



## RESEARCH PAPER

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## Prevalence, occurrence and biochemical characterization of *Xanthomonas campestris* pv. *vesicatoria* in District Swat, Pakistan and its management through host resistance

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### Abstract

*Xanthomonas campestris* pv. *vesicatoria* the causal organism of bacterial spot in tomato results in heavy losses both in the form of quality and. In this study a survey was carried out to report the incidence of bacterial spot disease of tomato in district Swat. We reported maximum disease incidence in tehsil Kabal (71.66%), followed by Charbagh (61.66%) and Barikot (58.33%). For resistant screening a total of 13 tomato germplasms were screened against the disease. The foliar severity ranged from 3.33% to 73.33%, while severity for fruits was ranged from 18.33% to 30.66%. In case of phenotypic data the highest numbers of fruits obtained were 34, plant height 79.5cm and fruit weight was 470 grams/ten tomatoes. While the lowest average numbers of fruits were 6.67, plant height 45.7cm and fruit weight recorded was 215.67 grams/ten tomatoes. Line 1288 showed highest level of resistance followed by Red-stone. However, line 9708 showed highest susceptibility when exposed to artificial inoculation. Our study showed that bacterial spot is a major issue in some part of Pakistan and germplasm screening are linked to increased host resistance and could offer an important contribution to future integrated bacterial spot management programs.

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## Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important vegetable and fruit crop (Akbar *et al.*, 2018) after potato both in consumption and production worldwide (Lemma *et al.*, 1992). It is consumed as a raw or as an ingredient in a number of dishes and drinks (Anonymous, 2009). Tomato contains essential amino acids, dietary fibers, minerals, vitamins and sugars (Akbar *et al.*, 2018). The metabolites of tomato have reducing effect on acidity of urine when compared to orange juice (Saywell *et al.*, 1933). Furthermore, lycopene contents in tomato act as an antioxidant and play great role in prevention of cancer heart diseases and many other health problems (Miller *et al.*, 2002) (Akbar *et al.*, 2018).

In terms of production China is in the list of top tomato producing country and accounts for one quarter of tomato acreage of the world. Furthermore Asia and Africa is shares approximately 65% to the world tomato production (FAO, 2008). Under divers agro-ecological conditions of Pakistan tomatoes are cultivated over 52.8 thousand hectares with the annual production of about 529.9 thousand tons Pakistan (Agriculture statistics of Pakistan, 2011-12). The tomato production of Baluchistan is more (45%) as compared to other provinces like KP which 35% followed by Punjab 14% and Sindh 10%. (Anonymous, 2007). In Khyber Pakhtunkhwa, tomato occupied an area of 16.5 thousand hectares with the average yield of 9.8 tons/ha (Federal Bureau of Statistics, 2012). Off season tomatoes are grown in the frost-free area of the Katha, Sughral in Punjab province and Dargai area in KP (Burney, 1995).

In Pakistan tomato yield is very low when compared to other leading tomato growing countries like USA, India and is much less than world average production which is 36 ton/ha. Low tomato production is attributed to several insect's attack and diseases. Different fungal, bacterial, and viral and nematodes diseases occur in tomato. Among those bacterial diseases, tomato spot is one of the destructive disease. Bacterial spot caused by *Xanthomonas vesicatoria* causes a heavy loss than any other bacterial disease.

In 1921 bacterial spot was reported by Doidge in USA and South Africa for the first time and later reported on capsicum for the first time in Florida in 1923 by Gardner (Jones *et al.*, 2004).

*Xanthomonas vesicatoria* is having rod shape, motile aerobic and gram negative and having single or polar flagellum (Thieme *et al.*, 2005). On Nutrients Agar colonies are circular, wet,

The pathogen can affect all above ground parts of tomato including stem, leaf, fruit, etc. Lesions on leaves are small (<3mm in diameter) brown and circular with water-soaked margins. Lesion on fruits begin as raised blisters. Older lesions are brown, scab like and sunken in the middle.

Keeping in view the importance of this disease in our province particularly in tomato growing areas of district Swat, the study was planned with the following objectives.

1. To determine the incidence of bacterial spot caused by *Xanthomonas vesicatoria* in tomato growing areas of District Swat.
2. To isolate and identify the pathogen through biochemical tests.
3. To screen tomato germplasm for resistance to bacterial spot.

## Material and methods

### *Assessment of disease incidence and samples collection*

Survey was conducted in tomato growing areas of district Swat for bacterial spot of tomato incidence. The district was divided into three different areas and in each tehsil three locations were surveyed and two fields in each location were selected at a well apart distance i.e., 5 to 10Km (Table 1). To find out disease incidence 10 plants from 2 to 3 spots in each field were randomly selected and their average is obtained. The disease incidence was determined by using the following formula.

$$\text{Disease incidence (\%)} = \frac{\text{No of diseased plants}}{\text{Total no. of plants observed}} \times 100$$

To isolate pure culture of the causal bacterium diseased samples were collected at the same locations. Samples were kept in bags, properly

labeled and brought to plant pathology laboratory at Agriculture Research Institute (ARI) Swat and stored at 4°C for further processing.

**Table 1.** Tomato growing areas of district Swat visited for disease incidence and samples collection.

SL	Locality	Tehsil
1	Jawand	
2	Nasrat	Kabal
3	Sogaley	
4	Ghari	
5	Parrhi	Barikot
6	Dadahara	
7	Charbagh	
8	Gulibagh	Charbagh
9	Alabad	

Experiment In-vitro

*Isolation and identification of pathogen*

The technique developed by Chiejina. (2008) which is used to isolate bacterium. 2mm thin sections were cut from infected fruits and leaves and sterilized in 70% ethyl alcohol. After sterilization, the pieces were washed three times with sterilized distilled water and blotted dried on paper towels aseptically. Then 4-5 sterilized pieces were placed on Nutrient agar (NA) plates. Plates were kept at 30°C in incubator for 24 to 48 hours upside down. To obtain pure culture a smear of well grown colony was streaked on NA medium and incubated for 24 to 48 hours. Then the isolates obtained from samples were subjected for identification using gram staining reaction (Schaad., 1988), Hypersensitive response test (James *et al.*, 1996), (Sahin *et al.*, 1996) and through use of various biochemical tests by (Holt *et al.*, 1994) such as KOH test (Ryu, 1940; Suslow *et al.*, 1982), Catalase test (He *et al.*, 1993), Oxidase test (Schaad, 1988) and Starch hydrolysis test (Sands, 1990).

*Experiment in-vivo for screening germplasms against Bacterial spot*

*Nursery preparation and transplantation for screening tomato germplasms*

For this purpose, diseased free seeds of different available tomato germplasms (13) were sown initially in trays. Trays were maintained in screen house for four weeks till germination of seeds.

After four weeks, transplantation was done in the field at ARI (Agriculture Research Institute) Mingora Swat. Six plants per plot were planted, the distance from plant to plant was 45cm and the rows distance was 90cm in a randomized complete block design. The management practices for fertilization, insect and foliar fungal diseases were done according to standard recommendations (Ivors *et al.*, 2013). Copper was sprayed on control plants to control bacterial pathogens.

**Table 2.** Tomato germplasms and its source, evaluated for resistance against bacterial spot.

SL	Germplasms	Source
1	AVTO 9802	ARI Swat
2	1405	--
3	1409	--
4	1455	--
5	AVTO 1003	--
6	AVTO 1130	--
7	9708	--
8	1429	--
9	1288	--
10	U-RO	--
11	Rio-Grande	Market
12	Red stone	--
13	Roma	--

*Inoculum preparation and inoculation*

A 3-days old culture of *Xanthomonas vesicatoria* was flooded with SDW (sterilized distilled water). The colonies were scrapped by using a glass slide. The colonies concentration in suspension was adjusted to OD 600 and abs 0.3 with spectrophotometer. Plants were inoculated with suspension in the field by using a sprayer after 30 days of transplantation. Inoculation process was done in morning time to increase the chances of infection. Bacterial spot symptoms developed in about 14 to 21 days on leaf surface.

Data on the following Parameters was recorded and statistically analyzed using statistic 8.1 software. The determined were subjected to ANOVA at the least significant difference Test (at 5% significance level) (steel *et al.*, 1997).

*Disease severity*

After four weeks of inoculation Plants were calculated for foliar bacterial disease severity according to the following scale, adopted by Suárez-Estrella1 *et al.*, 2014.

**Table 3.** Disease Severity Scale used for grading germplasm resistance.

Disease rating	Symptoms	Category
0	Symptomless Plants	Resistant
1	Mildly infected plants (<50% of spotted leaves)	Moderately Resistant
2	Highly infected plants (>50% of spotted leaves but plants not dead)	Susceptible
3	Dead plants	Highly Susceptible

(Suárez-Estrella *et al.*, 2014).

*Number of fruits per plant*

Number of fruits per plant was counted after each picking. Mean number of fruits per plant were calculated after the last picking and were analyzed statistically.

*Plant height*

The growth of a plant is highly reflected by plant height which contributes to the final yield. Statistical results of data pertaining to the plant height (cm) were measured at 4, 6 and 8 weeks after planting.

*Weight of fruits*

The disease affects the weight of the fruits as well, so data about the weight of fruits at maturity was calculated through digital balance.

*Statistical analyses*

Analyses of variance of RCBD was performed using Statistical Software statistix 8.1. Least Squares of Means were calculated, and lines were separated using LSD at P = 0.05 levels (Steel *et al.*, 1997). Disease severity on leaves and fruits were calculated on ten severely infected leaves and fruits of each plot.

**Results**

*Incidence of bacterial spot in tomato growing areas of Swat*

Survey was done for incidence of bacterial spot disease in different growing areas of Swat. A total of nine locations were visited in the district. Highest bacterial spot incidence was found for tehsil Kabal which was 71.6%, followed by tehsil Charbagh 61.6% and in tehsil Barikot 58.3% disease incidence was recorded (Table 4).

**Table 4.** Incidence of Bacterial Spot disease in Tomato growing areas of District Swat.

SL	Areas	Incidence (%)
1	Kabal	71.6 A
2	Charbagh	61.6 B
3	Barikot	58.3 B
4	LSD Value	3.60

*Experiments in vitro*

*Isolation and purification*

Small pieces of diseased leaves/fruits samples, placed on Nutrient Agar plates and re-streaking was done. Bacterial colonies were obtained at 30°C after 48 hours of incubation. (Fig.1). The visual observation was made visually to identify the pathogen (*Xanthomonas vesicatoria*) through colonies morphology. The color of colonies was light yellow to yellow. The colonies shape was found to be mucoid and convex. A total of 9 isolates were obtained which were used further (Table 5).

**Table 5.** Morphology of bacterial colonies obtained after 24 hours grown on NA medium.

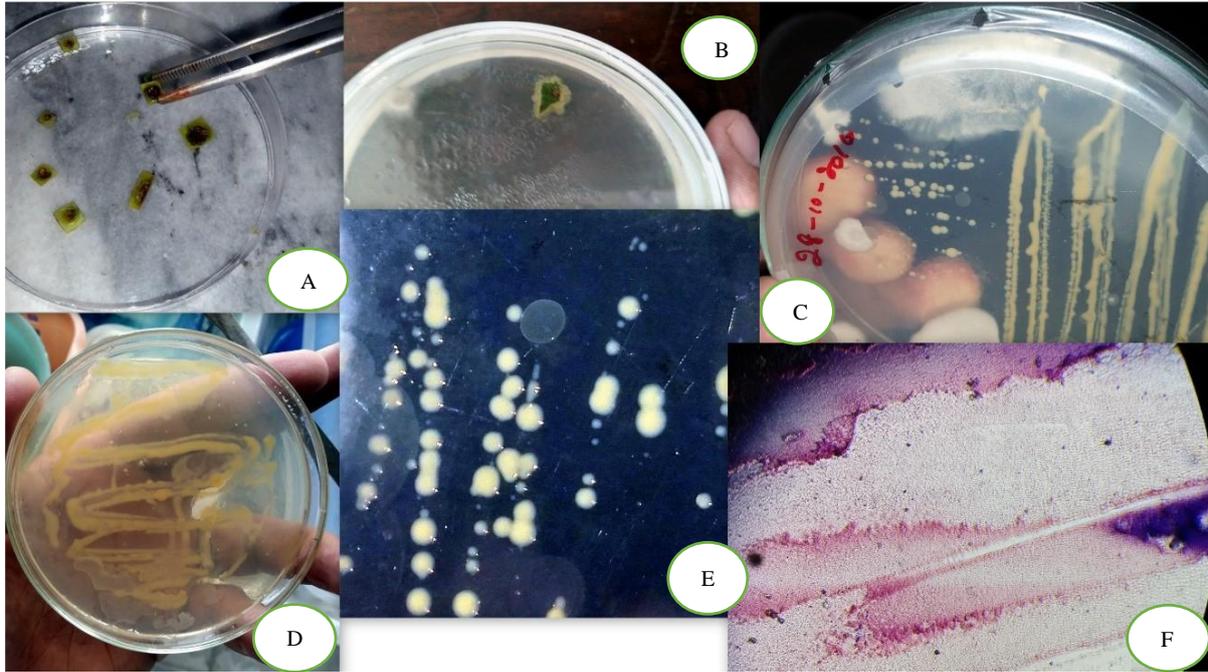
SL	Locality	Colony/24 hour Mucoïd, convex,	Yellow colony
1	Jawand	yes	+
2	Nasrat	yes	+
3	Sogaley	yes	—
4	Garrhi	yes	+
5	Pharri	yes	+
6	Dadahara	yes	+
7	Charbagh	yes	—
8	Gulibagh	yes	+
9	Alabad	yes	+

+ = *Xanthomonas vesicatoria* (Xv)

- = bacterial culture other than Xv

*Hypersensitive response test*

HR-inducing ability of the isolates was tested on tobacco plant. Complete collapse of tissue followed by necrosis after 24 hours was found as positive (Fig. 2) (Table 6). Out of nine isolates, seven showed H.R response. However, isolate representing Tehsil Kabal showed sever HR response that was used for inoculation in *In-vivo* experiments.

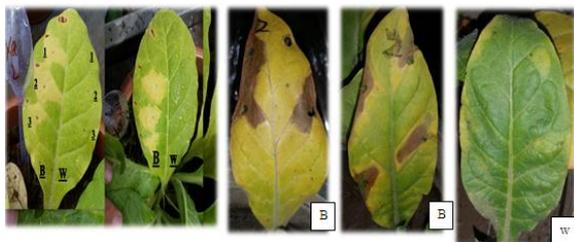


**Fig 1.** Isolation of pathogen from leaves and fruits samples and inoculation on NA medium. A & B (Isolation and inoculation), C & D (Bacterial culture), E (Bacterial colonies), F (Gram staining).

**Table 6.** Hypersensitive Response of bacterial isolates on tobacco plants 24 hours after inoculation.

SL	Isolates	Hypersensitive Response
1	I. k1.	+
2	I. k2	+
3	I. k3	+
4	I. c1	+
5	I. c2	+
6	I. c3	-
7	I. b1	+
8	I. b2	-
9	I. b3	+

The symbol “+” = express positive result that the bacterium is pathogenic while the symbol “-” denotes a negative result.



**Fig 2.** Hypersensitive Response on Tobacco plants 24 hours after inoculation with representative bacterial isolates. B(bacteria) W(water).

*Biochemical characterization of pathogen*

The biochemical characterization results have been shown (Table 7). The bacterial stained mounts were

observed under light microscope using oil immersion in gram staining. *Xanthomonas vesicatoria* isolates were found pinkish in color and termed as gram negative.

*KOH test*

Thread like slime was formed from viscous suspension when picked up by the toothpick during KOH test (Fig. 3a), indicating that the bacterium was Gram-negative.

*Catalase test*

The bacterium produced bubbles by dropping Hydrogen peroxide on smear of culture indicating the bacteria as catalase positive (Fig. 3b).

*Oxidase test*

The *Xanthomas vesicatoria* was characterized as oxidase negative that gave no color after 60 seconds (Fig. 3d).

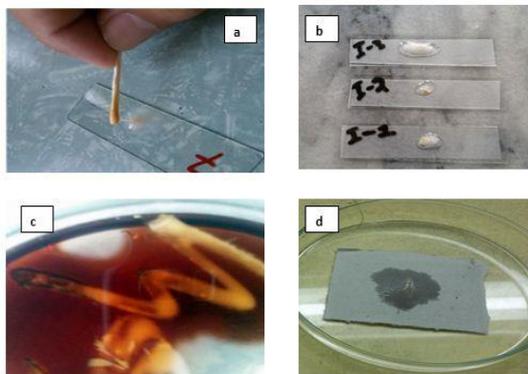
*Starch hydrolysis test*

After incubation of seven days at 30°C a clear zone around the colonies was formed on starch medium when Lugol’s Iodine was applied. This test indicated that the bacterium hydrolyzed the starch which was present in the nutrient agar medium (Fig. 3c)

**Table 7.** Bio-chemical characterization of bacterial isolates.

SL Isolates	Gram reaction	KOH	Catalase	Oxidase	Starch Hydrolysis
1 Isolate.k1	—	+	+	—	+
2 Isolate.k2	—	+	+	—	+
3 Isolate.k3	—	+	+	—	+
4 Isolate.c1	—	+	+	—	+
5 Isolate.c2	—	+	+	—	+
6 Isolate.c3	—	+	+	—	+
7 Isolate.b1	—	+	+	—	+
8 Isolate.b2	—	+	+	+	+
9 Isolate.b3	—	+	+	—	+

The symbol “+” = denotes a positive result while the symbol “\_” denotes a negative result.



**Fig. 3.** Identification of the bacterium through different bio-chemical tests i.e KOH Test (a), Catalase Test (b), Starch Hydrolysis (c), Oxidase Test (d).

*Experiment in vivo*

*Disease severity*

There was high Bacterial Spot disease pressure in the field. Disease symptoms were seen on all parts of a plant (Fig. 4). ANOVA of the field study demonstrated that there were significant differences among tomato lines for foliar ( $P=0.00$ ) (Table 8) and fruit disease scores ( $P=0.00$ ) (Table 9). Mean data exhibited that the foliar disease severity among the germplasm ranged from 3.333 to 73.333 and the mean data for fruit disease severity ranged from 18.333 to 30.667. LSD of the severity of tomato germplasm in the field for fruit was 2.0728 (Table 9) and LS means of BS disease score on leaves was 4.6685 (Table 8). Tomato lines 1288 showed highest level of resistance among the germplasm and followed by Redstone, Rio-Grande respectively, while in contrast the line 9708 was found highly susceptible to BS disease followed by AVTO-9802, AVTO-1130 etc. respectively.

**Table 8.** Disease severity of Leaves recorded on tomato lines.

SL	Germplasm	Severity (Foliar)	Conclusion
1	9708	73.333 A	Susceptible
2	AVTO-9802	63.333 B	Susceptible
3	AVTO-1130	53.333 C	Susceptible
4	1455	43.333 D	Moderately resistant
5	1409	36.667 DE	Moderately resistant
6	1405	33.333 EF	Moderately resistant
7	AVTO-1003	33.333 EF	Moderately resistant
8	U-RO	26.667 FG	Moderately resistant
9	Roma	23.333 GH	Moderately resistant
10	1429	16.667 HI	Moderately resistant
11	Rio-Grande	13.333 I	Moderately resistant
12	Redstone	10.000 IJ	Moderately resistant
13	1288	3.333 J	Moderately resistant
	LSD Values	4.6685	

**Table 9.** Disease severity of Fruits recorded on tomato lines.

SL	Germplasm	Severity (Fruits)
1	9708	30.667 A
2	AVTO-9802	30.000 AB
3	AVTO-1130	29.667 AB
4	1455	29.000 ABC
5	1409	27.667 ABC
6	1405	27.000 ABC
7	AVTO-1003	26.667 ABC
8	U-RO	26.000 BC
9	Roma	26.000 B C
10	1429	25.333 C
11	Rio-Grande	20.667 D
12	Redstone	19.667 D
13	1288	18.333 D
	LSD Values	2.0728



**Fig 4.** Tomato leaves and fruits showing typical bacterial spot symptoms.

*Number of fruits per plant*

The germplasm displayed highly significant difference ( $p=0.001$ ) for number of fruits per plant (Table 10). Mean data showed that number of fruits among the tomato germplasm ranged from 34 to 6.67. Maximum fruits were collected from line 1288 (34), followed by Redstone, (28.3), Rio-Grande (22.6).

While minimum number of fruits were picked from line 9708 (6.6), followed by AVTO-9802 (8.6), AVTO-1130 (9.6), 1455 (10.3) respectively.

**Table 10.** No of Fruits of 13 germplasms of tomato.

SL	Germplasms	No of Fruits/plant	Control
1	1288	34.000 A	35 A
2	Redstone	28.333 AB	31 B
3	Rio-Grande	22.667 BC	35 A
4	1429	19.667 BCD	25 DE
5	Roma	18.333 CDE	29 BC
6	U-RO	16.333 CDEF	26 CD
7	AVTO-1003	15.333 CDEFG	22 EF
8	1405	13.333 DEFG	21 FG
9	1409	12.667 DEFG	20 FGH
10	1455	10.333 EFG	18 GH
11	AVTO-1130	9.667 EFG	17 H
12	AVTO-9802	8.667 FG	18 GH
13	9708	6.667 G	10 I
	LSD Values	4.5031	1.6845

*Plant height*

Significant differences (0.00) among the germplasms were observed for plant height (Table 11). Plant height ranged from 45.7cm to 79.5cm. Minimum plant height was recorded for line 9708 (45.7cm), followed by AVTO-9802 (56.7cm), AVTO-1130 (60.9cm), 1455 (63.5cm) respectively. However, maximum plant height (79.5cm) was recorded for Redstone followed by line 1288 (78.7cm), and Rio-Grande (76.2cm).

**Table 11.** Plant height (cm) of 13 germplasms of tomato.

SL	Treatment	Plant height	Control
1	Redstone. 1	79.587 A	80 B
2	1288. 2	78.740 A	80 B
3	1429. 3	76.200 AB	77 BC
4	Rio-Grande. 4	76.200 AB	80 B
5	Roma. 5	74.507 ABC	76 BC
6	U-RO. 6	72.813 BCD	85 A
7	AVTO-1003. 7	71.967 BCD	72.46 CDE
8	1405. 8	70.273 CD	75 CD
9	1409. 9	68.580 DE	73 CDE
10	1455. 10	63.500 EF	71 DE
11	AVTO-1130. 11	60.960 FG	70 E
12	AVTO-9802. 12	56.727 G	73 CDE
13	9708. 13	45.720 H	65 F
	LSD Values	2.5598	2.2632

*Weight of fruits*

Mean squares revealed highly significant difference (0.00) among germplasms for fruits weight (Table 12). Weight of fruits ranged from 215.6 to 470.0 in which line 9708 displayed low weighted fruits

followed by AVTO-9802, AVTO-1130,1455 respectively, while in contrast, maximum fruits weight was found for line 1288 followed by Redstone, Rio-Grande.

**Table 12.** Weight (g) of Fruits of 13 germplasms of tomato.

SL	Treatment	Fruit weight	Control
1	1288	470.00 A	470 A
2	Redstone	460.00 A	476 A
3	Rio-Grande	405.00 B	402 B
4	U-RO	383.33 BC	380 CD
5	1429	381.67 BC	392 BC
6	1409	375.00 BCD	370 D
7	1405	372.00 CD	375 D
8	Roma	348.33 DE	340 E
9	AVTO-1003	316.6 EF	321 F
10	1455	305.0 F	306 FG
11	AVTO-1130	285.00 F	296 G
12	AVTO-9802	225.0 G	255 H
13	9708	215.67 G	237 I
	LSD Values	15.501	8.1072

**Discussion**

Survey was determined to find out disease incidence (%) in tomato growing areas of district Swat. Incidence percentage of bacterial spot was 71.6%, 61.6% and 58.3% for Tehsil Kabal, Charbagh and Barikot. Incidence of disease in Kabal was on top among the three regions of the district. This could be because of inoculum buildup due to successive tomato cropping in environmental conditions, mostly moisture and temperature favored bacterial spot disease as compared to Charbagh and Barikot.

Various biochemical tests like Gram reaction, Starch hydrolysis, Oxidase, Catalase and KOH tests characterized the *Xanthomonas vesicatoria* as gram negative, catalase positive and oxidase negative bacteria. Some of the isolates were not positive according to biochemical tests so may be those were the pathogens of bacterial speck or other bacterial diseases. In oxidase test the isolate (ib2) showed as oxidase positive which means that was pseudomonas spp the cause of bacterial speck. Our results correlate with the work of Mubeen *et al.* (2015), Vernière *et al.* (1998) and Suslow *et al.* (1982) who used Gram staining, Starch hydrolysis, Tween 80 hydrolysis, Gelatin Liquefaction, KOH test, Kovacs' Oxidase and Fluorescent Pigmentation tests for identification of

*Xanthomonas axonopodis* campestris pv. citri. Vernière *et al.* (1998) also used several biochemical tests to identify and differentiate pathotypes of citrus canker bacteria *Xanthomonas axonopodis* citri.

Bacterial spot symptoms were seen on leaves and fruits. The appearance of the fruit symptoms generally appears when the disease pressure is high. When hair of fruit fall from the fruit, there is an injury or opening left for the bacteria to enter through the fruit surface and cause raised black lesions. Significant differences among the germplasm were observed for disease resistance. Most of the lines were moderately resistant to susceptible according to the scale reported by Suarez *et al.* (2014). Ten lines, in which lines 1288, 1429, AVTO-1003, 1405, 1409 and 1455 and in commercial varieties Red-stone, Rio-Grande, Roma and U-RO were moderately resistant. These lines were resistant as compared to the rest of lines and variety because they may have resistance genes to bacterial spot while another three lines were found susceptible including lines 9708, AVTO-9802 and AVTO-1130. The symptoms were found more as compared to fruits because the plants were inoculated at the early stage and fruits were not produced and other reason for more symptoms on leaves could be the stomata through which the bacteria easily enter as compared to fruits. These results are in uniformity with the findings of Bhattarai *et al.* (2014) who screened 63 varieties for resistance to Bacterial spot, where eleven genotypes were found resistant. Similar results were obtained by Wang (1992) where different genotypes were tested both in greenhouse and field. Our results are also in similarity of Alballat *et al.* (2016), five out of 35 hybrids were found resistant.

Phenotypic traits were measured i-e, plant height, number of fruits, and weight of fruits. Significant differences were found for all the traits among the germplasm because the disease effects the plant height as well when compared with control. For plant height the best result was recorded (79.5cm) for commercial variety Red-stone followed by 1288, Rio-Grande, 1429, Roma, U-RO, AVTO-1003, 1405, 1409, and 1455. (78.7, 76.2, 76.2,74.5,72.8,71.9,70.2,68.5

and 63.5). While the minimum plant height was recorded 45.7cm for 9708 followed by AVTO-9802, AVTO-1130 and 1455. (56.7cm, 60.9cm, 63.5cm). More number of fruits were found for line 1288 which was 34 followed by Red-stone, Rio-Grande, 1429, Roma, U-RO, and 1405, while less number of fruits was recorded for line 9708 followed by AVTO-9802, 1455, and 1409. Fruit weight was found more for (470g) line 1288 while the minimum result for fruit weight was shown by 9708 (215.6). The results of these phenotypic traits are in similarity with the findings of Bhattarai *et al.* (2014), in which the traits measured were growth type, height, leaf type, leaf color, fruit shape, fruit size. The findings of this study demonstrated that screening is an effective step in the management of tomato bacterial spot. It's also reported that host genetic resistance is the most effective strategy in managing bacterial -speck and -spot diseases Jones *et al.* (1986) and Hulbert *et al.* (2001).

Survey was conducted in tomato growing areas of swat for bacterial spot incidence. Severe incidence was found in Tehsil Kabal (71.6%), Tehsil Charbagh (61.6%), and Tehsil Barikot (58.3%). Samples were collected from same tomato fields of District Swat, visited for bacterial spot incidence.

The experiment was carried out at Agriculture Research Institute Mingora Swat. The collected samples were brought to laboratory for further isolation and identification. Physiological and biochemical tests were done for the identification of the bacterium. The bacterium was confirmed as *Xanthomonas vesicatoria* through different biological tests, such as gram reaction, KOH, catalase, oxidase and starch hydrolysis test.

While in *in vivo study*, 13 germplasms were planted in a RCB design with 3 replications in the field. Nursery was raised in greenhouse and then transplanted into field after 27 days of sowing. For inoculation purpose, A 3-days old culture of *Xanthomonas vesicatoria* was flooded with SDW (sterilized distilled water). The colonies were scrapped by using a glass slide. The colonies concentration in suspension was adjusted to

$10^8$  CFU/ml, OD 600 with spectrophotometer. Plants were inoculated with suspension in the field after 30 days of transplantation. Bacterial spot symptoms developed in about 14 to 21 days on leaf surface.

Data were recorded according to the parameters. The variability for all the traits was studied. The germplasms revealed high significant difference ( $p=0.00$ ) for disease severity, incidence, plant height, fruit weight and number of fruits per plant. Average of varieties were moderately resistant according to scale for foliar severity but among 13 varieties the minimum disease severity was revealed (3.3%) by 1288 while the maximum disease severity was shown (73.3%) by 9708. For fruit severity, the germplasm 9708 showed severity of 30.6% while the severity of 1288 was 18.3%. The best result for number of fruits (34), fruit weight (470 gram) and plant height (78.4cm) after Redstone was displayed by 1288. Thus, it is concluded that the breeding line 1288 showed best result among all the germplasms evaluated and should be used in future to prevent significant losses of bacterial spot disease.

### Conclusions

In the light of present research work, it is concluded that the Bacterial spot disease is widespread in district Swat and severely affected tomato crop. In screening for disease resistance, the line 1288 exhibited more resistant among all the germplasms evaluated. 1288 was tallest, had heavy fruits and having more number of fruits per plant. The second resistant was commercial variety, Redstone and the then Rio-Grande. Further, most of the lines were moderately resistant to susceptible.

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