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ORIGINAL RESEARCH PAPER

# Fungi inhabiting the aboveground organs of sea buckthorn (*Hippophae rhamnoides* L.) in organic farming

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## Abstract

Sea buckthorn is becoming an increasingly popular medicinal plant. This plant material contains many nutrients and bioactive substances used in the food, cosmetic, and pharmaceutical industries. Compounds found in the organs of this plant have antiviral, antibacterial, and antifungal properties, but, despite these features, sea buckthorn is affected by many pathogens. As shown by studies on the health and presence of pathogenic fungi on the aboveground organs of this plant, no obligatory pathogens and Basidiomycota fungi were found in 2019. The mycological analysis of the aboveground organs, i.e. leaves, fruits, and shoots, showed that *Alternaria alternata* was the most frequent species of fungi isolated from all examined organs. Moreover, the *Monilinia fructigena* and *Botrytis cinerea* species were often isolated from fruits and *Cladosporium cladosporioides* fungus was found on leaves. Numerous isolates of the pathogenic *Fusarium sporotrichiodes* fungus and single isolates of *Phomopsis* spp., i.e. fungi causing gangrene and drying out of the bark of shoots and the bark of many fruit plant species, and *Nigrospora oryzae* causing leaf blotch of herbaceous plants and fruit trees were obtained from the examined organs of the sea buckthorn. The mycological analysis revealed no species of the genus *Verticillium*, universally recognized as the most dangerous to this plant.

## Keywords

sea buckthorn; fungi; diseases

## 1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.), belonging to the Elaeagnaceae family, is one of the oldest medicinal plants. Its properties were already known in ancient Greece, where the leaves and young shoots of this plant fed to horses accelerated their growth and increased their weight; additionally, their coat became shinier (Gut et al., 2008; Řezníček & Plšek, 2008). The sea-buckthorn was mentioned by Theophras of Ereos in the 3rd century BC and in the 1st century AD by Pedanios Dioscorides, the author of the herbalism textbook "De Materia Medica" used until the 17th century AD. The mention of sea buckthorn is also found in the Treaty of Tibetan Medicine entitled "The Four Tantras of Medicine" from the 8th century BC. At that time, sea buckthorn was recommended for people with circulatory problems, blood clots, chronic diarrhea, and cough (Gut et al., 2008; Krejcarová et al., 2015). In addition, the health-promoting properties of sea buckthorn berries were used in the folk medicine of our eastern neighbors, i.e. in Russia and the Far East (Turek et al., 2016). The Mongolian army of Genghis Khan used an oil extract called "blood from the emperor's heart" to heal wounds and as a sedative (Turek et al., 2016).

Sea buckthorn occurs in Europe and Asia; in Poland, it grows at the Baltic Sea in the north and in the Pieniny Mountains in the south. This plant is the only representative of the Elaeagnaceae family in Poland (Seneta & Dolatowski, 2009).

The cultivation of sea buckthorn is widespread in many countries in Europe, Asia, and also in North America (Drevinska & Moročko-Bičevska, 2022; Ivanišová et al., 2020; Kennedy, 1987). Poland's climatic and soil conditions meet all the requirements for the cultivation of this plant, also in ecological cultivation. Due to the multidirectional action of biologically active substances present in the sea buckthorn (in its leaves and fruits), among others, antioxidant, immunomodulatory, or antibacterial properties, and the widespread belief that both the plant and its fruits are resistant to infection by various pathogens, the research on sea buckthorn pathogens has so far been quite limited. The research on sea buckthorn agrophages has also been limited to date because the plant was considered as free from fungal diseases (Khovalyg et al., 2017). However, both recent literature reports (Drevinska & Moročko-Bičevska, 2022; Khovalyg et al., 2017) and our field observations carried out on plantations of this plant indicate that it is infected by numerous infectious agents, including fungi. Many fungal species belonging to the genera *Verticillium*, *Fusarium*, *Alternaria*, and *Botrytis* (Cotuna et al., 2014; Drevinska & Moročko-Bičevska, 2022; Li & Beveridge, 2003). Considering the above, the research signaled in the title of this work was undertaken. The research aimed to determine the health of sea buckthorn shrubs (Li, 2002) in organic cultivation and to identify fungi inhabiting the aboveground organs of this plant.

## 2. Material and methods

### 2.1. Research area

The field research was carried out on a private 2-ha ecological plantation of sea buckthorn, the *Hippophae rhamnoides* L. thornless variety, located in the village of Kolonia Czerniejów (51.4147°N 22.7333°E), Jabłonna commune, Lublin voivodeship. The research covered sea buckthorn shrubs in the third year of cultivation. The plantation is located on the southeastern slope surrounded by forests and arable fields. The Czerniejówka and Skrzyniczanka rivers flow near the plantation. The plantation was established in 2017 in an area of 2 ha. Rendzinas and sandy loam soil form the susbtrate. The plantation has an organic farm certificate.

### 2.2. Evaluation of plant health

Observations of the health of sea buckthorn shrubs were carried out twice during the growing season in 2019 directly on the production plantation. The research was carried out on 30 randomly selected sea buckthorn shrubs (10 in each of three rows) showing disease symptoms. The disease symptoms were detected on shoots, leaves, and fruits. Organs showing disease symptoms were collected for macro- and microscopic examination in the laboratory. During the observations, attention was paid to the presence of strict pathogens, i.e. powdery mildew and rust. Based on the observation of aetiological signs and mycological analysis as well as the frequency of occurrence, the most common species of fungi inhabiting the aboveground organs of this plant were determined.

### 2.3. Mycological analysis

The mycological analysis of the collected plant material was carried out using artificial cultures, according to Machowicz-Stefaniak and Zalewska (2000). Samples of plant material were placed under running water and rinsed for 20 minutes. The surface of the material prepared in this way was disinfected in a 10% sodium hypochlorite solution for 90 seconds. After this time, the material was washed three times for 3 minutes in sterile distilled water. The decontaminated plant material from individual organs was minced using a scalpel or scissors, and 100 three-millimeter sections of each organ and each plant sample were prepared. The material prepared in this way was placed in Petri dishes on solidified mineral medium. The composition of the

**Figure 1** Drying buds and yellowing leaves of sea buckhorn (photo. E. Zalewska).

mineral medium was 18 g of saccharose, 20 g of agar, 0.7 g of $NH_4NO_3$, 0.3 g of $KH_2PO_4$ and $MgSO_4 \times 7H_2O$, and trace amounts of $FeCl_3 \times 6H_2O$, $ZnSO_4 \times 7H_2O$, $CuSO_4 \times 7H_2O$, and $MnSO_4 \times 5H_2O$ in 1 L of distilled water autoclaved at 121 °C for 15 minutes. (Strzelczyk, 1968). Ten inocula were placed in each dish. The dishes lined with plant material were kept in a thermostat for 7 days at 22°C and protected from light. The fungal colonies that grew after this time were cleaved on slants prepared from PDA medium (Difco finished product). After bringing to pure cultures, the fungal isolates were determined with the method of multiple dilutions according to Raiłło (1950) on the medium used for cultivation or on standard media. The fungi of the genus *Fusarium* were determined on PDA and SNA media according to Kwaśna et al. (1991) and Nelson et al. (1983). Other species of fungi were identified as in the studies conducted by De Vries (1952), Ellis (1971), Gilman (1957), Seaver (1961), and Sutton (1980).

## 3. Results

### 3.1. Evaluation of plant health

The observations of the health of sea buckthorn shrubs in the first period, i.e. in the summer of 2019, showed the presence of shoots with disease symptoms in the form of wilting and yellowing of leaves as well as drying buds and entire drying shoots, which, depending on the shrub, constituted from 2 to 100% (Figure 1). Moreover, the death of single whole shrubs was found. During the observation of the health of sea-buckthorn berries in the second period of the study, single mummified fruits with sporodochia of the fungus *M. fructigena* on their surface were found (Figure 2). The presence of the pathogen was confirmed by the mycological analysis. In addition to yellowing, single necrotic spots with a brown border were found on the leaves, and numerous conidial spores of *A. alternata* and *Nigrospora oryzae* were detected in the microscopic specimen made of these leaves. In addition, there were pests from the order *Homoptera*, i.e. *Heteroptera*, which damaged and caused the fruits to dry out. In autumn, single fruits with brown spots of 1 mm in size were also observed, but no aetiological signs of fungi, hyphae, stalks, and conidial spores were found on their surface. During the health observations, no obligatory pathogens, i.e. powdery mildew and rust, were found. Moreover, there were no spores of *Basidiomycota* fungi.

### 3.2. Mycological analysis

The mycological analysis of the aboveground organs of the sea buckthorn carried out in the spring of 2019 provided 524 isolates of fungi belonging to 10 species

**Figure 2** Drying and mummified fruits of sea buckthorn from which *Monilinia fructigena* was isolated (photo. E. Zalewska).

(Table 1). The aboveground parts of the sea buckthorn were commonly inhabited by *Epicoccum nigrum* and *A. alternata*, constituting 27.8% and 22.9% of the total fungal isolates obtained. The frequently isolated species also included *A. radicina* - 11.1% of the total isolates, *C. cladosporioides* - 16.9%, and *B. cinerea* - 13.5% (Table 1). The most frequently isolated species of fungi from individual organs were *C. cladosporioides* - 35.5% of the total isolates obtained from the leaves and *Epicoccum nigrum*, *A. alternata*, and *B. cinerea* constituting 27.7%, 22.9%, and 21.7% of the total isolates, respectively, obtained from the fruits, and *E. nigrum* and *A. alternata* - 41.7% and 28.6% of isolates obtained from the shoots, respectively (Table 1).

In the summer of 2019, the mycological analysis of the examined aboveground organs revealed the presence of 542 fungal isolates belonging to 13 species (Table 1). The most frequently isolated species of fungi were *A. alternata*, *M. fructigena*, and *B. cinerea*. The isolates of these fungi constituted 68.8%, 13.5%, and 7.6% of the total number of all isolates, respectively (Table 1). *A. alternata* was most often isolated from the leaves – 87.2%, and *A. alternata* and *M. fructigena*, accounting for 42% and 38.4%, respectively, were most frequently isolated from the fruits. The species *A. alternata* was also the most frequently isolated fungus from the sea buckthorn shoots, as the isolates of this species constituted 78% of the total number of isolates. It is also worth noting that there were several isolates of *Phomopsis* spp., which are known to cause gangrene in many fruit and ornamental plants.

## 4. Discussion

The health studies of the sea buckthorn shrubs in organic cultivation showed various disease symptoms on all examined aboveground parts of this plant. Based on the macroscopic and microscopic observations, *A. alternata* and *A. radicina* were considered hazardous species of fungi for sea buckthorn, as they colonized the examined organs at all research dates, which was confirmed by the mycological analysis. Fungi of the genus *Alternaria* belong to saprophytic species occurring in all regions of the world. They are allergenic species and, in the case of plant infections, dangerous pathogens causing blotch-type disease symptoms. Moreover, they are dangerous species of toxic fungi, and consumption of infected food may cause disease symptoms in humans and animals (Chełkowski, 2009; Machowicz-Stefaniak & Zalewska, 2000). Equally dangerous are the fungi of the genus *Fusarium*, i.e. *F. graminearum* and *F. sporotrichioides*. They are dangerous pathogens of various species of cereals causing dry rot, especially in young specimens of these plants (Kiecana et al., 2011).

**Table 1** Fungi isolated from aboveground organs of sea buckthorn growing on an organic farm in 2019.

| Species/genus of fungi | Number of isolates | | | | | | Sum of isolates in each period of isolation; number (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | I | | | II | | | | | |
| | Leaves | Fruits | Stems | Leaves | Fruits | Stems | I | II | Total fungi; Number (%) |
| *Alternaria alternata* (Fr.) Keissl. | 32 | 38 | 50 | 163 | 78 | 132 | 120 (22.9) | 373 (68.8) | 493 (46.3) |
| *Alternaria radicina* Meier, Drechsler et E.D. Eddy | 34 | 24 | - | - | - | - | 58 (11.1) | - | 58 (5.4) |
| *Aureobasidium pullulans* (de Bary et Löwenthal) G. Arnaud | - | - | - | - | 11 | - | - | 11 (2.0) | 11 (1.0) |
| *Botrytis cinerea* Pers. | 14 | 36 | 21 | 23 | 14 | 4 | 71 (13.5) | 41 (7.6) | 112 (10.5) |
| *Chaetomium globosum* Kunze | - | - | - | - | - | 5 | - | 5 (0.9) | 5 (0.5) |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 65 | 8 | 16 | - | - | - | 89 (16.9) | 1 (0.2) | 90 (8.4) |
| *Colletotrichum gloeosporioides* (Penz.) Penz. Et Sacc. | - | - | - | - | - | 2 | - | 2 (0.4) | 2 (0.2) |
| *Epicoccum nigrum* Link | 27 | 46 | 73 | - | - | 3 | 146 (27.8) | 3 (0.6) | 149 (14.0) |
| *Fusarium graminearum* Schwabe | - | - | - | - | 9 | - | - | 9 (1.7) | 9 (0.8) |
| *Fusarium sporotrichioides* Sherb. | - | 6 | 2 | - | - | 1 | 8 (1.5) | 1 (0.2) | 9 (0.8) |
| *Ilyonectria destructans* (Zinssm.) Rossman, L. Lombard et Crous | 7 | 8 | 5 | - | - | - | 20 (3.8) | - | 20 (1.9) |
| *Monilinia fructigena* (Pers.) Honey | - | - | - | - | 71 | 2 | - | 73 (13.5) | 73 (6.8) |
| *Nigrospora oryzae* (Berk. et Broome) Petch | - | - | - | - | - | 3 | - | 3 (0.6) | 3 (0.3) |
| *Penicillium* spp. | - | - | - | - | - | 2 | - | 2 (0.4) | 2 (0.2) |
| *Phomopsis* spp. | - | - | 2 | 1 | 2 | 15 | 2 (0.4) | 18 (3.3) | 20 (1.9) |
| *Saccharomyces* spp. | 4 | 4 | - | - | - | - | 8 (1.5) | - | 8 (0.8) |
| *Sclerotinia sclerotiorum* (Lib.) de Bary | - | - | 2 | - | - | - | 2 (0.4) | - | 2 (0.2) |
| Total | 183 | 170 | 171 | 187 | 185 | 170 | 524 (100) | 542 (100) | 1066 (100) |

I – first isolation; II – second isolation.

These fungi may have caused the death of young sea buckthorn plants in the studied plantation, which was noticed during the observations.

Similarly, fungi of the genus *Fusarium* caused necrosis of the shoots of various varieties of sea buckthorn cultivated in Finland (Ruan et al., 2010, 2013). Moreover, several pathogenic fungi from the genus *Fusarium*, i.e. *F. sporotrchioides, F. oxysporum*, and *F. acuminatum*, were identified as a cause of dried-shrink disease of sea buckthorn in India and China (Malik, 2016; Xia et al., 2021). In northeast China, *F. sporotrichioides* caused stem wilt of twelve very sensitive Russian and Chinese varieties cultivated in this country and caused high (nearly 70%) mortality of young plants (Xia et al., 2021). *Fusarium* fungi are very strong toxic species contaminating plants with trichothecene compounds from group A, especially T-2 toxin and HT-2 toxin, as well as neosolaniol and diacetyldeoxyscirpenol. These mycotoxins are particularly harmful to warm-blooded organisms, but also have a phytotoxic effect, which can affect

the quality and quantity of plant material (Desjardins, 2006). Moreover, it was very interesting to obtain isolates of *F. graminearum*, which is a species infesting mainly grasses (Bottallico & Perrone, 2002). However, taking into account the source of the research material, i.e. an organic farming plantation located among cultivated fields, the occurrence of this species on fruit trees and shrubs should be taken into account. The presence of isolates of fungi belonging to the genus *Phomopsis* should also be considered dangerous. These fungi cause spots, necrosis, gangrene, and canker in the shoots of many species of ornamental, fruit, and berry plants as well as vegetables and herbs (Bielenin & Meszka, 2009; Khovalyg et al., 2017; Król, 2006; Król et al., 2017; Patkowska, 2012; Szmagara, 2009; Weingartner & Klos, 1975; Zalewska et al., 2013). Moreover, the fungus *Nigrospora oryzae* may play a significant role in causing the death of sea buckthorn leaves. Although this fungus is an endophytic species and filtrates made from its mycelium exhibit antiviral, antibacterial, and antifungal properties, the species is known to be harmful to various crops, as shown by studies on cotton leaf blotch fungi in China and date palm fungi in Iraq (Abass & Najlaa, 2014; Zhang et al., 2012).

The presence of the *M. fructigena* fungus should be considered particularly dangerous to sea buckthorn berries. During the plant health observations, mummified fruits with aetiological signs in the form of sporodochia and conidial spores were noticed directly on the plantation. Moreover, the presence of the pathogen was confirmed by the mycological analysis of the fruits. Fungi of the genus *Monilinia* are dangerous pathogens of the fruits of many species of fruit plants. Mass infestation of fruits can significantly reduce yields, with losses reaching 70 or 100% (Grabowski & Wiech, 2003; Zalewska, 2005). The absence of fungi of the genus *Verticillium* should be considered satisfactory, despite the observation of plant wilting and yellowing of the leaves. The species *V. aboatrum* and *V. dahlie* as well as representatives of the genus *Phytium* are hazardous pathogens of this plant and can cause its death within two years (Cotuna et al., 2014; Drevinska & Moročko-Bičevska, 2022).

During the observations of the health of the sea-buckthorn plants, no pathogens with the obligatory way of parasitism and no *Basidiomycota* fungi creating characteristic, differ in size and shape of fruiting bodies on the shoots of this plant, were found. It appears that the absence of hubs on the branches results from the young age of the plantation, as these fungi occur on older sea buckthorn branches, which have been repeatedly found on plants growing in natural positions in the northern part of Poland on the Baltic Sea (Wilga & Wantoch-Rekowski, 2013). Similarly, powdery mildew caused by *Phyllactinia hippophaës* (*Erysiphales*) on sea buckthorn leaves was found near Potsdam, Germany, in 2009 (Kummer et al., 2010).

## 5. Conclusion

Sea buckthorn has wide applications in many areas of human and animal life. Oil, which is extracted from its seeds and fruits, is an especially valuable and most widely used resource for pharmacy and cosmetic industry. Numerous *M. fructigena* isolates were obtained from mummified sea buckthorn berries. The fungus causes brown rot of fruits, which results in a reduction in the quality and quantity of the crop. One of the most allergenic fungi which colonized all the studied organs of the sea buckthorn was *A. alternata*. The presence of pathogenic fungi *F. sporotrichioides*, *Phomopsis* spp., and *S. sclerotiorum* should be considered alarming. These species are the causes of many dangerous diseases of fruit trees and shrubs. The studied sea buckthorn shrubs were free from fungi characterized by an obligatory way of parasitism. Due to the significant biodiversity of fungi obtained from the studied organs of the sea buckthorn, further studies on the pathogens of this plant are necessary and the initiated research should be continued.

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