

MICROBIAL ORGANISMS DETECTED ON *JUGLANS REGIA* IN EASTERN ANATOLIA, TURKEY

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ABSTRACT

From Eastern Part of Anatolia in (Erzurum and Erzincan) 2014-2016 (May-September) from 13 different places and 183 infected walnut trees were collected and kept in the laboratory. Herbarium specimens prepared and cultivated in the medium from fresh leaves, fruits and shoots at 25 °C. The collected fungal isolates investigated and identified under the light microscopy. The results of this survey showed that the majority of isolated microbes are fungi and also some different bacterial species isolated. Totally, 17 genera (*Alternaria*, *Aspergillus*, *Cladosporium*, *Chaetomium*, *Drechslera*, *Fusarium*, *Melanospora*, *Microstroma*, *Ophiognomonina*, *Penicillium*, *Phoma*, *Rhizopus*, *Mucor*, *Sclerotinia*, *Sepotofusidium*, *Stemphyllium*, and *Trichothecium*) and 23 species of fungi and 4 genera of bacteria (*Bacillus*, *Erwinia*, *Xanthomonas*, and *Pseudomonas*) have been identified. According to the survey, there is significant diversity on the walnut shoots which are able to effect on the walnut growth and yield.

KEYWORDS:

Juglans regia, fungal diversity, shoots, microfungi, Turkey.

INTRODUCTION

Walnut (*Juglans regia* L.), as an important dried food with a high value of economic benefiting in agriculture, is producing in different regions of the world. *J. regia* is native to Mediterranean regions to Central Asia but it is widely cultivated in temperate regions of the world for nut production. The high amount of walnut production is done by China, Iran, the USA, Turkey, and Mexico. Turkey increases the walnut production area from 59000 ha in 2000 to 99617 ha in 2012 and the walnut trees number reach to 6,526,028 plantations [1]. The walnut production area in Turkey extended in all over the country such as; Anatolia, Ege, Marmara, Mediterranean Sea, and the Black Sea regions. Recently, the cultivation of grafted walnut trees is growing rapidly. There are

limited studies on the microbial flora of walnut [2]. Some fungal pathogen reported on walnut from different countries [3]. Also, there are studies about the microbial biodiversity of European walnuts [2]. Besides that, there is a different report about microbial pathogens of walnut from Turkey [4]. The fungal pathogens such as; *Microstroma juglandis* [4]; *Ascochyta juglandis* [5]; *Ophiognomonina leptostyla* [5]; *Pestalotiopsis guepinii* [6]; *Phyllosticta juglandis* [5]; *Phytophthora chlamydospora*, *Fusarium moniliforme*, *F. solani*, *Alternaria alternata* [7]; some bacterial diseases such as; *Xanthomonas campestris* pv. *juglandis* [8] and *Xanthomonas arboricola* pv. *Juglandis* [7] have been reported on walnut trees from Turkey. This study was conducted to investigate the microbial biodiversity on leaves, shoots, and fruits on walnut trees in Anatolia region of Turkey to determination the diversity of fungal and bacterial species.

MATERIALS AND METHODS

Plant material was collected from a total of 183 walnut trees from 13 sites. Sampling the plant materials collected from Eastern Anatolia and different locations such as; Erzincan (Üzümlü, Kemah, Kemaliye, Çatal Armut Köyü, Merkez-Bahçe Bitkileri Araştırma Merkezi), Erzurum (Narman-Tortum, Tortum-Pazaryolu (Yedi Göller); Uzundere, Oltu, İspir, Pazaryolu-Yeşil vadi Aktaş, Maden Köy), Artvin (Yusufeli), and İstanbul (Sultan Ahmet Camisi). The height of walnut trees were about 20-30 m and their diameter about 0.2-1 m. The area covered with snow from 15 December-30 April. There are daily and partly rainfall between May-June and then the rainfall start from September to early December.

The trees were not sprayed with pesticide and fungicides. At least ten leaves from each tree collected and transformed into the laboratory. From infected areas of leaves and shoots cut to 5×5 mm pieces and with surface sterilization with 70% ethanol for one minute and cultivated on PDA (LAB) medium and incubated at 25 °C and darkness condition. The fungal isolates investigated under the

TABLE 1
The Fungal isolates from walnut

| Fungus Species | Leaves | Fruit | Shoots | Number of Observed Isolates |
|---------------------------------------|--------|-------|--------|-----------------------------|
| 1 <i>Alternaria alternata</i> | * | * | * | 50 |
| 2 <i>Alternaria solani</i> | * | * | | 39 |
| 3 <i>Aspergillus falvus</i> | * | * | * | 3 |
| 4 <i>Aspergillus niger</i> | * | * | | 5 |
| 5 <i>Aspergillus ochraceus</i> | * | | | 9 |
| 6 <i>Aspergillus parasiticus</i> | * | | | 2 |
| 7 <i>Cladosporium cladosporioides</i> | * | * | * | 1 |
| 8 <i>Chaetomium globosum</i> | | * | | 1 |
| 9 <i>Drechslera</i> sp. | * | * | | 1 |
| 10 <i>Fusarium solani</i> | | * | | 1 |
| 11 <i>Fusarium incarnatum</i> | * | | * | 1 |
| 12 <i>Melanospora zamiae</i> | * | | | 1 |
| 13 <i>Microstroma juglandis</i> | * | | | 7 |
| 14 <i>Penicillium chrysogenum</i> | * | | | 6 |
| 15 <i>Penicillium citrinum</i> | * | | | 12 |
| 16 <i>Penicillium expansum</i> | * | | | 5 |
| 17 <i>Penicillium italicum</i> | * | | | 3 |
| 18 <i>Phoma glomerata</i> | | * | * | 10 |
| 19 <i>Rhizopus stolonifer</i> | * | * | | 13 |
| 20 <i>Mucor hiemalis</i> | * | * | | 2 |
| 21 <i>Sclerotinia sclerotiorum</i> | * | | | 3 |
| 22 <i>Septofusidium</i> sp. | * | | | 1 |
| 23 <i>Stemphyllium</i> sp. | * | | | 1 |
| 24 <i>Trichothecium roseum</i> | * | * | | 4 |
| 25 <i>Ophiognomania leptostyla</i> | * | * | * | 111 |
| Total Fungal Isolates | | | | 292 |

TABLE 2
The bacterial isolates from walnut

| Bacteria species | Leaves | Number of isolates |
|--|--------|--------------------|
| 1 <i>Bacillus subtilis</i> | * | 51 |
| 2 <i>Erwinia carotovora</i> | * | 12 |
| 3 <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> | * | 33 |
| 4 <i>Pseudomonas</i> sp. | * | 18 |
| Total Bacteria Isolates | | 104 |

light microscope (BH2) with sterile water, lactophenol, and acid fusion slide microscopic preparation. To identification the fungal isolates at least 50 spores measured and different identification key and fungal description have been used [8-11]. Also, the bacterial isolate identified with the GC-MS apparatus library in Phytobacteriological Laboratory in Department of Plant Protection, Faculty of Agriculture, at Ataturk University.

RESULTS AND DISCUSSION

During the investigation of walnut leaves and shoots, different fungal and bacterial species isolated in mediums and microscopical sampling. According to the identification keys, the fungal species classified in 17 genera and 23 species and total 292 fungal isolates collected. Also, four bacterial species from

104 bacterial isolates were identified by GC-MS apparatus library in Phytobacteriological Laboratory, Department of Plant Protection, at Ataturk University.

Although the studied area has cold climate and covered with snow for nine months in a year, the results of this survey showed us there is significant fungal biodiversity on walnut shoots and leaves in Eastern Anatolia. The leaves of walnut trees in this area start to emerge in mid-April. The leaf spots on walnut observed in last of May and early June and the last of September and early of December, the walnut leaves fall down the trees. In short period of growing season, different fungal propagation could infect and colonization on the walnut leaves. Among the fungal species, the asexual stage of *Ophiognomania leptostyla* was made most abundant leaf spots on the leaves, shoots, and fruits of trees. The infected trees with walnut anthracnose casual agents showed

a high percentage of infection. Almost all the walnut leaves were infected with this pathogen and the leaf spots were covered until to 10-50 % of each leaf. There were acervulus and conidia of fungi in most of the leaf spots and because of this disease, there were limited numbers (20-50) of the nut on each old tree. Another most abundant fungal species on the leaves were *Alternaria alternata*, *A. solani*, *Aspegillus ochraceus*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Microstroma juglandis*. Except for three fungi (*Fusarium solani*, *Chaetomium globosum*, and *Phoma glomerata*), all other fungal species were isolated from walnut leaves, too (Table 1). Besides that, four bacterial isolates have been identified from walnut leaves such as; *Bacillus subtilis*, *Erwinia carotovora*, *Xanthomonas arboricola* pv. *juglandis* and *Pseudomonas* sp. (Table 2). All the bacterial isolates were isolated from walnut leaves.

In previous studies on walnut in Turkey, some fungal (*Alternaria alternate*, *Ascochyta juglandis*, *Fusarium moniliforme*, *F. solani*, *Microstroma juglandis*, *Ophiognomonina leptostyla*, *Pestalotiopsis guepinii*, *Phyllosticta juglandis*, *Phytophthora chlamydospora*) and bacteria (*Xanthomonas campestris* pv. *juglandis* and *Xanthomonas arboricola* pv. *juglandis*) species have been reported [5-8, 12]. According to the result, there are significant fungal and bacterial diversity on the walnut in Eastern Anatolia, Turkey and some of the have potential to be a disease problem in walnut production.

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Received: 11.05.2019
Accepted: 24.06.2019

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