

Detection of *Pseudomonas Syringae* pv. *Lachrymans* Associated with the Seeds of Cucurbits

S. J. Shila, M. R. Islam, N. N. Ahmed, K. M. G. Dastogeer*, M. B. Meah

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
*Corresponding Author: gdastbau@gmail.com

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Abstract Seed-borne bacteria that impair germination and emergence of seedlings and reduce yield and quality of cucurbits are of major concerns for cucurbit production throughout the world. The present investigation has been carried out to detect bacteria from the seeds of eight cucurbits viz. cucumber, bottle gourd, wax gourd, sweet gourd, ridge gourd, bitter gourd, sponge gourd and snake gourd collected from the different shops of Mymensingh town during July to November 2011. Seed samples were tested to detect seed-borne bacteria by nutrient agar plate method, cassette holder and liquid assay method. Only one bacterium was isolated and identified through nutrient agar plate and liquid assay method. The bacterium was identified as *Pseudomonas syringae* based on creamy white color colonies on the nutrient agar plate. The Pathogenicity test revealed that the bacterium was able to cause leaf spot symptom on the seedlings of all cucurbits confirming that the bacterium was *Pseudomonas syringae* pv. *lachrymans*. Hypersensitive response (HR) test on tobacco leaves caused partial death of local cell of tissues (HR) within the infiltrated area of tobacco leaves (cv. Bankete -A1). Gram staining and KOH test indicated the bacterium was gram negative and it was not able to grow at temperature 37°C and above. Positive reactions were observed in levan test, catalase test and sugar utilization test, but soft rot test, arginine dehydrolase and Kovac's oxidase was negative.

Keywords Biochemical tests, cucurbit, hypersensitive response, pathogenicity, seed-borne bacteria

during rainy season and crop field in winter are cultivated with cucurbit. This group of vegetable, is either used as salad or for cooking (gourds), for pickling (cucumber), or as desert fruits (musk-melon and water-melon) [2]. In Bangladesh the area denoted for cucumber cultivation is estimated to about 13000 acres with annual production of 230000 metric tons [3]. However, the yield of cucurbit vegetable in the country is very low due to various reasons. One of the major concerns for cucurbit production in Bangladesh is the infestation with seed-borne fungal and bacterial diseases that impairs germination and emergence yield and quality of cucurbits. Cucurbits are subjected to attack by more than 24 different diseases, 14 of them are seed borne-pathogen [4-6]. Cucurbits bacterial leaf spot, bacterial rind necrosis, bacterial soft rot are the most important bacterial disease infecting cucurbit plants. Recently, angular leaf spot symptoms caused by a bacterium *Pseudomonas syringae* pv. *lachrymans* [7,8] have become apparent on cotyledons, leaves and fruits in Bangladesh, that inflicts up to 37- 40 % reduction in fruit production and renders some fruits unmarketable. The disease poses threat not only to cucumber but also to a number of cultivated and wild cucurbits throughout the world. But no systematic study was carried out to identify and characterise the pathogen in Bangladesh. Hence, efforts were made to identify and isolate the harmful pathogen. Therefore, the present investigation deals with the detection and identification of the strain, *Pseudomonas syringae* pv. *Lachrymans* from the seeds of eight cucurbits namely cucumber, bottle gourd, wax gourd, sweet gourd, ridge gourd, bitter gourd, sponge gourd, snake gourd.

1. Introduction

Cucurbits are the member of the family cucurbitaceae. Cucurbits are tropically adapted creeper type plants which provide plenty of vegetable in summer especially in rainy season when most of the vegetable crops are out of production. In Bangladesh, cucurbits are most popular and cheap vegetables. They have nutritive status due to presence of carbohydrates, protein, fats, vitamins & minerals [1]. A commendable area of highland, specially the homestead area

2. Materials and Methods

2.1. Collection of Seed Sample

The experiment was conducted in the Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh. Seed samples of eight cucurbits, namely sweet gourd, bottle gourd, snake gourd, sponge gourd, wax gourd, bitter gourd, ridge gourd and cucumber, were collected in July 2011 from different shops in Noton Bazar, Mymensingh.

200 g of seed for each cucurbit species was sampled randomly from each. Then the collected seed samples were mixed to make a composite sample for each cucurbit. Then the composite samples were made into small fraction for working sample (around 100 seeds). The samples enclosed in polythene bags with proper labeling were brought directly to Seed Pathology Centre (SPC) and kept in the refrigerator at $4 \pm 1^\circ\text{C}$ until used for subsequent studies.

2.2. Detection of Bacteria

2.2.1. Nutrient Agar Plate Method

External surfaces of Seed samples for each cucurbit species were sterilized with 10% Clorox for 1 minute. Then the samples were washed with sterile water and the excess water was removed using blotter paper. Five seeds of sweet gourd, bottle gourd, snake gourd, bitter gourd and 10 seeds of sponge gourd, wax gourd, ridge gourd and cucumber were placed on the Nutrient Agar plate. Plates were incubated in an inoculation chamber at 27°C for 2 days to allow the growth of bacteria associated with the seeds.

2.2.2. Cassette Holder Method

Four seeds for each sample were taken randomly and placed between two layers of filter paper ($4.5 \times 4.5\text{cm}$) lined every second slit of the slide cassette holder. The cassette holder with the filter paper was put in a tray filled with 230 ppm urea solution until it covered cover 2 cm of the cassette. Symptoms of brown coleoptile, water soaked and yellow discolorations of leaves were also observed.

2.3. Liquid Assay Method

To identify seed borne bacteria associated with seeds of different cucurbits species, liquid assay method as described by Moura and Romeiro [9] was used. Five to ten seeds from each sample were grinded by mortar and pestle into powdery masses. The seed powder was mixed in 50 ml sterile water and incubated for 2 hrs at room temperature with periodical shaking. 1 ml. of the suspension was added in 9 ml of sterile water to make 10^{-1} dilution. Then 1 ml. aliquot from first dilution (10^{-1} dilution) was added in 9 ml. sterile water to prepare second dilution (10^{-2} dilution). Similarly, 10^{-3} and 10^{-4} dilutions were made by taking 1 ml. aliquot from previous dilution and mixing with 9 ml. sterile water in each case. A L- shaped glass spreader was used to smear the suspension on nutrient agar plate that was incubated in an incubator at 27°C for 2 days. Single colonies were transferred another nutrient agar plates for purification. From the pure colony of bacteria on NA media bacterial slant was then prepared in NA slant for future identification.

2.4. Pathogenicity Test

Pathogenicity test was performed using one month old cucurbits viz. cucumber, bottle gourd, wax gourd, sweet gourd, ribbed gourd, bitter gourd, sponge gourd and snake

gourd seedlings as described by Lelliot and Stead [10]. Bacterial suspension (approximately 10^6 - 10^7 cells/ml) was prepared from the 24 hrs old culture of different isolates of *Pseudomonas* sp. The suspension was sprayed onto the leaves of cucurbit seedlings in 3-4 true leaf stage grown in pots by hand sprayer. Inoculated cucurbit seedlings were covered with a black polythene sheet to create 80-90% relative humidity for 48 hrs. Then black polythene sheets was replaced by a transparent polythene sheet to conserve moist condition. Then the seedlings were kept on the net house. The symptoms were noted up to 10 days. During the experiments plants were watered regularly and wetted with hand sprayer. Check plants were sprayed with distilled water.

2.5. Tobacco Hypersensitive Response (HR) Test

At 30-40 days old tobacco plants, bacterial suspension (approximately 10^6 CFU/ml) of isolate representing a group was injected in the leaves. Bacterial suspensions were injected into the intracellular space of the leaf with a hypodermal syringe. Hypersensitive reaction was observed daily and continued up to five days of infiltration. Necrotic lesion on the leaves were recorded positive [2].

Soft rot test: Well washed, firm potatoes were surface sterilized, peeled aseptically and three standard slices were peeled in sterile petridishes, the bottom of which was lined with moist filter paper (What man no. 1). Two slices were then inoculated with loopful of bacteria previously grown on Nutrient Agar culture of each isolate and incubated at room temperature for 24-48 hrs for the detection of soft rot symptoms. A control for each isolate was maintained using loopful of sterile water. Pectolytic activity was performed to observe the condition of the sliced potato [10].

Temperature sensitivity test: The ability of bacterial isolates to grow at different selected temperature at 27°C , 37°C and 41°C were tested by initially growing isolate on NA for 24 hrs. The bacterial suspension of isolate was prepared from 24 hrs NA culture to 5 ml of nutrient broth. 2-3 drops of bacterial suspension was taken in nutrient broth contained in test tubes which were placed at temperature 27°C , 37°C and 41°C .

2.6. Biochemical Tests for Identification of *Pseudomonas Syringae* Pv. *Lachrymans*

Pseudomonas syringae pv. *lachrymans* detected in the seeds of eight cucurbits was characterized by several biochemical tests viz. Gram staining, Potassium hydroxide solubility, Kovac's, Levan, Sugar fermentation, Catalase and Arginine dihydrolase test

Gram staining reaction: 24 hrs old fresh bacterial cultures for each group of isolates were taken to perform the gram reaction test. Bacterial mass were taken out from culture, spread on clean glass slide followed by drying in air without heat and was flamed it lightly and gently to settle the bacteria. Crystal violet was then poured over the bacterial

smear on the slide for 2-3 minutes and washed in tap water for 1 minute and lightly blot dry on a paper towel to remove crystal violet. The smear was flooded with iodine solution for 1 minute and washed in tap water for few seconds and then blot dried. It was decolorized with solvent e.g. alcohol, until the solvent flowed colorlessly from the slide (about 2-4 seconds) and rinse in tap water for 5 seconds. Then the smear was counterstained for about 1 minute with safranin solution and washed briefly in tap water followed by blot dry. One drop emulsion oil was then added on the slide and placed it on compound microscope with 100 x magnification for observation.

Potassium hydroxide solubility test: On glass slide a loopful of bacteria from a well grown colony was mixed with 1-2 drop of 3% KOH solution was placed on microscope slides, bacterial isolates was slowly stirred in the KOH solution by means of a loopfull and the loopfull was slowly raised from the solution. If the solution was mucoid and it was stretched as fibres it was recorded as positive. If the solution was watery, not mucoid and fibres formation was not occurred while the loopfull was raised it was recorded as negative [12].

Kovac's oxidase test: A filter paper was placed into a clean petri dish and impregnated with 1% aqueous solution of Nitrogen, (Nitrogen tetramethyl-p-phenol-diamine dihydrochloride). Then, a loopful inoculum from pure culture was picked up by sterilized platinum loop. The inoculum was smeared over the area of filter paper containing oxidize reagent to develop deep blue or purple color within ten seconds indicating the oxidation of the reagent [13].

Levan test: Bacteria that have the enzyme levan sucrase, can break down sucrose and produced levan (Poly-fructose), a capsular material. Sucrose peptone agar or a nutrient agar medium with 5% sucrose is suitable substrate for Levan test. For the test a single colony for each isolate was stabbed with a sterilized tooth pick on NA medium containing 5% sucrose. Then the plates were incubated at 28°C for 2 to 4 days to have distinctive dome shaped colonies.

Sugar Utilization test: The medium consists of peptone water to which fermentable sugar was added in the proportion of 1%. Peptone water was prepared by adding 1 g of Bacto peptone (Difco, USA) and 0.5 g of sodium chloride in 100 ml distilled water. The medium was boiled for 5 minutes, adjusted to pH at 7.0, cooled and then filtered through filter paper. Phenol red, an indicator at the strength of 0.2% solution was added to peptone water and then dispensed 5 ml in Durham's fermentation tubes, placed inversely. These were then sterilized in autoclave at 121°C maintaining at 15 PSI pressure for 15 minutes. The sugars used for fermentation test were prepared separately as 10% solution in distilled water (10 g sugar was dissolved in 100 ml of distilled water). A little heat was given to dissolve the sugar completely. The sugar solutions were sterilized using filters. An amount of 0.5 ml of sterile sugar solution was added aseptically in each culture tubes containing sterile peptone water and the indicator. Before use, the sugar media

was sterilized and incubated it for 24 hours at 37°C. The carbohydrate fermentation test was performed by inoculating a loop full of nutrient broth culture of the organisms into the tubes containing different sugar media (four basic sugars such as dextrose, sucrose, lactose, and manitol) and incubated for 24 hours at 37°C. Acid production was indicated by the color change from reddish to yellow in the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes.

Arginine dihydrolase activity: The test was performed to observe the presence of two enzymes that permits certain Pseudomonads to grow under anerobic conditions. The enzymes generate ATP by the degradation of arginine to ornithine with the generation of CO_2 and NH_3 . The two enzymes are arginine desmidase which degrades arginine to citrulline + CO_2 + NH_3 . It is the alkaline reaction of NH_3 production that is detected by the test. To prepare the medium 0.2g peptone, 1g NaCl, 0.06g K_2HPO_4 , 0.6g Agar, 0.02mg Phenol red and 2g Arginine HCl was taken in a conical flask and mixed with 200mL water. The conical flask was shaken gently for mixing the chemicals properly. The pH was adjusted at 7.2. These were then sterilized in autoclave at 121°C maintaining at 15 PSI pressure for 15 minutes. A fresh culture was stabbed into a soft agar tube of Thornley's medium, sealed with sterile mineral oil or melted agar and incubated at 28°C. A color change from faint pink to red within four days is positive reaction [14].

Catalase Test: This test was performed to isolates to check their liveliness. Standard cultures are used for control purpose. One milliliter of 3% hydrogen peroxide was placed on the microscope slides. The bacterial isolates collected from the leaf samples were tested for catalase test. Bacterial isolates developed in NA medium for 24 hrs were added by a loopful. Bubbles' arising from the solution was recorded as positive reaction. This test is used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism. Catalase breaks H_2O_2 down into water and O_2 .

3. Results and Discussion

3.1. Detection of *Pseudomonas syringae* pv. *lachrymans* on cucurbit seeds

Both Nutrient Agar Plate and Liquid assay method revealed the presence of only one bacterium in all the seeds of cucurbits. Creamy white color colonies grown on NA plate from different cucurbit seeds as tested by both nutrient agar plate and Liquid assay method primarily indicated that the bacterium was *Pseudomonas* (It's a repletion). Smith [15] reported that *Pseudomonas lachrymans*, isolated from cucumber or muskmelon, formed white, slightly raised, transparent, smooth surfaced and circular colonies with irregular edges on beef-extract agar producing abundant green fluorescent pigment but the colonies were slightly

raised, filiform, white and butyrous on potato dextrose agar. Palleroni [16] reported that King's medium B is frequently used for direct isolation of fluorescent pseudomonads and their colonies can be identified on the plates by characteristic diffusible pigments. Saprophytic pseudomonads are reported to produce more fluorescent pigment on King's medium B than the pathovars of *Pseudomonas syringae*, which fluoresce blue by visualizing the colonies under UV light [17]. *Pseudomonas syringae* pv. *lachrymans* and other pathovars of this species are reported to form levan type colonies on nutrient sucrose agar within three days, which constitutes as a distinguishing character of the species [8].

3.2. Potato Soft Rot Test

Potato soft rotting test was performed using the bacterium obtained from different samples of cucurbit seeds did not able to cause soft rot symptoms in potato (Table. 1). Hence the bacterium might be *Pseudomonas syringae*. The temperature sensitivity test revealed that none of the isolates can grow at 37°C and 41°C and but can grow at 27°C (Table. 1). Schaad *et al* [18] also reported that *Pseudomonas syringae* pv. *lachrymans* has no ability to degrade the pectolytic substance of potato while the test was observed positive in case of *Pseudomonas viridiflava*, *P. marginalis* and *E. carotovora* subsp. *carotovora*. Ohuchi *et al.* [19] studied biochemical characters of 110 Japanese isolates of *P. syringae* pv. *lachrymans* pathogenic to cucumber, and found

that all of these were negative for potato soft rot activity.

3.3. Pathogenicity and Hypersensitive Response Test

All isolates produced typical symptoms of angular leaf spot/leaf blotch symptoms in seedlings of eight cucurbits tested, 15 days after inoculation, causing greasy and individual spots (Fig.1 and Table 2). The first symptoms appeared three days after inoculation and no differences in pathogenicity were found between other isolates. Therefore, it may be confirmed that the bacterium detected in the seeds of cucurbits was *P. syringae* pv. *lachrymans*. This result is an agreement with the findings of Aksoy [20] who confirmed *P. syringae* pv. *lachrymans* as the causal organism of bacterial leaf spot of cucurbits by pathogenicity test. *P. syringae* pv. *lachrymans* detected from all cucurbit seeds were tested for hypersensitive reaction in tobacco. Results of this test showed that the isolates were able to cause partial death of local cell of tissues (HR) within the infiltrated area of tobacco (cv. Banket-A1) leaves (Fig. 2). Gvozdyak *et al.* [21] also showed the hypersensitive reaction on the tobacco leaf and the hypersensitive reaction was most distinctly manifested when 1-3 days old bacterial cultures of *P. syringae* pv. *lachrymans* were used. Several other findings such as those reported by Misaghi and Grogan [22] and Bradbury [8] also pointed out that *P. syringae* pv. *lachrymans* pathogenic to cucurbits and showed positive reaction with respect to hypersensitivity to tobacco.

Table 1. Morphological and Physiological Characteristics of *Pseudomonas syringae* pv. *Lachrymans*

Name of the seed sample	Colony morphology on nutrient agar plate	Potato soft rot test	Temperature sensitivity test			Inference
			27°C	37°C	41°C	
Sweet gourd	Creamy White	-	+	-	-	<i>Pseudomonas syringae</i> pv. <i>lachrymans</i>
Wax gourd	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>
Bottle gourd	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>
Bitter gourd	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>
Snake gourd	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>
Sponge gourd	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>
Ridge gourd	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>
Cucumber	Creamy White	-	+	-	-	<i>P. syringae</i> pv. <i>lachrymans</i>

“+” = Positive Reaction and “-” = Negative Reaction

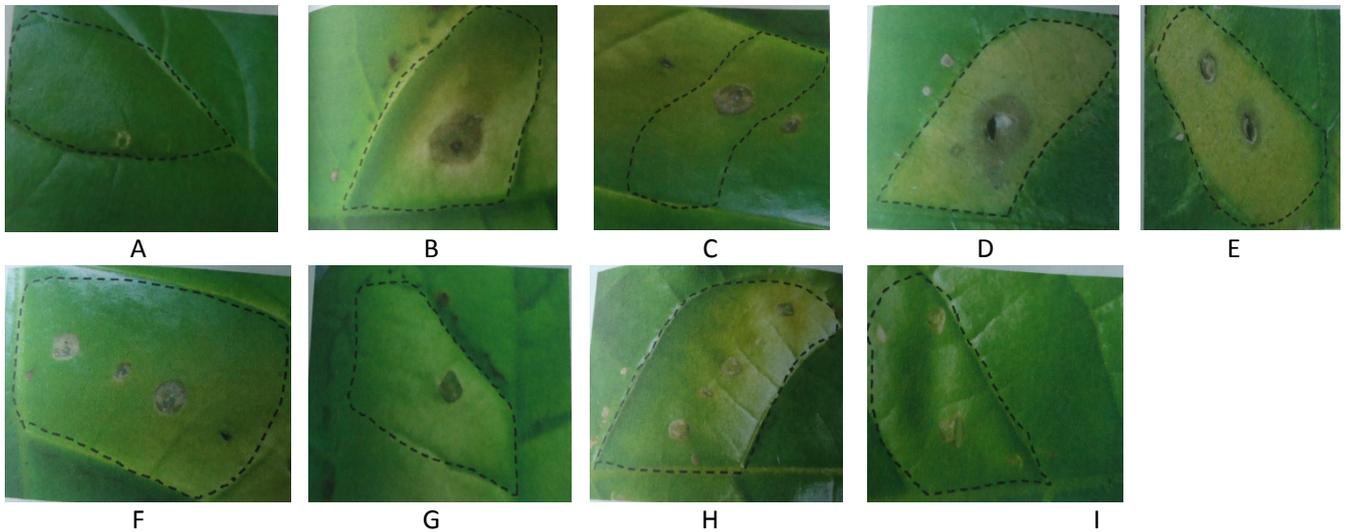


Figure 1. Hypersensitive response test on tobacco leaves infiltrated with isolates of *P. syringae* pv. *lachrymans* seeds of B. Cucumber, C. Bottle gourd, D. Sweet gourd, E. Ridge gourd, F. Ash gourd, G. Snake gourd, H. Bitter gourd, I. Sponge gourd. Areas inside the dotted line infiltrated with bacterial suspension (B-I) and control (A)

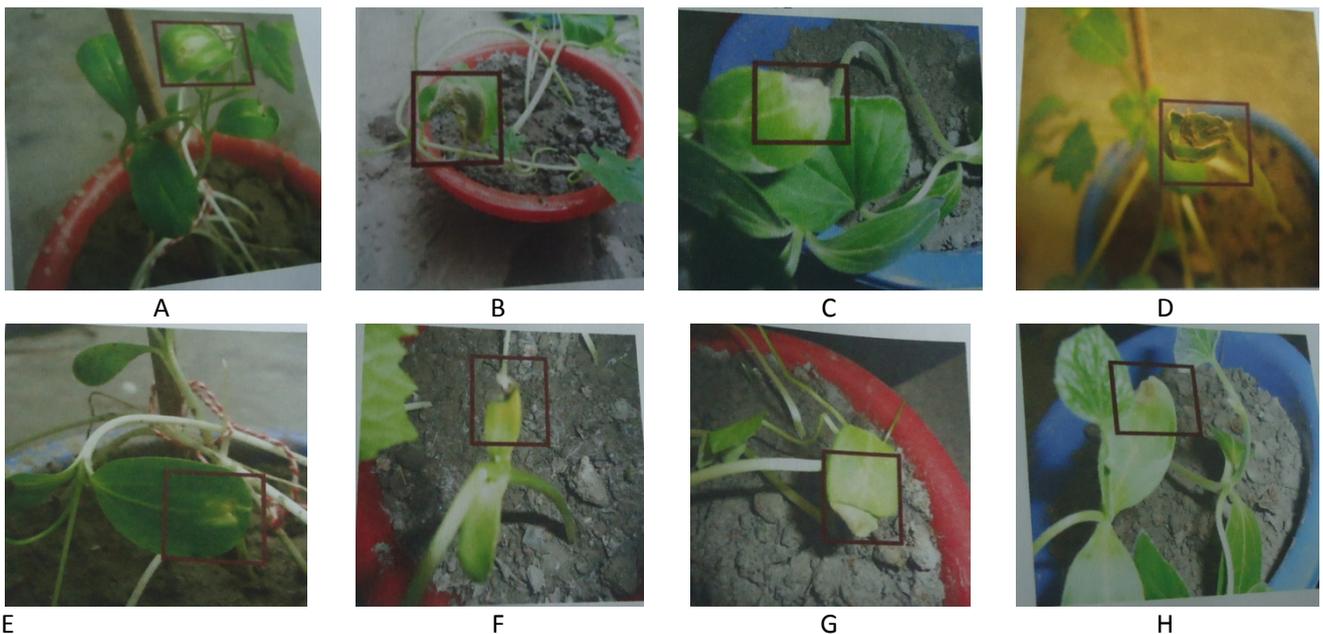


Figure 2. Pathogenicity test of *P. syringae* pv. *lachrymans* isolated from seeds of A. Cucumber, B. Bottle gourd, C. Sweet gourd, D. Ridge gourd, E. Ash gourd, F. Snake gourd, G. Bitter gourd, H. Sponge gourd (Red box shows lesion on leaves)

3.4. Biochemical Tests

Biochemical tests viz. gram staining test, KOH test, levan test, catalase test, oxidase test were performed to characterize the isolates of *Pseudomonas syringae* pv. *lachrymans* detected in the seeds of eight cucurbits. KOH solubility test and gram staining reaction showed the bacterium was gram negative (Table. 2). With respect to the Kovac's oxidase test *Pseudomonas syringae* pv. *lachrymans* did not give the positive reaction revealing that it has no oxidizing ability. The isolates of *Pseudomonas syringae* pv. *lachrymans* detected in the seeds of eight cucurbits were confirmed by levan test and sugar utilization test. The isolates were able to produce round or circular dommed

shaped colonies in sucrose medium. The acid production in sugar fermentation test by the bacteria were indicated by the color change from reddish to yellow; gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes and the oxidation of sugar manitol by isolates indicated by the production of red to yellow color. All the bacterial isolate were also subjected to test with arginine dihydrolase test. Results of this test showed that the isolates were able to raise the pH and change the color of the medium from faint pink to red. This results clearly indicated that the bacterium was *Pseudomonas syringae* pv. *lachrymans*. Again, catalase test for *Pseudomonas syringae* pv. *lachrymans* isolates detected in the seeds of cucurbits showed positive reaction by the appearance of arising bubbles (Table. 2).

Suslow *et al.* [23] reported that the KOH technique is far easier and faster to distinguish gram negative and gram positive bacteria than the traditional Gram-stain in which dyes are employed. Smith [15] studied the morphological characters of *Pseudomonas lachrymans* and reported that the bacterium was Gram-negative, rod shaped with rounded ends, nonacid fast, motile with one to three polar flagella. Kagiwata [24] studied the morphological characters of several isolates of *P. syringae* pv. *lachrymans* isolated from all over Japan and reported that all were Gram negative, aerobic, non-sporeing, straight rods and motile with one to five polar flagella. Bradbury [8] in his detailed account of genus *Pseudomonas*, its species and pathovars reported that pseudomonads are Gram-negative, aerobic rods, straight or curved and range $0.5-1.0 \times 1.5-4.0 \mu\text{m}$ in size. Smith [15] reported that *P. lachrymans* was negative for nitrate reduction, production of indole and hydrogen sulphide, and starch hydrolysis but was positive for ammonia production. He further reported that the bacterium utilized arabinose, xylose, sucrose, dextrose, levulose, galactose, mannose and sorbitol but not rhamnose, maltose, lactose, trehalose, raffinose, melizitose, melibiose, cellobiose, starch, inulin, dextrin, glycogen, inositol, glycerol, dulcitol, erythritol, salicin, esculin and arbutin.

Our result is in line with the findings of Aksoy [20] who reported that the isolates of *Pseudomonas syringae* pv. *lachrymans* were able to produce round or circular domed shaped colonies in sucrose medium. Lelliot *et al.* [25] reported that *P. Lachrymans* as positive for Levan production, tobacco hypersensitivity and sucrose utilization but negative for oxidase, potato rot, arginine dihydrolase and nitrate reduction with different isolates giving varied results in case of tyrosinase activity and aesculin utilization. Our present result is in accordance with the findings of Aksoy [20] who reported that *Pseudomonas syringae* pv. *lachrymans* shows negative reaction in arginine dihydrolase and Catalase test as gram negative bacteria. A negative reaction in arginine dihydrolase test was also reported by Schadd *et al* [18]. Misaghi and Grogan [22] reported that *P. lachrymans* isolates were negative for

oxidase, potato rot, and arginine hydrolase tests. Ohuchi *et al.* [19] studied biochemical characters of 110 Japanese isolates of *P. syringae* pv. *lachrymans* pathogenic to cucumber, and found that all of these were positive, production of Levan, liquefaction of gelatin, utilization of tartarate and sucrose, but were negative for growth at 37°C , arginine dihydrolase, oxidase, tyrosinase, casein hydrolysis, cotton oil hydrolysis, nitrate reduction and potato soft rot activity. Bradbury [8] summarized the biochemical description of *P. syringae* pv. *lachrymans* as positive for levan production from sucrose and tobacco hypersensitivity and negative for arginine dihydrolase, oxidase, starch hydrolysis and potato rot. Scortichini *et al.* [26] reported that the capability of aesculin utilization by *P. syringae* pv. *lachrymans* disappeared after serial transfers on King's B medium as well as after the passage through non-host plants and as such suggested that carbohydrate utilization ability of the bacterium would vary according to the different environments in which it had lived. Variation due to frequent subculturing of *P. syringae* pv. *lachrymans* in utilization of glycerol, ribose, D-xylose, rhaminose, inositol, and mannitol has also been reported [26]. In a comparative study of phytopathogenic pseudomonads, Braun-Kiewnick and Sands [17] reported that *P. syringae* pv. *lachrymans* was positive for pectate lyase, beta-glucosidase, polygalacturonase, aesculin hydrolysis, arbutin hydrolysis, gelatin liquefaction, levan production and ice nucleation, and could utilize D-mannitol, inositol, D-sorbitol, trigonalline, D-quinic acid, erythritol, L(+) tartarate, glutarate and DLglycerate but not adonitol, D(-) tartarate, L-lactate, anthranilate and DL-homoserine for growth.

The findings of the present study give an indication of the presence of a bacterium in cucurbit seeds. The bacterium isolated and identified as *P. syringae* pv. *syringae* is a pathogenic bacterium. Therefore, cucurbit seeds available in the markets should be subject to health test for bacterium before sowing. Further studies need to be undertaken in order to get the detailed information pertaining to the identity and mode of function of the bacterium with a view to developing pathogen targeted and sustainable management approaches.

Table 2. Pathogenicity and Biochemical tests of *Pseudomonas syringae* pv. *Lachrymans*

Name of the seed sample	Pathogenicity test	Tobacco Hypersensitivity reaction	Gram staining reaction	KOH solubility test	Kovac's oxidase test	Levan test	Sugar fermentation test				Arginine dehydrolyse Test	Catalase test	Inference
							Dextrose	Sucrose	Manitol	Lactose			
Sweet gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Wax gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Bottle gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Bitter gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Snake gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Sponge gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Ridge gourd	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>
Cucumber	+	+	Gram negative	Gram negative	-	+	+	+	+	+	-	+	<i>P. syringae</i> pv. <i>lachrymans</i>

“+” = Positive Reaction, “-” = Negative Reaction

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