were donated with different letters (Table 1). The analyses were performed with Minitab statistical Software (MINITAB Inc.814-238-3280 WS 112102553).

**Table 1.** Infected leaves percentage and leaf infection degree of various hazelnut cultivars inoculated with *X. arboricola pv. corylina*

***Çizelge 1.*** *Xanthomonas arboricola pv corylina ile enfekte edilmiş çeşitli fındık bitkilerinin enfeksiyona uğramış yaprak yüzdesi ve yaprak lezyon oranı*

| Cultivar | Infected leaves percentage (%) | Leaf infection degree |
|---|---|---|
| Acı | 35.40 bc* | 1.75 bcde* |
| Çakıldak | 42.24 bc | 1.80 bcde |
| Foşa | 46.87 b | 2.64 ab |
| İncekara | 17.57 c | 1.47 de |
| Kalınkara | 50.55 b | 2.33 abcd |
| Kan | 51.28 b | 2.40 abc |
| Kargalak | 32.77 bc | 1.66 cde |
| Kuş | 36.49 bc | 1.92 bcde |
| Mincane | 29.95 bc | 1.97 bcde |
| Palaz | 33.08 bc | 1.77 bcde |
| Sivri | 40.35 b | 1.85 bcde |
| Tombul | 36.43 bc | 1.68 cde |
| Uzunmusa | 31.70 bc | 1.34 e |
| Yassı Badem | 77.51 a | 3.00 a |
| Yuvarlak Badem | 48.83 b | 2.21 abcde |
| LSD | 23.44 | 0.7512 |

* Different letters indicate significant plant response differences between the cultivars (Duncan %5).

## RESULTS and DISCUSSION

Yassı Badem showed the highest percentage of infected leaves (%77.51) followed by Kan (%51.28), Kalınkara (%50.55), Yuvarlak Badem (%48.83) and Foşa (%46.87) cultivars (Table 1). The remaining hazelnuts infected leaves range was between % 17.57-42.24 with İncekara being the most tolerant to *X. arboricola* pv. *corylina* (Table 1).

Allahverdi, Çakıldak, Foşa, Mincane, Palaz, Sivri and Tombul hazelnut cultivars grown in the Black Sea Region of Turkey were investigated against susceptibility to filbert blight. Allahverdi showed highest leaf lesions after infection with *Xanthomonas arboricola pv. corylina*. The infection rate in this cultivar was %37.57 and %21.43 in 1994 and 1995 year, respectively, and Mincane was the most tolerant (Ecevit et al., 1996).

Leaf lesion degree which was scored between 1-4 (1 the least affected leaf area up to 4 completely affected leaf area) was evaluated and Yassı Badem with a score of 3.00 was the most susceptible to the

pathovar, followed by Foşa (2.64), Kan (2.40), Kalınkara (2.33), and Yuvarlak Badem (2.21). Leaf lesion degree for the remaining hazelnut cultivars ranged between 1.34-1.97, with İncekara being the most tolerant to *X. arboricola* pv. *corylina* (Table 1).

The results from both of the evaluations (total infected leaf percentage and infected leaf area) suggested that Yassı Badem cultivar was the most susceptible and İncekara the most resistant to the pathogen. Figure 2 shows Yassı Badem sapling infected with the pathogen.

**Figure 2.** Appearance of Yassı Badem sapling infected with *X. arboricola* pv. *corylina.*

***Şekil 2.*** *X. arboricola pv. corylina ile enfekte olan Yassı Badem çeşidinin görünüm .*

Various hazelnut cultivars from Italy including Tonda Gentile delle Langhe, Tonda Gentile Romana, Tonda di Giffoni, Camponica, Mortarella and San Giovanni were found infected with *X. arboricola* pv. *corylina* and this condition significantly affected overall plant growth (Pisetta et al., 2016).

Antimicrobial efficacy of the tested hazelnut cultivars was evaluated and Mincane with 20 mm diameter showed the highest activity, followed by Kan (17 mm) and Foşa (15 mm). (Table 2). Acı, Kargalak, Sivri and Uzunmusa cultivars didn't show any antimicrobial activity. Antimicrobial efficacy of the remaining hazelnut cultivars ranged between 1.5-14 mm (Table 2). The results suggest no linear relationship between tolerance to the pathogen and antimicrobial activity (Table 1 and 2).

Antimicrobial activity of various walnut cultivars' green husks was evaluated against Gram negative and Gram-positive bacteria. All of the green husks extracts of the corresponding walnut cultivars inhibited the growth of Gram positive bacteria (Oliveira et al., 2008).

Bacterial blight is a major disease of young hazelnut orchards and nurseries causing severe mortality (Pisetta et al., 2016). Therefore, it is crucial to develop hazelnut genotypes with improved tolerance to the pathogen. The current study is intended to be a baseline for the future breeding projects to improving resistance in hazelnuts against the pathovar *X. arboricola* pv. *corylina* causing agent of bacterial blight.

**Table 2.** Antimicrobial efficacy area of various hazelnut cultivars
***Çizelge 2.*** *Fındık çeşitlerinin antimikrobiyal etkinlik alanı*

| Cultivar | Area of antimicrobial efficacy (mm) |
|---|---|
| Acı | 0.0 |
| Çakıldak | 10.0 |
| Foşa | 15.0 |
| İncekara | 1.50 |
| Kalınkara | 11.0 |
| Kan | 17.0 |
| Kargalak | 0.0 |
| Kuş | 10.0 |
| Mincane | 20.0 |
| Palaz | 14.0 |
| Sivri | 0.0 |
| Tombul | 17.0 |
| Uzunmusa | 0.0 |
| Yassı Badem | 12.0 |
| Yuvarlak Badem | 10.0 |

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