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DETERMINATION OF *Pestalotiopsis sp*. CAUSING DISEASE ON FRUIT CLUSTERS IN HAZELNUT GROWING AREAS OF ORDU, GİRESUN AND TRABZON PROVINCES IN TURKEY

SUMMARY

Turkey is the leader in the production and export of hazelnuts. Hazelnut production in Turkey in the Black Sea Region extends from the east of the Georgia border to Istanbul. In this study conducted in hazelnut growing areas in Ordu, Giresun and Trabzon provinces in Black Sea Region between 2008-2009, determination of Pestalotiopsis sp. causing disease on fruit clusters and isolation rate of the agent in these provinces, was investigated. Surveys were carried out on total 95,600 fruit clusters corresponding a field of 1555.5 da in July-August. The most predominant symptom was determined as blight.

The incidence of Pestalotiopsis sp. was found as 6.28% in Ordu, 7.33% in Giresun and 7.41% in Trabzon. The agent was isolated from symptoms of cluster blight and fruit necrosis with highest rates. Species identification was done according to the basis of culture and conidial morphology, growth rate and rDNA sequences. The pathogenicity tests using detached leaf and fruit clusters revealed various rates of disease severity for the isolates in the genera of Pestalotiopsis. Disease severity was 37.5-100% on detached leaves, 66.7% on severity of disease on detached twigs and clusters in pathogenicity tests.

Keywords: *Pestalotiopsis* sp, *Corylus avellana*, blight, Turkey

INTRODUCTION

Hazelnut (*Corylus avellana*) is one of the most important tree nut crops in Turkey, as having the 80% of world production, is the leader in the production and export of hazelnuts (Anonymous, 2011). It is not only one of the most important export crops of Turkey, but also the main economical activity of nearly 400 000 households under the form of family farmer in the Black Sea Region (Tanrıvermiş et al., 2006). Hazelnut production in Turkey in the Black Sea region extends from the east of the Georgia border to Istanbul. In terms of provinces Ordu has the largest hazelnut production area followed by Giresun in the country (Anonymous, 2013).

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Insect pests, diseases, environmental factors and growing techniques can be major constraints to production of hazelnut. Many fungal, bacterial, and viral diseases cause crop losses in hazelnut orchards when environmental condition favour disease development and spread. Main fungal hazelnut fruit diseases in the world are brown rot caused by *Monilia fructigena* and *M. laxa*, anthracnose caused by *Gloeosporium coryli*, fruit rot caused by *Botrytis cinerea* (Belisario and Santori,2009), gray fruit necrosis caused by *Fusarium lateritium* (Santori et al.,2010). In Turkey, *Botrytis cinerea*, *Monilia fructigena* or *M. coryli* were previously determined as hazelnut fruit diseases agents in Bolu, Zonguldak and Bartın provinces (Yürüt et al.,1994). Recently it was suggested that *Botrytis cinerea* is the most important and the most prevalence disease agent for hazelnut fruit clusters in Ordu, Giresun and Trabzon provinces (Sezer and Dolar, 2012).

Species of *Pestalotiopsis* are common in tropical and temperate ecosystems and may cause plant disease, they are often isolated as endophytes or occur as saprobes (Maharachchikumbura et al., 2011). *Pestalotiopsis* spp., on hazelnut was reported from different country (Akça and Tuncer,2005; Mirhosseini-Moghaddam and Taherzadeh, 2007). *Pestalotiopsis macrospora*, was found to be pathogen on leaves and twigs of hazelnut in İran (Mirhosseini-Moghaddam and Taherzadeh, 2007). *Pestalotiopsis guepinii* was isolated from diseased hazelnut twigs (Yürüt et al.,1994) and reported that the fungus causing blight on hazelnut and walnut twigs in Turkey (Karaca and Erper, 2001).

The objective of this research was to determine of *Pestalotiopsis* sp. causing disease on fruit clusters of hazelnut.

MATERIAL AND METHODS

Survey and fungal isolation

In order to determine the hazelnut fruit cluster diseases, surveys were carried out 221 hazelnut orchards in 2008 and 124 in 2009 growing seasons in Ordu, Giresun and Trabzon provinces in Black Sea Regions (Figure 1).

Fifure 1. Survey area for fruit cluster diseases of hazelnut in Turkey

Total 95,600 fruit clusters were examined for any disease symptom between July and August. Infected fruit clusters were surface-sterilized [0.5-1% (w/v) sodium hypochlorite] for 1-2 min before they were placed onto potato dextrose agar (PDA, Merck) containing streptomycin. Single spore isolates were obtained from fungal cultures incubated at 23±1°C with a 12-h photoperiod for 3-5days, and stored on PDA slant tubes at 4°C. Disease incidence was determined as the percentage of diseased fruit clusters calculated by counting the number of fruit clusters with symptoms.

Identification of fungus

For identification, culture morphology, growth rate and conidial morphology were observed from 7-10 day-old cultures grown on PDA (Sutton, 1980). The shape, length and width of 100 conidia were determined; and mean length and width were calculated. Fungal isolates were sent to CABI-UK for species identification by sequencing the internal transcribed spacer (ITS) of the rDNA of two representative isolates.

Pathogenicity tests

Pathogenicity tests were performed on detached leaves and detached twigs in the greenhouse.

Pathogenicity tests on detached leaves: Approximately, two-thirds the normal size and unhardened leaves were collected from Tombul and Palaz hazelnut cultivars and then detached leaves were surface disinfested with a cotton ball with 70% ethanol (Merck). The leaves were put in 9 cm glass petri dishes containing sterile filter paper moistened. Plant tissue was inoculated by placing a mycelial disc (5 mm) from an actively growing edge of the 10-day-old fungal culture. Leaves were incubated for 14 days at 20-25°C, with a 12 h photoperiod. Six fungal isolates were tested on leaves of two cultivars. PDA discs were used as controls. Symptoms were assessed and reisolations were performed from artificially infected leaves.

Pathogenicity tests on detached twigs and clusters: Detached twigs (15-20 cm long) each bearing one healthy fruit cluster (approximately at half fruit size) were surface sterilized with 70% ethanol for 2-3 min followed by three washes with sterile distilled water and kept in 50-ml Falcon tubes filled with tap water. Approximately 10 to 15 ml conidial suspension ($1x10^6$/ml) prepared in sterile distilled water using 10-day-old fungal culture were sprayed onto each fruit cluster. Controls were sprayed with sterile distilled water. Detached twigs and clusters kept in Falcon tubes were placed inside the containers covered with transparent plastic bags and incubated in the greenhouse at 20-25°C. Bags were removed after 48 h and symptoms were monitored daily for a week. Two fungal isolates which causes different disease severity on detached leaves was tested on clusters of Tombul hazelnut cultivar. Reisolations were performed.

Disease assessment: For disease assessment, percentage surface area covered by lesions on leaf or fruit cluster of hazelnut was determined. Diseases severity was calculated from estimated size of lesions. Lesions size was assessed on a scale of 0 to 3, where 0 indicated no lesions and 1, 2 and 3 indicated about

1-30%, 31-60% and more than 60% of all leaf or fruit cluster tissue, respectively. These scale values were converted to disease severity values (Xi et al., 1990) using the following formula:

Disease Severity= [∑ (no. of plant in category x category value)] x 100 / Total no. of plants x max. Category value).

RESULTS AND DISCUSSION

A total of 221 hazelnut orchards were surveyed in Ordu, Giresun and Trabzon provinces in 2008. 70,800 fruit clusters were examined and infection rate was determined as 18.15%.The most predominant symptom was found as blight. During 2009 year surveys 24,800 fruit clusters were examined. A total of 1603 isolates belonging 17 fungal genera were isolated from diseased fruit clusters in 2008 and 2009 years. The incidence of *Pestalotiopsis* sp. was found as 6.28% in Ordu,7.33% in Giresun and 7.41% in Trabzon, total of 112 Pestalotiopsis isolates. The agent was isolated from symptoms of cluster blight and fruit necrosis with highest rates: 27.68% of total was from blight clusters in late stage of fruit development, 9.82% from blight clusters in early stage of fruit development and 23.21% from fruit necrosis in early stage of fruit development. Isolation rate from fruit cluster spots or lesions was 16.96%.

Pestalotiopsis isolates were divided into two groups based on colony characteristics on PDA. In the first group, the colony color is white, sometimes very light pink. Aerial mycelia were dense, conidia masses seen as ink drop usually were in acervuli close to center. In the second group, aerial mycelium was less compact, more effuse (Figure 2). Conidia were spread all over the colony, so colony color was seen markedly black. The two groups were similar in terms of conidia morphology. Conidia were 22.9 x 6.4 µm, 5 celled with three central cells brown, first two darker than the other. Apical and basal cells were hyaline, the apical one with 2 or 3 appendages (7.0–21.0µm long) and the basal one with a single 2.0–8.0µm long appendage (Figure 3). Based on these morphological characteristics, the fungus genus was identified as *Pestalotiopsis* (Sutton, 1980).

For species identification two representative isolates, one of each group, were sent to CABI, UK. According to sequencing the internal transcribed spacer (ITS) of the rDNA, two isolates were in the same species different from *P. quepinii* reported from hazelnut before. However species identification could not be made. It was not surprizing, as Maharachchikumbura et al. (2011) explained recently that *Pestalotiopsis* was taxonomically poorly understood both at the inter- as well as the intra specific level. It was not clear whether Pestalotia was really distinct from Pestalotiopsis, since strains of the type of the former had not been sequenced. Nomenclature of the genus was confusing and most host based names in data bases might be synonyms. Molecular data had still not been success fully applied for species-level differentiation and names applied to data in GenBank are doubtful, as they were not linked to any type materials.

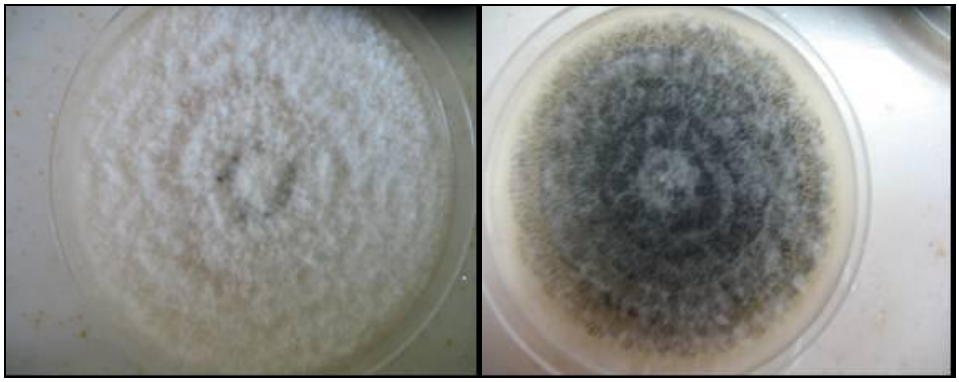

Figure 2: Figure 2.10-day old colonies of *Pestalotiopsis* sp. isolates on PDA (a, from firstgroup; b, from second group)

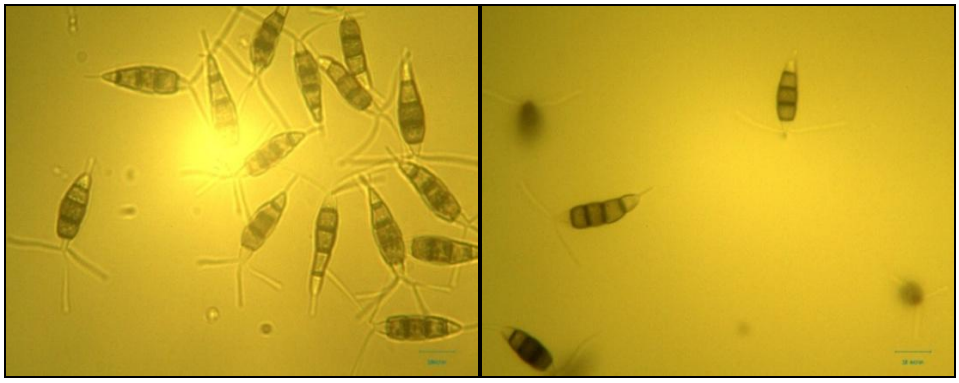

Figure 3: Conidia of *Pestalotiopsis* sp. isolates(x400), (a, from first group; b, from second group)

For pathogenicity test on detached leaves, 6 Pestalotiopsis sp. isolates were used on Tombul and Palaz cultivars. Isolates caused blight on leaves and disease severity values were determined 37.5%-100.00%.Two of these isolates, one caused 37.5% other one caused 100% disease severity were used for pathogenicity test on detached twigs and clusters. Both caused 66.67 % disease severity on clusters.The pathogen was reisolated from diseased plant parts.

Previously, Yürüt et al. (1994) and Karaca and Erper (2001) have determined Pestalotiopsis guepinii from hazelnut twigs, Akça and Tuncer (2005) reported that Pestalotiopsis sp. isolated from some hazelnut fruits with necrosis in Turkey. Pestalotiopsis macrospora was identified as a pathogen on leaves and stems of hazelnut in Iran (Mirhosseini-Moghaddam and Taherzadeh, 2007).The current study was the detailed survey on hazelnut fruit cluster diseases in Turkey. The incidence of Pestalotiopsis sp. was found as 6.28% in Ordu,7.33% in Giresun and 7.41% in Trabzon. Pestalotiopsis sp. caused the high and moderate disease severity on leaf and fruit clusters in pathogenicity tests.

CONCLUSIONS

Species of P*estalotiopsis* are common in tropical and temperate ecosystems in the world. There are some reports in Turkey that it caused disease on hazelnut twigs and fruits. In present study the *Pestalotiopsis* isolates obtained from hazelnut fruit and fruit clusters causing the yield reduction directly. Due to the economic importance of hazelnut, it is required further study of *Pestalotiopsis* sp.

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