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Full Length Article

Potential of *Ampelomyces* as a Biological Control Agent against Powdery Mildew in Hazelnut Orchards

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Abstract

This study was carried out during 2016–2017 for determining the natural occurrence of *Ampelomyce*s and observing its potential as biocontrol agent against powdery mildew in the Western Black Sea and Marmara Region. The infected hazelnut leaves taken from the surveyed orchards were evaluated under the microscope for presence of powdery mildew and *Ampelomyces*. In addition, the effect of *Ampelomyces* on the formation of chasmothecia of powdery mildews and the parasitized chasmothecia were also determined. As a result of the microscopic examination, the pathogen of powdery mildew found in the leaves taken from the hazelnut orchard was *Erysiphe corylacearum*. As a result of these studies, it was found that the infection frequency of *Ampelomyces* ranged from 0 to 100% on the hazelnut leaves with powdery mildew. Similarly, the infection of *Ampelomyces* ranged from 0 to 87.5%. The mean chasmothecia numbers in the leaves with powdery mildew were 46 and 435 in 2016, 40.45 and 344 in 2017, respectively, with and without *Ampelomyces*. At the same time, it was determined that 40 and 23.33% of the chasmothecia in 2016 and 2017, respectively, selected from the samples of leaves with powdery mildew containing hyperparasite was parasitized with *Ampelomyces*. Future research should be aimed at determining the isolates of *Ampelomyces*, their biology and the possibilities of using these against powdery mildew. © 2018 Friends Science Publishers

Keywords: *Ampelomyces*; Biological control; Powdery mildew, Pathogen

Introduction

Turkey possesses approximately 70% of hazelnut production in the world. Cultivation of hazelnut in Turkey is widespread mainly in the Eastern Black sea, Western Black Sea and Marmara Regions (Tanrıvermiş *et al*., 2006). *Phyllactinia guttata* (Wallr.: Fr.) Lev. and *Erysiphe corylacearum* are important fungal pathogens causing powdery mildew in the hazelnut orchards (Sezer *et al*., 2017).

Chemical fungicides are the most commonly used method in the control of powdery mildew (Zhao *et al*., 2012). However, due to the negative effects of fungicides on the natural balance, there is an increasing interest in the use of biological control agents in recent years. Hyperparasites or antibiotic producers are generally known as the biological control agents of powdery mildew (Belanger *et al*., 1997). Hyperparasitism is one of the important mechanisms of biological control against plant diseases.The most common hyperparasite of powdery mildew is *Ampelomyces* (Sztejnberg *et al*., 1989; Jeffries, 1995). *Ampelomyces* that is anaturally occurring hyperparasite of powdery mildews. *Ampelomyces* affects mycelium, conidial spores and ascocarps of powdery mildews (Kiss *et al*., 2004). Until now, large number of *Ampelomyces* isolates has been isolated from *Erysiphaceae* species and are common in nature. The obtained isolates are successfully used in many agricultural products (Falk *et al*., 1995; Kiss, 2003; Kiss *et al*., 2004; Zhao *et al*., 2012; Junaid *et al*., 2013).

In the recent years, a new powdery mildew disease has become widespread in hazelnut orchards in Turkey. This disease, which is devastating, leads to significant crop losses. The causative agent of powdery mildew disease has been identified as *E. corylacearum* (Lucas *et al*., 2017).

Determining the natural occurrence of *Ampelomyces* is important for assessing its potential as a biological control agent against powdery mildews. In order to be successful in the biological control against pathogens, it is important to determine the locally adaptive hyperparasites and determine their natural prevalence. In this case, it is important to determine the natural prevalence of biological control agents in hazelnut orchards. For successful natural occurrence of *Ampelomyces*, both favorable conditions (e.g., high humidity or moisture, temperature around 25°C) and the presence of the host are required. It is believed that, there are favorable conditions at the Western Black Sea and Marmara Region for the successful natural occurrence of *Ampelomyces*.

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Until now very little is known about the natural occurrence of *Ampelomyces* in hazelnut orchards in Turkey. The main objectives of this study were to determine the occurrence of *Ampelomyces* in powdery mildew fungi infecting hazelnut leaves, and to determine the percentage infection and infection frequency in powdery mildew for potential as a biological control agent against powdery mildews. The study was also aimed to determine number of chasmothecia on infected leaves by *Ampelomyces* and the percentage of parasitized chasmothecia.

Materials and Methods

Determination of Natural Occurrence of Ampelomyces in Hazelnut Orchards

This study was conducted on hazelnut orchards in Western Black Sea and Marmara Regions during vegetative seasons of 2016 and 2017. Sampling was done in July–August, when powdery mildew was most common in hazelnut gardens.Each examined hazelnut orchard is considered as an example. In each garden, 10 pieces of hazelnut leaf covered with powdery mildew from 60 to 100% were randomly collected. The leaves that had powdery mildew were examined under the microscope. Afterwards, we determined the presence or absence of brownish intracellular pycnidia and hyphal fragments of *Ampelomyces* on hyphae, conidiophores and conidia of the powdery mildew agent. *Ampelomyces* spp. was identified according to Belsare *et al.* (1980), Kiss (1998).

To evaluate the natural occurrence of *Ampelomyces* in powdery mildew fungi in hazelnut orchards, the percentage infection and percentage infection frequency of *Ampelomyces* in the powdery mildew were determined. In the evaluation of percentage infection of *Ampelomyces*, it was determined how much of the area covered by the powdery mildew on the hazelnut leaves is parasitized by *Ampelomyces*. For the same, 0–4 scale has been used (0 – lack of infestation; 1) up to 10% infestation; 2) 11–25% infestation; 3) 26–50% infestation; 4) 51–100% infestation).

Percentage infection of *Ampelomyces* was calculated according to Mc Kinney's formula (Dynowska, 1994; Sucharzewska *et al*., 2011).

$$R\ Ampelomyces = \frac{\sum(c \times d) \times 100\%}{N \times 4}$$

Where: R *Ampelomyces* – an index of powdery mildew mycelium infestation by *Ampelomyces* expressed in percent;

$\Sigma\ (c \times d) \times 100\%$

(c) the sum of products obtained by multiplying the number of collected hazelnut leaves infested with powdery mildew (d) by a given degree of infestation

N–the total number of hazelnut leaves infested powdery mildew

4–the highest degree of infestation in the five-degree scale.

Determination of Chasmothecia Numbers Formed on Powdery Mildew with Ampelomyces

The effect of *Ampelomyces* on the formation of chasmothecium was determined. Evaluation was done on the 20 pieces of hazelnut leaves with *Ampelomyces*. In each leaf, chasmothecia on a one cm^2 areaof powdery mildew colony (that was infected with *Ampelomyces*) were counted. Chasmothecia were also counted using the same method in the hazelnut leaves which were not infected with *Ampelomyces* (Sucharzewska *et al*., 2012). Counts were made under a stereomicroscope.

Determination of İnfestation of Chasmothecia with Ampelomyces

Total of 30 chasmothecia were randomly selected in the powdery mildew leaf samples which were infected with *Ampelomyces*. Chasmothecia were gently crushed by pressing the cover slip over the glass slide on which the sample was spread to allow the release of *Ampelomyces* conidia and pycnidia (Falk *et al*., 1995; Angeli *et al*., 2009; Younes *et al*., 2016). The incidence of *Ampelomyces* in each powdery mildew species was calculated as the percentage of powdery mildew species chasmothecia parasitized by *Ampelomyces*.

Data Analysis

The prevalence of *Ampelomyces* was determined as the ratio of the number of hazelnut orchards with parasitism to the total number of hazelnut orchards sampled. To determine the percentage infections frequency of *Ampelomyces* in each hazelnut orchards, the number of parasitic leaves is proportional to the total number of leaves sampled in the orchard. Percentage infection of *Ampelomyces* was calculated according to Mc Kinney's formula. Chasmothecia numbers are evaluated in graph form in MS Excel. Rate of contaminated chasmothecia are shown graphically using MS Excel.

Results

Sampling was done in total 72 hazelnut orchards in the West Black Sea and Marmara Regionin 2016 and 2017. As a result of studies made under microscope, the powdery mildew pathogen found in hazelnut orchards was determined as *E. corylacearum.*

Brownish pycnidia were observed under the microscope on the powdery mildew colonies on hazelnut leaves. These pycnidia were determined in the powdery mildew mycelia.The pycnidia were pale, golden, and brown with different shapes, and the mycoparasite conidia were hyaline and smooth with round, straight or slightly curved

ends. In the measurements made, it was determined that the length and width of the conidia are between 12 and 14 μm and between 2.5 and 3.5 μm, respectively. As a result of the measurements,it was determined that this hyperparasite on the powdery mildew agent is *Ampelomyces*.

According to results of sampling, *Ampelomyces* was detected in 36 of 47 hazelnut orchards in 2016 and 21 of 25 hazelnut orchards in 2017. Prevalence rate of *Ampelomyces* has been determined as 76.59 and 84% in the region during 2016 and 2017, respectively (Table 1).

The mean infection frequency of *Ampelomyces* were determined as 33.2% in 2016 and 44.4% in 2017 (Table 2). When *Ampelomyces* was evaluated in terms of infection frequency, it was found 100% in a orchard in 2016. The mean percentage infection of *Ampelomyces* were determined as 20.3% in 2016 and 27.2% in 2017 (Table 2). The highest infection rate of *Ampelomyces*, which was hyperparasitic on the leaves of hazelnut with powdery mildew, was recorded as 87.5% in 2016 and 85% in 2017.

Avarage number of chasmothecia on leaves that were infected and not infected with *Ampelomyces* were counted. The mean chasmothecia numbers on the leaves with powdery mildew that were non-infected with *Ampelomyces* were counted as 435 and 344 units in 2016 and 2017, respectively. The mean chasmothecia numbers on the leaves with powdery mildew that were infected with *Ampelomyces* were counted as 46 and 40.45 units in 2016 and 2017, respectively.

As a result of the investigation, it was determined that 40 and 23.33% of the chasmothecia were contaminated with *Ampelomyces* in 2016 and 2017, respectively.

According to climate data, it was seen that weather conditions are suitable from May to end of August for the natural spread of *Ampelomyces* (Table 3). In the Western Black Sea and Marmara Region, the average temperatures during the months of May, June, July and August were 16.2°C, 22.1°C, 23.3°C, 24.2°C in 2016, and 16.6°C, 21.6°C, 23.7°C, 23.8°C in 2017 (Table 3). Total rainfall for the same months was 139.9 mm, 65.6 mm, 19.2 mm, 60.5 mm for the year 2016, and 60.8 mm, 113.5 mm, 28.2 mm ve 53.2 mm for the year 2017.

Discussion

There are about 40 biological control agent against the powdery mildew (Kiss, 2003). These have mechanisms of action such as mycoparasitism and antibiosis. Hyperparasitism is one of the important mechanisms of biological control against plant diseases (Jeffries, 1995). *Ampelomyces* is the best-known hyperparasite of powdery mildew (Kiss, 2003). Hyphae of *Ampelomyces* enters hyphae of powdery mildew and continues to develop into hyphae. *Ampelomyces* produces pycnidia in conidiophores, immature chasmothecia and in hyphae of fungal host (Falk *et al*., 1995; Kiss, 1997; Kiss, 2003; Angeli *et al*., 2009).

Previous research work reported that *E. corylacearum* was the causative agent of powdery mildew since 2013 in hazelnut orchards located in the Black Sea and Marmara Regions of Turkey (Sezer *et al*., 2017). The same has been observed in our research work.

Ampelomyces can be found on more than 64 powdery mildews that infect 256 plant species (Kiss, 1998; Kiss, 2003; Kiss *et al*., 2004; Younes, 2009). There are two types of powdery mildew on hazelnut; (*Phyllactinia guttata* (Wallr.: Fr.) Lev.), and *E. corylacearum*. In the latest studies, the presence of *Ampelomyces* has been determined on the *Phyllactinia guttata* on hazelnut (Szentivanyi and Kiss, 2003). As far as we know, the presence of *Ampelomyces* on *E. corylacearum* in hazelnut has not been determined. Our study is the first in this subject.

Ampelomyces isolates are divided into two groups according to the rate of growth in culture; rapidly growing (3–4 mm in diameter per day at room temperature) and slower ones (Kiss and Vajna, 1995; Kiss, 1997; Kiss and Nakasone, 1998; Kiss *et al*., 2004). Real *Ampelomyces* isolates usually develop slowly in culture and produce intracellular pycnidia in powdery mildew scales (Kiss *et al*., 2004). *Ampelomyces* conidia of the different strains also varied in size. They ranged from 5.5 to 14.5 μm in length and from 2.3 to 3.5 μm in width (Angeli *et al*., 2011). These results support the data that have been obtained in our work.

Ampelomyces parasitizes different powdery mildew at different rates.In previous studies infection rates range from 0 to 65% (Kiss, 1998). The highest infection rate in our study was 87.5 and 85% in 2016 and 2017, respectively.

Chasmothecia of powdery mildew are important for overwintering. *Ampelomyces* suppress the formation of chasmothecia on powdery mildew colony. The formation of chasmothecia on the powdery mildew colony with infected *Ampelomyces* is considerably reduced (Czernaiwska, 2001). In our study,we found that there were quite a few number of chasmothecia in the powdery mildew colonies that were contaminated with *Ampelomyces*. This situation will cause significant decrease in the inoculum source for the next year.

The evolution of fungicide resistance in powdery mildews, and the envrionmental damages caused by fungicides have increased the interest of scientist in biological control agents (Kiss *et al*., 2004). Having a wide range of hosts and compatibility with fungicides makes *Ampelomyces* the suitable candidate for use as a biological control agent (Sztejnberg *et al*., 1989; Falk *et al*., 1995). Efficacy of *Ampelomyces* in the control of the powdery mildew is highly variable. Relative humidity and temperature are important environmental conditions in their activity and spread of *Ampelomyces* (Szentivanyi and Kiss, 2003; Kiss *et al*., 2004). *Ampelomyces* infection requires free water and warm temperature (20–30°C).

Table 1: The number of samples examined and the mean of prevalence of *Ampelomyces*

	The total number of orchard examined	Number of orchard infected with *Ampelomyces*	Prevalence rate of *Ampelomyces* (%)
2016	47	36	76.59
2017	25	21	84

Table 2: Infection frequency and percentage infection of *Ampelomyces* on powdery mildew on hazelnut leaves

2016						2017		
Orchard number	Infection frequency (%)	Percentage infection (%)	Orchard number	Infection frequency (%)	Percentage infection (%)	Orchard number	Infection frequency (%)	Percentage infection (%)
1	90	87.5	26	40	10	1	60	30
2	40	12.5	27	60	35	2	90	85
3	20	5	28	70	37.5	3	60	40
4	90	65	29	10	7.5	4	70	35
5	70	47.5	30	60	15	5	70	60
6	100	72.5	31	10	2.5	6	80	57.5
7	90	57.5	32	20	5	7	60	25
8	50	25	33	30	20	8	30	10
9	10	12.5	34	0	0	9	80	52.5
10	0	0	35	30	10	10	50	30
11	0	0	36	0	0	11	0	0
12	40	10	37	50	42.5	12	40	27.5
13	50	35	38	10	2.5	13	40	27.5
14	50	35	39	30	15	14	30	12.5
15	10	7.5	40	40	22.5	15	40	30
16	60	17.5	41	40	27.5	16	0	0
17	10	2.5	42	0	0	17	70	32.5
18	20	5	43	80	65	18	40	17.5
19	30	20	44	90	87.5	19	30	22.5
20	0	0	45	0	0	20	50	20
21	20	7.5	46	10	2.5	21	20	17.5
22	0	0	47	0	0	22	0	0
23	30	22.5				23	0	0
24	0	0				24	40	20
25	0	0				25	60	27.5
	2016					2017		
	Mean of infection frequency (%)±SE		Mean of percentage infection (%)±SE			Mean of infection frequency (%)±SE	Mean of percentage infection (%)±SE	
	33.2±4.43		20.3±3.53			44.4±5.29	27.2±4.09	

SE:Standard Error

Table 3: Climate data of Western Black Sea and Marmara Region 2016 and 2017

Months	Average temperature (°C)		Relative humidity (%)		Total rainfall (mm)	
	2016	2017	2016	2017	2016	2017
March	10.2	9.4	70	72.8	66.2	50
April	14.6	11.6	67.9	69.2	47.8	81.6
May	16.2	16.6	77.8	75	139.9	60.8
June	22.1	21.6	70.5	75.4	65.6	113.5
July	23.3	23.7	72.1	72.4	19.2	28.2
August	24.2	23.8	75.1	74.6	60.5	53.2
September	19.1	20.9	73.5	72.6	65.8	24.2

Under optimal conditions, infcetion can ocur in less than 24 h. For this reason, successful results were obtained in greenhouse where temperature and relative humidity could be controlled and some vegetable cultivation. The Western Black Sea and Marmara Region where the study is conducted has moist, rainy and temperate climate. The climate conditions in the Western Black Sea and Marmara Region are suitable for the development of *Ampelomyces*. Hence, high rate of infection, infection frequency and prevalence of *Ampelomyces* were determined in hazelnut orchards in the region. Although *Ampelomyces* can develop as saprophytic in nature, it cannot survive for a long time unless it has a host. Therefore, manual application of *Ampelomyces* may be required during the season in order to raise its population for the effective control of powdery mildew (Kiss *et al*., 2004). The study has shown the natural occurrence of *Ampelomyces* in hazelnut orchardsin the Western Black Sea and Marmara Region. This *Ampelomyces* can possibly control powdery mildews.

Conclusion

Further, our research work concluded that climatic conditions in the regions are favorable for the *Ampelomyces*; these are warm temperatues, high relative humidity and high rainfall. The occurence of *Ampelomyces* can be developed into a potenail biocontrol means against powdery mildews. This may require application of *Ampelomyces* a few times during the growing season.

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