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CROP HOST SPECIFICITY OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* PREVALENT IN NASHIK DISTRICT IN INDIA

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ABSTRACT
The bacterial leaf spot and blight disease pathogen of tomato prevalent extensively in tomato growing areas of Nashik district causes up to 40 percent yield losses. The bacterial pathogen was isolated from infected tomato leaves sample, purified and identified as *Xanthomonas campestris* pv. *vesicatoria* based on various morphological ,biochemical and host specificity test. The isolated bacterial colonies were yellow, raised, round and translucent. The bacterium was gram negative with single polar flagellation. The bacterium was oxidative, did not produce the fluorescent pigment, did not reduce the nitrate and did not produce oxidase. It induced the HR on tobacco. The bacterium was positive for hydrolysis of esculin, gelatinase activity, H_2S production, Tween esterase activity and amidan. Similarly the bacterium was able to utilize the sugar arabinose, glucose, galactose and cellobiose. This bacterial pathogen was specific for the infection to the tomato crop plant and does not infect its other natural host chilli and capsicum. The significance of this study is that the bacterial pathogen X.c.pv.vesicatoria prevalent in Nashik district on tomato crop is not a threat to chilli and capsicum crops grown in the region as the strain is host specific.

INTRODUCTION

Tomato is an important crop of Nashik district occupying an area of 1.25 lakh hectare with production of around 2.4 thousand metric tonnes. Bacterial leaf spot and blight caused by *Xanthomonas campestris* pv.*vesicatoria* in tomato crop is a major disease prevalent in the district causing the damage to the tune of 40 per cent yield losses under favourable climatic condition (Borkar, 1997). The bacterium *Xanthomonas campestris* pv.*vesicatoria* is known to infect tomato, chilli and capsicum as its natural host (Ritchie, 2000) to cause the damage and yield losses (Pohronezny and Volin,1983); however some strains of the pathogen is known to be specific to one host and not the other due to pathogenic variation (Mote, 2011). Chopada *et al*. (2014) reported pathogenic variation among *fusarium oxysporium* f.sp.*lycopersici* isolates and used these in varietal screening of tomato against wilt under south Gujarat,India. Host plant specificity in the infection by exophytes and endophytes is reported by Vera Lucida *et al*. (1980) and Bhagya and Shalini (2017). To manage the tomato disease sustainable integrated approach is proposed by Yogesh *et al*. (2016) while use of disease resistance chemical elicitor to induce disease resistance and to promote plant growth parameters was proposed by Raut *et al*. (2014). If a prevalent pathogen's strain is pathogenic to all its natural host in the area, it may cause catastrophic effect on cropping system and cropping pattern. Therefore, the studies on host specificity of *Xanthomonas campestris* pv. *vesicatoria* prevalent in tomato growing area of Nashik district was undertaken.

MATERIALS AND METHODS

Survey for bacterial leaf spot disease of tomato

A survey was undertaken in the Niphad taluka of Nashik district to assess the occurrence and losses due to bacterial leaf spot pathogen *Xanthomonas campestris* pv. *vesicatoria* in tomato as this crop is extensively cultivated in this taluka. The survey was carried in the month of August 2017, when the atmospheric condition was favourable for the occurrence and spread of the disease. The weather condition prior to survey period was intermittent rainy days coupled with cloudiness and humid weather.

Isolation, purification and Identification of bacterial leaf spot disease pathogen

The isolation of bacterial leaf spot pathogen from infected disease tomato leaves were carried out by routine method of isolation of bacterial plant pathogen (Borkar, 2018). The isolated bacterial colonies were purified and tested for their pathogenicity on tomato leaves. The pathogenic bacterial culture was identified by using the identification system given by Gardan and Luisetti (1981). Further differentiation of the genus *Xanthomonas* into species was based on a set of biochemical tests as per the protocol (Borkar, 2018). For identification of *Xanthomonas campestris* into pathovar, the bacterial cultures were infiltrated into the leaves of tomato, chilli and cabbage , since *Xanthomonas campestris* are the pathogen on these host plants.

Host specificity of *Xanthomonas campestris* pv. *vesicatoria of* tomato crop

Xanthomonas campestris pv. *vesicatoria* is reported to infects

tomato, chilli and capsicum as its natural hosts but sometimes a strain of one host plant is specific to that host plant only and does not infect the another host plant. Therefore the three host plants of *Xanthomonas campestris* pv. *vesicatoria viz*. tomato, chilli and capsicum were tested for their susceptibility. Some other plants belonging to solanaceous family particularly eggplant, tobacco and stramonium were tested for their reaction to the bacterium.

RESULTS AND DISCUSSION

Occurrence and losses due to Bacterial Leaf Spot and blight Pathogen *Xanthomonas campestris* pv. *vesicatoria*.

The survey observations revealed that most of the fields have about 35-40 per cent infection with disease severity of 30 per cent in the month of August. In subsequent period the disease spread rapidly and caused yield losses up to 40 per cent in the tomato production as expressed by the farmers (Fig 1).

Pernezny *et al*. (1996) conducted three large-scale field trials of tomato under commercial growing conditions in south-western Florida to quantify yield losses associated with foliar diseases of fresh market tomato. Marketable and extra-large fruit yields were reduced to 30-43 per cent in these trials. In our observations also the yield losses was up to 40 per cent as expressed by farmers. Patel *et al*. (1950) reported the bacterial leaf spot disease on chilli leaves and unripe tomato fruits caused by *Xanthomonas campestris* pv. *vesicatoria* at agricultural college farm, Pune, India in August, 1948. Our observation for the occurrence of this disease also confirmed that the disease occur prominently in the month of August when the

Figure 1: Disease severity of bacterial leaf blight infection on tomato.

Figure 2: Symptoms of bacterial leaf spot of tomato leaf

Table 1: Identification of bacterial isolates obtained from diseased tomato leaves into bacterial genus

Bacterial isolate of	Tests Gram reaction	Utilization of glucose	Flagella	fluorescence	Reduction of nitrates	Oxidase	HR on tobacco	Genus
Tomato	-	Oxidative	+	-	-	-	+	*Xanthomonas*

Table 2 :Identification of *Xanthomonas* genus into species

Genus	Tests Hydrolyse Esculin	Gelatinolyse	H_2S	Tween esterase	Urease	Amidan	Utilization of Arabinose	Glucose	Galactose	Cellobiose	Species
Xanthomonas	+	+	+	+	-	+	+	+	+	+	*campestris*

Table 3: Identification of Xanthomonas campestris pv vesicatoria isolate into pathovars

Sr. No	*X. campestris* isolate	Host infectivity Chilli	Tomato	Cabbage	Pathovar
1	*X. campestris*	_	+	_	*vesicatoria*

Table 4: crop host specificity of *Xanthomonas campestris* pv. *vesicatoria* prevalent in Nashik district.

*X.c.*pv. *vesicatoria* of tomato	Reaction of *X.c.*pv. *vesicatoria* on host plant			Reaction of *X.c.* pv. *vesicatoria* on non-host plant		
	Tomato	Chilli	Capsicum	Brinjal	Tobbaco	Stramonium
	+	HR 72 hrs	HR 72 hrs	HR 48 hrs	HR 48 hrs	HR 72 hrs

+ = Disease water soaking reaction; HR = hypersensitive browning reaction.

Figure 3: Colony morphology of *Xanthomonas campestris* pv. *vesicatoria* isolated from bacterial leaf spot sample

weather were cloudy and humid. Morton (1966) studied the bacterial leaf spot development at several temperatures and concluded that optimum temperature for rapid development on excised leaves was 27.5ºC. Symptoms were maximum at 22.5-25ºC. Incubation of similar leaves under variable conditions in the glass house resulted in more rapid and severe appearance of symptoms. Dieb *et al*. (1982a,b) also reported that bacterial leaf spot of tomato disease was favoured by heavy rainfall, high humidity and further reported that the disease is favoured by temperatures above 30ºC but not over 35ºC.

Jones *et al*. (1991) reported that bacterial leaf spot disease of tomato causes significant losses especially when warm temperature and rainy weather occurs. Tai *et al*. (1999) reported that bacterial leaf spot disease of tomato and pepper caused by *Xanthomonas campestris* pv.*vesicatoria* (*Xcv*), can be devastating to commercial production of these crops in areas of the world with high humidity and heavy rainfall. Ritchie (2000) reported bacterial leaf spot as one of the most devastating diseases of pepper and tomato grown in warm, moist environment. Once present in the crop, it is almost impossible to control the disease and prevent major fruit loss when environmental conditions remain favourable. When it occurs soon after transplanting and weather conditions remain favourable for disease development, the results are usually total crop loss. Abbasi *et al*. (2002) reported that bacterial leaf spot disease development is favoured by high moisture and temperature conditions prevalent during the growing season of tomato and pepper in Ontario. This disease can cause serious economic losses to these crop growers by reducing yield and fruit quality. Quezado-Duval and Maria (2003) reported that both processing and fresh-market tomatoes may be severely affected by bacterial leaf spot disease when environmental conditions are conducive to the disease. No resistant cultivars are available and chemical control is frequently reported as ineffective. Falico (1973) reported bacterial spot of tomato and capsicum in Corrients province in 1971 and in 1972 in tomato and capsicum nurseries which were severely attacked by *Xanthomonas vesicatoria* for the first time in the province. Golenia and Oajewska (1992) reported *Xanthomonas campestris* pv. *vesicatoria, Clavibacter michiganensis* sub sp. *michiganensis* from tomatoes grown in Poland. Tamayo *et al*. (1993) reported incidence of *Xanthomonas campestris* pv. *vesicatoria* from tomato cv. Santa Cruz in the city area of Rionegro, Antioqula, Colombia. Pohronezny and Volin (1983) reported that bacterial leaf spot did not reduce the total number of fruits, however the economic losses result from unmarketable infected fruit and sun scalded fruit due defoliation caused by the bacterial leaf spot disease. In the absence of effective disease management practices, this disease presents a continued threat to tomato and pepper production. Thieme *et al*. (2005) reported the disease results in defoliation and severely spotted fruits, both of which cause massive yield losses.

Symptoms of leaf spot disease in tomato

The symptoms of bacterial infection in tomato were observed on all the above ground parts of tomato plant. Initially the spots appeared as small, water soaked and light to dark green areas on young infected leaves. Individual lesions on leaves developed rapidly, 0.2 cm in diameter and appear black and greasy. The lesions were with or without yellowish halo and irregular (Fig 2).

When leaf spot were numerous the affected tissues often turn brown and the whole leaf died. Under wet and cloudy weather conditions, the spot intermingle with each other to produce black necrotic areas on the leaves. The infected leaves dry and remain attached to the plant thus giving a brown-reddish scorchy appearance to the infected plants and field.

Similar symptoms were also reported by Zapata (1995). These symptoms included necrotic lesions with chlorotic halos, raised hyperplastic pustule lesions, raised canker like lesions with chlorotic margins, raised canker lesions with necrotic borders and progressive water soaking due to infection of *Xanthomonas campestris* pv. *vesicatoria*. Ritchie (2000) studied the effect of bacterial attack on pepper and tomato crop. The bacteria infect the foliage, stems, and fruits of peppers and tomatoes. Generally, defoliation is more common for peppers than for tomatoes. Because the diseased foliage often remains on tomato plants, affected plants may have a scorched appearance. Sun *et al*. (2012) reported that the symptoms of bacterial infection in tomato and pepper can be found on all above ground parts of these plants. Initially, spots appear as small, water-soaked and light to dark green areas on the young infected leaves. On tomato, individual lesions on leaves develop rapidly to a size of about 0.2 cm in diameter and appear to be black and greasy. Lesions on pepper may enlarge rapidly to a size of 0.25 to 0.5 cm in diameter and become tan and brownish-red. Lesions on both hosts are with or without a yellowish halo and generally irregular, while leaf spots caused by phytopathogenic fungi or chemical injuries are usually round. When leaf spots are numerous, surrounding tissue often turns brown and the whole leaf will die. Under very wet weather conditions, spots may grow together, producing large black necrotic areas on the leaf. Lesions on stems and petioles are slightly more elongated than leaf spots. On peppers, defoliation occurs more commonly on heavily infected leaves, while infected tomato leaves remain on the plant and the whole diseased plant appears to be scorched. Abbasi *et al.*(2002) reported that the pathogen, *Xanthomonas*

campestris pv.*vesicatoria* (*Xanthomonas vesicatoria* and *Xanthomonas axonopodis* pv. *vesicatoria*), infects all the above ground plant parts, causing necrotic lesions on leaves, stems, and fruits in pepper and tomato plants.

Colony morphology of isolated bacterial pathogen from Leaf Spot Disease of tomato prevalent in Nashik district.

The bacterial colonies obtained during the isolation of bacterial pathogen responsible for leaf spot disease of tomato were yellow, round, raised and translucent. These bacterial colonies appeared in the isolation plates on nutrient agar media after 72 hours of incubation (Fig3).

The bacterial pathogen was easily being isolated from the leaf spot disease symptoms on tomato leaves. Several workers (Mohammed and Al-Saleh, 2011; Araujo *et al.,* 2012; Buonarurio *et al.,* 1994; Chand *et al.,* 1994; Dieb *et al.,* 1982; Falico, 1973; Mote 2011; Patel *et al.,* 1950) observed the same colony morphology for *Xanthomonas campestris* pv. *vesicatoria*.

Identification of bacterial leaf spot pathogen of tomato.

The bacterium were gram negative having single polar flagella.

The results of biochemical test (Table 1) revealed that the bacterium was oxidative, did not produce the fluorescent pigment, did not reduce the nitrate and did not produce oxidase. It induces the HR on tobacco. On the basis of these results the bacterium was identified of the genus *Xanthomonas*.

The results (Table 2) showed that the bacterium was positive for hydrolysis of esculin, gelatinase activity, H_2S production, Tween esterase activity and amidan. Similarly the bacterium was able to utilize the sugar arabinose, glucose, galactose and cellobiose. On the basis of these tests the bacterium was identified as *Xanthomonas campestris*.

The results (Table 3) indicate that the bacterium *Xanthomonas campestris* belong to pathovar *vesicatoria* as it infects only the tomato and not the cabbage plant.

Jones *et al*. (2000) reported the taxonomy and evolutionary relationships among members of the genus *Xanthomonas* associated with tomato and pepper. These have been a matter of considerable controversy since their original description in 1921. These bacteria, which are a major affliction of tomato and pepper crops in warm and humid regions, were originally described as a single species, but subsequent research has shown the existence of at least two genetic groups differentiated by physiological, biochemical and pathological characteristics**.** Mohammed and AL-Saleh (2011) isolated five bacterial isolates from the infected tomato seedlings growing in Al-Kharj area of Saudi Arabia. The pathogen's identification was confirmed by biochemical and physiological tests, hypersensitive reaction in tobacco plant and pathogenicity test on tomato.

Samiah and Al-Mijalli (2014) recovered 43 bacterial isolates from naturally infected green pepper fruits (38 samples) showing dark brown, irregular-shaped blotches. Out of 43 bacterial isolates six isolates were related to *Xanthomonas vesicatoria* on the basis of biochemical tests. Roach *et al*. (2017) studied the outbreaks of bacterial leaf spot of tomato in most commercial growing regions in Australia. The study describes the identification and diversity of pathogenic and non-pathogenic *Xanthomonas* spp. associated with bacterial leaf spot in Australia based on biochemical and molecular analysis.

Host specificity of *Xanthomonas campestris* pv. *vesicatoria* prevalent in Nashik district.

The result (Table 4) indicates that the bacterium *Xanthomonas campestris* pv. *vesicatoria* isolated from tomato leaf spot was specific to the tomato host plant. It did not cause disease to the chilli and capsicum. However the strain induces HR on chilli and capsicum plant at 72 hours. Similarly the bacterium also induced HR in eggplant and tobacco at 48hours and in stramonium at 72hours.

Cook (1973) reported the hypersensitivity in *Capsicum annum* induced by the tomato strains of *Xanthomonas vesicatoria*. Gardner and Kendrick (1923) reported the host range of *Xanthomonas campestris* pv. *vesicatoria* which included 73 varieties of tomato; 4 types of pepper (Pimento, Tabasco, Chilli and bell peppers); Potato leaves, current tomato (*Lycopersicum pimpinnellifolium*), *Solanum nigrum*, *S. dulcamara*, *S. rostratum*, *Datura tatula* (*Datura stramonium*), *Physalis minima*, *Lyciumchinese*, *L. halimifolium*, *Hyoscyamus aureus*, *H. Niyer* and *Nicotisna rustica*, while atomizer inoculation under greenhouse conditions with the tomato organism gave negative results with turnip, cabbage, eggplant, tobacco, Datura, *Solanum carolinense*, *S. integrifolium* and *S. opacum*. Mote (2011) studied the reaction of *Xanthomonas campestris* pv. *vesicatoria* capsicum isolate and tomato isolate in 21 different non-host plants. Among 21 non-host plants taken, only potato showed water soaking reaction and all others gave hyper sensitive reaction.

The bacterial leaf spot and blight pathogen of tomato prevalent in Nashik district is specific to tomato crop only and this specificity may be due to different factors. Luke Barrett and Martin Hall (2012) gave a unifying concept and mechanism in the specificity of plant-enemy interaction. Schulez-Lefert and Panstruga (2011)reported a molecular evolutionary concept connecting non host resistance, pathogen host range and pathogen speciation. Killiny and Almeida (2011) suggested gene regulation mediates host specificity for a bacterial pathogen. Da Silva *et al*. (2002) identified several group of strain specific genes and on the basis of these group propose mechanism that may explain the differing host specificities and pathogenic processes. cDNA –AFLP analysis unravels a genome-wide hrpG- region in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (Noel *et al.,* 2001). Wengelnik and Bonas (1996) suggested HrpXv, an Ara C-type regulator activates expression of five of the six loci in the hrp cluster of *X.c.* pv.*vesicatoria*. Fenselau and Bonas(1995) reported sequence and expression analysis of the hrpB pathogenicity operon of *X.c.* pv.*vesicatoria* which encodes eight protein with similarity to components of the Hrp, Ysc, Spa, and FII secretion system. Yang and Gabriel (1995) reported intragenic recombination of a single plant pathogen gene to provide a mechanism for the evolution of new host specificities. Bonas (1991) isolated a gene cluster from *X.c.* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. The strain of *X.c.* pv. *vesicatoria* prevalent in Nashik area has developed host specificity for tomato plant and non-pathogenic to chilli and capsicum indicating that the

cultivation of these two crop in this region has no threat from *X.c.* pv. *vesicatoria*.

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