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Biochemical characterization of bacteria responsible for bacterial black pit disease of lime (*Citrus aurantifolia*) and their control system

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Abstract
The present investigation was design to isolate and characterize the *Pseudomonas syringae,* bacteria responsible for citrus black pit disease and its biological control. Deviating fruits test assay in the petri dishes is more convenient and most reliable for determining the pathogenicity of *Pseudomonas syringae* on citrus. The bacteria isolated from infected fruits of citrus was gram-negative which showed pink color and rod shaped in gram staining procedure and the isolated bacteria was Motile. In biochemical test, Triple Sugar Iron agar test, Simmons citrate test, Methyl Red was positive. Catalase test, MacConkey agar test, Kligler Iron Agar test, Tween 80 hydrolysis test, King's B test was negative. Tetracycline antibiotic revealed the highest activity with inhibition zone of 22.0±0.5mm diameter. In antibacterial activity assay, *Allium sativum* plant extract showed intermediate susceptible activity against the isolated bacteria with 12.0±0.5mm diameter zone of inhibition. Antagonistic activity of soil bacteria was found 8.0±0.5mm diameter inhibition zone against the isolated bacteria. This research would be help to identify the causal agent of the citrus black pit disease and antibiotic sensitivity assays perform its working efficiency.

Keywords: *Citrus aurantifolia*, bacterial black pit, characterizations, biological control

1. Introduction
Lime (*Citrus aurantifolia*) is a broadly cultivated genus of the family Rutaceae [1, 2] and most popular fruits in our country. The genus *Citrus* was established by Carl Linneaus in 1753. The genus Citrus is generally divided into two subgenera, *Citrus* and *Papeda*. Citrus originated in northern India and spread later to China, and that the sweet orange originated in southern China, where both mandarins and pumelos were planted together, spreading later to India. They are widely grown in the tropical, subtropical, and borderline subtropical/ temperate areas of the world. The subgenera *Citrus* contains edible citrus fruits, while the *Papeda* consists of the papedas, which have fruits with high concentrations of acrid oil, citric acid, causing a bitter, unpleasant flavor and thus rendering them inedible. Lime is grown 127 species in 140 countries of the world and have higher nutritional value such as it contains vitamin C, carbohydrate, providing 35% of the daily Value per 100 g serving. It is a good source of dietary fiber and contains numerous other nutrients in small quantities. Many citrus fruits, such as orange, tangerine, grapefruits and clementine's (type of mandarin), are generally eaten fresh. Its play an important role for strong ligament, dentin, skin blood vessels and bone. It is a good source of antioxidant and minimize the risk of chronic disease and cardiovascular disease and prevention facts such as cancer, neural tube defects, reduce the risk of birth defects [3].
Most of the black pit disease observed to citrus fruits and little in other parts of the plant. Black pit disease of citrus fruit caused by *Pseudomonas syringae* is widespread on the citrus foliage, although their presence does not always lead to disease development. Other reasons the occurrence of disease symptoms is influenced by several factors such as temperature, humidity, oxygen depletion, varietal susceptibility and the virulence of conditions. Symptoms of the disease, initially water soaked lesions turning to brown to black necrotic areas. Black pit appears as small light brown to black pits or larger sunken spots (5-20mm) on fruit. Most commonly begins on young leaves and twigs. Leaf lesion extend through the petiole to the stem and expand in both directions Expansion of the lesions often leads to girdling of the affected branch and withering of the portions distal to the lesion [4]. Members of the genus display these defining characteristics. Rod shaped, Gram negative, providing motility, Aerobic, Non-spore forming, Catalase-positive and Oxidase-positive. The *P. syringae* colonies on LB agar plate were white, convex, mucoid and different in colony size [5].
The aim of the research work was to investigate to identify causal agent of lime bacterial black pit disease through morphological, biochemical test and set up antibiotic sensitivity test and others biological control methods against isolated bacteria.

2. Materials and Methods
2.1 Collection of infected plant materials
At first, infected fruits of citrus were collected from local market of Rajshahi and used as research plant material. Then several steps were followed, which described in following heads.

2.2 Isolation of bacteria
Firstly the infected leaves were surface sterilize using a dilute sodium hypochlorite (NaOCl) solution (10%) and rinsed thoroughly. Infected fruit portions were cut and grinded using a sterile mortar and pestle. The extract was poured into Luria and Bertani (LB) liquid medium and incubator at 37ºC for 12-16 hours. The next day, culture

bacteria was taken from LB liquid medium with the help of a sterile loop to streak the bacteria onto a solid agar medium very carefully. The bacteria were allowed to grow for at least 12 hours at 37ºC temperature and the single colony were observed onto ager medium.

2.3 Morphological and Biochemical characterization

Gram staining (+/-) test: Gram staining is a test that was done to evaluated size, shape, and staining reaction of the isolated bacteria. In this test, we used four reagents such as crystal violet, gram iodine, decolorizers (alcohol), and safranin to perform this test. At first step isolated bacterial culture was heat fixed onto a glass slide. Then crystal violet was added to the bacterial sample and incubated for 1 min. Again, washing the slide, iodine was added in the medium. Then safranin was added to counterstaining. Carefully after all these steps the slide was observed under the light microscope at 100X using oil immersion.

Motility test: Mild agar media were used to determine the isolated bacteria was motile or not. Single colony from the plate was picked and inoculated the motility agar medium by stabbing the center to a depth of 2 inch [6].

Biochemical tests

SIM-medium test (Sulfide-Indole-Motility medium): SIM medium test was used to perform the test for hydrogen sulfide, indole, and motility of the organism. At first in conical flask 50 ml distilled water and 1.5 gm ready-made media was taken. Then the medium, conical flask, two test tube, cotton were autoclaved at 121°C for 30 min. After autoclaving all the materials transfer in the laminar air flow, 25 ml media was taken in two test tubes. After cooling, a single bacterial colony was taken and inoculated in medium. Finally, test tubes were kept for incubation at 37°C for 24 hrs. It will show the reaction with the sodium thiosulfate in the medium and the indicator, ferric ammonium citrate, to produce ferrous sulfide which falls out of solution as a blackish precipitate shape. The indole portion of the test was performed by adding Kovac's reagent to the inoculated medium. The Kovac's reagent reacts with the indole to produce a pinkish-red or reddish-purple ring shape around the top of the medium [7].

Simmons citrate agar test: This test to detect the ability of a bacterium to utilize citrate as side carbon source [8]. In the laminar air flow, medium was poured in two test tubes and kept in slant condition. When the medium was cooled a single pure isolated colony was picked with a needle and the slant surface of one test tube was lightly streaked. The incubation was done at 37°C for 16-18 hours. Citrate-positive result was set out on the basis of intense deep blue and in case of citrate –negative result no color change occurred in medium.

Catalase test: This test is used to identify organisms that produce the enzyme, catalase or not. This enzyme detoxyfies hydrogen peroxide by breaking it down into water and oxygen gas [9]. A single colony was taken on a clean slide and hydrogen per oxide was added, smeared carefully. Catalase positive result was elaborated on the basis of production of oxygen bubbles.

MacConkey agar test: MacConkey agar test was used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram negative bacteria [10]. First of all, appropriate amount of MacConkey agar kits were taken in conical flask and pH was adjusted to 7.0 and boiled to dissolve agar and sterilized at 15 lbs and 121°C for 20 min. Then MacConkey agar was inoculated with bacteria using streak plate technique and was incubated the plate in incubator at 37°C for 24 hrs.

Kligler Iron Agar (KIA) test: Kligler Iron Agar test used as an aid in the differentiation of enteric gram-negative on the basis of carbohydrate fermentation and H_2S production This test was also done to see the bacteria can ferment carbohydrate sucrose as a carbon source. If the bacteria were able to ferment sucrose then the media changes its color to indicate acid production [11].

Urease test: Urea Agar was developed by Christensen in 1946 for the differentiation of enteric bacilli. The urease test is used to evaluate the ability of an organism to split urea, through the production of the enzyme urease. At first media were autoclaved at 121°C for 30 minutes. Then autoclaved medium was poured into two test tubes and kept in slant condition. When medium was cooled, a bacterial single colony was taken. Then a sterilized inoculating loop was used for streaking back and forth along the surface of the slant. Streak the surface of a urea agar slant with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture and incubate at 37°C in inclusive air for 3 to 7 days and observed for the development of a pink color [12].

Triple Sugar Iron (TSI) agar test: The TSI agar slant test containing agar, a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose as well as sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate. This test was performed to check the ability of an organism to attack a specific carbohydrate incorporated in a basal growth medium with or without the production of gas, along with the determination of possible hydrogen sulfide (H_2S) production. With a sterilized inoculation needle, the top of a well isolated single colony was touched. Bacteria was inoculated on agar medium by first stabbing through the center of the medium to the bottom of the tube and then streaked on the surface of the agar slant. The TSI agar slant were streak with inoculums and incubate at 37°C for 18 hours [13].

Tween 80 hydrolysis test: Tween 80 hydrolysis test is the test that performed the milky white precipitation and it assured that the isolated bacteria was able to positive tween 80 reagents [14].

Methyl red (MR) test: Methyl red solution was made and the medium was assumed to equilibrate at room temperature. Firstly (MR) test, 2-3 drops of MR reagent was added. Bacteria were inoculated into the MR broth medium in the test tubes and were incubated at 37°C for 16-18 hrs. After inoculation the suspension was poured 1/3 into a clean non sterile tube then run the MR test in the tube with 2/3

medium. In this test, a distinct red color indicated positive result and yellow color indicated negative result [15].

King's B agar test: A medium for detection and differentiation of *Pseudomonas syringae* from other Pseudomonads based on fluorescein production. Mention to pack label for quantities and appropriate volume required. Prepare MAST Pseudomonas agar base (King's B) suspending the powder in distilled. For sachet packs dissolve the entire contents of the sachet in the volume shown on the label. Add 10ml of glycerol per litre of medium. Autoclave at 121°C for 30 minute. Mix well, poured culture plates and allowed to set. Prepare culture plates may be used immediately or stored in an upright position in the plastic bags at 2-8°C for up to one week before use. Inoculate plates directly by surface plating, streaking out of single colonies. Inoculate using an inoculum obtained from pure 18-24 hour cultures of *Pseudomona*s species. Incubate at 37°C for 24 hours.

2.4 Antibiotic susceptibility test against isolated bacteria

The isolated bacterial strains were grown overnight in nutrient broths that were placed in the shaker at 37^0C temperature and 150 rpm for the antibiotic sensitivity test. A serial dilution technique was done for the test respective. At first we prepared LB agar medium, then for preparing culture plates, when autoclaved sterile liquid medium temperature cooled down to 40-50^0C. At that time 15-20 ml of the medium was poured in each petri-dish and left the airflow cabinet for solidification. Using a loop we streaked the colony on LB agar culture. Commercially available and frequently prescribed antibiotics were received as antibiotic disks. 10units, 10mcg, 5µg, 15µg, 10mcg, 30mcg, 30mcg/disk concentration of Ampicillin, Amoxycillin, Azithromycin, Carbenicillin, Cefotaxime, Chloramphenicol, Doxycycline, Erythromycin, Gentamycin, Kanamycin, Neomycin, Tetracycline, respectively was used to test antibiotic sensitivity of the isolated bacteria. Antibiotic disks were placed center on the plates and incubate overnight at 37^0C. After overnight incubation then the inhibition of zone was observed of the plate and measured zone by the millimeter (mm) scale [16].

2.5 Antibacterial activity screening tests

The Antibacterial activity of the test organisms to the 10 plant extracts such as *Zingiber officinale, Allium sativum, Allium cepa, Azardica indica, Cinnamomum cassia, Spondias mombin, Datura metal, Coccinia grandis, Hibiscus rosa-sinensis,* was screened by using the disc diffusion method [17]. An inoculum suspension was swabbed uniformly to solidified 20 mL LB agar for bacteria and the inoculum was allowed to dry for 5 min. The discs was made by what-man filter papers. Each discs of 6 mm in diameter were used. Aliquot of 10, 20, 30µl from each plant crude extract was added into each disc on the seeded medium and allowed to stand on the bench for 30 min for proper diffusion and thereafter incubated at 37ºC for 24 h. The resulting inhibition zones were measured by millimeters (mm) scale. Scale for identification of resistant, intermediately resistant and susceptible are given below. If diameter of zone inhibition with disc diameter is less than 10 mm then it's show resistant, if diameter of zone inhibition with disc diameter is greater than 10 and less than 15 mm then its show intermediately resistant, if diameter of zone inhibition with disc diameter is getter than 15 mm then its show susceptible.

2.6 Antagonistic effect of soil bacteria

The Antagonistic activity test two isolated soil bacteria were screened by using the disc diffusion method. Two isolated soil borne bacterial strains were collected from Microbiology lab, GEB, Rajshahi University, which was previously collected and identified. For antagonistic activity study, they were indicated as soil borne bacteria *Rhizobium* from *Cicer arietinum* (RCV) and *Rhizobium* from *Vigna mungo* (RVM). The discs (6mm diameters) were made by cutting the What-man filter paper with the help of punch machine. Briefly, 20ml nutrient agar medium were placed in a petri-dish with 0.1ml of a 10-2 dilution of isolated bacterial culture. We made dics 6mm in size. 10µl, 20µl and 30µl/disc of each soil bacterial culture was taken by micropipette and were incubated at 37°C for 16-18 hrs. The antagonistic activities of the two soil bacteria against the isolated bacteria were evaluated by measuring zone of inhibition with the help of mm scale [18].

3. Results

3.1 Isolation and culture of bacteria

At first the infected fruit of citrus was collected then surface sterilized it by distilled water then grind it by mortar and pestle and made it juicy form by the laminar air flow cabinet. Then placed 10-20 µl grinded juice into previously prepared 50ml LB liquid medium by micropipette and incubate it at 37°C for overnight. Our isolated bacteria grow in LB liquid medium after 16 hours of incubation. The isolated each single colonies were creamy in color. The size and shape of each colony was found to be small, medium, mucoid and Convex onto agar plate (Fig. 1).

Fig 1: Showing bacterial infected fruit of lime sample A) Liquid culture in LB medium B) Isolated colonies C).

3.2 Morphological and Biochemical characteristics

In gram staining procedure, the isolated bacterium was gram-negative, rod shaped and pinkish color under microscope [Fig. 2 A]. Isolated bacterium was motile because the growth area formation extending away from the inoculation line [Fig.2 B]. In SIM medium test the isolated bacteria was motile, indole and sulfide production negative because the bacteria did not produce red/pink color band on the top of tube when adding kovacs reagent and H2S did not produced as no black precipitation formed. Simmon's citrate test was positive because the medium green turns to the dark blue color the medium [Fig. 2 C]. In Tween 80 hydrolysis test performed negative result because no color change from orange yellow to red [Fig. 2 D]. In catalase test, there is no the presence of bubbles from production of oxygen gas it's confirmed a catalase negative result against isolated bacteria. KIA in this test, the isolated bacterium there was no glucose and lactose fermenting because no butt formation and slant did not change the color [Fig.2 E]. After inoculate the bacteria into Methyl Red medium it produce acid and give pink color. So isolated bacteria showed positive result, because it turns a pink color [Fig. 2 F]. In TSI test, isolated bacterium did not produce any gas and hydrogen sulfide and was glucose, lactose and or sucrose fermenting because acidic slant and yellow color were found in medium [Fig. 2 G]. In Urease test, isolated bacteria did not hydrolyze urea [Fig. 2 H]. The isolated strains were grown on the media it shown negative result. There is no produced pink color around the colony and did not produce lactose fermentation [Fig. 2 I]. In King" B ager test, Isolated bacteria showed a negative result because, of did not produce fluorescence on KB ager medium and did not trurns color [Fig. 2 J]. Different test results is summarized in Table 1.

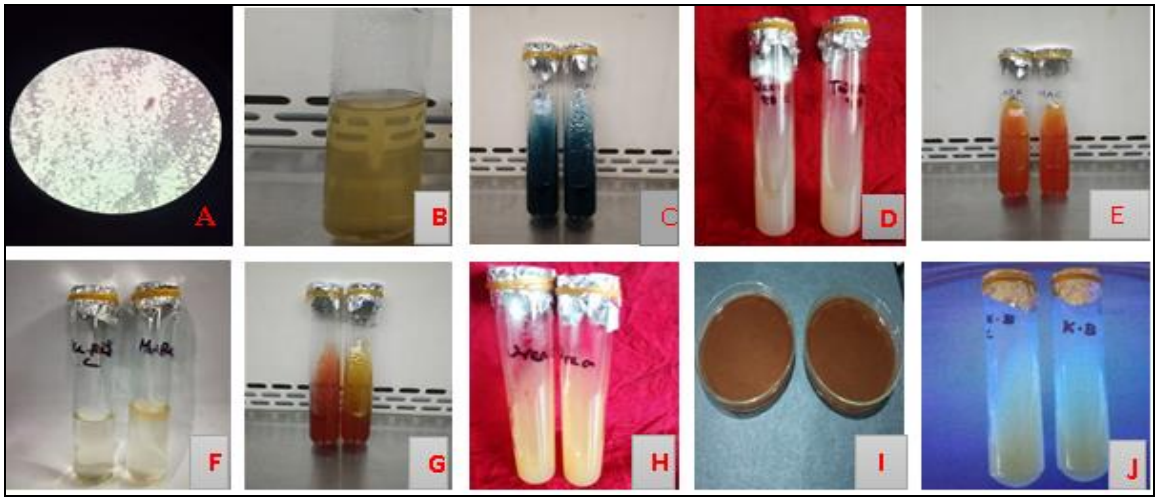

Fig 2: Biochemical characteristic of isolated bacteria (A) gram stain (B) Motility test (C) Citrate utilization test (D) Tween 80 hydrolysis test (E) Kligler iron agar (F) Methyl red test (G) Triple sugar iron test (H) Urease test (I) MacConkey agar test (J) King ' B ager test.

Table 1: Responses of the isolated bacteria in different biochemical test media

Name of the test	Reaction	Indication sing	Remarks
Gram staining	-ve	Small, rod shaped, pinkish in color	Gram staining showed gram negative bacteria
Motility	+ve	growth area extending away from the inoculation line	Gram negative bacteria showed motile did extending growth area formation
Sulphide –Indole-Motility (SIM	+ve, (-ve)	motile, no H2S and indole production	Gram negative bacteria performed motile and did not produce any indole and H2S
Simmon's citrate	+ve	Color changed from green to the dark blue	Citrate metabolizing gram negative bacteria
Methyl Red	+ve	Color turns from yellow to red ring	Isolated bacteria had the ability to utilize glucose
catalase test	-ve	There was no oxygen bubbles	Isolated bacteria did not form bubbles resulting from production of O2 gas
Tween 80 hydrolysis	-ve	There was no turbid zone around the colonies	Gram negative bacteria no hydrolyzed the tween 80
TSI test	+ve	Color changed from red to yellow	Bacteria didn't produce H2S and confirming lactose fermenting
King" B ager test	-ve	Did not trurns color and no fluorescence production	Isolated bacteria showed a negative result because, of did not produce fluorescence on KB ager medium and did not trurns color.

3.3 Antibiotic susceptibility test against isolated bacteria

Tetracycline revealed the highest significant antibacterial activity with inhibition zone 22.0±0.5mm. The attainted results showed that Erythromycin, Oxytetracyline, Penicillin, kanamycin, Gentamycin revealed a wide antibacterial spectrum against test bacterial strains. Vancomycin, Neomycin, Doxycycline, Azithromycin, Amoxycillin showed intermediate activity with their average inhibition zone of 14.0±0.5mm [Fig.3]. Lowest antibacterial activity was showed by Cefixime and cefotaxime with inhibition zone of 6.0±0.5 mm. Antibiotic sensitivity test results is summarized in Table 2.

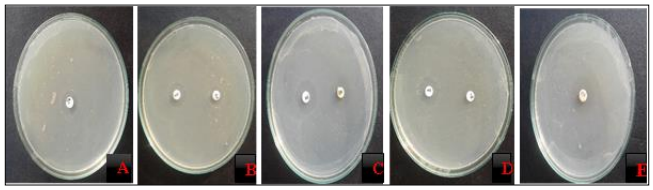

Fig 3: Some antibiotic test against isolated bacteria (A) Azithromycin (B) Erythromycin, Neomycin (C) Oxytetracycline, Gentamicin (D) Kanamycin, and Rifampicin (E) Tetracycline.

Table 2: Antibiotic sensitivity test

Name of Antibiotic	Disc potency (µg)	Zone of inhibition (mm)	Sensitivity pattern
Vancomycin	30	14.0±0.5	Intermediate
Neomycin	30	12.0±0.5	Intermediate
Cefixime	5	6.0±0.5	Resistant
Tetracycline	30	22.0±0.5	Susceptible
Doxycycline	30	15.0±0.5	Intermediate
Oxytetracycline	30	19.0±0.5	Susceptible
Cefotaxime	30	6.0±0.5	Resistant
Nalidixic acid	30	7.0±0.5	Resistant
Azithromycin	15	14.0±0.5	Intermediate
Erythromycin	15	16.0±0.5	Susceptible
Rifampicin	5	9.0±0.5	Resistant
Penicillin	10	18.0±0.5	Susceptible
Kanamycin	30	16.0±0.5	Susceptible
Gentamicin	30	19.0±0.5	Susceptible
Amoxycillin	10	15.0±0.5	Intermediate

Note: Resistant=<10 mm; Intermediate =10-15 mm; Susceptible=>15 mm

3.4 Antibacterial activity screening tests

Ten plant extract species were used where raw extract of *Allium sativum* showed the highest significant antibacterial activity with inhibition zone 12.0±0.5mm [Fig. 3A]. *Datura metal* showed lowest antibacterial activity with mean inhibition zone of 0.0±0.5mm [Picture not given].

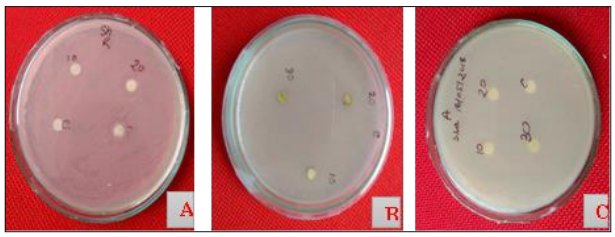

Fig 4: Antibacterial activity of some plant exract against isolated bactera, (A) *Allium sativum*, (B) *Cinnamomum cassia* (C) *Allium cepa.*

Table 3: Antibacterial activity of some plant extracts

Name of plant extract	Dose of plant extract (zone in mm)			Sensitivity pattern
	10 µl	20 µl	30 µl	
Allium sativum	8.0±0.5	8.5±0.5	12.0±0.5	Intermediate
Allium cepa	0.0±0.5	7.0±0.5	8.0±0.5	Resistant
Spondias mombin	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Cinnamomum cassia	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Azadiracta indica	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Coccinia grandis	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Datura metal	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Hibiscus rosa-sinensis	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Zingniber officinale	0.0±0.5	0.0±0.5	0.0±0.5	Resistant
Momordica charantia	0.0±0.5	7.5±0.5	8.0±0.5	Resistant

Note: Resistant=<10 mm; Intermediate =10-15 mm; Susceptible=>15 m

3.5 Antagonistic effect of soil bacteria

Soil bacteria *Rhizobium* from *Vigna mungo* displayed highest activity with inhibition zone of 8.0±0.5mm and *Rhizobium* from *Cicer arietinum* showed 7.5±0.5 mm zone of inhibition against the isolated bacteria.

4. Discussion

The isolation, identification and characterization of microorganisms, useful as bio-control agents or producers of bioactive compounds, are great relevance for the modern and compatible agriculture. In the present research project, the morphological, physiological and biochemical characterization were conducted for bacterial identification. The Gram staining is the test that's always the first step in the identification of a bacterial organism. In gram staining procedure, the isolated bacteria performed gram negative as well as small rod shaped and pinkish color. The isolated bacteria from *Citrus aurantifolia* by Abubaker *et al*. [19] also showed similar results by different biochemical test. In biochemical test, the motility test indicates, the isolated bacterium was motile, because growth area formation extending away from the inoculation line. *P. syringae* did not showed any color in the media plate which indicates our bacterium were not able to ferment lactose. Similar results were showed by Brodsky and Nixon (1973) in *P. aeruginosa* bacterium the isolated bacteria from citrus fruits. King's B agar medium test were confirmed by Abdellatifa al. [20]. Eight of the isolates failed to produce fluorescence on KB medium, although most of the bacterial species belonging to the genus *Pseudomonas* are well known for their capability to produce siderophores that fluoresce under UV light at 365 nm. However, the ability of these bacteria to enhance the pigment excretion when grown on synthetic media depends on the composition of the growth medium. This is confirmed by the results, because it was found that all isolates, including those that do not produce fluorescence on KB, were able to change the colour of the chrome azurol S (CAS) agar medium from blue to orange. This is a result of siderophore removal of iron from the used dye confirmed by Sreedevi *et al*. [21]. He found isolated from citrus plants does not produce fluorescence on KB nutrient agar medium. And also the bacteria isolated from citrus fruits showed positive results to Simmon's citrate, Triple Sugar Iron Agar, Methyl Red test and negative results to Urease, Tween 80 hydrolysis. Our results confirmed the work of Ali *et al* [7], Islam *et al*. [22] and Hussain *et al*. [23]. Catalase test demonstrate the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). In this research we found when we added H_2O_2, to our isolated bacteria did not produce air bubbles that clearly indicates it was negative to catalase it was against to Islam *et al*.[24], which also reported positive result of Islam [24].

In the present study, Tetracycline performed highest antibacterial activity against isolated bacterial with inhibition zone 22±0.5 susceptible. Bharathi *et al*. [25] found by Erythromycin against *Pseudomonas aeruginosa*. Hasan and Sikdar [26] performed the similar zone of inhibition by kanamycin against *Pseudomonas sp*. Sumi *et al*. [27] also found the similar zone of inhibition results against *Pseudomonas syringae*. These results confirm our present findings. This result showed that the isolated bacteria is highly sensitive against Erythromycin, Oxytetracyline, Penicillin, kanamycin, Gentamycin revealed antibacterial activity with more than 18±0.5 mm zone of inhibition

against test bacterial strain and lowest antibacterial activity with zone of inhibition 6±0.5 by cefixime.

Antimicrobial activity of 10 medicinal plants have been evaluated *in vitro* against isolated species. *Allium sativum* revealed the highest significant antibacterial activity with inhibition zone more than 12±0.5 mm which were intermediate susceptible. Sumi worked on *Allium sativum* extract against *Pseudomonas syringae* at 50% concentration [27]. Likewise, raw extract of *Allium cepa*, *Spondias mombin*, *Cinnamomum cassia*, *Azadiracta indica*, *Datura metal*, *Zingenifera officinale*, *Hibiscus rosa-sinensis*, *Coccinia grandis*, *Momordica charantia* showed antibacterial activity with inhibition zone equal to 8±0.5 mm and 0±0.5 mm, 0±0.5 mm, and 0±0.5, 0±0.5,0±0.5,0±0.5,0±0.5,8±0.5 mm respectively which were resistant activity against isolated bacteria which are quite similar Bharathi *et al*. [25] studied antimicrobial activity of *Datura metel* in 2010. In this investigation, *Daura metel* showed resistant antibacterial activity against isolated bacteria with inhibition zone 0±0.5mm. Antagonistic activities of two soil bacteria have been evaluated *in vitro* against isolated bacteria from black pit disease of citrus fruits. *Rhizobium* from *Cicer arietinum* and *Rhizobium* from *Vigna mungo* both revealed the resistant antagonistic activity with their inhibition zone one was 8±0.5 and another 8±0.5. This study confirms us the competency of some antibiotic, plant extracts and soil bacteria as natural antimicrobials and suggests the possibility of employing them in drugs for management of infectious diseases caused by *Pseudomonas syringe.*

5. Conclusion

In the present investigation, the isolated bacteria was gram-negative, pink color, rod shaped and Motile. Triple Sugar Iron agar test, Simmons citrate test, Methyl Red is positive. Catalase test, MacConkey agar test, Kligler Iron Agar test, Tween 80 hydrolysis test, King" s B test is negative. Tetracycline, revealed the highest significant antibacterial activity with inhibition zone 22.0±0.5 mm. Antibacterial activity study suggested that the plant extracts from *Allium sativum*, have a broad spectrum of intermediate antimicrobial activity against isolated bacteria with the inhibition zone 12.0±0.5 mm. Antagonistic activity were displayed of soil bacteria with inhibition zone 8.0±0.5mm. The above tests provided detail information about the isolation, characterization, and antibiotic sensitivity assay against *Pseudomonas. syringae*. The present investigation would be good source of information for molecular detection of this devastating bacteria and design a suitable control technique.

6. Acknowledgments

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7. Author Contributions

MSA, SN, MFH, MAI and BS designed the experiments, developed the methodology and prepared the manuscript. MSA, MEKC, BS and MFH collected the data and carried out analysis. SN, MAI, MEKC and MFH assisted with manuscript preparation.

8. Conflict of interest

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

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