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Prevalence of Bacterial Leaf Spot of Bottle Gourd and Pumpkin in Subtropical Zone of Himachal Pradesh

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Authors' contributions

This work was carried out in collaboration among all authors. Author KJ designed the studies and conducted survey during 2018 and 2019. She also finalized this manuscript. Author SK performed all experiments including survey and scanned the literature. Authors RSJ, SJ and DS helped author KJ in designing the experiments. Author RSJ also conducted survey with KJ and SK. All the authors collaboratively helped in finalizing the manuscript.

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ABSTRACT

To assess the prevalence and severity of bacterial leaf spot on bottle gourd and pumpkin, a survey was conducted in Hamirpur, Una and Bilaspur districts of sub tropical zone of Himachal Pradesh, India during the years 2018 and 2019. Data were recorded in terms of disease severity and fruit rot incidence. The associated pathogen from bottle gourd and pumpkin was isolated on nutrient sodium chloride agar medium and identified on the basis of morphological, biochemical and pathogenicity tests on bottle gourd and pumpkin seedlings. Disease was found to be prevalent at all the locations surveyed exhibiting a mean disease severity from 24.70 to 87.55 and 5.30 to 52.92

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per cent in bottle gourd and pumpkin, respectively. Fruits of bottle gourd were recorded to be affected badly exhibiting a mean fruit rot incidence of 10.23 to 95.32 to per cent, while, no fruit rot incidence was recorded in pumpkin fruits. The colonies of the isolated bacterium were mucoid, circular, smooth textured and yellow in colour having a diameter of 2-4 mm. The pathogen was found to be Gram–ve and tested positive for esculin hydrolysis as well as protein digestion test. During pathogenicity tests, incubation period of 2 and 4 days was recorded on bottle gourd and pumpkin, respectively. Based on these studies, the identity of the pathogen was confirmed to be *Xanthomonas cucurbitae*(ex Bryan) Vauterin *et al.*

Keywords: Xanthomonas cucurbitae; occurrence; pathogenicity; bacterial leaf spot; bottle gourd; pumpkin.

1. INTRODUCTION

Family cucurbitaceae is of high economic value being a major source of food for man [1]. During cultivation, various cucurbits are attacked by several pathogens including straminopiles,fungi, bacteria, viruses and nematodes. Out of these, *Xanthomonas cucurbitae* (ex. Bryan) Vauterin *et al.* (Syn.: *X. campestris* pv. *cucurbitae*) causing bacterial leaf spot is emerging as an important pathogen leading to huge crop losses especially to bottle gourd, pumpkin and squashes [2,3]. The disease was first described by Bryan in 1926 on 'Hubbard' squash in a garden in New York [4]. The pathogen was earlier identified as *X. campestris* pv. *cucurbitae*. The genus *Xanthomonas* belonging to the class Gammaproteobacteria and comprising of 27 species causes many economically important diseases in plants [5]. *Xanthomonas* was first described as *Bacterium vesicatorium* as a pathogen of pepper and tomato in 1921 [6]. Later it was reclassified as *Xanthomonas* [7]. Members of genus *Xanthomonas* are single celled, rod shaped, obligatory aerobic bacteria bearing a single polar flagellum. Most of the species of *Xanthomonas* (except *X. axonopodis* pv. *manihotis*) produce an exopolysaccharide known as "xanthan gum" on any sugar containing medium and appear as shiny yellow mucoid colonies [8]. Vauterin et al. [9], described the strains of *Xanthomonas* attacking cucurbits at a species level as *X. cucurbitae* (ex Bryan) Vauterin et al. on the basis of DNA hybridization studies, as these were sufficiently different from other species. The disease has been reported to cause significant losses in different cucurbits [2,9,10,11]. Keeping in view the importance of disease, it was thought worthwhile to study the prevalence of disease in sub tropical region of Himachal Pradesh and identify the pathogen associated with the disease.

2. MATERIALS AND METHODS

2.1 Survey

To record the occurrence and severity of bacterial leaf spot on bottle gourd and pumpkin, a survey was conducted in Hamirpur, Una and Bilaspur districts of sub tropical zone of Himachal Pradesh, India during the year 2018 and 2019. The data were recorded in terms of disease severity on plants and disease incidence on fruits.

The disease severity was recorded as per the scale given by Jarial et al. [2] and disease index was further calculated as per the formula given by Mc Kinney [12] as follows:

$$\text{Disease Index (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings x maximum disease grade}} \times 100$$

Disease incidence was recorded on the fruits as per the following formula:

$$\text{Disease Incidence (\%)} = \frac{\text{Number of rotten fruits}}{\text{Total number of fruits observed}} \times 100$$

2.2 Symptomatology

The symptoms of the disease were recorded both on bottle gourd and pumpkin and compared with the available literature.

2.3 Isolation and Purification of the Causal Organism

Infected leaves of bottle gourd and pumpkin exhibiting characteristics symptoms were brought to laboratory for isolation of the pathogen. The associated bacterium was isolated from the diseased samples on nutrient sodium chloride agar (NSA) medium by using standard procedures and isolated colonies of the associated bacterium were maintained on nutrient agar slants.

2.4 Identification of the Pathogen

The pathogen was identified on the basis of cultural and biochemical characters as suggested by Society of American Bacteriologists [13] and Schhad and Stall [14] which was further confirmed by pathogenicity tests and available literature.

2.4.1 Cultural characters

To study the cultural characters of the bacterium, a dilute cell suspension was prepared by suspending a loopful of 48 h old culture grown in 50 ml sterilized nutrient sodium chloride broth and plated (1 ml/plate) on NSA plates (90 mm diameter) after serial dilution upto a factor 10^{-7}. These plates were incubated at 30°C for 72 h and colony characters were recorded.

2.4.2 Biochemical tests

Following biochemical tests were performed to ascertain the identity of the pathogen.

2.4.2.1 Gram's staining

Gram staining was performed by the procedure given by Aneja [15]. Observation was taken under microscope to see the colour reaction.

2.4.2.2 Esculin hydrolysis test

Three Petri plates poured with sterilized Esculin agar for each isolate were streaked with 72 h old bacterial culture. These Petri plates were incubated at 30°C for 7 days to see the development of dark brown colour in the Petri plates and compared with the un-inoculated control.

2.4.2.3 Protein digestion test

Forty gram powdered skimmed milk was added to beaker containing 100 ml of distilled water and bromocresol purple (4 mg) was added to it so as to obtain the concentration of 0.004 per cent. Eight test tubes each containing 10 ml of this solution were subjected to fractional sterilization. Subsequently, the test tubes, each inoculated with a loopful of bacterial cells of both the isolates were incubated at 30°C for 10 days to observe the clearing reaction. Two un-inoculated test tubes one each for both the isolates containing the same solution were kept as check.

2.4.3 Pathogenicity test

2.4.3.1 Preparation of bacterial inoculums

One loopful of bacterial colonies from 72 h old culture of each isolate was inoculated in 50 ml of sterilized nutrient sodium chloride broth contained in 150 ml capacity Erlenmeyer flasks separately and shaken in orbital shaker for 5-10 minutes. Thereafter, 1 ml suspension from each flask was further inoculated into two different flasks containing 50 ml of sterilized nutrient sodium chloride broth. These flasks were incubated at 30°C for 48 h so as to get the inoculum concentration of approximately 10^8 colony forming units per millilitre (cfu/ml).

2.4.3.2 Inoculation and Pathogenicity

Pathogenicity of the isolated bacterium was proved by inoculating the bacterial suspension on the bottle gourd and pumpkin plants raised in pots. The leaves of both the plants were swabbed with spirit and washed off with distilled water for their surface sterilization. These were then inoculated by application of bacterial suspension (10^8 cfu/ml) at leaf margin in the morning hours. Inoculated plants were covered with polythene bags so as to maintain the desired relative humidity. The relative humidity was further maintained by frequent spray of sterile distilled water in the polythene bags. Simultaneously, uninoculated plants sprayed with equal volume of sterile water were kept as check. Leaves were observed periodically for appearance of symptoms. The pathogen was re-isolated and compared with the original culture.

3. RESULTS AND DISCUSSION

3.1 Survey

Bacterial leaf spot was found to be prevalent in moderate to severe form on both bottle gourd and pumpkin at various locations surveyed (Tables 1 & 2). In case of bottle gourd (Table 1), mean disease severity was recorded to be minimum (24.70%) at Dhundla (586.0 m amsl) in district Una and maximum (87.55%) at Haar (522.0 m amsl) in district Hamirpur. However, in case of pumpkin, (Table 2) minimum (5.30%) disease severity was recorded at Jalari (544.0 m amsl) and maximum (52.92%) at Haar in district Hamirpur. As far as disease incidence on fruits was concerned, it ranged between 10.23 per cent at Punchvati (522.0 m amsl) in district Hamirpur to 95.32 per cent at Chakmoh (775.0m amsl) in the same district in case of bottle gourd. However, in pumpkin, no incidence of disease was recorded on fruits at any of the locations surveyed except at Neri (630.0 m amsl) during 2018 cropping season where only 2.00 per cent fruits were recorded to be infected with the disease. In general, mean disease severity as well as disease incidence was higher during 2018 crop season as compared to 2019 crop season in both the crops under study. However, on an average disease severity (56.60% and 24.47% in bottle gourd and pumpkin, respectively) as well as incidence (69.76% and

Table 1. Occurrence and magnitude of bacterial leaf spot of bottle gourd in the sub-tropical zone of Himachal Pradesh

Location	Approx. Elevation (m) above mean sea level	Mean disease severity (%) per vine			Mean disease incidence (%) on fruits		
		2018	2019	Mean	2018	2019	Mean
Hamirpur							
Salauni	976.0	64.20	60.45	62.33	80.23	78.62	79.43
Karer	903.0	50.20	46.40	48.30	60.17	62.84	61.51
Bijhari	870.0	68.20	65.00	66.60	80.58	70.39	75.45
Chamned	868.0	51.50	45.20	48.35	100.00	75.47	87.74
Ukhali	858.0	58.20	50.00	54.10	80.62	65.35	72.99
Lambloo	812.0	54.15	58.40	56.28	80.33	60.24	70.29
Bohani	810.0	51.55	44.40	47.98	100.00	80.66	90.33
Chakmoh	775.0	57.75	49.15	53.45	100.00	90.63	95.32
Bhota	772.0	77.15	65.00	71.08	100.00	87.72	93.86
Kangoo	648.0	44.00	38.00	41.00	80.59	54.85	67.72
Neri	630.0	71.45	78.20	74.83	100.00	55.49	77.75
Lathiani	576.0	29.50	35.70	32.60	0.00	43.87	21.94
Jalari	544.0	47.00	48.25	47.63	40.95	22.36	31.66
Haar	522.0	98.20	76.90	87.55	100.00	83.59	91.80
Punchvati	522.0	49.20	50.00	49.60	20.46	0.00	10.23
Modhun	519.0	64.40	63.50	63.95	100.00	76.18	88.09
Mean		58.54	54.65	56.60	76.49	63.02	69.76
Una							
Thana Kalan	598.0	46.30	48.30	47.30	75.38	50.29	62.84
Bangana	590.0	49.30	43.00	46.15	60.71	42.18	51.45
Dhundla	586.0	21.10	28.30	24.70	40.35	43.41	41.88
Tanoh	569.0	51.20	42.80	47.00	40.69	23.96	32.32
Una	385.0	48.70	45.35	47.03	60.16	48.22	54.19
Mean		43.32	41.55	42.44	55.46	41.61	48.54
Bilaspur							
Berthin	664.0	50.40	44.85	47.63	40.23	71.65	55.94
Shah-Talai	615.0	46.55	50.70	48.63	100.00	83.78	91.89
Ghumarwin	610.0	44.10	43.50	43.80	60.37	52.12	56.25
Mean		47.02	46.35	46.69	66.87	69.18	68.02
Overall Mean		49.62	47.51	48.57	66.27	57.94	62.11

Table 2. Occurrence and magnitude of bacterial leaf spot of pumpkin in the sub-tropical zone of Himachal Pradesh

Location	Approx. Elevation (m) above mean sea level	Mean Disease severity (%) per vine			Mean Disease incidence (%) on fruits		
		2018	2019	Mean	2018	2019	Mean
Hamirpur							
Salauni	976.0	30.00	28.65	29.33	-	-	-
Karer	903.0	30.00	20.47	25.24	-	-	-
Bijhari	870.0	25.00	18.35	21.68	-	-	-
Chamned	868.0	20.00	19.69	19.85	-	-	-
Ukhali	858.0	15.20	18.43	16.82	-	-	-
Lambloo	812.0	12.25	5.75	9.00	-	-	-
Bohani	810.0	11.40	13.5	12.45	-	-	-
Chakmoh	775.0	50.4	40.34	45.37	-	-	-
Bhota	772.0	35.00	30.25	32.63	-	-	-
Kangoo	648.0	28.00	25.36	26.68	-	-	-
Neri	630.0	21.20	25.59	23.40	2.00	-	1.00
Lathiani	576.0	5.70	8.69	7.20	-	-	-
Jalari	544.0	6.35	4.25	5.30	-	-	-
Haar	522.0	56.90	48.93	52.92	-	-	-
Punchvati	522.0	10.93	12.54	11.74	-	-	-
Modhun	519.0	43.50	20.58	32.04	-	-	-
Mean		25.11	23.83	24.47	0.13	-	0.07
Una							
Thana kalan	598.0	13.00	15.12	14.06	-	-	-
Bangana	590.0	13.30	10.38	11.84	-	-	-
Dhundla	586.0	18.30	15.61	16.96	-	-	-
Tanoh	569.0	15.35	10.27	12.81	-	-	-
Una	385.0	28.80	20.54	24.67	-	-	-
Mean		17.75	14.38	16.06	-	-	-
Bilaspur							
Berthin	664.0	30.70	25.95	28.33	-	-	-
Shah-Talai	615.0	24.85	28.50	26.68	-	-	-
Ghumarwin	610.0	13.50	10.25	11.88	-	-	-
Mean		23.02	21.57	22.30	-	-	-
Overall Mean		21.96	19.93	20.95	0.04	-	0.02

- No incidence was recorded on fruits

0.07% in bottle gourd and pumpkin, respectively) was recorded in district Hamirpur in both the years.

The disease was found to be prevalent in moderate to severe form on both bottle gourd and pumpkin at different locations surveyed in Himachal Pradesh. The findings were in accordance with Jarial et al. [2] who conducted survey in 64 locations of five districts of Himachal Pradesh and reported the variation of disease severity in bottle gourd from 12.50 to 78.33 per cent causing around 10.07 to 70.61 per cent yield losses at various locations surveyed. These findings are further supported by Sharma [16] who conducted a survey in 27 locations of three districts of Jammu and Kashmir and reported that the mean disease severity ranged from 14.5 to 33.4 per cent at different locations surveyed.

3.2 Symptomatology

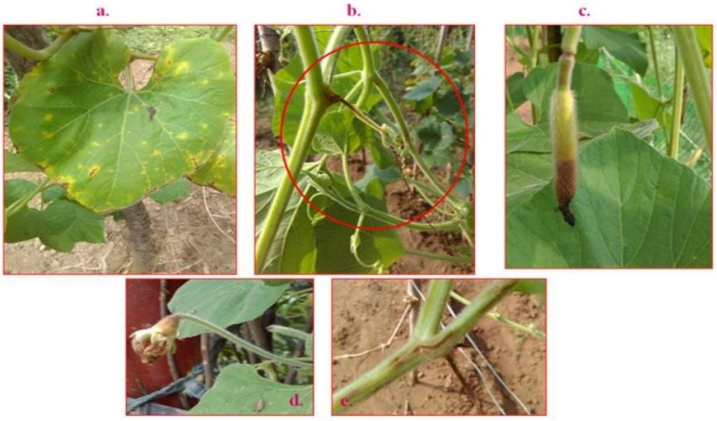
Symptoms of the disease were recorded on both bottle gourd and pumpkin. On bottle gourd as well as pumpkin, symptoms were observed as small marginal chlorotic spots which increased in size towards the centre of the leaves. Later, these chlorotic areas converted in to necrotic spots, increased in size, merged with each other, occupied almost complete leaf lamina and formed sites with dead brown tissues. On young fruits of bottle gourd, the symptoms appeared as

water soaked spots which developed into a rot finally leading to decaying of the fruit whereas on the fruits of pumpkin, the symptoms developed rarely as water soaked spots from styler end of the fruit ultimately leading to rotting of the fruits (Plate 1). These symptoms typically resembled with those described by Jarial et al. [2], Babadoost and Ravanlou [3] and Altin et al. [17].

3.3 Identification of the Pathogen

The colonies of the pathogen were mucoid, circular, smooth textured and yellow in colour having a diameter of 2-4 mm (Plate 2). The pathogen was found to be Gram–ve and tested positive for esculin hydrolysis and protein digestion tests (Plates 3 & 4). These tests were in confirmity with the characters documented by Society of American Bacteriologists [13] and Schaad and Stall [14] for genus *Xanthomonas*. The findings were also in accordance with many researchers who worked on *X. cucurbitae* and reported that the bacterium produced yellow, mucoid and smooth colonies and was positive for esculin hydrolysis as well as protein digestion tests and Gram–ve [16,17,18,19,20,21]. Based on these cultural and biochemical characters, the identity of the pathogen was confirmed to be *Xanthomonas* sp.

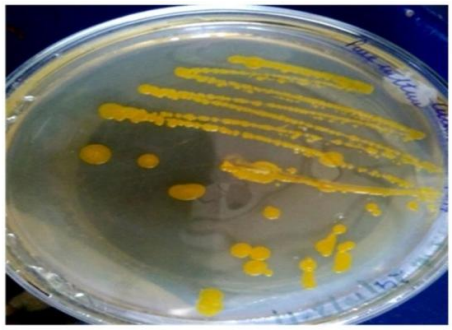
Incubation period of 2 and 4 days was recorded during the pathogenicity tests conducted on bottle gourd and pumpkin, respectively (Table 3). The pathogen was successfully re-isolated from these artificially inoculated plants thus proving the Koch's postulates. These results are in accordance with Jarial et al. [2] who reported an incubation period of 3 to 5 days on the bottle gourd and pumpkin plants during pathogenicity tests. These results are further supported by the findings of Zhang and Babadoost [22] who reported that bacterial lesions appeared on inoculated leaves in pumpkin cultivars after 3 and 4 days of incubation during pathogenicity tests.



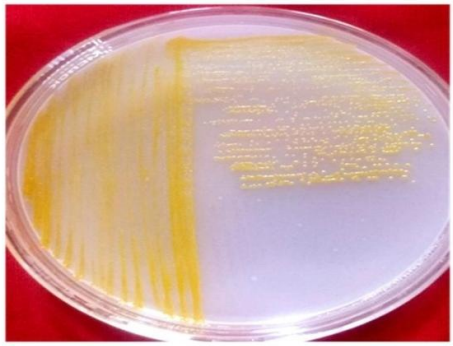
Symptoms observed on different plant parts on bottle gourd (a. on leaves, b. on tendrils, c. on fruit and d. on flower e. on stem)

Symptoms observed on different plant parts on pumpkin (a. on leaves, b. symptoms on tendrils, c. on fruit and d. on stem)

PLATE 1

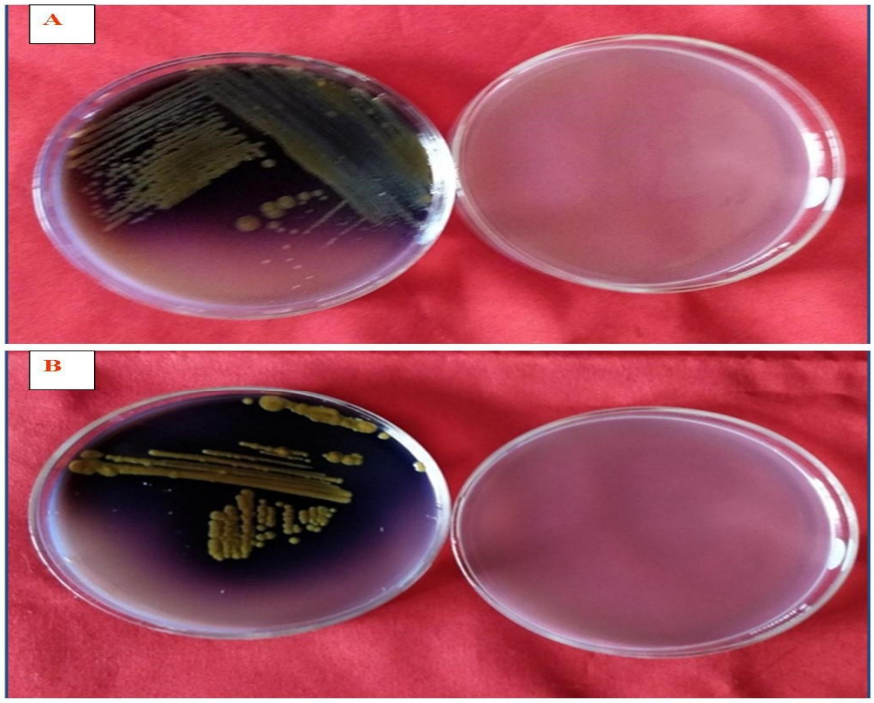


(i). Bottle gourd isolate

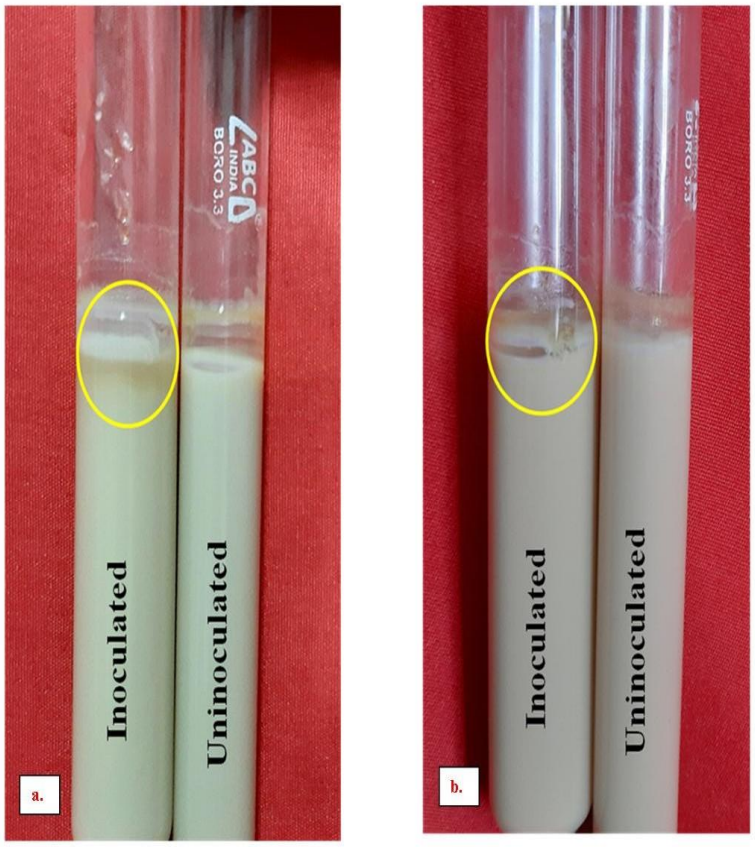


(ii). Pumpkin isolate

PLATE 2: PURE CULTURE OF *Xanthomonas cucurbitae*



Esculin hydrolysis test (A. Bottle gourd isolate
B. Pumpkin isolate)
PLATE 3



Protein digestion test showing the positive result as appearance of protein digestion ring in the inoculated test tubes for both the isolates (a.) bottle gourd isolate and (b.) pumpkin isolate

PLATE 4

Table 3. Incubation period and symptoms observed during pathogenicity test

Isolates	Incubation period (in days)	Symptoms developed
Bottle gourd	2	Symptoms developed as marginal brown spots on the leaves.
Pumpkin	4	V-shaped marginal spots on the leaves which invaginate towards the mid vein.

Based on symptomatology, cultural, biochemical and pathogenecity tests as well as scanning of available literature, the pathogen associated with the disease was identified as Xanthomonas cucurbitae (Ex Bryan) Vauterin et al.

4. CONCLUSION

Bacterial leaf spot was found to be prevalent in moderate to severe form on both bottle gourd and pumpkin at various locations surveyed in Himachal Pradesh. Typical symptoms of bacterial leaf spot were observed in both the hosts. The isolated bacterium produced yellow, mucoid colonies, tested negative for Gram's stain and positive for esculin hydrolysis and protein digestion tests. The isolated bacterium successfully produced symptoms on artificially inoculated bottle gourd and pumpkin plants within 2-4 days and the bacterium was successfully re-isolated from the inoculated plants. On the basis of symptomatology, culture, biochemical and pathogenecity tests as well as scanning of available literature, the pathogen

associated with the disease was identified as *Xanthomonas cucurbitae* (Ex Bryan) Vauterin *et al.*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ajuru M, Nmom F. A review on the economic uses of species of cucurbitaceae and their sustainability in Nigeria. Am J Plant Biol. 2017;2(1):17-24.
2. Jarial K, Dogra BS, Mandradia RK, Kumar S, Sharma D, Gupta AK. Investigations on a new bacterial disease of bottle gourd in sub-tropical zone of Himachal Pradesh. Plant Dis Res. 2011;26(1):68-75.
3. Babadoost M, Ravanlou A. Outbreak of Bacterial Spot (*Xanthomonas cucurbitae*) in Pumpkin Fields in Illinois. Plant Dis. 2012;96:1222.
4. Bryan MK. Bacterial leaf spot of squash. J. Agric. Res. 1930; 40(4):385-391.
5. Ryan RP, Vorhalter FJ, Potnis N, Jones JB, Van Sluys M, Bogdanove AJ, Maxwell J. Pathogenomic of *Xanthomonas*: understanding bacterium-plant interaction. Annu. Rev. Phytopathol. 2011;9:344-436.
6. Doidge EM. A tomato canker. Ann App Biol. 1921;7(4):407–30.
7. Dowson WJ. On the systematic position and generic names of the Gram-negative bacterial plant pathogens. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1939; 100:177–193.
8. Kado CI, St Paul MN. Plant Bacteriology. The American Phytopathological Society. 2010;336.
9. Vauterin L, Hoste B, Kersters K, Swings J. Reclassification of *Xanthomonas*. Int J Syst Bacteriol. 1995;45:472-489.
10. Larazev AM. Diseases: *Xanthomonas campestris* pv.*cucurbitae* (Bryan) dye-bacterial leaf spot of cucurbits in: 2003-2009 Project "Interactive agricultural ecological atlas of Russia and neighbouring countries: Economic plants and their diseases, pests and weeds". 2009;
Accessed 17th October, 2020.
Available:http://www.agroatlas.ru/en/content/diseases/Cucurbitae/Cucurbitae_Xanthomonas_campestris_pv_cucurbitae/index.html
11. Salamanca LR. Bacterial diseases of pumpkins: An old enemy and an emerging bacterial disease. Michigan State University Extension Bulletin; 2014. Accessed 15th July, 2019.
Available:https://www.canr.msu.edu
12. McKinney HH. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum.* J Agric Res. 1923;26:195-217.
13. Society of American Bacteriologists. Mannual of microbiological methods. New York, McGraw Hill Book Company. 1957;315.
14. Schhad NW, Stall RE. *Xanthomonas* in: Laboratory guide for identification of plant pathogenic bacteria by N.W. Schaad (ed.). American Pythopathogical *Society*, St. Paul, Minnesota. 1988;81-94.
15. Aneja KR. Experiments in microbiology, Plant pathology and biotechnology. 4th revised edition. New Age International (P) Limited, Publishers. 2015;607.
16. Sharma RR. Integrated management of bacterial spot of bottle gourd caused By *Xanthomonas cucurbitae*. M.Sc. Thesis. Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu main campus, Chatha, Jammu-180009 INDIA. 2016;81.
17. Altin I, Casoli L, Stefani E. First report of bacterial spot caused by *Xanthomonas cucurbitae* on pumpkin in Italy. New Dis Rep. 2020;41:21.
Available: http://dx.doi.org/10.5197/j.2044-0588.2020.041.021
18. Lamichhane JR, Varvaro L, Balestra GM. Bacterial leaf spot caused by *Xanthomonas cucurbitae* reported on pumpkin in Nepal. New Dis. Rep. 2010; 22:20.
19. Dutta B, Gitaitis RD, Lewis, KJ, Langston DB. A new report of *Xanthomonas cucurbitae* causing bacterial leaf spot of watermelon in Georgia, USA. Plant Dis. 2013;97:556.

20. Ravanlou A, Babadoost M. Development of bacterial spot, Incited by *Xanthomonas cucurbitae*, in Pumpkin Fields. Hort. Sci. 2015;50(5):714-720.
21. Kumari S, Jarial K, Jarial RS, Jandaik S. Cultural parameters of *Xanthomonas cucurbitae* causing bacterial leaf spot of bottle gourd and pumpkin. Int J Biores Str Manage. 2020;11(3):214-218.
22. Zhang X, Babadoost M. Characteristics of *Xanthomonas cucurbitae* isolates from pumpkins and survival of the bacterium in pumpkin seeds. Plant Dis. 2018;102(9): 1779-1784.

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