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First Report of *Xanthomonas euvesicatoria* Causing Bacterial Spot Disease in Tomato in Blue Nile State, Sudan

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Abstract: Bacterial spot caused by *Xanthom*onas *vesicatoria* is considered to be a major constraint of tomato production all over the world in particular warmer and humid parts.A disease disorder was reported in autumn season 2015within the Blue Nile State in Sudanaffecting all aerial parts of tomato plant. Symptomatic tomato leaves were collected and sent to Plant Pathology Laboratory, Faculty of Agriculture, University of Khartoum. An investigation on whether a phytopathogen was associated with symptoms of bacterial spots damage on tomato was conducted.The pathogen was isolated and characterized morphologically, biochemically and at molecular level. The bacterium wasidentified as *Xanthomonaseuvesicatoria* and confirmed by PCR and sequence analysis of the 16S RNA gene (GenBank Accession Nos MH047248, MH051263 and MG686236).The disease incidence was remarkably high in ALrosserisand Aldamazein which recorded

49.27 and 52.17%, respectively, while the disease severity was 28.28 and 30.32%, respectively. To our knowledge, this is the first report of *Xanthomonaseuvesicatoria* causing bacterial spot disease in tomato in Sudan.

Key words: Xanthomonas, tomato, Bacterial spot, molecular characteristics, Blue Nile

Tomato plants are attacked by many diseases and pests in Sudan. The important diseases of tomato in Sudan include; seedling damping-off, tomato yellow leaves curls (TY LC), powdery mildew, late blight and Fusarium wilt (Juha 2009).

More recently , it has been shown that bacteria belonging to four distinct groups (previously designated A , B , C , and D) cause bacterial spot on tomato and paper (Jones *et al*. 2004); *i.e.* X. *euvesicatoria* group A, X. *vesicatoria* group B, X. *perforans* group C and X. *gardneri* group D. Group A is particularly more aggressive against pepper (Ignjatov *et al*. 2010). No pepper strains have been found in group C ; however , strains from all four groups have been isolated from tomato(Jones *et al*. 2004).In Sudan, Bouzar *et al*. (1994) reported that *Xanthomonas campestris* pv. *vesicatoria* was the causal agent of bacterialspot of tomato and pepper in central Sudan. Bacterial spot of tomato is considered to be a major constraint of tomato production all over the world in particular warmer and humid parts. The bacterial spot affects all aerial parts of the plant. Yield reduction due to the disease is a result of the direct infection on the photosynthetic leaf area, the drop of buds and flowers and reduction in commercial fruit value (Araújo *et al.* 2012) .Profits also decrease with the cost of chemical control. Infected seeds, volunteer crop plants and diseased plant debris may serve as inoculum sources of the disease. Estimates of total marketable yield losses ranged from 17 % to 52 % due to late and early bacterial spot infections, respectively. The disease also reduces seed germination, as well as causing defoliation and flower and young fruits drop (Kebede *et al.* 2013).

In autumn season of 2015, eight tomato fields in two localities in the Blue Nile State were surveyed;four fields (ALrosseris, ALgrif, Tiba and Bdoos) in ALrosseris locality and four fields (ALseriow, Abormad, ALregiba and

Haroon) in ALdmazein locality. A disease disorder was reported affecting all aerial parts of tomato plant; the primary symptoms were necrotic lesions that occur on leaves, stems, flower parts, and fruits. Symptoms of the disease were characterized by small water–soaked, with light to dark green areas at first and becoming brown, then necrotic on infected leaves. Individual irregular lesions on leaves develop rapidly to a size of about 0.2 cm in diameter and appear to be black and greasy. Lesions on leaves or fruits are surrounded by large chlorotic halos; infected tomato showed the typical bacterial spot symptoms (Dong *et al*. 2009; Aiello *et al.* 2013). Hundred plants were randomly chosen from each field and inspected for the disease. Ten leaves were taken from each plant to calculate the severity. Symptomatic tomato leaves were collected and sent to the Plant Pathology Laboratory, Faculty of Agriculture, University of Khartoum. An investigation on whether a phytopathogen was associated with symptoms of bacterial spots damage on tomato was conducted by pathogenicity test. The diseased parts were washed under running water and cut into small pieces. They were surface sterilized with 0.5% NaOCl for 30 sec, and rinsed three times in sterilized distilled water and blotted dry on sterilized filter paper. Specimens were soaked for 2 hours in normal saline and serial dilutions were made in 10 tubes. A volume of one ml was taken from each of the last three dilutions (10^{-8}, 10^{-9} and 10^{-10}) and spread on Nutrient Agar medium (NA). The inoculated plates were incubated at 25-30 $^{\circ}$C for 5 days. Well isolated single colonies were picked and streaked on slant of nutrient agar and kept in the refrigerator for further use. Then the bacterium was characterized morphologically and biochemically. At molecular level the identity of the bacteria isolates was confirmed by PCR and sequence analysis of the 16S RNA gene using primers fd1 5'AGAGTTTGATCCTGGCTCAG 3' and rp^{2}5'ACGGCTACCTTGTTACGACTT 3' as described by Weisburg *et al.*(1991). The survey conducted in the fields of tomato in Blue Nile State displayed some characteristic symptoms on foliage and fruits. The observed symptoms were diagnostic for the tomato bacterial spot disease (Fig.1). The disease incidence was remarkably high in ALrosseris and ALdamazein which recorded 49.27 and 52.17 %, respectively, while the disease severity was

28.28 and 30.32 %, respectively. The morphological and biochemical tests showed that the isolates were rod shaped, obligate aerobic, Gram negative, catalase positive, oxidase negative, can hydrolyse starch and gelatine and yellow colonies were observed on nutrient agar and Yeast Dextrose Calcium carbonate (YDC) media. In the pathogenicity test the three isolates showed pathogenic reaction on tomato plants (Fig.2) showing typical bacterial spot symptoms. The bacteria isolated from the infected tomato plants produced 1500 bp amplicon (Fig. 3). A BLAST search in the NCBI GenBank database (GenBank Accession Nos MH047248, MH051263 and MG686236) revealed that both sequences had 100 % identity to the 16S RNA sequences of *Xanthomonas euvesicatoria.* Accordingly, the isolates were identified as *Xanthomonas euvesicatoria*.

High severity was reported in ALdamzein locality compared to ALrosseris. These results could be attributed to the high humidity which enhancing development of the disease at Aldamazine. The symptoms of tomato bacterial spot (TBS) displayed on tomato plants in different locations in Blue Nile State were typical to those described in the literature (Aiello *et al.*2013). In this study, the generated sequencing data of the studied isolates, yielded unique matches with GeneBank sequences database for most isolates. However, sequence similarity of all isolates was 100 %. These results were in agreement with (Rashid *et al.* 2016). To our knowledge, this is the first report of *Xanthomonas euvesicatoria* causing bacterial spot disease in tomato in Sudan**.**

Fig.1. GeneralSymptoms on leaves and fruit of tomato plant

Fig. 2.Tomato leaf shows typical symptoms of bacterial spot

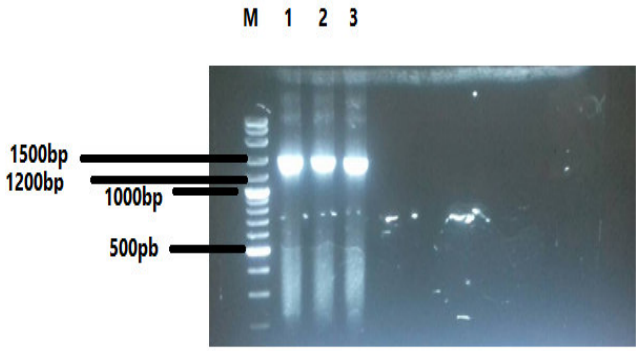

Fig. 3. Gel electrophoresis (Agarose 1.5%) of PCR products using universal primerfd1 rp2 designed to amplifya fragment of 1550bp.

Lane M, 100bp DNA ladder marker; lanes1-3 tested samples.

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أول تقرير عن تسبب بكتريا *Xanthomonas euvesicatoria* لمرض التبقع البكتيري للطماطم بولاية النيل الأزرق، السودان

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المستخلص: يعتبر مرض تبقع الاوراق البكتيري الذي تسببه البكتريا *Xanthomonas vesicatoria* مهددا"رئيسيا" لانتاج الطماطم في العالم خاصة المناطق الدافئة الرطبة. في موسم خريف 2015 لوحظت اعراض مؤثرة على كل الأجزاء الهوائية لنبات الطماطم المزروعة في ولاية النيل الازرق،جمعت الاوراق المصابة وارسلت الى معمل أمراض النبات، قسم وقاية المحاصيل كلية الزراعة ، جامعة الخرطوم. أجريت اختبارات لتحديد ما اذا كان المسبب المرضي ذو صلة بمرض تبقع الاوراق البكتيري. تم عزل المسبب المرضيو تمييزه ظاهريا، وعلى مستوي الاختبارات الكيموحيوية و الجزيئية.عرفت البكتيريا على انها *Xanthomonas euvesicatoria* وتم تأكيد هوية السلالات بواسطة تفاعل البلمره المتسلسل وتحليل تسلسل تتابعات الجين 16sRNA(رقم ايداع في بنك الجينات:MH047248و MH051263 و MG686236).كانت نسبة الاصابة عالية في كل من الروصيرص والدمازين بلغت 49.27 و 52.17%، على التوالي، بينما كانت شدة الاصابة 28.28 و 30.32%، على التوالي. حسب معرفتنا، لاول مرة ان مرض تبقع الاوراق البكتيري في الطماطم تسببه بكتريا *Xanthomonas euvesicatoria*.