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**FIRST REPORT OF THE OCCURRENCE OF *PESTALOTIOPSIS* SP. ON BLUEBERRY IN BULGARIA – MORPHOLOGY AND PATHOGENICITY**  
(SHORT COMMUNICATION)

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**Abstract**

The cultivation of blueberry (*Vaccinium* sp.) in Bulgaria has increased in recent years, but the knowledge of its diseases is almost nonexistent.

During monitoring for the sanitary status of blueberry crops in Southern Bulgaria (Plovdiv region), symptoms of stem cancer, twigs necrotic lesions, leaf spots and low vigour were observed on 3-year-old Duke cv. Four isolates were obtained from damaged stems and leaves and were single-spored. The isolates were recognized as *Pestalotiopsis* sp. according to the cultural characteristics of the fungal colony (colony color and the presence of fruiting bodies) and morphological characteristics of conidia (color, length, width, number and size of the apical and basal appendages). In a controlled experiment, the representative isolate was pathogenic to the Duke cv.

Based on morphological characteristics and pathogenicity, this study is the first report of the *Pestalotiopsis* sp. associated with cancer stem and leaf spots on blueberries in Bulgaria.

**Keywords:** *Pestalotiopsis* sp., blueberry, morphology, pathogenicity

**SHORT COMMUNICATION**

Blueberry (*Vaccinium* sp.) cultivation expanded in Bulgaria in recent years to cover about 70 ha and yield to 110 t in 2021 (FAO, 2021). This fact is determined by the high nutritional and economic values, the naturally occurring antioxidants of blueberry fruits and the favourable conditions for growing. The increasingly cultivated blueberry crops and the introduction of new varieties in the country have resulted in periodic monitoring and adequate control of the blueberry diseases.

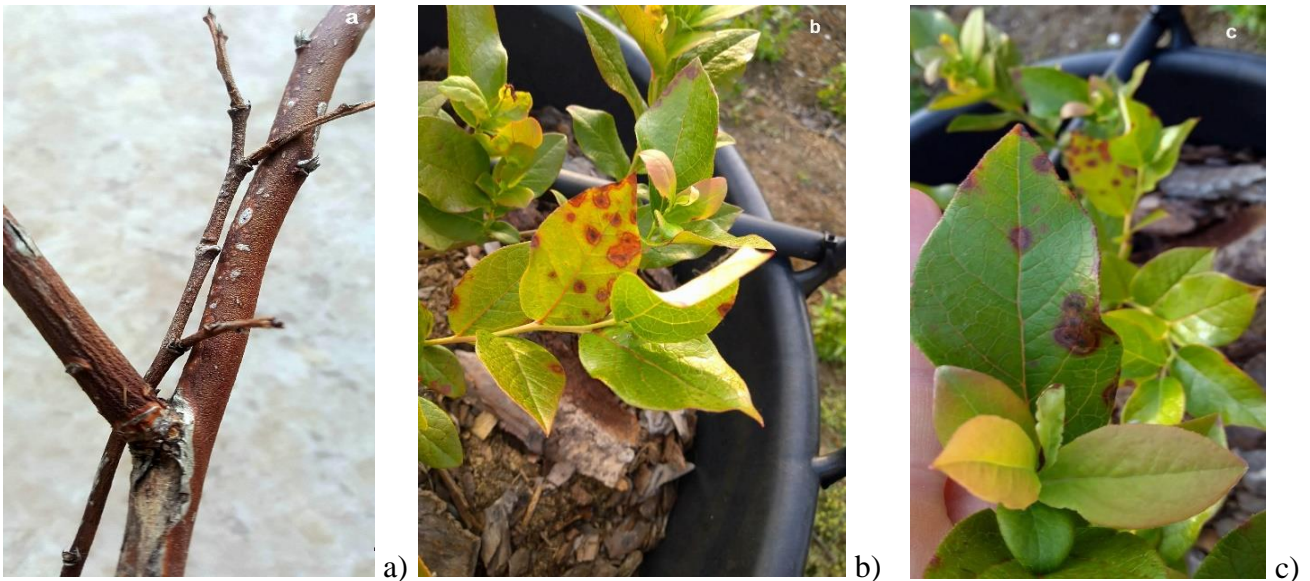
In May 2019, unusual symptoms were observed in 3-year-old of "Duke" blueberry plants near Krichim (Southern Bulgaria, Plovdiv region). Diseased plants (20 to 40%) showed brown stems and twigs lesions, cancer lesions and low vigour. Necrotic vascular damage was observed, too. In July 2020, in addition to the symptoms described above, red-brown to dark brown, small to enlarge, and

circular to irregular leaf spots were observed on the blueberry plants. Black acervular conidiomata were developed on the spots.

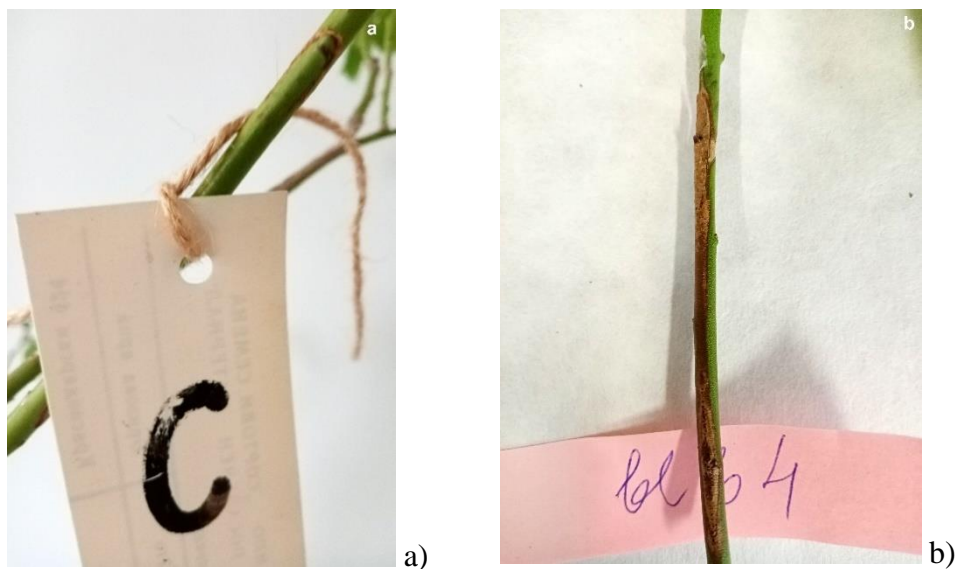
Isolations were made from twigs lesions, stem cancer, brown vascular tissues, and leaf spots on potato dextrose agar (PDA), supplemented with 0.5 g L-1 streptomycin. The isolates were cultured at 23±1 °C for ten days. Four isolates showed *Pestalotiopsis*-like colonies – white cottony mycelia covered with black fruiting bodies (acervuli), filled with black spore masses. The representative isolate (Blb4) was single-spored and cultured on PDA for characterization. The micromorphological observation using Leica DM750, software of Leica ICC50 HD camera, showed typical *Pestalotiopsis* sp. 5-celled conidia with three dark brown middle cells and two pale brown end cells. Conidia were fusiform, strained or slightly curved, 17.19(20.75±1.62SD) 24.20 µm x 6.31 (7.54 ±0.58 SD) 8.83 µm (n=30). Apical conidial cells were conical in shape and hyaline

to pale brown, sometimes bearing one appendage or with two and mainly three, rarely four appendages,  $18.55 (18.55 \pm 2.80SD) 24.11 \mu m$  long ( $n=30$ ). Basal cells had the same shape and color but with a single appendage  $2.86 (4.07 \pm 0.85SD) 6.6 \mu m$  long ( $n=30$ ). Koch's postulates were fulfilled according to Sarrocco et al. (2009). Pathogenicity test was conducted by inoculating mycelial plugs from 10-days old culture of Blb4 isolate into incisions made on

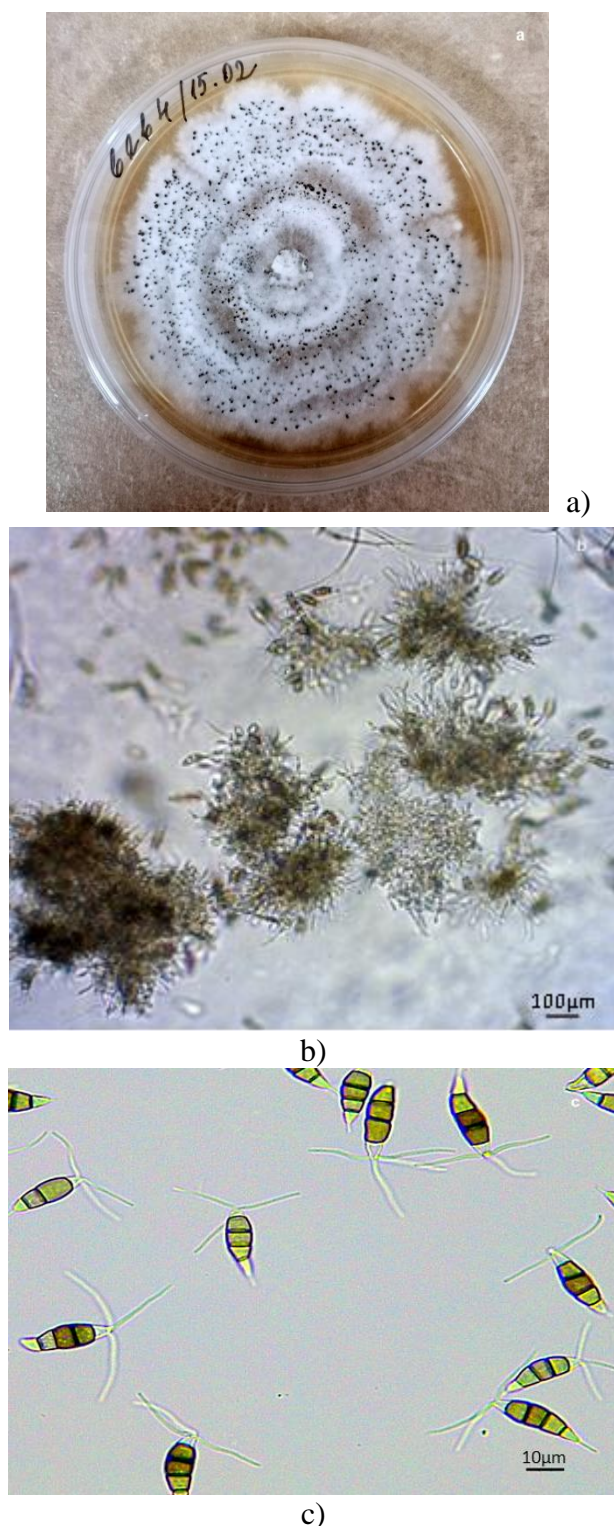
disinfested stems of healthy blueberry plants cv. Duke. Incisions, inoculated with sterile PDA plugs serving as control. The experiment was performed in three replications. After 14 days, necrotic lesions with acervular conidiomata and small cankers were observed. The three control plants remained symptomless. The *Pestalotiopsis* sp. (Blb4 isolate) was reisolated from all inoculated plants except the controls to confirm Koch's postulates.



**Fig.1** Symptoms in blueberry associated with *Pestalotiopsis* sp. Stem cancer (a), leaf spots with acervular conidiomata (b, c).



**Fig. 2.** Pathogenicity test of *Pestalotiopsis* sp. (blb 4 isolate). Control (a) and stem cancer on the inoculated plant (b).



**Fig. 3.** Fungal cottony colony with dark acervular conidiomata (a) and morphology of *Pestalotiopsis* sp. - microphotograph of acervuli fruiting bodies (b), scale bar = 100  $\mu$ m, manure conidia (c), scale bar = 10  $\mu$ m.

The *Pestalotiopsis* genus is common plant pathogens in tropical and temperate areas, associated with stem cancer, twig lesions, and leaf spots. In recent years, isolated *Pestalotiopsis* endophytes are known to riches in a wide range of chemically diverse metabolites (Maharachchikumbura et al., 2011; Maharachchikumbura et al., 2014).

In Bulgaria, Dobрева et al. (2016) reported *Pestalotiopsis* sp. as a pathogen that damages the needles of *Pinus* species and Bencheva & Belyova (2017) reported it as the causative agent of poplar bark necrosis.

Based on the cultural and morphological characteristics and the pathogenicity test the causal agent from symptomatic blueberry plants belongs to *Pestalotiopsis* sp. A molecular assay of the obtained isolates is currently in progress. To our knowledge, this is the first report of *Pestalotiopsis* sp. in blueberries in Bulgaria.

#### ACKNOWLEDGEMENTS

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