International Journal of Multidisciplinary Research and Publications
ISSN (Online): 2581-6187

Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure

Afrin Akter[1], Mst. Sabina Eyesmin Sumi[1], Md. Roushan Ali[1, 2], Rizwoana Sharmin Lia[1], Md. Faruk Hasan[1], M. Asadul Islam[1], Biswanath Sikdar[1], M. Khalekuzzaman[1,*]
[1]Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh
[2]Department of Microbiology and Immunology, University of Science and Technology of China, China
*Corresponding author: kzaman63@gmail.com

***Abstract*—** *Bacterial speck is a prominent disease of tomato, caused by Pseudomonas syringae pv. tomato. The present investigation was carried out to isolate and characterize the bacterial disease as well as their biological control management by antibiotic and antimicrobial sensitivity assay. Creamy colored bacterial colonies were observed on yeast peptone glucose agar medium after streaking and incubation at 37°C for 16 hours. The isolated bacterium was identified as Pseudomonas syringae pv. tomato on the basis of morphological, physiological and biochemical tests method. The isolated bacterium was gram negative, rod shaped and motile. It showed positive result to catalase, MacConkey agar, potassium hydroxide, methyl red, Simmon citrate test and negative result to Kovac oxidase, and urease test. Triple sugar iron agar and Kligler iron agar tests showed positive result in isolated bacteria. Gentamycin revealed the highest inhibition zone with 24.5±0.2 mm against isolated bacterial strain. Terminalia arjuna plant extract showed highest 11.2±0.2 mm zone of inhibition against the isolated bacteria. The present investigation could be helpful for further molecular detection of the isolated bacteria and their biological control technique.*

***Keywords*—** *Tomato, Pseudomonas syringae pv. tomato, Biochemical characterization, Sensitivity test.*

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is economically the most important and popular vegetables throughout the world including Bangladesh, belonging to the Solanaceae family. Tomatoes are native to South America and Central America (Jenkins 1948). Tomato consumption reduces the risk of certain types of cancer, cardiovascular diseases and age-related mascular degeneration (Giovannucci et al. 2002; De Stefani et al. 2000, Muller et al. 2002). It also gives relief from diabetes, skin problems, urinary tract infections and provide essential antioxidants (Bhowmik et al. 2012). Total 40 types of carotenoids are found in the human diet, among them fresh and processed tomato contains 9–20 types of carotenoids such as lycopene α-carotene and β-carotene, lutein, zeaxanthin and β-cryptoxanthin (Dorais et al. 2008). Tomato contains ascorbic acid which is most effective antioxidant in plants (Smirnoff 1995).

Bacterial speck is a common disease of tomato that occurs worldwide wherever tomatoes are grown. *Pseudomonas syringae* pv. *tomato* is one of 50 pathovars belonging to the heterogeneous species of *Pseudomonas syringae* (Young et al. 1996). It is most severe in early spring plantings that are exposed to cool and moist conditions. Early infection may result in significantly considerable economic losses (Schneider and Grogan 1977). Yields lose varied in different condition. Up to 75% lose occur if the disease develop on the early stage of growth but 5% lose occur if it develop on the later stage (Yunis et al. 1980). This bacterium may also be seed borne and can over-winter on plant debris in soil and on the roots of many perennial plants. Though this disease is devastating for tomato, there is no suitable report on the pathogen and their control management. Therefore, the present investigation was designed to isolate and characterize the pathogenic bacteria and their proper control measure.

II. MATERIALS AND METHODS

Bacterial speck infected tomato plants is identified according to symptoms such as small black lesions, often with a discrete yellow halo, tend to have a greasy appearance, curl leaves, lesions are superficial.

Plant Material Collection

Bacterial speck disease infected leaves of tomato collected from Khorkhori bypass region of Rajshahi and the disease were identified by Bangladesh Council of Science and Industrial Research (BCSIR), Binodpur, Rajshahi-6205, Bangladesh. These infected leaves used as plant material source.

Isolation of Bacteria

Infected plant leaves were surface disinfested using a dilute sodium hypochlorite solution (10%) and rinsed thoroughly. The infected area of leaves was cut and crushed using mortar and pestle. The fluid oozes out from the pest of infected leaves inoculated into Luria and Bertoni (LB) liquid medium for 14 hour. After the bacteria have grown in LB liquid medium, the bacteria were streak onto a solid agar medium using a sterile loop and incubate overnight at 37°C.

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.

Characterization of the Isolated Bacteria

Gram Staining: Gram staining test was performed according to Vincent and Humphrey (1970). Crystal violet, iodine, ethanol and saffranin were used. At first the isolated bacterial culture was heat fixed onto a glass slide. Then crystal violet was added to the bacterial sample and incubated for 1 min. After washing, iodine was added on the slide. Then saffranin was used to counterstaining (Jacquelyn 1993). After all these steps the slide was used to observe under the light microscope at 100X using oil immersion.

SIM test: SIM medium (Kirsop and Doyle 1991) was used to detect sulfide production, indole formation and motility of isolated bacteria. The medium contains pancreatic digest of casine, peptic digest of animal tissue, ferrous ammonium sulfate and sodium thiosulfate. One isolated bacterial colony was inoculated into SIM medium by stabbing and the medium was incubated at 35°c for 24 hour. Kovac's reagent was added to the media to detect indole formation capability of isolated bacteria.

Simmons citrate test: Simmons citrate test was performed to detect gram negative bacteria by the citrate metabolism capability of isolated bacteria (Difco 2016). If the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity that turns the bromthymol blue indicator in the medium from green to blue above pH 7.6. A single pure colony was picked with a loop, lightly streaked on slant surface of medium and incubated at 35^0C for 24 hours.

Catalase test: This test was used to identify organisms as described by Hayward (1960). It produce catalase enzyme that protect cell from oxidative damage by breaking down hydrogen peroxide into water and oxygen gas (Chelikani et al. 2004). Isolated bacterial colony was taken onto a glass slide and hydrogen peroxide was added with it and mixed with a loop.

MacConkey agar test: MacConkey agar test (MacConkey 1905) was performed to detect gram negative bacteria and lactose fermentation capability. After preparing MacConkey agar plate one pure bacterial colony was picked by a loop and streaked as well as control without streaking. The plates were incubated at 37^0C for 48 hours. MacConkey agar contains bile salts which selectively facilitate the growth of gram negative bacteria.

Kligler Iron Agar test: KIA medium (Kligler 1917) contains a large amount of lactose and a very small amount of glucose, a pH indicator (yellow in acid and red in base), and iron, which is precipitated as a black sulfide if H_2S is produced. Lactose positive organisms yield a yellow slant and lactose negative organisms yield a red slant. Cracks, splits, or bubbles in the medium indicate gas production. Sterilized media 121°C for 15 min was poured into a test tube and kept it slant position. Isolated bacterial colony was streaked on the slant surface of KIA medium and incubated at 37°C for 48 hours.

Urease test: This test is used to differentiate organisms based on their ability to hydrolyze urea with the enzyme urease (Christensen 1946). The color of the slant changes from yellow to pink when urease enzyme produced by bacteria in this media. Sterilized Urease media was poured into two test tube and kept them slant position. Single colony was streaked on slant surface of the medium and incubated at 37°C for 48 hours.

KOH test: The KOH test (Suslow et al. 1982) was performed to detect gram negative bacteria. This test generally uses to confirm test of gram staining. Some bacterial colony were taken from pure culture and mixed with one drop of 3% KOH by a loop.

TSI test: Triple Sugar Iron Agar (TSI) medium contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate bacteria based on the ability to reduce sulfur and ferment carbohydrates. After preparing media, it was sterilized at 121°C for 15 min. Sterilized media was poured into test tubes and kept on slant position. Single colony was streaked on the slant surface and incubated at 37°C for 48 hours.

Methyl Red test: Methyl Red test (MR test), is used to identify the bacteria that utilize glucose present on medium and formed stable acid (Jennens 1954). The stable acid change pH and convert yellow color methyl red into red color. One bacterial colony was taken from pure culture of bacteria and inoculated it into sterilized Methyl Red medium. It was incubated for 16 hours at 37°C. After 16 hours 2 drop of methyl red were added into it to observe.

Antibiotic Sensitivity of Some Antibiotics against Isolated Bacteria

Antibiotic sensitivity test was done to identify those antibiotics which can be used to control this isolated bacteria. The isolated bacterial strain was grown in liquid nutrient broths at 37°C for 14 hours at 150 rpm. The LB agar medium was prepared and sterilized. Sterilized media was poured into agar plates and cooled them. Then some commercially available and frequently prescribed antibiotic discs were used to perform this sensitivity test. 500 µl of liquid culture of isolated bacteria was taken by a micropipette and spread by a spreader on LB agar plates and waited until air dry. Then a disk for each plate was taken and put in the middle position of spread plates and incubated those plates for 16 hours at 37°C. After 16 hours the zone of inhibition were measured by mm scale. For this purpose 100 mcg/disk, 30 mcg/disk, 10 mcg/disk, 10 units/disk, 10 mcg/disk, 15µg/disk, 10µg/disk, 30 mcg/disk, 15 mcg/disk, 30mcg/disk, 30 µg/disk, and 10mcg/disk concentration of carbenicillin, doxycycline, streptomycin, penicillin, ampicillin, clarithromycin, amoxycillin, kanamycin, erythromycin cefotaxime, chloramphenicol, and gentamicin respectively were used by moderate disk diffusion method.

Antibacterial Activity of Some Plant Extract against Isolated Bacteria

Six different plant parts like, bark of *Terminalia arjuna*, leaves of *Ocimum tenuiflorum*, flower of *Dutura metal*, leaves of *Justicia adhatoda*, leaves of *Centella asiatica*, leaves of *Mentha arvensi* were collected from different place of Rajshahi University campus. They were carefully washed and crushed by mortar and pestle to squeeze out fluid from it. This row extracts were kept on different test tubes and kept it for

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.

one hour in 4°C. The supernatant of each extract were separated and used to make three concentrations (10µl, 20µl, and 30µl per disc) of disk with each extract. Then LB agar plates were prepared and 500µl of liquid culture of isolated bacteria was taken into the agar plates. Three disk of different concentration of each extract were placed on each agar plates after spreading. Then they were incubated for 16 hours at 37°C. After 16 hours the zone of inhibition were measured by millimeter (mm) scale.

Statistical Analysis

All the above experiments of the present study were repeated threes for consistency of results and statistical purpose. The data were expressed as mean and standard error (M±SE). The data were calculated using Microsoft Excel 2010 software.

III. RESULTS

Isolation and Purification

Isolated bacterial colonies of pure streak plate from LB liquid culture were small in size, convex, mucoid, and creamy in color. (Fig. 1)

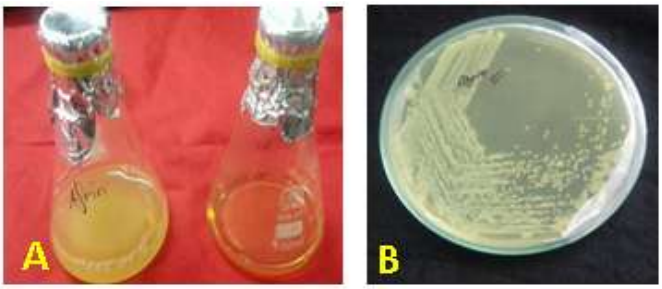

Fig. 1. Showing isolation of causal organism, A) Liquid culture of bacterium B) Pure culture of isolated bacterium.

Morphological and Biochemical Characteristics of Isolated Bacteria

In gram staining test isolated bacteria showed rod shaped and pink color. In Kligler Iron Agar test isolated bacteria converted red orange color of media into yellow color that indicate isolated bacteria can ferment lactose. Bubble and crake formed that indicate gas formation but hydrogen sulfide produced (Fig. 2A).

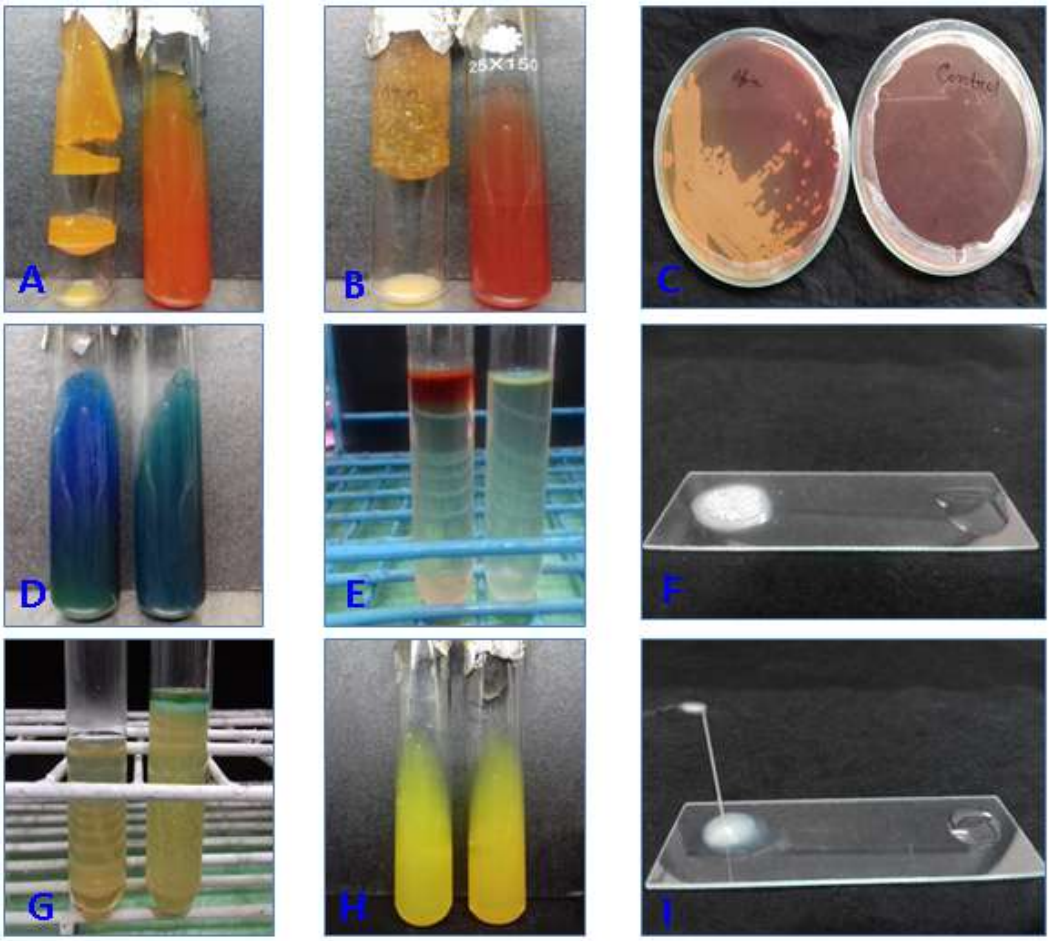

Fig. 2. Showing biochemical test of isolated bacteria, A) Kligler Iron Agar test, B) Triple Sugar Iron Agar test, C) MacConkey agar test, D) Simmon's citrate test, E) Methyl Red test, F) Catalase test, G) SIM test, H) Urease test, I) KOH test.

In Triple Sugar Iron (TSI) agar test isolated bacteria converted red color of the media into yellow color (both butt and slant) that means these bacteria can ferment glucose, sucrose, and lactose. Bubble and crake formed that indicate

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.

gas production but no hydrogen sulfide produced (Fig. 2B). Isolated bacteria grew on MacConkey agar medium and form red color colonies. So this bacteria is gram negative and lactose fermentive bacteria (Fig. 2C). Color of simmon citrate medium changed from green to the royal blue after inoculating bacteria that indicate that the isolated bacteria can utilize citrate and change pH (Fig. 2D). In the presence of bacteria methyl red medium produced stable acid and formed red color (Fig. 2E). The isolated bacteria is catalase test positive. Because it formed oxygen bubbles with hydrogen peroxide by detoxifying it (Fig. 2F). In Sulfide-Indole-Motility medium isolated bacteria showed motility and formed indole with kovacs reagent. But no black precipitate formed that means no hydrogen sulfide produced (Fig. 2G). In urease test the color of media remain unchanged. Isolated bacteria cannot produce ammonia (Fig. 2H). In KOH test, isolated bacteria formed viscous string with KOH. So the isolated bacterium is gram negative bacteria (Fig. 2I). The result of morphological and biochemical test are summarized in table 1.

Antibacterial Activity of Some Antibiotic against Isolated Bacteria

Twelve antibiotics has been evaluated in-vitro against isolated bacteria. Among them Gentamycin revealed highest antibacterial activity against isolated bacteria with inhibition zone 24.5±0.4 mm in the concentration of 10 mcg/disc followed by 23.8±0.2 mm of zone of inhibition by Streptomycin at the concentration of 10 mcg/disc. On the other hand Penicillin G, and Ampicillin revealed lowest antibacterial activity against isolated bacteria with inhibition zone 8.2±0.2 mm in the concentration of 10 units and 10 mcg/disc respectively (Fig. 3). The result is summarized in table 2.

Antimicrobial Activity of Some Row Plant Extract Against Isolated Bacteria:

Row pant extract of six plants has been used to test the antibacterial activity against isolated bacteria. The results of antibacterial test showed that the extract of *Terminalia arjuna* revealed highest antibacterial activity against isolated bacteria with inhibition zone 11.2±0.2 mm at 30 µl/disc concentration. *Dutura metel* showed second highest antibacterial activity against isolated bacteria with inhibition zone 10.5±0.4 mm at 30 µl/disc concentration. On the other hand, *Centella asiatica* revealed lowest antibacterial activity against isolated bacteria with inhibition zone 7.1±0.2 mm in the 10 µl/disc concentration (Fig. 4). The result is summarized in table 3.

TABLE 1. Morphological and biochemical test of isolated bacteria is summarized below.

Name of the Test	Reac- ion	Appearance	Remarks
Gram staining	-ve	Small, rod shaped, pink color colony	Isolated bacteria is a Gram negative bacteria
KIA	+ve	Convert red orange color into yellow color	This gram negative bacteria confirm lactose fermentation, gas production but cannot produce H_2S
TSI	+ve	Color changed from red to yellow	This gram negative bacteria cannot produce H_2S and confirm lactose, sucrose and glucose fermenting
MacConkey agar	+ve	Pink color around the colony	Gram negative and lactose fermenting bacteria
Simmons citrate	+ve	Color changed from dark green to the royal blue	It is citrate metabolizing gram negative bacteria
Methyl Red	+ve	Color changed from yellow to Red ring	This gram negative can utilize glucose
Catalase	+ve	Bubbles formation	Gram negative bacteria formed O_2 bubbles
SIM	+ve, -ve, +ve	Motile, no H_2S formation but Indole formation occur	This gram negative bacteria is motile and form indole but cannot produce H_2S
Urease	-ve	No color change	This gram negative Bacteria cannot hydrolyze urea
KOH	+ve	Sticky string formed	Isolated bacteria is a Gram negative bacteria

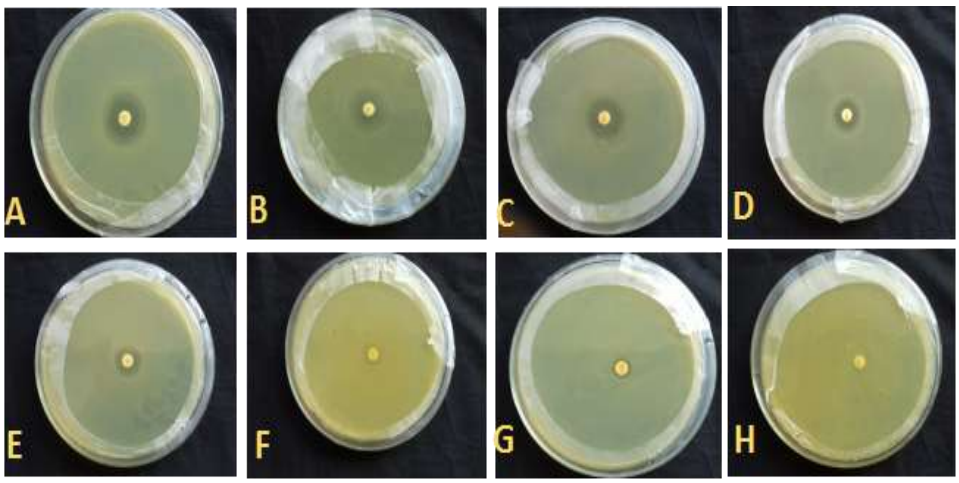

Fig. 3. Showing antibiotic sensitivity test of isolated bacteria, A) Gentamicin 10, B) Streptomycin 10, C) Chloramphenicol 30, D) Kanamycin 30, E) Carbenicillin 100, F) Clarithromycin 15, G) Doxycyclin 30, H) Penicillin 10.

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.

TABLE 2. Result of antibacterial activity of some antibiotic against isolated bacteria.

Name of antibiotic	Disc conc.	Zone of inhibition of diameter (in mm) with SE	Sensitivity pattern
Carbenicillin	100 mcg	16.5 ± 0.4	Susceptible
Doxycycline	30 mcg	9.8 ± 0.2	Resistant
Streptomycin	10 mcg	23.8 ± 0.2	Susceptible
Penicillin G	10 units	8.2 ± 0.2	Resistant
Ampicillin	10 mcg	8.2 ± 0.2	Resistant
Clarithromycin	15 µg	9.2 ± 0.2	Resistant
Amoxicillin	10 µg	8.5 ± 0.4	Resistant
Kanamycin	30 mcg	19.7 ± 0.5	Susceptible
Erythromycin	15 mcg	9.2 ± 0.2	Resistant
Cefotaxime	30 mcg	18.5 ± 0.4	Susceptible
Chloramphenicol	30 µg	17.7 ± 0.5	Susceptible
Gentamicin	10 mcg	24.5 ± 0.4	Susceptible

Note: R = Resistant (5-10 mm), I = Intermediate (11-15 mm), S = Susceptible (16 mm ≥)

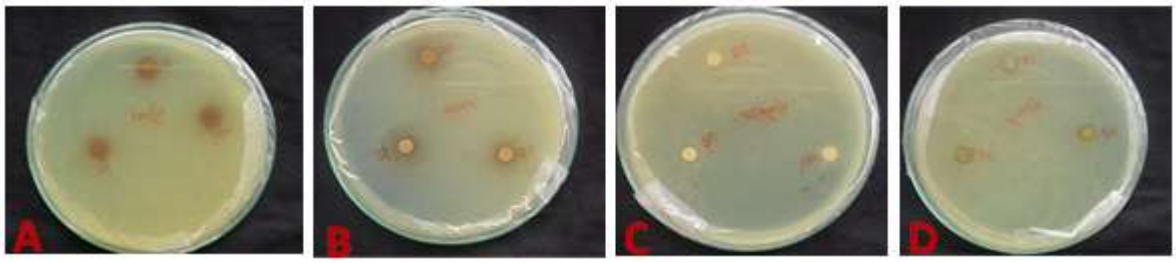

Fig. 4. Showing antibacterial activity of some plant extract against isolated bacteria, A) Terminalia arjuna, B)Justicia adhatoda, C)Centella asiatica, D)Ocimum tenuiflorum.

TABLE 3. Antibacterial activity of some row plant extract against the isolated bacteria.

Name of plant extract	Zone of inhibition with three doses of plant extract and SE (in mm)			Sensitivity pattern
	10µl/disc	20µl/disk	30µl/disk	
Terminalia arjuna	9.2±0.2	10.2±0.2	11.2±0.2	Intermediate
Ocimum tenuiflorum	7.5 ± 0.4	8.2± 0.2	8.5± 0.4	Resistant
Dutura metel	9.2 ± 0.2	9.3± 0.2	10.5± 0.4	Resistant
Justicia adhatoda	7.3± 0.2	7.5± 0.4	8.2± 0.2	Resistant
Centella asiatica	7.1± 0.2	7.3± 0.2	7.8± 0.2	Resistant
Mentha arvensis	8.2± 0.2	8.5± 0.4	9.8± 0.2	Resistant

Note: R = Resistant (5-10 mm), I = Intermediate (11-15 mm), S = Susceptible (16 mm ≥).

The result of sensitivity pattern concluded on the basis of 30µl/disk concentration.

IV. Discussion

The bacterial speck disease spread between the temperatures of 13 to 28°. High relative humidity and free water helps to develop the disease. Lesions of bacterial speck disease of tomato surrounded by dark green to yellow halos and fruit are only infected when green (Jones et al. 2014, LeBoeuf et al. 2005). *Pseudomonas syringae* produces smooth, mucoid, creamy color colonies on nutrient agar with 5% sucrose and causes a hypersensitive reaction on tobacco leaves (Lelliott and Stead 1987). Isolated bacterium was identified as *Pseudomonas* syringae depending upon the creamy white color colonies on nutrient agar medium.

Shila et al. (2013) reported that *Pseudomonas syringae* associates with the cucurbits are gram negative bacteria. In this investigation Gram staining test also showed similar result. In 2000 Preston reported the morphological characteristics of *Pseudomonas syringae* pv. *tomato* as gram negative, aerobic, motile, rod shaped, polar flagella and elicit hypersensitive response on tomato.

In Suslow et al. (1982) reported that potassium hydroxide (KOH) test is easier and faster method of differentiating gram negative and positive bacteria. They differentiate *Pseudomonas syringae* pv. *tomato* as a garm negative bacteria. The isolated bacteria of present investigation corroborate with the result.

SIM test was done according to Kirsop and Doyle 1991 method. In 1986 Oliveira and Santa-Marta reported that *Pseudomonus syringae* pv. *tomato* negative for H_2S production. This investigation showed similar result. Isolated bacterium was a motile and also positive in indole formation.

Simmons' citrate agar test performed according to Simmons (1926) and the isolated bacteria showed positive result to Simmon citrate agar. In 1986 Oliveira and Santa-Marta also reported that *Pseudomonus syringae* pv. *tomato* positive in catalase test and negative in urease test. The present investigation corroborate with their result. MacConkey agar (MacConkey 1905) was used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting Gram-negative bacteria. The isolated bacteria grew on the medium and appeared as pink color. So the isolated bacteria was lactose fermenting bacteria. In Triple Sugar Iron agar test and Kligler

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.

Iron Agar test, the bacteria fermented carbohydrate; lactose, sucrose, glucose (dextrose). In methyl red test isolated bacteria showed positive result. The result of present investigation was confirmed the work of (Crown and Gen 1998) who used methyl red test to differentiate bacteria. The sensitivity test of isolated bacteria against 15 antibiotics was done by disc diffusion method, isolated bacteria response differently on different antibiotics. Among them Gentamycin revealed highest antibacterial activity against isolated bacteria with inhibition zone 24.5±0.4 mm at the concentration of 10 µg/disc followed by 23.8±0.2 mm of zone of inhibition by Streptomycin at the concentration of 10 µg/disc.

The use of medicinal plant as traditional medicine has been started several 1000 years ago (Chang et al. 2016). All part of plant such as seeds, root, stem, leaves, and fruit, contains bioactive components potentially (Jiang et al. 2014; Mandave et al. 2014). The main bioactive components present in different part of medicinal plant are considered to be union of secondary metabolites (Singh et al. 2010). The antibacterial activity some row plant extract were screened using agar disc diffusion method (Perez et al. 1990). Six row extract of plant is used to test sensitivity. Among them *Tarminalia arjuna*, exhibite broad spectrum activity against different bacteria such as *B. cereus*, *S. aureus* and *Pseudomonas aeruginosa* (Shan et al. 2007; Arora and Kaur 1999; Sofia et al. 2007). In this investigation the row extract of it also revealed highest antibacterial activity isolated bacteria with inhibition zone 11.2±0.2 mm at 30 µl/disc concentrations. Bharathi et al. Studied antimicrobial activity of *Dutura metel* in 2010. In this investigation *Dutura metel* showed second highest antibacterial activity against isolated bacteria with inhibition zone 10.5±0.4 mm at 30 µl/disc concentration. By improving the quality of these plant extract the activity of these extract can be increase and can be used as drugs to treatment different infectious diseases.

V. Conclusion

Bacterial speck disease of tomato is an important disease of tomato. The morphological and biochemical test methods that are used to detect the isolated bacteria in this method can be helpful for molecular identification. The result of antibiotic sensitivity test can be used to control the bacteria. The extract of *Terminalia arjuna, Dutura metel* and *Mentha arvensis* can be used to contol the bacteria. By improving the quality of these plants extract we can be used them as an alternative for antibiotics.

Acknowledgements

The authors wish to thank S. M. Zia Hasan, Professor Joarder DNA and Chromosome Research Lab., Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, for her technical supports during the whole research work.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Arora DS, Kaur J (1999) Antimicrobial activity of spices. Int J of Antimicro Ag 12(3):257–262
[2] Bhowmik D, Kumar KS, Paswan S, Srivastava S (2012) Tomato-a natural medicine and its health benefits. Journal of Pharmacognosy and Phytochemistry. 1(1):33-43.
[3] Chang JD, Mantri N, Sun B, Jiang L, Chen P, Jiang B, Jiang Z,Zhang J, Shen J, Lu H, Liang Z (2016) Effects of elevated CO_2 and temperature on *Gynostemma pentaphyllum* physiology and bioactive compounds. J Plant Physiol 19:41–52
[4] Chelikani P, Fita I, Loewen PC (January 2004) Diversity of structures and properties among catalases. Cell and Mol Life Sci 61 (2):192–208
[5] Christensen WB (1946) Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bacteriol 52:461–466.
[6] Crown ST, Gen J (1998) Micromethod for the methyl red test Microbiology. 9:101-109
[7] De Stefani EP, Boffetta P, Brennan P, Deneo-Pellegini H, Carzoglio JC, Ronco A, Mendilaharsu M (2000) Dietary carotenoids and risk of gastric cancer: a case–control study in Uruguay. Eur J Cancer Prev 9:329–334
[8] Difco and BBL Manual (PDF) (2nd ed)(2009) Sparks, Maryland: Difco Laboratories, B Dic Com pp. 508 ISBN 0-9727207-1-5 Retrieved Jan 14, 2016
[9] Dorais M, Ehret DL, Papadopoulos AP (2008) Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytoche Rev 7(2):231
[10] Giovannucci E, Rimm E, Liu Y, Stampfer M, Willett W (2002) A prospective study of tomato products, lycopene, and prostate cancer risk. J Natl Cancer Inst 94:391–398
[11] Hayward AC (1960) A method for characterizing *Pseudomonas solanacearum*. Nature 186:405. doi: 10.1038/186405a0
[12] Jacquelyn G (1993) Microbiology: Principles and Explorations. Black Prentice Hall, p. 65
[13] Jiang W, Jiang B, Mantri N, Wu Z, Mao L, Lu H, Tao Z (2014) Comparative ecophysiological analysis of photosynthesis, biomass allocation, polysaccharide and alkaloid content in three *Dendrobium candidum* cultivars. Plant Omics J 7:117–122
[14] Jenkins JA (1948) The origin of the cultivated tomato Economic Botanyume 2(4):379–392
[15] Jones J, Zitter T, Momol T, Miller S (2014) Compendium of tomato diseases and pests, second edition. American Phytopathol Soc, St Paul, MN
[16] Kirsop BE, Doyle A (1991) Maintenance of Microorganisms and Cultured Cells: A Manual of Laboratory Methods, 2nd Edn. London: Academic Press
[17] Kligler IJ (1917) A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am J Public Health 7: 1042- 1044.
[18] LeBoeuf J, Cuppels D, Dick J, Loewen S, Celetti M (2005) Bacterial diseases of tomato:bacterial spot, bacterial speck, bacterial canker. OMAFRA FactSheet, ISSN 1198-712X.
[19] Lelliott RA, Stead DE (1987) Methods for the diagnosis of bacterial diseases of plants. In: Preece TF, ed Methods in Plant Pathol, Oxford, UK: Black Sci Pub 2:44–56
[20] MacConkey AT (1905) lactose fermenting Bacteria in Faeces. J Hyg (Lond) 5 (3): 333–79
[21] Mandave P, Pawar P, Ranjekar P, Mantri N, Kuvalekar A (2014) Comprehensive evaluation of in vitro antioxidant activity, total phenols and chemical profiles of two commercially
[22] Muller K, Carpenter KL, Challis IR, Skepper JN, Arends MJ (2002) Carotenoids induce apoptosis in the T-lymphoblast cell line Jurkat E6.1. Free Radic Res 36:791–802
[23] Oliveira MH, Santa-Marta J (1986) *Pseudomonas syringae pv. Tomato* (Okabe, 1933) Young, Dye and Wilkie, 1978. A new bacterial disease of tomato in portugal. Acta Horti (Netherlands)
[24] Perez C, Pauli M, Bazerque P (1990) An antibiotic assay by agar-well diffusion method. A Biol M Exp 15:113-115
[25] Preston GM (2000) *Pseudomonas syringae pv. tomato*: the right pathogen, of the right plant, at the right time. Mol plant pathol 1(5):263-275
[26] Schneider RW, Grogan RG (1977) Bacterial speck of tomato: sources of inoculum and establishment of a resident population. Phytopathol 67:388-394

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.

[27] Shan B, Cai YZ, Brooks J D, Corke H (2007) The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J of Food Microbiol 117(1):112–119
[28] Shila SJ, Islam MR, Ahmed NN, Dastogeer KMG, Meah MB (2013) Detection of *pseudomonas syringae pv. Lachrymans* associated with the seeds of cucurbits. Uni J Agric Res 1(1):1-8
[29] Simmons JS (1926) A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J Infect Dis 39:209
[30] Singh V, Amdekar S, Verma O (2010) *Ocimum Sanctum* (tulsi): Bio-pharmacological Activities. WebmedCentral Pharmacol 1:WMC001046 10.9754/j wmc 2010.001046
[31] Smirnoff N (1995) Antioxidant systems and plant response to the environment. In: Smirnoff (ed) Environment and plant metabolism: flexibility and acclimation. BIOS Sci Pub Ltd, Oxford pp. 217–243
[32] Sofia PK, Prasad R, Vijay VK, Srivastava AK (2007) Evaluation of antibacterial activity of Indian spices against common foodborne pathogens. Int J Food Sci Technol 42(8):910–915
[33] Suslow TV, Schroth MN, Isaka M (1982) Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathol 72: 917-918
[34] Vincent JM and Humphrey B (1970) Taxonomically significant group antigens in Rhizobium. J Gen Microbiol 63: 379–382. doi: 10.1099/00221287-63-3-379
[35] Young JM, Saddler GS, Takikawa DSH, Vauterin L, Gardan L, Gvozdyak RI, Stead DE (1996) Names of plant pathogenic bacteria 1864–1995, Review of Plant Pathol 75: 721–63
[36] Yunis H, Bashan Y, Okon Y, Henis Y (1980) Weather dependance, yield losses and control of bacterial speck of tomato caused by *pseudomonas tomato*. Plant Dis 64:937-939

Afrin Akter, Mst. Sabina Eyesmin Sumi, Md. Roushan Ali, Rizwona Sharmin Lia, Md. Faruk Hasan, Md. Asadul Islam, Biswanath Sikdar, and Md. Khalekuzzaman, "Detection of Bacterial Speck Disease of Tomato (*Solanum lycopersicum* L.) Through Biochemical Approaches and Evaluation of Its Biological Control Measure," *International Journal of Multidisciplinary Research and Publications (IJMRAP)*, Volume 1, Issue 8, pp. 31-37, 2019.