



Research Paper

## Prevalence and characterization of *Xanthomonas axonopodis* pv. *cyamopsidis* causing bacterial blight of clusterbean in the semi-arid region of North Gujarat

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Received: 12 April 2024

Accepted: 13 June 2024

Handling Editor:

Dr. PR Sabale, ICAR-Indian Institute of  
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### ABSTRACT

The bacterial blight caused by *Xanthomonas axonopodis* pv. *cyamopsidis* is the most widespread and destructive disease of clusterbean. The present study aimed to survey the major clusterbean growing areas, expanding in five districts i.e., Banaskantha, Patan, Sabarkantha, Mehsana and Aravali of North Gujarat. The study found that bacterial blight disease is prevalent in Idar, Ambaji, Danta, Sanali, Khedbramha, Shamalaji, Patan, Bhiloda, Visnagar, Deesa and Amirgadhd. The highest mean disease incidence (28.44%) was recorded in Idar, Sabarkantha. Based on the gram straining it was observed that the test bacterium was gram-negative and rod-shaped. Further, the biochemical tests, such as starch hydrolysis, gelatin liquefaction, hydrogen sulphide production, catalase, and acid production from different sugars viz., sucrose, maltose, and dextrose showed a positive reaction, except, acid production from mannitol was negative. Based on the morphological, biochemical, and pathogenic characteristics, the pathogen was identified as *Xanthomonas axonopodis* pv. *cyamopsidis*.

**Key words:** Bacterial blight, Cluster bean, Biochemical characterization, Disease survey, *Xanthomonas axonopodis* pv. *cyamopsidis*.

### INTRODUCTION

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] commonly known as 'guar' is an important legume and vegetable crop belonging to the family *Fabaceae* (Kumar and Rodge 2012). It is a major agricultural crop with great social and economic value. It is widely grown in arid and semi-arid regions for multi-purpose uses such as vegetables, green manure, guar-gum production, fodder, medicine, industrial and religious purposes (Patel *et al.* 2010, Choudhary *et al.* 2014). Interestingly, India is the largest producer of clusterbean in the world and it contributes about 75 to 80 percent to the global market (Dhaka *et al.* 2019). Vavilov (1951) suggested India as the geographic center of variability for clusterbean. Rajasthan is the country's leading producer of guar gum and seeds, making up between 70 and 75 percent of India's total production (Mishra 2008, Shabarishrai *et al.* 2012). Gujarat, Haryana, and Punjab are also known as guar-producing states in India (Bhatt *et al.* 2017). The changing climatic scenario with a capricious rainfall distribution pattern leads to the attack of several pathogens, like *Xanthomonas axonopodis* pv.

*cyamopsidis* which has culminated in heavy crop losses. The pathogen becomes a major constraint for the cultivation of clusterbeans. Initially, it was reported for the first time in 1953 from Patna (Bihar), Khopoli, and Bombay (Maharashtra) (Patel *et al.* 1953). Reclassification of *Xanthomonas axonopodis* pv. *cyamopsidis* was done by Vauterin *et al.* (1995).

The bacterial blight of clusterbean occurs almost every year and mostly during monsoon season. The pathogen attacks all the above-ground parts and sometimes, severe infection results in the death of well-established plants (Srivastava and Rao 1963, Sain and Gour 2009). Initially, symptoms appear as water-soaked spots on leaves. In the later stage of disease development, inter-veinal necrosis on leaves and black streaks on petioles, stems and pods with stunted plant growth are quite evident in the field (Ren *et al.* 2014), which is more confusing with the infection and symptomatology of *Tobacco streak virus* in clusterbean (Sivaprasad *et al.* 2012). The bacterial blight pathogen is internally seed-borne and it acts as the primary source of inoculum for secondary spread (Lodha 2001). Cluster bean suffers from bacterial blight disease which is responsible for

50 to 70 percent yield loss depending on the disease severity (Bagri *et al.* 2019). Because of the destructive nature of the pathogen and drastic change in the climatic situation, the severity of the bacterial blight increases and will be the major limiting factor for achieving the desirable production of clusterbean. Thus, a study was carried out to predict the present status of the bacterial blight disease and identify the disease etiology for better crop management.

## MATERIALS AND METHODS

### *Disease survey*

The survey of major clusterbean growing areas in five different districts *i.e.*, Banaskantha, Patan, Sabarkantha, Mehsana and Aravali of North Gujarat was undertaken to record the bacterial blight incidence during *Kharif* season over three years from 2019-2021. From each district, observations for bacterial blight incidence on cluster bean crops were recorded from randomly selected locations, and in each location, 5 fields were selected randomly. Infected plants were diagnosed from an area of 1m×1m marked diagonally across the field at five random spots. The total number of plants and the number of plants showing the typical symptoms of bacterial blight were recorded. The first symptoms observed at the seedling stage of the crop as a water-soaked spots on leaves. As the infection progressed, the colour of the spots became dark and necrotic; gradually reaching up to the stems and pods. In the later stage, the large, angular necrotic lesions and black streaks are observed on stems and defoliation occurs along with a split stem that results in marginal necrosis (Fig. 1). The percent disease incidence (PDI) was calculated by using the following equation,

$$PDI = \frac{\text{Total number of plants infected in field}}{\text{Total number of plants examined in field}} \times 100$$

### *Collection and isolation of disease samples*

Clusterbean plants that had typical symptoms of bacterial blight in leaves, pods, and stems were collected from Pulses Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The infected samples were brought to the laboratory and subjected to isolation. The causal agent was isolated from infected parts by extracting the ooze in sterile distilled water taken in a test tube followed by a dilution plate technique on nutrient agar media under aseptic conditions. After the incubation period, well-defined light yellow, small bacterial

colonies were selected for purification of the pathogen. The suspected bacterial colonies were picked up with the help of a sterilized inoculating loop and streaked onto the surface of nutrient agar media. The inoculated plates were incubated for 72 hours at  $28 \pm 2^\circ\text{C}$  and after the incubation period, the development of well-separated light yellow, small bacterial colonies were observed. The pure colonies were streaked onto slants containing the nutrient agar media and incubated for 72 hours at  $28 \pm 2^\circ\text{C}$ . After that, pure bacterial culture was stored in the refrigerator at  $5^\circ\text{C}$ , which served as a stock culture. After that, the biochemical characteristics such as hydrolysis of starch, gelatin liquefaction, hydrogen sulphide production, catalase and acid production from different sugars *viz.*, sucrose, maltose, dextrose and mannitol were studied.

### *Biochemical characters*

The biochemical characteristics of a pathogen such as hydrolysis of starch, gelatin liquefaction, hydrogen sulphide production, catalase and acid production from different sugars *viz.*, sucrose, maltose, dextrose and mannitol were studied as per the methods described by Salle (1961) and Schaad (1992).

### *Gram staining reaction*

A loop full of the bacteria was smeared on a clean glass slide, air fixed by gentle heating on the flame of the spirit lamp. Aqueous crystal violet solution (0.5%) was spread over this smear for 30 seconds and then rinsed with a gentle stream of running tap water for a minute. This stained smear was later flooded with Gram's iodine solution for a minute and rinsed in tap water. Later decolorized with 95 percent ethanol until colour runoff, rinsed gently with water and treated with safranin as a counterstain for 10 seconds, again rinsed in tap water and got dried using air/blotting paper. The result was observed under a research microscope at 100X by using oil immersion.

### *Gelatin liquefaction*

The nutrient gelatin media was prepared using the proportion of peptone (10.0 g), beef extract (5.0 g), gelatin (20.0 g), and agar (10.0 g). After mixing all the ingredients, one litre of media was prepared by adding distilled water, and pH was adjusted to 7.0. The media was heated over a water bath until the gelatin was dissolved. After that media was sterilized for 15 minutes at  $121^\circ\text{C}$  temperature

and 15 p.s.i. pressure. Once the temperature was reduced, media was poured into the Petri plates and cooled down until it became solid. Then media was spot inoculated with 48 hours old bacterial culture and plates were incubated for 48 hours at  $28 \pm 2^\circ\text{C}$ . After the incubation period, the Petri plates were flooded with 'acidic mercuric chloride' solution for 5 to 10 minutes and the excess solution was removed. On completion of the above process, if a clear zone around the colonies appeared, indicated the positive result for proteolytic hydrolysis of gelatin by the enzyme gelatinase.

#### **Catalase tests**

A colony from pure culture was smeared on a slide using a sterilized loop and covered it with one drop of 20 percent hydrogen peroxide. Within a minute, gas bubbles appeared on a slide that indicated the positive result of the reaction.

#### **Hydrogen sulphide ( $\text{H}_2\text{S}$ ) production**

The peptone water medium was prepared using the proportion of 10 g peptone, 5.0 g NaCl, 1 litre distilled water, and pH adjusted to 7.0. After that media was dispensed in 5 ml quantity in each test tube and autoclaved for 15 minutes at  $121^\circ\text{C}$  temperature and 15 p.s.i. pressure. Lead acetate test strips used to detect  $\text{H}_2\text{S}$  were prepared using the following method. Filter paper (Whatman No. 42) was cut into  $5 \times 50$  mm strips which were soaked in a saturated solution of lead acetate. Once the strips dried, they were autoclaved for 15 minutes at  $121^\circ\text{C}$  temperature and 15 p.s.i. pressures. After autoclaving, the strips were dried in the oven at  $60^\circ\text{C}$ . In each test tube media was inoculated with a loop full of 48 hours old bacterial culture and the test strips were hung in such a way that they did not touch the broth. The test tubes were incubated at  $28 \pm 2^\circ\text{C}$  temperature and observations were recorded at regular intervals, up to 14 days. Black coloured reaction on the test strips confirmed the liberation of  $\text{H}_2\text{S}$ .

#### **Starch hydrolysis**

The media was prepared by adding 0.2 percent soluble starch into a nutrient agar medium, and pH was adjusted to 7.0. The media was sterilized for 15 minutes at  $121^\circ\text{C}$  temperature and 15 p.s.i. pressure. Once the temperature reduced, media was poured into the Petri plates and cooled down until it became solid. Then the plates were inoculated with 48 hours old bacterial culture and incubated for 48

hours at  $28 \pm 2^\circ\text{C}$ . After the incubation period, Petri plates were flooded with 'Lugol's iodine' solution to react. On completion of the above process, a clear zone around the colonies appeared that indicated the positive result for the hydrolysis of starch by the action of amylases.

#### **Acid production from Sucrose, Maltose, Dextrose and Mannitol**

The acid production by the pathogen was tested using the basal media of Dye (1962). For that, 10 ml of medium was dispensed in each test tube and sterilized in an autoclave for 15 minutes at  $121^\circ\text{C}$  temperature for 15 p.s.i. pressure. After that filter-sterilized carbohydrates *viz.*, sucrose, maltose, dextrose, and mannitol were added in each tube at 0.1 per cent concentration. The tubes were inoculated with 0.1 ml of 24-hour-old bacterial culture and incubated for three days at room temperature. The change in the colour of a media confirmed the acid production by *Xanthomonas*.

#### **Urease test**

A urease test is used to check the ability of an organism to produce a urease by hydrolyzing urea into ammonia and carbon dioxide. The media turns alkaline due to the accumulation of ammonia resulting in a colour change from yellow to bright red or pink. For this bacterial pathogen, the basal media of Dye (1962) was dispensed 9 ml in each test tube and autoclaved. Once the temperature of the media was reduced to  $50^\circ\text{C}$ , 1 ml of filter-sterilized 20 percent urea solution was added into each test tube and mixed well. Also, the control test tube was kept without adding urea. For solidification of the media, test tubes were placed in a slanting position. The test tubes were inoculated with 24 hours-old culture and incubated at a temperature of  $28 \pm 2^\circ\text{C}$ . Pertaining observations were recorded at regular intervals up to 14 days. The change in the colour of media from yellow to bright red/pink confirmed the urease production by *Xanthomonas*.

#### **Oxidase test**

A tetramethyl-p-phenylenediamine dihydrochloride was used as the substrate and it was saturated onto a filter paper disc. After that, a loopful of culture was inoculated onto a filter paper. The observation was made based on the change in the colour of the paper from deep blue to purple in 20-30 seconds.

### Pathogenicity test

The pathogenicity test was carried out to confirm whether the isolated bacteria are capable of producing typical symptoms of bacterial blight on clusterbean under artificial inoculation conditions. Cluster bean plants were raised in a steam-sterilized potting mixture with soil, sand, and FYM in 3:1:1 ratio. The isolated bacteria multiplied in nutrient broth taken in Erlenmeyer's flask by inoculating a loopful of bacteria from a pure culture. The inoculated flask was incubated for 72 hours at  $28 \pm 2^\circ\text{C}$ . The bacterial suspension was prepared by adjusting cell concentration to  $5 \times 10^6$  cfu/ml using a spectrophotometer at 420 nm (O.D.=0.5). Twenty to twenty-five days old cluster bean plants were pre-incubated for 24 hours in the humid tent made up of plastic sheets in which humidity was maintained between 60 to 80 percent before the inoculation. After that, the leaves of cluster bean plants were slightly injured with sterilized pins and the bacterial suspension was sprayed on injured leaf surfaces and stems with a low-pressure sprayer. The inoculated plants were kept in the plastic tent in which high humidity was maintained by spraying sterilized water inside the tent for five days. The plants were taken out from the plastic tent and kept in the greenhouse at  $25$  to  $30^\circ\text{C}$ . Observations were made for the development of symptoms of bacterial blight. Similarly, injured plants sprayed with only sterilized water were kept to serve as control. After the development of the typical symptoms of the disease, the pathogen was reisolated and compared with the original culture to prove the pathogenicity.

## RESULTS AND DISCUSSION

### Disease survey

A roving survey was conducted in major clusterbean growing areas of 5 districts of North Gujarat during *Kharif* season over three years (2019-2021) and found that the problem of bacterial blight disease is highly serious mainly in Idar, Ambaji, Danta, Sanali, Khedbramha, Shamalaji, Patan, Bhiloda, Visnagar, Deesa and Amirgadh. The highest mean disease incidence (28.44%) was recorded in Idar, Sabarkantha (Table 1). The mean disease incidence was 21.36 percent during *Kharif* 2019. However, the clusterbean grown at Idar, Sabarkantha were found to be affected more severely with the maximum mean disease incidence (28.67%) and lowest mean disease incidence 15.20% recorded at Siddhpur, Patan during *Kharif* 2019. During the *Kharif* 2020, the mean disease incidence was 22.16

percent. The maximum disease incidence of 29.33% was observed at Ambaji, Banaskantha, and the lowest mean disease incidence 16.33% was recorded at Vijapur, Mahesana. In the season *Kharif* 2021, 23.11 percent mean disease incidence was observed. Where, the maximum mean disease incidence of 30.00% was observed at Idar, Sabarkantha and the minimum disease incidence of 15.66% was observed at Vijapur, Mahesana.

The survey was carried out at six different locations in Banaskantha district *viz.*, Deesa, Amirgadh, Ambaji, Danta, Palanpur and Sanali. On average per cent disease incidence 21.98, 23.33 and 24.94 were observed in *Kharif* 2019, *Kharif* 2020 and *Kharif* 2021, respectively. The pooled data revealed that the highest disease incidence 27.56 percent was recorded from Ambaji followed by Danta (26.67%), Sanali (25.78%), Deesa (22.19%), Amirgadh (20.44%) and Palanpur (19.19%).

A survey on disease incidence was conducted at three locations in Patan district *viz.*, Radhanpur, Patan city and Siddhpur. An average per cent disease incidence of 18.40, 21.00 and 21.40 were observed in *Kharif* 2019, *Kharif* 2020 and *Kharif* 2021, respectively. The highest disease incidence (22.67%) was recorded from Patan city followed by Radhanpur (20.32%) and Siddhpur (18.40%).

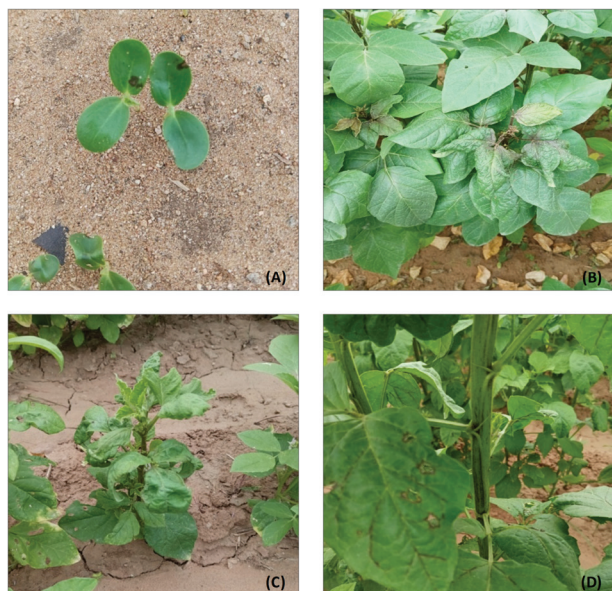
Three different locations in Sabarkantha district *viz.*, Idar, Khedbramha and Bhiloda. An average disease incidence of 25.33%, 23.56% and 26.67% were observed in *Kharif* 2019, *Kharif* 2020 and *Kharif* 2021, respectively. The highest disease incidence (27.11%) was recorded from Idar followed by Khedbramha (25.33 %) and Bhiloda (21.78%).

In Mahesana district, the survey was conducted in four different locations *viz.*, Unjha, Unava, Visnagar and Vijapur. A mean per cent disease incidence of 19.33, 19.42 and 18.75 were observed in *Kharif* 2019, *Kharif* 2020 and *Kharif* 2021, respectively. The highest disease incidence was recorded from Visnagar (20.89%) followed by 19.37% at Unjha, 18.89% at Unava and 17.33% at Vijapur. Only one location *viz.*, Shamalaji was surveyed from the Aravali district and 24 percent of disease incidence was recorded.

The results were consistent with the earlier study published by Chakravarth *et al.* (2005), who reported 30.00 to 40.00 percent disease after surveying clusterbean growing areas of Karnataka during the *Kharif* 2002 and 2003. Likewise, Sandipan *et al.* (2017) surveyed the districts of Surat, Bharuch, and Narmada; they found that the percent incidence

**Table 1.** Survey of bacterial blight disease of cluster bean in five districts of North Gujarat during *Kharif* season from 2019- to 2021.

Sr. No.	District	Locations	Longitude (°N)	Latitude (°E)	Percent bacterial blight disease incidence			Mean PD (%)
					<i>Kharif</i> 2019	<i>Kharif</i> 2020	<i>Kharif</i> 2021	
1	Banas-kantha	Deesa	24.2610	72.1858	18.67	20.00	24.00	20.89
		Amirgadh	24.4070	72.6402	17.33	21.33	22.67	20.44
		Ambaji	24.3310	72.8517	26.67	29.33	26.67	27.56
		Danta	24.1930	72.7659	28.00	25.33	26.67	26.67
		Palanpur	24.1724	72.4346	17.24	18.67	21.67	19.19
2	Patan	Sanali	20.0648	79.8017	24.00	25.33	28.00	25.78
		Radhanpur	23.8286	71.6139	18.67	21.33	19.21	19.74
		Patan	23.8500	72.1210	21.33	23.00	23.67	22.67
3	Sabar-kantha	Siddhpur	23.9309	72.3621	15.20	18.67	21.33	18.40
		Idar	23.8395	73.0006	28.67	26.67	30.00	28.44
		Khedbramha	24.0291	73.0435	25.33	24.00	26.67	25.33
4	Mehsana	Bhiloda	23.7633	73.2441	24.00	20.00	21.33	21.78
		Unjha	23.8035	72.3902	17.33	21.33	20.00	19.56
		Unava	23.7630	72.3637	18.67	17.33	20.67	18.89
		Visnagar	23.7024	72.5426	21.33	22.67	18.67	20.89
5	Aravali	Vijapur	23.5609	72.7511	20.00	16.33	15.66	17.33
		Shamalaji	23.6875	73.3863	22.67	25.33	24.00	24.00
		General mean			21.36	22.16	23.11	22.21



**Fig. 1.** Symptomatology of bacterial blight in cluster bean. (A) Initially, water-soaked, necrotic spots appeared on cotyledonary leaves at the seedling stage. (B) Necrotic brown leaves and buds podvisualized in the severely infected plants. (C) It results in severe stunting with very less flowers and pods. (D) In mid-infected plants, the veinal necrosis of the leaves with black necrotic streaks on the stems is very common.

of bacterial leaf blight ranged from 0.0 to 5.5% in Surat, 0.0 to 3.0% in Bharuch and 0.0 to 24.0 percent in Narmada district. Similarly, Isameldin *et al.* (2018)

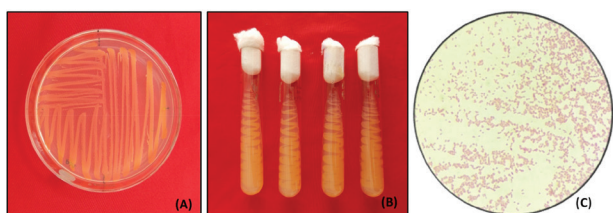
surveyed clusterbean-producing areas grown under rain-fed and irrigated and found that the disease incidence was higher in rain-fed conditions than in irrigated areas. It indicates the significance of rainfall with increasing incidence of bacterial blight in clusterbean under changing climatic scenarios.

#### *Collection and isolation of disease samples*

Clusterbean plants that had typical symptoms of bacterial blight with small, round, water-soaked spots on leaves and blackening or cracking on the stem were collected from Pulse Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The infected samples with typical symptoms were brought to the laboratory and subjected to isolation. The causal agent was isolated from infected samples such as leaves of clusterbean plants by extracting the ooze in sterile distilled water taken in a test tube followed by a dilution plate technique on nutrient agar media under aseptic conditions. After the incubation period, well-separated light yellow, small bacterial colonies (Figure 2) were obtained and further biochemical characterization was done.

#### *Gram staining reaction*

After the Gram staining test, the stained bacterium was observed in 100X using oil immersion lens, and observed that the test bacterium did not retain the violet colour of the primary stain



**Fig. 2.** Pure culture of *Xanthomonas axonopodis* pv. *Cyamopsidis* was maintained in Petri plate (A) and test tubes (B). Gram staining showed rod-shaped, gram negative bacteria.

(crystal violet), but looked pinkish colour due to counter-stain safranin. Hence, it was concluded that the test bacterium gram-negative and rod-shaped (Fig. 2). The results of the Gram staining reaction are consistent with the findings of Jonit *et al.* (2016) who reported that all isolates were stained as gram-negative with rod shape. Similarly, Jadhav *et al.* (2018) also reported the Gram staining test as negative for the pathogen *Xanthomonas axonopodis* pv. *citri*.

### Biochemical characteristics

**Table 2.** Biochemical characteristics of the *Xanthomonas axonopodis* pv. *cyamopsidis*

S. No.	Biochemical tests	Reaction
1	Gelatin liquefaction	+ ve
2	Catalase test	+ ve
3	Hydrogen sulphide (H <sub>2</sub> S) production	+ ve
4	Starch hydrolysis	+ ve
5	Acid production from :	
	(a) Sucrose	+ ve
	(b) Maltose	+ ve
	(c) Dextrose	+ ve
	(d) Mannitol	- ve
6	Urease test	+ ve
7	Oxidase test	- ve

### Gelatin liquefaction

In the gelatin liquefaction test, the formation of white precipitation observed around the growth of the bacterium indicated that gelatin was liquefying in the presence of gram-negative bacteria. In the present study, the bacterium showed a positive reaction to the gelatin liquefaction test (Fig. 3A).

### Catalase test

The release of gas bubbles when a loopful of the test bacterium was smeared on a glass slide and covered with a few drops of hydrogen peroxide

(H<sub>2</sub>O<sub>2</sub>) indicated a positive reaction to the catalase test (Fig. 3B).

### Hydrogen sulphide (H<sub>2</sub>S) production

The test bacteria produced gas that reacted with lead acetate strips; hence, the test strips turned black. The blackening of strips indicated the release of H<sub>2</sub>S gas. Thus, the bacteria showed a positive response to this test (Fig. 3C).

### Starch hydrolysis

A clear zone around the colonies appeared that indicated the hydrolysis of starch by the action of amylases. Thus, a positive result was obtained by the test bacterium (Fig. 3D).

### Acid production from Sucrose, Maltose, Dextrose and Mannitol

The ability of acid production by using different carbon sources, *viz.*, sucrose, maltose, dextrose and mannitol (Table 2) was determined by changes in colour from dark purple to yellow. Whereas, mannitol showed a negative reaction without changing colour (Fig. 3E).

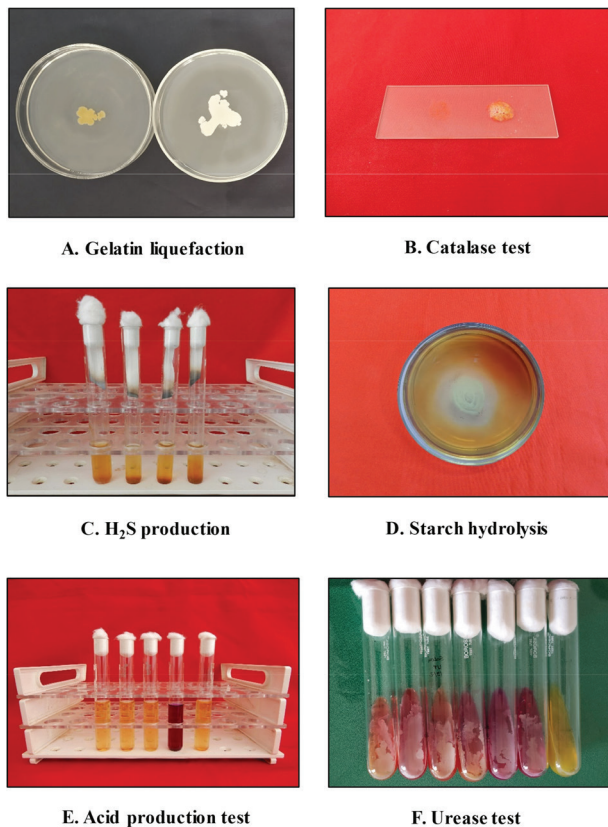
### Urease test

A urease test was conducted to check the ability of the pathogen *Xanthomonas axonopodis* pv. *cyamopsidis* to produce urease which is necessary for hydrolysis of urea into ammonia and carbon dioxide. During the experimentation, the change in colour from yellow to bright red or pink indicated alkaline state of media due to the accumulation of ammonia. This showed a positive reaction and confirmed the urease production by *Xanthomonas* (Fig. 3F).

### Oxidase test

The results revealed that the test bacterium was unable to carry out the colour changes, which indicated a negative reaction for the oxidase test.

The results for different biochemical tests are in line with the findings of Patil *et al.* (2017c) who reported that all the isolates of *Xanthomonas oryzae* pv. *oryzae* showed positive reactions for ammonia production, hydrolysis of starch, H<sub>2</sub>S production, liquefaction of gelatin and catalase test. Similarly, fifteen isolates of *Xanthomonas oryzae* pv. *oryzae* were obtained and subjected to different biochemical tests by Jonit *et al.* (2016) who reported that biochemical tests like starch hydrolysis and acid production



**Fig. 3.** Biochemical characteristics of *Xanthomonas axonopodis* pv. *cyamopsidis* were identified through different in vitro biochemical assay

from carbohydrates varied among the isolates. In the starch hydrolysis, nine isolates showed a positive reaction, and for the acid production test from carbohydrate five isolates showed a positive reaction. Jadhav *et al.* (2018) studied the biochemical characteristics of *Xanthomonas axonopodis* pv. *citri* and revealed that all isolates were found negative to Gram's reaction. While, positive to catalase oxidation, KOH, and starch hydrolysis test.

#### Pathogenicity test

The pathogenicity test was conducted to prove the pathogenic nature of *Xanthomonas axonopodis* pv. *cyamopsidis*. The clusterbean var. GG1 plants were raised and inoculated with bacterial cell suspension. After 8 to 10 days of inoculation, the typical symptoms were observed on inoculated clusterbean leaves as small, water-soaked, brown to black-coloured lesions. Later, they developed into irregular shaped spots along the veins and veinlets of the leaves which led to marginal necrosis (Fig 4). After the development of the typical symptoms, the pathogen was reisolated from the artificially inoculated clusterbean plants and compared with



**Fig. 4.** Pathogenicity of *Xanthomonas axonopodis* pv. *cyamopsidis* in clusterbean showed typical symptoms on different plant parts

the original culture. The bacterial colonies isolated from artificially inoculated plants were found to be identical to the original culture of *Xanthomonas axonopodis* pv. *cyamopsidis*.

The similar result was recorded by Yousif *et al.* (2018) for proving the pathogenicity of *Xanthomonas axonopodis* pv. *cyamopsidis* on clusterbean. They observed symptoms reproduced on stems and leaves of inoculated clusterbean plants within 10 days of inoculation were similar to those of the original one. On reisolation, yellow bacterial colonies of the pathogen were obtained from inoculated plants. Kumar and Doshi (2018) also carried out a pathogenicity test for *Xanthomonas axonopodis* pv. *vignae radiatae*. The pathogenicity was proved on

one-month-old greengram plants cv. SML-668 by spraying bacterial suspension method gave quicker symptom expression in potted plants as well as field conditions.

## CONCLUSION

Nowadays, the prevalence of clusterbean bacterial blight is increasing due to heavy rainfall in recent years. The disease poses a significant threat to clusterbean production, particularly in semi-arid regions of North Gujarat. The increasing trends in the disease prevalence were recorded from different locations such as Idar, Ambaji, Danta, Sanali, Khedbramha, Shamalaji, Patan, Bhiloda, Visnagar, Deesa, and Amirgadh. Based on morphological, and biochemical pathogenic characteristics, the pathogen was identified as *Xanthomonas axonopodis* pv. *cyamopsidis*. But symptomatology is very similar to the *Tobacco streak virus*. Thus, there is a need for continuous research and surveillance on changing host susceptibility, disease epidemiology, and disease development pattern of clusterbean bacterial blight. In this regard, the futuristic research will contribute to the development of adaptable strategies that reduce the effects of bacterial blight disease in an era of climate change.

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