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Biological Control of Bacterial Leaf Spot Disease of Papaya (*Carica papaya*) through Antagonistic Approaches using Medicinal Plants Extracts and Soil Bacteria

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ABSTRACT

The present investigation was carried out to isolate the causal agent of bacterial leaf spot disease of papaya and evaluation of its biological control through different extracts from selected medicinal plants and antagonistic microorganisms. The causal organism was isolated on nutrient agar medium at 37^0C for incubation period of 16 hours. Molecular detection of the isolated bacteria confirmed by PCR using specific primers 27F (5'-AGAGTTTGATCCTGGCTC-3') and 1391R (5'-GACGGCGGTGTGTRCA-3') which amplified approximately 1600 bp DNA fragment of the isolated bacteria. Four different types of solvents Methanol, Ethanol, Acetone, Petroleum ether were used to prepare the extract from Lantana camara, Pongamia pinnata, Rauwolfia serpentina, Cymbopogon citrates, Tagetes erecta. Among them the ethanol extract of Lantana camara showed highest 13.5 mm zone of inhibition at 60µg/ml concentration and methanol extract showed 17mm zone of inhibition at 100µg/ml concentrations respectively against isolated bacteria. Antagonistic soil bacteria were isolated from different soil samples from Rajshahi University premises. Isolate 1 and 2 of soil bacteria showed highest 12±0.2 mm inhibition zone at 40µl/disc concentration in disc diffusion method and isolate 3 of soil bacteria showed 16±0.5 mm inhibition zone against bacterial leaf spot disease causing pathogen at 50 µl/well concentrations in well diffusion method. This study will be helpful for effective biological control of bacterial leaf spot disease of papaya causing less harm to the environment compare to the traditionally available chemical control.

Key words: *Papaya, Bacterial leaf spot, Biological control, Antagonistic, Pseudomonas carica papaya.*

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INTRODUCTION

Carica papaya L. commonly called papaya is a well-known plant with edible fruit. Papaya (*Carica papaya* L.) is grown all over the world. It grows mainly in the wetter parts of West Africa, tropical and subtropical regions[1]. Papaya belongs to the family caricaceae. The plant is native to tropical America and was introduced to India in 16th century. The papaya is a large, tree- like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the trunk[2]. Papaya fruit is highly appreciated worldwide for its flavor, nutritional qualities and digestive properties[3]. Both the green and ripe papaya fruits contain nutritive and medicinal value. Both leaf and fruit of the *Carica papaya* Linn. possess carotenoids namely β- carotene, lycopene, anthraquinones glycoside, as compared to matured leaves and hence possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities have also been established. Leaves being an important part of several traditional formulations are undertaken for standardization for various parameters like moisture content, extractive values, ash values, swelling index, etc[4].

Bangladesh is an agricultural country. Most of the peoples of Bangladesh depend on agriculture. Different types of crops and fruits are grown throughout the Bangladesh. Among them papaya is the widely cultivated but the hindrances behind the large scale production of papaya in Bangladesh are different types of diseases. Papaya leaves and fruits are affected by different types of bacterial, fungal and viral diseases. Bacterial diseases like bacterial leaf spot, bacterial wilt, bacterial canker, erwinia decline are some of the diseases of papaya. Among them bacterial leaf spot is very common in Bangladesh. Bacterial leaf spot of papaya is caused by the *Pseudomonas caricapapayae.* It is one of the most important bacterial diseases of papaya. It appears mainly on leaves as small spot. *Pseudomonas caricapapayae* is a gram-negative soil bacterium that is pathogenic to plants. It was originally isolated on papaya (*Carica papaya*) in Brazil. Based on 16S rRNA analysis, *P. caricapapayae* has been placed in the *P. syringae* group[5]. It greatly hampers the production of papaya. Significant loss of papaya production occurs due to this disease. In Bangladesh various types of chemical pesticides and bactericides are used to control the bacterial disease of papaya. Continuous use of chemical pesticide has lead to an increase of health hazards due to their phytotoxic residual and pollution effect. To cope with this problem biological control have been applied. Biological control offers an environmentally sound alternative to chemical pesticides and is an attractive method for plant protection against soil-borne diseases. Many biological control agents are found by screening large numbers of micro-organisms against plant pathogens in vitro or in planta[6,7]. The mechanisms underlying these bacterial antagonisms for plant pathogens involve antibiosis, competition for nutrients or space, enhancement of root and plant development, induction of plant resistance and/or inactivation of the pathogen's enzymes[8]. Extracts and essential oil from medicinal plants are also used as important agent for biological control. Contrary to the synthetic drugs antimicrobials from plants don't have many side effects and have enormous therapeutic potential to heal many infectious diseases[9]. Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attribute. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized. Therefore the present study was designed to screen the antagonistic activity of soil bacteria and extracts from selected medicinal plants to biological control of bacterial leaf spot disease of papaya.

MATERIAL AND METHODS

Plant Materials

In the present study bacterial leaf spot infected papaya plants were collected from Rajshahi University premises and were identified by

Bangladesh Council of Science and Industrial Research (BCSIR), Binodpur, Rajshahi. Five medicinal plants *Lantana camara* (choitra), *Pongamia pinnata*, *Rauwolfia serpentina*, *Cymbopogon citrates* (lemon grass), *Tagetes erecta* were collected from the Botanical garden of Rajshahi University for the Preparation of plant extracts.

Isolation of causal organism

Disease infected leaves of papaya were first washed using distilled water and disinfested using a dilute sodium hypochlorite solution (10%) and rinsed thoroughly. We cut the infected area and placed on LB liquid media and incubated for 12 to 16 hours at 37^{o}C for allow to growing bacteria. After the bacteria have grown into LB liquid medium, used a sterile loop to streak the bacteria onto a solid nutrient agar media plates and incubated for 12 hours at 37^{o}C. One of creamy white colony was picked by wire loop and streaked on another media plate for pure culture.

Molecular identification of causal organism (Isolated bacteria)

For genomic DNA isolation and purification, the isolated bacterial single colony was cultured in LB broth medium at 37ºC for overnight. The culture was centrifuged and the liquid was discarded. The total genomic DNA were isolated from bacterial mass by heat lyses and selective precipitation of cell debris and polysaccharides with CTAB (cetyltrimethylammonium-bromide), and the procedure was maintained as similar to Ausbel[10] with slight modification in the incubation period and amount of chemicals which was used. The DNA was resuspended in TE buffer and quantified using a spectrophotometer then electrophoresed on 1% agar gel by comparison with DNA samples of known concentration. The amplification of 16S rRNA gene from the isolated DNA was done by PCR reaction in a thermo cycler (Nyx, Technic, Inc., USA), using the primers 27F (5′-AGAGTTTGATCCTGGCTC-3′) and 1391R (5′-GACGGCGGTGTGTRCA-3′). PCR was performed in volumes of 25µl, containing nuclease free ddH_2O 15µl, dNTP mix 1.0µl, forward primer 1.0µl, reverse primer 1.0µl, DNA template 1.5µl, $MgCl_2$ 2.5µl, *Taq* buffer B 2.5µL and *Taq* polymerase (Takara, Japan) 0.5µl. The procedure was as following: initial denaturation at 95ºC for 5min; 35 cycles of denaturation for 40s at 95ºC, annealing for 1min at 65ºC, and extension for 2min at 72ºC; the final extension at 72ºC for 10 min, followed by cooling to 4ºC until the sample was recovered. Gel electrophoresis was used to visualize the PCR products lengths. 0.5x TBE buffer was used in agar gel and visualized under a UV transilluminator.

Isolation of antagonistic microorganism (Soil bacteria)

Soil samples were collected in the month of January 2017 from rhizopheric region of leguminous plants from Rajshahi University region and brought to Lab. Samples were collected to a depth of 5-7 inches from rhizopheric region. After collection, soil samples were immediately dried at room temperature for two to five days. 200gm of soil samples was taken for microbiological analysis and the remainder was discarded. Soil sample was collected from four different regions. Analysis of each soil samples was done separately. Bacterial-flora from the soil samples were isolated by serial dilution, plating and streaking on suitable growth medium[11]. Colony was found after incubation at 37^{0}C for 16 hours.

Screening of antagonistic activity of soil bacteria

Screening of antagonistic activity was done by disc diffusion method and well diffusion method. Agar disc-diffusion testing developed in 1940 is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing[12]. Disc diffusion method was done according to Hasan and Sikdar[13]. In disc diffusion method we inoculated causal pathogen on to the agar plate. Then we placed the filter paper discs (about 6 mm in diameter), containing the soil bacteria at 10µl, 20µl, 40µl and 50µl per disc concentration, on the centre of agar surface. The Petri dishes were incubated at 37^{0}C for 16 hours. Then diameter of zones of inhibition was measured by millimeter (mm) scale.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts[14]. Similarly to the procedure used in disc-diffusion method, the agar plate surface was inoculated by spreading a volume of the causal pathogen over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a tip, and a volume 10µl, 20µl, 40µl, 50µl of the soil bacteria was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. Then diameter of zones of inhibition was measured by millimeter (mm) scale.

Screening of antagonistic activity of plant extracts

Screening of antagonistic activity of plant extracts against causal pathogen was done by moderate disc diffusion method according to Hasan and Sikdar[13]. Five different medicinal plants *Lantana camara*, *Tagetes erecta* ,*Cymbopogon citrates*, *Pongamia pinnata*, ***Rauwolfia serpentina*** *and four solvents methanol, ethanol, acetone and petroleum ether were used for the preparation of extract.* Plant materials were washed and air-dried for 15 days. The dried plant materials were grind to a fine powder in a grinding machine. A measured quantity of 20g of dried powder was soaked in 200ml methanol, ethanol, acetone and petroleum ether in round bottom flask at room temperature for seven days with occasional shaking. The extract was filtered by cotton white cloth followed by Whatman No.1 filter paper. The filtrate was evaporated at 45^0C to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use. An inoculum (causal pathogen) suspension was swabbed uniformly to solidified 20mL LB agar media for bacteria and the inoculum was allowed to dry for 5 minutes and 6mm diameter paper discs were used. Extracts from methanol, ethanol. acetone and petroleum ether were used at 20µg/ml, 40µg/ml 60µg/ml 100µg/ml concentrations and added into each disc on the seeded medium and allowed to stand on the bench for 1 hour for proper diffusion and thereafter incubated at 37^oC for 24 hours. The resulting inhibition zones were measured in millimeters (mm).

Antibiotic sensitivity test against the isolated bacteria

Sensitivity of causal pathogen to different antibiotics was determined *in-vitro* by employing a modified disc diffusion method[13]. The isolated bacterial strain was incubated overnight in nutrient broths that were placed in the shaker at 37^0C and 150 rpm for the antibiotic sensitivity test. A serial dilution technique was made for the test respective. At first we prepared LB agar medium, then for making culture plates, the sterile liquid medium was distributed in sterile conical flasks when the temperature cooled down to $40\text{-}50^0$C. Approximately 15-20 ml of the medium was poured in each petridish and left the airflow cabinet for solidification. Using a loop we streaked the colony on LB agar culture. Commercially available and frequently prescribed standard antibiotics namely, Penicillin, Tetracycline, Amoxycillin, Doxycycline, Cefotaxime, Carbenicillin, Clarithromycin, Streptomycin, Azithromycin, Ampicillin, Gentamycin, Rifampicin, Oxytetracycline, Vancomycin, Cefixime, Nalidixic acid, Kanamycin, Neomycin and Erythromycin were used to test antibiotic sensitivity of the isolated bacteria. Antibiotic discs were placed centrally on the respective plates and incubated overnight at 37^0C for 16 hours. After overnight incubation the diameter of zone of inhibition was observed and measured with the help of millimeter scale (mm)**.**

Statistical analysis

All experiments were performed at least three times. Data represent the means and standard errors (Mean ± SE) from at least three replicates of a representative experiment. The data were calculated using Microsoft Excel 2010 software.

RESULTS

Isolation of causal organism

Bacterial liquid culture from bacterial leaf spot infected papaya plant was obtained after 16 hours of incubation at 37°C. From liquid culture, subculture was done by streaking onto the Lauria and Bertani (LB) agar medium in 90mm petridishes. Visual observation was identified the colony morphology of the bacteria (Fig. 1A).

Molecular identification of causal organism (Isolated bacteria)

In the present experiment, the pathogen of bacterial leaf spot disease of papaya was detected by PCR in a naturally infected papaya plant. As expected, the pathogen was detected by PCR assay from infected papaya leaves. The purified genomic DNA was quantified using a spectrophotometer and electrophoresed on 1% agar gel which showed clear band in lane 1, and 2 (Fig. 1B). In PCR analysis, the DNA amplified PCR products showed a 1600bp length clear band in lane 1 in agar gel electrophoresis (Fig. 1C).

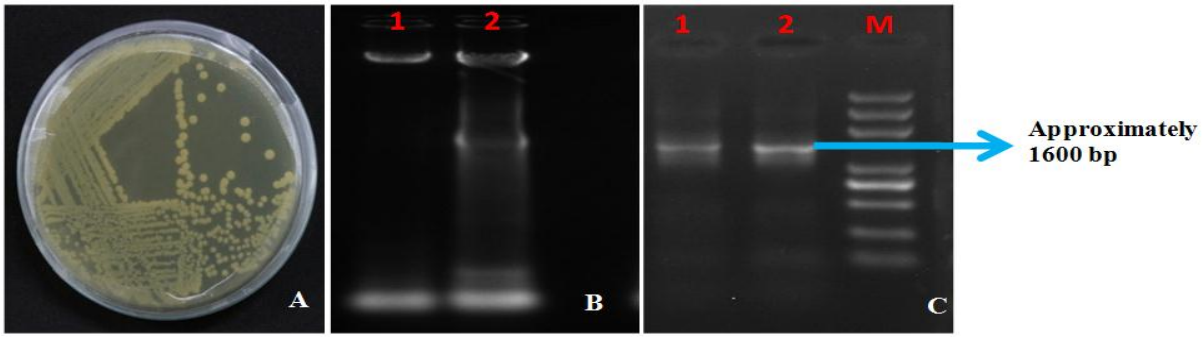

Fig. 1: Showing the colony of isolated causal pathogen, total genomic DNA of causal pathogen and PCR product of genomic DNA. (A) Colony morphology of causal pathogen (B) Total genomic DNA (C) PCR analysis. (M= 1kb molecular marker, 1, 2= clones of causal pathogen)

Isolation of antagonistic microorganism (Soil bacteria)

We isolated soil bacteria from four different places. Bacterial colonies were observed after 16 hours of incubation at 37°C. Isolate 1 of soil bacteria showed yellow color colonies (Fig. 2A) on agar plate, isolate 2, 3 and 4 showed creamy white colonies on agar plate (Fig. 2B, Fig. 2C, Fig. 2D).

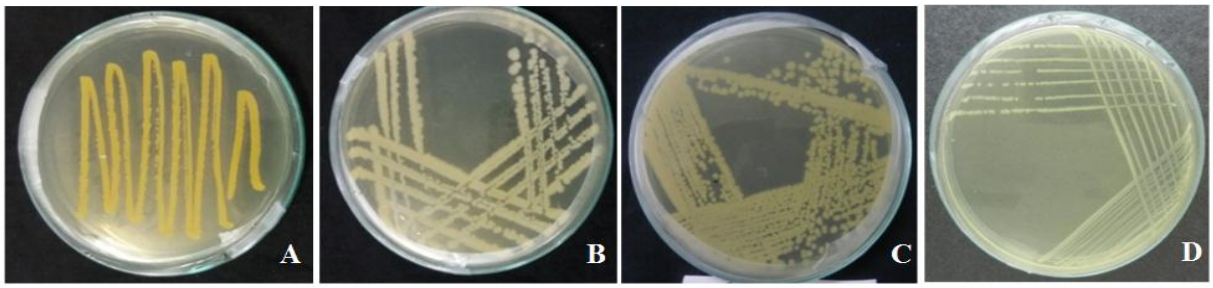

Fig. 2: Showing the four isolates of soil bacteria. (A) Isolate 1; (B) Isolate 2; (C) Isolate 3 and (C) Isolate 4

Screening of antagonistic activity of soil bacteria

In the present study we used disc diffusion and well diffusion method for screening antagonistic activity of soil bacteria against causal pathogen of bacterial leaf spot disease of papaya. Isolate 1 and 2 showed highest 12±0.2 mm zone of inhibition (Fig.3A and Fig.3B), at 40µl/disc concentration, isolate 3 and 4 showed highest 11±0.2mm and 9±0.4mm zone of inhibition (Fig.3C and Fig.3D) at 40µl/disc concentration in disc diffusion method. In well diffusion method isolate 3 showed highest 16±0.5mm zone of inhibition (Fig. 3E) at 50µl/well concentration. Results of antagonistic activity of soil bacteria are given in Table 1.

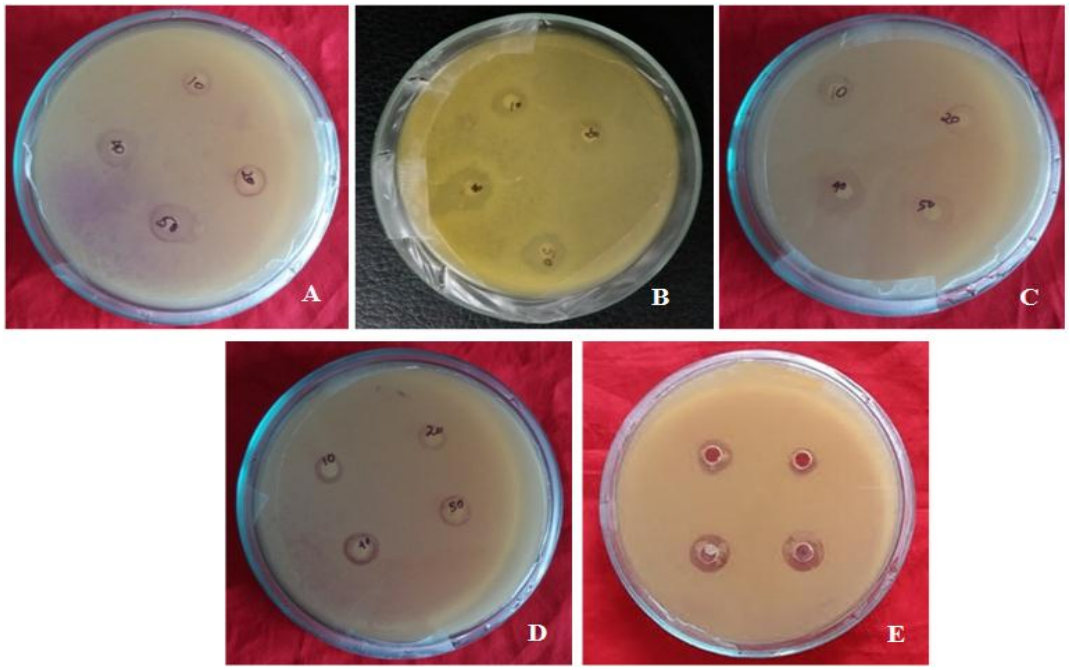

Fig. 3: Showing antagonistic activity of soil bacteria against causal pathogen. (A), (B), (C) and (D) showed DOZOI for isolate 1, 2, 3 and 4 respectively in disc diffusion method. (E) Showed DOZOI for isolate 3 in well diffusion method. DOZOI= Diameter of Zone of Inhibition

Table 1: Screening of antagonistic activity of soil bacteria

Soil bacteria	Method used	Diameter of zone of inhibition (in mm) Mean ± SE			
		Doses in µl			
		10µl/disc	20µl/disc	40µl/disc	50µl/disc
Isolate 1	Disc diffusion	9±0.2	9±0.5	12±0.2	10±1.2
Isolate 2	Disc diffusion	8±0.5	9±1.2	12±0.2	9±0.5
Isolate 3	Disc diffusion	8±0.2	9±0.2	11±0.2	8±0.2
Isolate 4	Disc diffusion	7.5±0.2	7±0.5	9±0.4	8±1.2
Isolate 3	Well diffusion	9±0.8	8±1.2	14±0.2	16±0.5

Abbreviation: Mean ± SE=mean and standard error

Screening of antagonistic activity of plant extracts

In the present study extracts from selected medicinal plants showed broad spectrum of antagonistic activity against the causal pathogen of bacterial leaf spot of papaya. The antibacterial sensitivity of different solvents of *Lantana camara*, *Tagetes erecta*, lemograss, *Rauwolfia serpentina*, *Pongamia pinnata* showed significant reduction in the growth of causal pathogen of bacterial leaf spot of papaya in terms of zone of inhibition around the disc. Present study showed that methanol and ethanol extracts exhibited effective response against the causal pathogen. Among the five medicinal plants, methanol extract of *Lantana camara* showed highest 17mm zone of inhibition at 100µg/ml concentrations (Fig. 4A) and ethanol extract showed 13.5mm zone of inhibition at 60µg/ml concentration (Fig. 4B). Methanol extract of Lemongrass inhibited 8mm zone (Fig. 4C) around the disc of causal pathogen at 100µg/ml concentration while Petroleum ether extract of *Tagetes erecta* exhibited highest 11mm zone of inhibition at 60µg/ml (Fig. 4D). Among the four solvents petroleum ether extract of *Rauwolfia serpentina* showed highest 15mm zone of inhibition at 60µg/ml concentration (Fig. 4E). Methanol extract of *Pongamia pinnata* showed 8mm zone of inhibition at 100µg/ml (Fig. 4F). All the results of antagonistic activity of plant extract against the causal pathogen are given in Table 2.

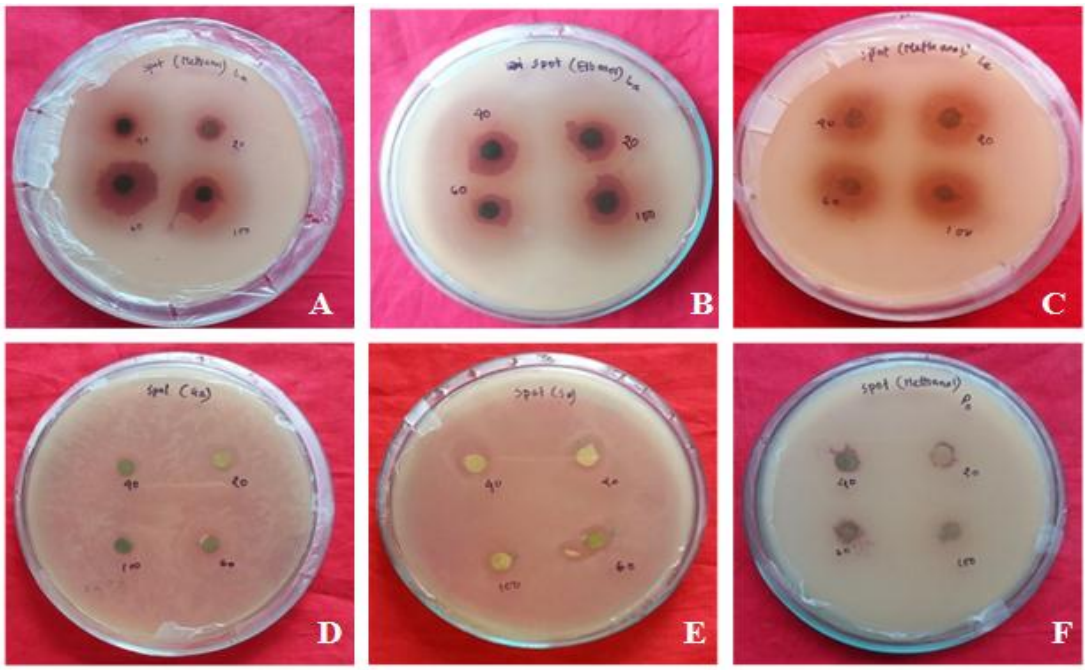

Fig. 4: Showing the antagonistic activity of plant extracts of five medicinal plants. (A) Methanol extract of *Lantana camara* showed DOZOI, (B) Ethanol extract of *Lantana camara* showed DOZOI, (C) Methanol extract of lemon grass showed highest DOZOI at 100µg/ml concentration, (D) Petroleum ether extract of *Tagetes erecta* exhibited highest 11mm zone of inhibition, (E) Petroleum ether extract of *Rauwolfia serpentina* showed DOZOI. (F) Methanol exract of *Pongamia pinnata* showed DOZOI

Table 2: Summarized result of antibacterial effect of five selected plants against isolated pathogen

Name of test plants	Plant parts	Diameter of zone of inhibition (in mm)				
		Solvents	20µg/ml	40µg/ml	60µg/ml	100µg/ml
Lantana camara (Choitra)	Leaf	Methanol	9	7.5	11	17
		Ethanol	12.5	11.5	13.5	10
		Acetone	7.5	7	7	8.5
		Petroleum ether	7.5	7	7	6
Tagetes erecta (Gandha)	Leaf	Methanol	6	7.5	8	7.5
		Ethanol	6	6	6	6
		Acetone	6	6	7	8
		Petroleum ether	10	8	11	8
Cymbopogon citrates (Lemon grass)	Leaf	Methanol	7	7.5	7.5	8
		Ethanol	6	6.5	6	8
		Acetone	7	7	6	6
		Petroleum ether	6	6	6	6
***Rauwolfia serpentina* (Sarpagandha)**	Root	Methanol	7	6.5	7	7
		Ethanol	7.5	7	6.5	6
		Acetone	6	6	7	8
		Petroleum ether	11	12.5	15	9
Pongamia pinnata	Leaf	Methanol	6	7	6	8
		Ethanol	7.5	6.5	7.5	8.5
		Acetone	6	6	6	6
		Petroleum ether	6	6	6	6

Antibiotic sensitivity test

In the present study isolated causal pathogen showed different sensitivity pattern against the nineteen antibiotics. Isolated causal pathogen showed highest 24mm diameter of zone of inhibition against erythromycin followed by 23mm, 22mm zone of inhibition for clarithromycin and gentamycin. On the other

hand isolated causal pathogen of bacterial leaf spot of papaya showed lowest 6mm diameter of zone of inhibition for both nalidixic acid and carbenicillin followed by 8mm for kanamycin. Results of antibiotic sensitivity of causal pathogens are given in Table 3.

Table 3: Results of sensitivity pattern of isolated causal pathogen against some antibiotics

Name of antibiotic	Diameter of zone of inhibition (in mm)	Sensitivity pattern
Penicillin	7	Resistant
Tetracyclin	15	Susceptible
Amoxycillin	6	Resistant
Doxycyclin	17	Susceptible
Cefotaxime	12	Intermediate
Carbenicillin	8	Resistant
Clarithromycin	23	Susceptible
Streptomycin	18	Susceptible
Azithromycin	17	Susceptible
Ampicillin	8	Resistant
Gentamycin	22	Susceptible
Rifampicin	11	Intermediate
Oxytetracyclin	13	Intermediate
Vancomycin	14	Intermediate
Cefixime	6	Resistant
Nalidixic acid	6	Resistant
Kanamycin	15	Susceptible
Neomycin	17	Susceptible
Erythromycin	24	Susceptible

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

DISCUSSION

Bacterial leaf spot of papaya is the most devastating disease which caused significant yield losses throughout Bangladesh. *Pseudomonas caricapapayae* is the causal agent of this serious disease. Different types of cultural and mechanical practices have done to control this disease all over the country. Varieties of chemicals are available in the local market to control this disease. All of these chemicals are hazardous to animal diversity and also for human beings. Therefore biological controls of the disease have been applied to overcome the problem of chemical control. In the present study bacteria isolated from the bacterial leaf spot infected leaves of papaya showed different types of morphological characteristics. Isolated bacteria showed creamy white colonies on agar plate. Isolated bacteria showed characteristics of the genus *Pseudomonas* and confirmed all the criteria for inclusion in the group of plant pathogenic *Pseudomonas*[15]. In the present study molecular identification of the isolated bacteria was done. In PCR analysis we found 1600 bp amplified clear band for isolated bacterial genomic DNA. *Pseudomonas aeruginosa* was isolated from Bacterial leaf spot of tobacco and showed 1491bp amplified sequenced product which had 98% similarity to 16S rDNA sequences of *P. aeruginosa* (AY486350) by using BLAST[16]. This work confirmed our present findings. The rhizosphere is an environment that the plant itself helps create and where pathogenics and beneficial microorganisms constitute a major influential force on plant growth and health[17].

In the present experiment we isolated bacteria from rhizopheric region of the soil sample for screening antagonistic activity against the causal plant pathogen. We isolated four isolate of soil bacteria. They showed wide range of antagonistic activity against the plant pathogenic bacteria. Isolate 1 and isolate 2 of soil bacteria showed highest 12±0.2mm zone of inhibition against the causal plant pathogen

in disc diffusion method at 40µl/disc concentration. In well diffusion method isolate 3 showed highest 16±0.5mm zone of inhibition The secondary metabolites produced by certain species and strains of the genus Bacillus have been found to show antibacterial activity against phytopathogenic bacteria[18,19,20]. Attempts made by different workers to control bacterial leaf spot with beneficial microorganism reveals that certain bacterial species are able to reduce the growth of *Pseudomonas spp*.[21, 22]. All of these works confirmed our findings. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[23]. Plant extracts are highly efficient against microbial infections. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time to other for medicinal purposes[24] Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties[25].

In our experiment five different types of plant showed broad spectrum of antibacterial activity against causal pathogen. Among different types of solvent extract, methanol extract of *Lantana camara* showed highest 17mm zone of inhibition at 100µg/ml concentrations and ethanol extract showed 13.5mm zone of inhibition at 60µg/ml concentration. Inhibitory action of methanol extract of *Lantana camara* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* was found[26]. The antibacterial properties of the *L. camara* were due to the presence of phenolics, anthocyanins and proanthocyanidins in their leaves[27]. Antibacterial activity of ethanol extract of *Lantana camara* against *E. coli* and *S. aureus* was also reported[28]. We also found similar antibacterial effect of methanol and ethanol extract of *Lantana camara* against the causal pathogen of bacterial leaf spot of papaya. Among the four solvents petroleum ether extract of *Rauwolfia serpentina* showed highest 15mm zone of inhibition at 60µg/ml concentration. Recently antibacterial activity of petroleum ether extract of *Rauwolfia serpentina* was reported[29]. Similar result was found for leaf and root petroleum ether extract of *Rauwolfia serpentina* that showed a good antibacterial activity against *Salmonella typhi, E. coli* and *Pseudomonas aeruginosa*[30]. These works confirmed our present work. We found that *Lantana camara* showed more antibacterial activity than other medicinal plants. Antibiotic sensitivity test was done to utilize specific monitoring techniques to evaluate the susceptibility of a microbe to different antibiotics. In our experiment we found that, erythromycin showed highest 24mm zone of inhibition against causal pathogen followed by 23mm and 22mm zone of inhibition for clarithromycin and gentamycin. Similar zone of inhibition by standard kanamycin against *Pseudomonas sp*. was found[13]. Analogous diameter of zone of inhibitions was also reported by erythromycin against *Pseudomonas aeruginosa*[31]. These works suggested our present findings.

CONCLUSION

Bacterial leaf spot of papaya is very demolishing disease in Bangladesh. It can cause serious damage to papaya production and greatly hampers the economy. *Pseudomonas caricapapaya*e caused this disease. Different types of chemical control have used to control this disease but it cause serious danger to animal diversity. In our study we used different types of medicinal plant and soil bacteria to inhibit the growth of causal pathogen of this disease *in vitro* as biological control. We found significant result for methanol and ethanol extract of *Lantana camara* as well as for soil bacteria in disc diffusion method regarding the growth inhibition of the causal pathogen. So findings of our present study would be helpful to biologically control the bacterial leaf spot disease of papaya.

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Authors' contribution: MFH, SMZH, ZFZ, MFH and BS designed the experiments and developed the methodology. MFH, SMZH, and MFH prepared the manuscript. MFH, SMZH, ZFZ, MFH collected the data and carried out analysis. MAI assisted with data analysis and manuscript preparation.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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