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Preliminary report on stem and root rot, a novel disease of cultivated Mursalitza mountain tea (*Sideritis scardica* Griseb.), first observed in Bulgaria

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Abstract

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A new disease of commercially cultivated Mursalitza mountain tea (*Sideritis scardica* Griseb.) was discovered and diagnosed for the first time in Bulgaria. Disease symptoms included yellowing and stunting, stem and root rot, sudden wilt and premature death on predominantly young newly replanted seedlings and less frequently on mature one- and two-year-old plants. Disease incidence increased throughout the season and in 2021 the disease totally caused 35% and 45% crop losses in the two surveyed fields. All isolates obtained from symptomatic plants were identified by microscopic observation of their morphological characteristics as *Fusarium culmorum* (Wm.G.Sm.) Sacc. and *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen. Pathogenicity of all isolates was confirmed on 30-day-old Mursalitza mountain tea seedlings grown in pots with artificially inoculated soils. All inoculated plants showed symptoms identical to those observed on "naturally" infected plants grown in the surveyed field sites. The inoculated pathogens were consistently reisolated from symptomatic tissues. Further efforts are needed to evaluate the full range of biotic and abiotic components involved in the etiology of the stem and root rot which results in early decline of the crop; an effective disease control program should be designed and implemented. Commercial and semi-commercial cultivation is an important means of protection for indigenous populations of Mursalitza mountain tea in the country. Hence, apart from the economic losses; the disease is likely to compromise the conservation of this endangered botanical species and to impede the restoration of its natural habitats in the wild.

Keywords: Greek mountain tea; ironwort; *Fusarium culmorum*; *F. oxysporum*; soilborne pathogens; root rot; stem rot; early dieback

Introduction

(*Sideritis scardica* Griseb.), Lamiaceae (Labiatae), known as Mursalitza tea, and also called Greek mountain tea, Alibotush tea, Mountain tea, Mursal tea, Pirin tea, Sharr Mountain tea, shepherd's tea, etc. is an endemic plant species occurring in the Balkans, including countries of Bulgaria, Greece, former Yugoslavia, and probably Albania (POWO, 2022). In nature, this species is generally found in mountain areas at altitudes between 1000 and 2200 m. In Bulgaria, Mursalitza mountain tea is being grown outdoor commercially on larger or smaller scales at relatively lower altitudes (Evstatieva and Alipieva, 2012), as well as domestically in kitchen gardens for home consumption or landscape. Field cultivation is also considered to be an efficient approach to promoting conservation and restoration of natural habitats and protection of indigenous populations of this endangered species in the country.

To the best of our knowledge, no infectious diseases or any other health issues have been reported in the literature for this botanical species till now. In 2020 and 2021, newly planted seedlings and mature, one-year-old plants in two adjacent commercial ~~/replanted~~ fields located near village of Druzhevo (43°13′72.69″ N 23°35′49.09″ E), Svoge municipality, West Balkan Mountains died in large numbers. The plants exhibited yellowing, sudden wilt, stem and root rot, and thus appearing to suffer from damping-off. The cause of the disease was not known; yet it was attributed to infection by soilborne fungal or fungal-like pathogens.

The purpose of this study was to report a newly discovered disease, diagnosed for the first time in cultivated Mursalitza mountain tea plants. The objectives were to (1) describe the symptoms of the diseased plants, (2) isolate and identify the causing agent(s), and (3) determine the pathogenicity of the isolated species.

Materials and Methods

Field surveys, observations and sampling

Surveys were conducted in June and July 2021 in the above mentioned production fields at an altitude of approximately 1000 m, where Mursalitza mountain tea had been continually cultivated for six to eight years. Disease symptoms on intact plants, detached stems and roots were observed and described. Samples of symptomatic plants were collected from each field survey, placed in labeled plastic bags and transferred to the laboratory where the plans were examined for presence of putative pathogens.

Pathogen isolation and identification of suspect pathogens

The isolations of potential pathogens were made from the stems and the roots of diseased Mursalitza tea plants either immediately or within 36 hours after sampling. Following the standard procedures, sections of the respective organs were washed under running water for 60 min. Small pieces (2–3 mm) of necrotic tissue were excised from the edge of the lesions and dipped in 70% ethanol for 60 sec., then rinsed three times with sterile distilled water and blotted dry between two sterile filter papers. The surface sterilized tissue segments were placed on non-selective agar media such as oatmeal (OA), potato dextrose (PDA) or water (WA) agar in Petri plates. The plates were incubated at 25–26°C in the dark for 7 to 10 days. *Fusarium* spp. isolates were readily obtained from all examined plants with disease symptoms. Pure cultures of isolates were grown in 90 mm Petri plates containing PDA medium. After 10 days of cultivation at 25–26°C in the dark and four days of cultivation at room temperature in the daylight, colonies were examined for colour, mycelium growth pattern – scarce, moderate or abundant and examined under a light microscope for identification of *Fusarium* species based on the fungal morphological characteristics (Booth, 1977; Nelson et al., 1983; Leslie & Summerell, 2006).

Pathogenicity test

Pure cultures of all *Fusarium oxysporum* and *F. culmorum* isolates were grown on OA in Petri plates at 25-26°C in the dark for 7 days. When the cultures filled the plates, the content of each plate was thoroughly mixed with 1L of sterilized Alluvial-Meadow soil (Fluvisol) containing 30% sand, 17.5% silt, 52% clay, and 1.25% organic matter; pH 7.95. The inoculated soils were placed in individual pots of 2.5 L. Each pot was planted with one to three 30-day-old Mursalitza tea seedlings, developed from high-quality seeds. In another experiment pots were planted with three Mursalitza mountain tea seeds. After germinating the plants were thinned to one in each pot. In both experiments six replicate pots were used for each isolate. Six pots prepared identically, but without pathogen served as a control. The experiments were carried out twice in two successive years. Development of symptoms on the inoculated Mursalitza tea plants was tracked for a three-month period.

Results

Field surveys and observations

Disease symptoms were observed predominantly on young Mursalitza mountain tea plants, 45 (7–67) days after replanting and less frequently on mature one- and two-year-old plants. Yellowing leaves, stunted and wilted plants in small patches in the field were the first symptoms to occur. Brown necrotic lesions appeared on the stems of the infected plants (Figure 1). Premature death came when stems were girdled; plants dropped on the soil, turned brown in colour and died. Single generally mature plants suddenly dropped and died without any prior symptoms. Uprooted plants revealed poorly developed root system, partly discolorated or entirely rotted away. Disease incidence increased throughout the season and in 2021 the disease totally caused 35 and 45% crop losses in the both surveyed fields.

Pathogen isolation and identification of suspect pathogens

In 2020 and 2021 a total of 28 isolates were obtained from Mursalitza tea plants with root and stem rot symptoms. Cultural, morphological and microscopic characteristics of the fungal isolates revealed the occurrence of two *Fusarium* species: some of the isolates of *Fusarium* spp. formed white

Fig. 1. Necrotic areas on the roots of young Mursalitza mountain tea plants. Stem lesions are hardly visible here

to pale brown spore mass and red to brown pigment where the colony contacts the agar. The macroconidia were long 22.7–52.8 µm and 4.5–8.6 µm wide, with rounded and blunt apical cell. Macroconidia were most 3- to 4-septate, formed in branches of the aerial mycelium, rarely in sporodochia. Microconidia were absent. After three weeks of cultivation chlamydospores were abundant in aerial hyphae and hyphae in the agar, found either singly, in chains or in clumps. The fungus was identified as *F. culmorum* (Wm. G. Sm.) Sacc. based on morphology and sporulation patterns (Booth, 1977; Nelson et al., 1983; Leslie & Summerell, 2006).

Other isolates of *Fusarium* spp. formed white to pale orange spore mass and pale yellow to pink-red pigment where the colony contacts the agar. Microscopic observations revealed macroconidia 28.4–36.6 µm long and 3.9–4.8 µm wide having a distinctive tapered apical cell and foot-shaped basal cell. Macroconidia were most 3- to 4-septate, formed in branches of the aerial mycelium, rarely in sporodochia. The isolates were producing oval or slightly curved microconidia with 1–2 septa and 5.5–9.7 µm × 2.7–4.1 µm in size. After two weeks of cultivation chlamydospores were abundant in aerial hyphae and hyphae in the agar, found either singly, in chains or in clumps. The fungus was identified as *F. oxysporum* Schlecht. emend. Snyder & Hansen based on morphology and sporulation patterns (Booth, 1977; Nelson et al,. 1983; Leslie & Summerell, 2006) (Figure 2). The two pathogens were discovered either individually or in combination in both younger and mature Mursalitza tea plants.

Pathogenicity test

During the course of the pathogenicity tests symptomatic plants were found in each inoculated pot. Initially the inoculated plants were apparently stunted compared to the control plants (Figure 3).

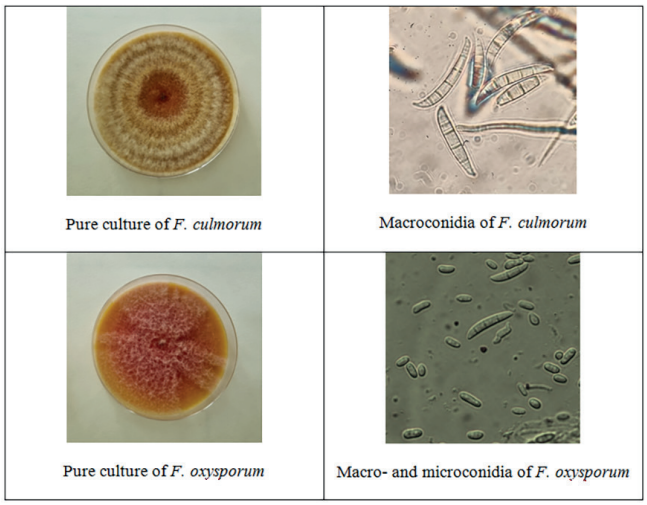

Fig. 2. Morphological characteristics of *Fusarium* pathogens isolated from roots and stems of Mursalitza mountain tea plants

Severe disease symptoms were expressed as brown necrotic lesions on the stems. Necrotic zones developed into extensive stem rot; roots severely decayed. Leaves turned yellow and brown, diseased plants wilted, lied on the ground and died (Figure 4). These symptoms were identical to those observed on "naturally" infected Mursalitza tea plants grown in the field. Fungi morphologically identical to the inoculated pathogens were consistently reisolated from symptomatic roots and stems, thus confirming pathogenicity of all *Fusarium* isolates and completing Koch's postulates. None of the control plants developed symptoms of the disease.

Fig. 3. Inoculated (*left*) and non-inoculated (*right*) Mursalitza mountain tea plants

Fig. 4. Inoculated (*left*) and non-inoculated (*right*) Mursalitza mountain tea plants

Discussion

A new disease causing stem and root rot, sudden wilt and premature death of field cultivated Mursalitza mountain tea was discovered and diagnosed for the first time in Bulgaria. The two identified causal agents of the disease, *F. oxysporum* and *F. culmorum*, are widespread soilborne phytopathogenic fungi that can infect a broad range of herbaceous and woody plant species (Okungbowa & Shittu, 2012; Yanashkov et al., 2016). The two pathogens may coexist in the same soil. It is not possible to differentiate between symptoms incited by each of the causing agents in single infections on Mursalitza tea plants. Yet, it is not known if any synergistic or additive effects occur in the case of mixed infection; neither has it been clear whether any other pathogenic species are involved in the etiology of the stem and root rot and premature death of cultivated Mursalitza mountain tea plants.

Until recently, there has been no reported infectious disease, if any, associated with this plant species. The emergence of stem and root rot disease could be a result from the anthropogenic movement of Mursalitza mountain tea from its natural range to new cultivation area. Growing this plant species outside its natural habitat may have resulted in unfavourable living conditions that allowed disease causing agents to develop on the weakened plants and to the emergence of a new disease complex (Anderson et al., 2004).

Mursalitza mountain tea production is relatively new in Bulgaria. The crop is usually grown without rotational change which may allow selection and build-up of pathogenic populations to levels that might become problematic for farmers. Indeed, according to the latest information available from local producers, a disease with similar symptoms appears to cause premature death or early decline of the crop in other regions of the country where Mursalitza mountain tea has been commercially or semi-commercially cultivated. Thus, apart from the economic losses, the disease is likely to compromise the conservation of Mursalitza mountain tea in the wild.

Conclusion

This study reported the emergence of a new infectious disease of cultivated Mursalitza mountain tea. Disease symptoms included stem and root rot followed by sudden wilt and death of severely damaged plants. Two pathogenic species inciting the disease were identified. *Fusarium culmorum* and *F. oxysporum* were pathogenic on 30-day-old Mursalitza tea seedlings in potted trials. The efforts should continue to evaluate the full range of biotic and abiotic components involved in the etiology of the stem and root rot causing early decline of the crop. Immediate measures should be taken to prevent further spread of the disease in the country and beyond. Immediate eradication measures should be initiated in locations where the disease appears and, if needed in the future, an effective disease control program should be designed and implemented.

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