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DISEASE NOTE

First report of *Xanthomonas euvesicatoria* pv. *euvesicatoria* causing bacterial leaf spot of pepper in Kosovo

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In August 2023, symptoms of bacterial spot were observed on pepper leaves cv. Somborka in two commercial fields located in the villages of Gexhë (42°21'57.9"N 20°32'18.4"E) and Krushë e Madhe (42°19'52.0"N 20°36'08.0"E), municipality of Rahovec, Kosovo. Necrotic lesions of irregular shape, surrounded by a chlorotic halo, were observed on leaves. In both affected fields, disease incidence was rated at approximately 30%. Isolation from symptomatic leaf tissues (6 samples) on yeast–glucose–calcium carbonate agar (YGCA) (EPPO, 2023) produced bright yellow and mucoid colonies, with entire margins that developed after 48–72 h at 28 °C. Single colonies of 12 isolates were further purified on the same medium and identified. An end-point PCR assay was performed on the putative *Xanthomonas* spp. colonies by using the Bs-XeF/Bs-XeR primer set (Koenraadt et al. 2009): the amplified bacterial DNA displayed a 173 bp amplicon fragment, specific for *Xanthomonas euvesicatoria* pv. *euvesicatoria* (*Xee*). The identity of two representative isolates (one for each field) was confirmed by amplification and sequencing of the *16 S rRNA*, *rpoD* and *fyuA* genes (Young et al. 2008). Gene sequences of both isolates showed 99.85–100% identity with *Xee* type strain NCPPB2968^T and the *Xee* strains ICMP 5051 and LMG930). Sequences were deposited in GenBank, under Accession Nos. PP587764 and PP587759 (*16 S rRNA*), PP747254 and PP747255 (*rpoD*), PP751931 and PP751932 (*fyuA*) for DLS2092 and DLS2098 isolates, respectively. Moreover, sequences of *16 S rRNA*, *rpoD* and *fyuA* were used to construct phylogenetic trees by means of the Maximum-likelihood method with 1,000 bootstrap replicates using MEGA 11 software. The results revealed that both representative isolates clustered with the type strain *Xee* NCPPB2968^T and the strain *Xee* LMG930 (Supplementary Fig. 1).

Koch's postulates were fulfilled using eight-weeks-old pepper plants cv. Somborka, sprayed with a bacterial suspension (5×10^7 CFU/ml) of the identified isolates and *Xee* NCPPB2968^T as the positive control. Inoculated and control plants (sprayed with sterile distilled water) were kept at 27 ± 1 °C and 90% relative humidity with a 16 h photoperiod. After 21 days, inoculated plants showed typical leaf spot symptoms, similar to those observed in the fields. No symptoms developed on control plants. *Xee* isolates were consistently reisolated from the inoculated pepper plants and identified by using a PCR assay (Koenraadt et al. 2009). The assay was repeated twice with the same results. To our knowledge, this is the first report of bacterial leaf spot on pepper caused by *Xee* in Kosovo.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42161-024-01797-8.

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Data availability The partial sequences of the *16S rRNA*, *rpoD* and *fyuA* genes generated in this study can be found in NCBI-GenBank under accession Nos. PP587764, PP587759, PP747254,PP747255, PP751931 and PP751932.

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Declarations

Ethical approval The authors confirm that there are no ethical issues in publication of the article.

Consent for publication Publication has been approved by all co-authors.

Conflict of interest The authors declare no conflict of interest.

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