Original Research Article

Characterization of Crown Rot Disease of Banana Fruit and Eco-Friendly Quality Improvement Approach During Storage

ABSTRACT

Introduction: Banana is one of the most popular andimportant fruit crop all over the world.A complex of fungal pathogen is responsible for crown rotdiseases of banana.

Aims: The present study was designed to detect and characterize thecrown rot disease of post-harvestbanana (*Musa paradisiaca)* andalso develop an alternative quality improvement approach to improve banana shelf-life during storage period.

Study design:A simplest general factorial experiment that was designed tocontrol crown rot disease of banana using different biological factors, including plant extract, antagonistic agents and commercial fungicide.

Place and Duration of Study: Disease infected bananas were collected from Rajshahi city, Rajshahi,Bangladesh in 2017 and the experiment had been conducted from April 2017 to April 2018.

Methodology: Different morphological, biochemical and molecular techniqueswere used to characterize and detect the liable fungi. Responsible fungi were subjected to antifungal activity screening test and*in vitro* antagonism test. Effect of carbendazim and kanamycin B against the mycelial growth of the isolates was determined by disc diffusion method.Qualityparameters including disease incidence and severity, pH, TSS, TTA and AA of the treated banana were also analyzed after application of treatments in the packing stage through standard estimation techniques.

Results: Two fungi, isolated from the infected portion were further identified as *C.musae*and *L.theobromae*. *D. metel*and *A. sativum* extract was better in inhibiting mycelial growth of all the test pathogen in culture. *B. cereus* and *T. harzianum* moved and attached to fungal isolates, affecting mycelial growth and *A. sativum* extractsignificantly affecting conidial germination on artificial medium. Satisfactory mycelia inhibitory effect was recorded from kanamycin B. Quality analysis after storage of banana showed minor measurable differences among treatments.

Conclusion: Post-harvest application of *A. sativum* extract (Conc. 25% w/v) improve the overall quality of harvested banana fruits and reduced the incidence and disease severity of crown rot to a level significantly lower than in fungicide treated or control fruits.

Keywords:*Musa paradisiaca, molecular technique,antagonism, mycelial growth, antifungal activity, quality analysis,disease severity.*

1. INTRODUCTION

Banana is one of the most important tropical crops and is affected by several fungal diseases, including crown rot postharvest disease [1]. Ripe banana mixed with rice and milk is atraditional dish for Bangladeshi. Banana also has several medicinal uses[2].Although banana fruits are highly demanded as nutritious and economically important fruits, they experience a different marketing problem, including post-harvest decay and physiological deterioration[3].Crown rot

is responsible for significant losses in banana fruits [1] and [4]. The fruit contains high levels of sugars and nutrient elements, and their low pH values make them particularly vulnerableto fungal decay[5].Crown rot begins with mycelium development on the crown surface, followed by internal development [4]. Crown rot affects tissues of the crown, which includes the peduncle and subsequently, development of fruit necrosis occurs and the main stalk decays rapidly. Common microorganisms isolated from crown rot are *Colletotrichum musae, Lasiodiplodiatheobromae, Nigrosporasphaerica, Penicillium* spp., and *Aspergillus*spp[6]. Postharvest fungicidal treatments are applied to control crown rot disease but severely affected banana fruits are still found in consumer markets [7]. There are different types of techniques for controlling the crown rot disease of banana and all are chemical based. There is no suitable report of an antagonistic control system for crown rot disease of banana. Therefore, the study was designed to isolate the pathogen responsible for crown rot disease of storage banana along with its molecular detection and control of this devastating disease by antagonistic activities.

2. MATERIALS AND METHODS

2.1 Infected banana collection: Banana fruits at 70-80 per cent maturity with typical symptoms of crown rot disease (black to brown lesion) were collected from different markets in various locations of Rajshahi, Bangladesh. The hands were thoroughly washed in running tap water to remove dusts and other impurities.

2.2 Collection and extraction of plant material: Selected plant specimens were dried under shade, milled using laboratory mill, and extracted with methanol. The extract was filtered through folded filter paper into a 500 mL round bottom flask and reduced to dryness on a rotary evaporator at 40°C water bath temperature.Fifty grams of each milled plant specimens (*Datura metel*, *Faidherbiaalbida*, *Acacia catechu, Allium sativum*, *Solanum torvum*, *Solanum* spp., *Persicariastagnina*and *Azadirachtaindica*) were extracted using 250ml methanol with continuous stirring for 15days using a magnetic stirrer[8].

2.3 Collection and isolation of antagonistic agents*:* Pure culture of *Trichoderma harzianum* was obtained from the central laboratory of institute of biological sciences, University of Rajshahi, Rajshahi-6205, Bangladesh. *Bacillus* was isolated from rhizosphere soil samples by plating a dilution series on nutrient agar medium[9]. Colony morphology of the isolated bacteria was recorded after 16 h of growth on LB agar plate at 37^0C. Gram staining tests (A loop full of the bacteria was spread on a glassslide and fixed by heating on a very low flame) and a series of biochemical testsincluding Triple Sugar Iron (TSI), Simmons citrate, Kligler-Iron Agar (KIA) test, tween 80 hydrolysis tests, methyl red and mannitol tests were performed for the characterization of the isolated bacteriafollowing the company manual instructions [10].The chemicals of different biochemical tests were collected from Oxido Ltd. Basingstoke, Hampshire, England.

2.4 Isolation of fruit rot fungi:First of all, Infected portions from the collected banana fruits were thoroughly washed in running tap water to remove dusts and other impurities. After that,they were surface sterilized with 0.2 per cent sodium hypochlorite solution and air dried for 6 hours at room temperature. Finally, Infected portions were transferred to the surface of potato dextrose agar (PDA) plates using sterile forceps[11].The PDA plates were incubated at 25±1°C for seven daysand the isolates obtained were purified by transferring monosporic isolates to fresh PDA medium.

2.5Growth profiling of fungi: Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), Nutrient Agar (NA), Sabouraud Brain Heart Infusion Agar (SBHIA) and finally Corn Meal Agar (CMA) media were used to examined cultural characteristics of the fungal isolates. The composition and preparation of the media were obtained fromAinsworth and Bisby's 'Dictionary of the Fungi' by Hawksworth et al., (1983)[12].Cotton blue staining slide was visualized for fungal spore detection [13]A wet mount of the fungi was prepared by suspending a little bit of fungal culture collected using a spatula and a needle in a few drops of lacto-phenol cotton blue solution on a microscope slide and then cover with a cover slip and view under x40 magnification.Colony morphology, spore characters and measurements were determined manually after incubation on PDA at 25 ± 1°C for 10 days.At the end of incubation period, the resulting growth of fungus was harvested and filtered through pre-weighed Whatman No.1 filter paper and washed thoroughly with distilled water. It was dried at 40^{o}C for two days in hot air oven and the dry weights were recorded.The optimum pH for mycelial growth was determined on PD broth, where pH was adjusted from 3.0,to 9.0 at 2.0 intervals. The fungal discs were put on the centre of all culture vessels containing PD broth. After seven days colony morphology and mycelia dry weight was recorded using previously described procedure.

2.6Molecular characterization

2.6.1 DNA extraction and PCR amplifications:After seven days of incubation, mycelium from the two pure fungal isolates (Isolate-1 and Isolate-2) were used for DNA extraction. Here, Maxwell® 16 LEV Plant DNA Kit (AS1420, Promega, USA) was used for the isolation of the genomic DNA. The isolated DNA was amplified via thePolymerase Chain Reaction (PCR) technique using universal primers ITS5F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') [14] and Hot Start Green Master Mix (Promega, USA). PCR was performed in a 50µl reaction mixture containing 25µl of Hot Start Green Master Mix (2X), 2.0 µL of each forward and reverse primer, 2.0 µL of genomic DNA and rest of the PCR water. The PCR program was as follows: pre heat at 95°C for 2 min, followed by 32 cycles of denaturation step at 95°C for 30 sec, primer annealing at 48°C for 30 seconds, primer extension at 72°C for 45 sec. After that, the temperature of final extension was at 72°C for 10 min and lastly, hold at 4°C for overnight. The amplicons were separated by 1% agarose (V3125, Promega, USA) gel electrophoresis.

Soil bacterium genomic DNA isolation was performed with the cetyl-trimethyl ammonium bromide (CTAB) method [15]. PCR amplification of isolated soil bacteria was performed in the same technique of fungal DNA isolation and amplification using specific primers27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT -3').

The quality and quantity of isolated DNA were checked by Nanodrop Spectrophotometer (ND2000, Thermo Scientific, USA). Finally, The PCR products were purified and used for sequencing analysis in Malaysia Ltd. via Invent Biotechnologies, Bangladesh. The sequence data were analyzed using similarities of nucleotide sequences between isolates through the BLAST procedure (http://blast.ncbi.nlm).

2.7Pathogenicity test of isolated fungi: Wound inoculated and non-inoculated fruits (green banana, ladies' finger and apple fruits) were separately subjected to pathogenicity tests.For the pathogenicity test, healthy green banana fruits, ladies finger fruits and apple fruits were surface-sterilized with 70% ethanol, and wounds were made in each fruit using sterilized wooden rod. Each wound was inoculated with mycelia plugs (3 mm) from a 7 days old culture of each isolate, and one was treated with uncultured pure PD (Potato Dextrose) broth as a control. Inoculated fruits were covered with plastic, and incubated at 25 ± 1°C for 5 days [16].

2.8Effects of commercial fungicide: Two different conc. (25 and 50mg/disc) of fungicide (carbendazim) and a novel antifungal agent Kanamycin B (maybe fungicidal) were tested against the fungal isolates for radial growth inhibition on PDA media using modified paper disc diffusion method under *in vitro* condition.Mycelia discs of 5 mm size from actively growing culture of the isolated fungus were cut out by a sterile cork borer and one such disc was placed at the center of each agar plate. Control was maintained without adding any fungicides to the medium.The efficacy of a fungicide and kanamycin was expressed as per cent inhibition of mycelia growth over control.

2.9Antifungal activity screening: Antifungal activity screening was performed using apaper disc diffusion method [17]. About 50mg of the MeOH extract of each plant were weighed, dissolved in 1ml of the extraction solvent and then tested for antifungal activities.The measurements were taken manually as the zone of inhibition of radial mycelia growth relative to a positive control.

2.10*In vitro* effect of *Allium sativum* extract against conidial suspension:Vogel's (minimal) medium [18] wasusedto detect the *in vitro* effects of garlic bulb extract against conidial suspensions of the isolates.Conidial suspensions were adjusted to 10^5 conidia/mL using a hemacytometer.10µl of garlic bulb extract and 90µl of the conidial suspension of the fungal isolates were mixed and the mixtures were added to the surface of depression slides or group slides. The slides were then placed on a glass rod in petri dish layered with moistened filter paper and incubated at 25°C for 24h. Conidial suspension mixed with an equivalent amount of the solvent served as control.After that, the treated and

control samples were spread inseperatepetridishes containing PDA medium and incubated overnight for evaluating antifungal activity.

2.11Determination of different quality parameters after *in vivo* application:

Banana fruits were surface-sterilized by dipping in 1% sodium hypochlorite solution for 10 min, rinsed in sterile distilled water and artificially inoculated by dipping into spore suspension of both the fungus. After incubation for 15 h covered by plastic sheet until conidia germinated, artificially inoculated banana fruits were dipped into methanol extracts of *Allium sativum* (Conc. 25% w/v), while the control fruits were dipped into methanol[19]. Five fruits were used for each of the treatments. Standard estimation formulae were used to calculated percentage of disease incidence [20] and disease severity [21].After 10 days, fruit quality parameters including, pH (pH of banana fruit juice was determined with standard electric pH meter hy211, Hanna, USA),Total Soluble Solid (TSS was determined as °Brix by placing a drop of banana juice on an ATAGO-automatic refractometer Smart-1, Japan), total titratable acidity (Using one to two drops of phenolphthalein (1%) as indicator, 5 mL of the filtrate was titrated using 0.1N NaOH to an endpoint pink)of the banana fruits were measured[22]. Ascorbic acid was determined using a dye method [23] and expressed as mg $100g^{-1}$ of fresh fruits.

2.12Antagonistic assay: The antagonistic activity of *T. harzianum*and *B. cereus* wasevaluated against the isolated crown rot fungi.5 mm diameter mycelia disc from an actively growing *T. harzianum* culture was placed on the agar surface opposite to the target pathogen[24].*B. cereus* at a conc. of 250µl/well was screened against test pathogen following agar well diffusion method.The plates were incubated together with the experimental controls at 28±2°C.Radial growth (in mm) of the antagonistic agents over both the reported isolates were recorded after 4 and 7 days of co-cultivation.

2.13Statistical Analysis

All the above investigations were conducted in triplicate and repeated three times for consistency of results and statistical purposes. The data were expressed as mean±SE and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett 't' test using SPSS software version 10. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Isolation of banana fruit rot fungi: Two types of fungi, were obtained from the infected portion, one forming pinkish white colony (Fig.1A) and the other showed gray to moss dark colony (Fig.1B) on PDA medium.

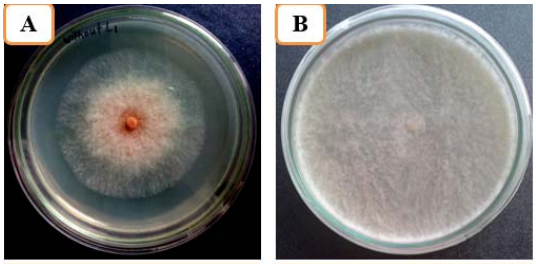


Figure-1: Isolation of responsible fungus

Legend: (A) Isolate-1 and (B) Isolate-2

3.1.2 Growth profiling of fruit rot fungi: Fungal isolates showed best aerial growth on PDA medium and cylindrical conidia that were septate withslightly rounded ends were observed under light microscopy, (Fig.2: A-B) while no growth observed on CMA medium (Graphs: C-F). GraphC-F also showcolony diameter and dry mycelia weights of the fungal isolates. The optimum pH for mycelia growth of the isolates were pH 5.0-7.0 and 7.0 respectively (Graph: G).

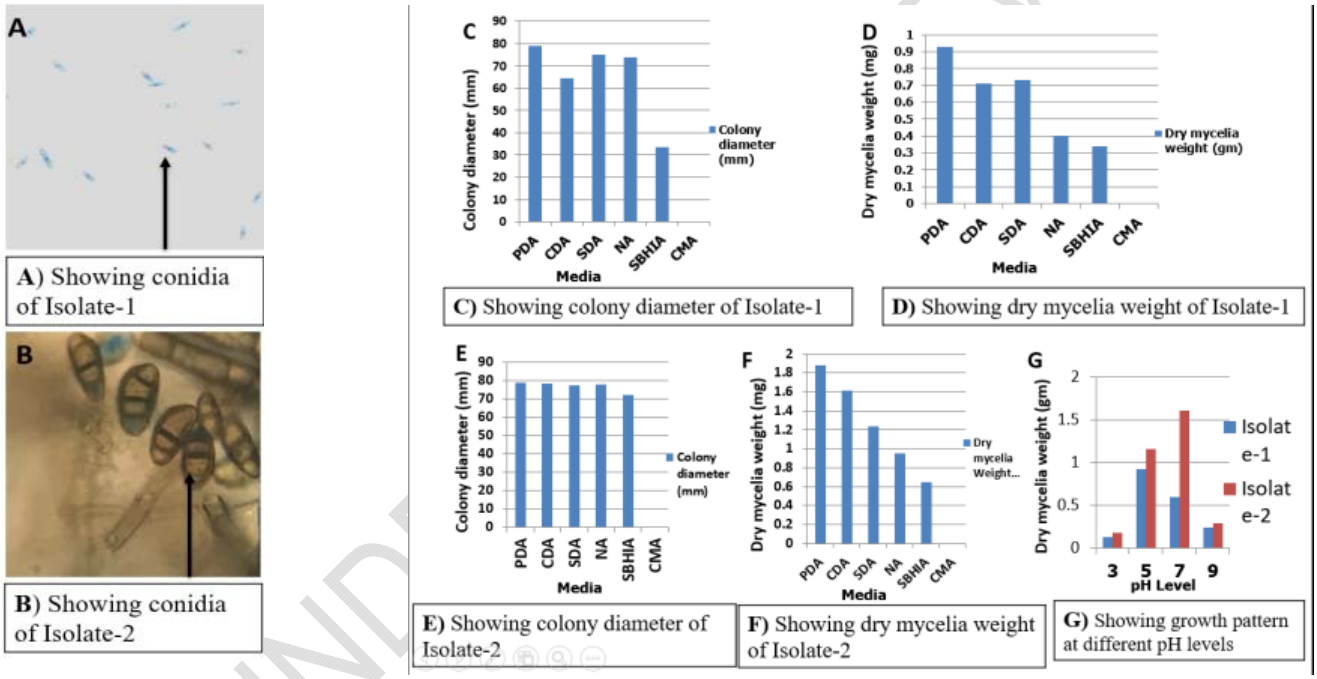


Figure-2: A & B showing Microscopic evaluation of isolate1 & 2

Graph C-G: Showing Growth profiling of fruit rot fungi.

Legend: Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), Nutrient Agar (NA), Sabouraud Brain Heart Infusion Agar (SBHIA), Corn Meal Agar (CMA)

3.1.3 Characterization of antagonistic agent:*T.harzianum* showed greenish colony morphology (Fig.3A) on PDA medium while isolated soil bacteria showed whitish creamy color and formed small to medium circular colonies (Fig.3B) on LB agar plates.

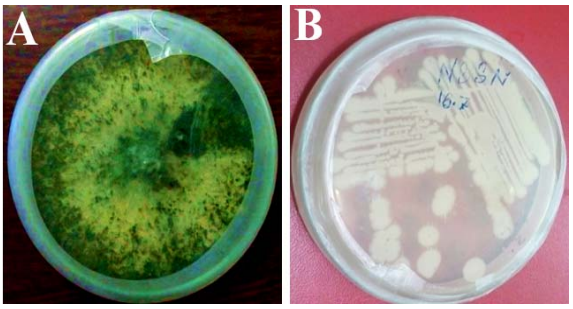


Figure-3: Showing culture condition of *T. harzianum*and soil bacteria.

Legend:(A) *T. harzianum* (B) Soil bacteria (Whitish creamy color colony).

Morphological and biochemical test confirmed thatisolated bacterium are gram-positive, rod-shape and motile. Carbohydrate fermenting (TSI), Simmons citrate, KliglerIronAgar (KIA) test, and tween 80 hydrolysis tests were positive, while methyl red and mannitol tests werenegative (Table 1).

Table 1. Morphological and biochemical characteristics of the isolated soil bacterium

Name of the test	Results
Gram staining	Gram-positive and rod-shaped
Motility	+(Pos.)
Simmons citrate	+ (Pos.)
Triple Sugar Iron (TSI)	+ (Pos.)
Methyl Red test (MR)	- (Neg.)
Klinger Iron Agar (KIA)	+(Pos.)
Tween 80 hydrolysis test	+(Pos.)
Mannitol test	- (Neg.)

Legend: +=positive (presence), - =negative (absence)

3.1.4Molecular characterizati3.1.4.1 PCR amplification: The genomic DNA isolated from the fungal isolates showed higher molecular weight and bright band on 1% agarose gel electrophoresis where 1kb DNA ladder was used as a marker. The universal primers, ITS-4 and ITS-5, were used to amplify a region of fungal genome of ribosomal DNA gene of both isolate-1 and isolate-2. The PCR amplified fragments of both the isolates yielded single bands of around 600bp (Fig. 4). The1492R and 27F primers were used to amplify a region of bacterial 16S ribosomal RNA gene. The bacterial isolates yielded a 1500bp molecular weight single band on 1% agarose gel electrophoresis where 1kb DNA ladder was used as a marker (Fig.5).

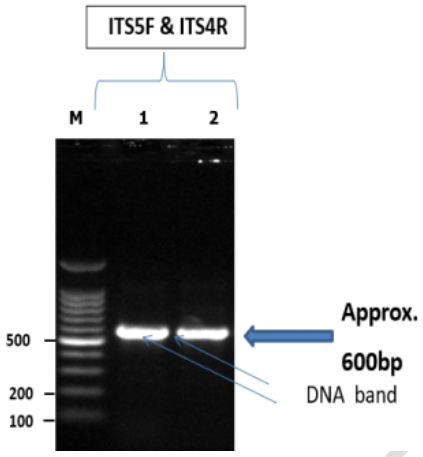


Fig.4: PCR amplification of both isolated fungi using ITS4/ ITS-5 primers; (M) DNA ladder (Marker), **(1)** Isolate 1 and **(2)** Isolate 2 fungi

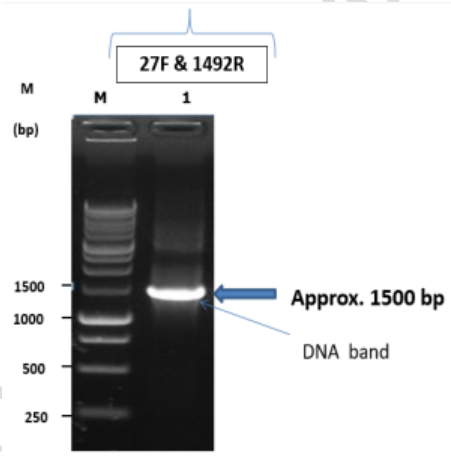


Fig.5: PCR amplification of soil bacteria using 1492R and 27F primers; (M) DNA ladder (Marker), **(1)** Isolated soil bacteria.

3.1.4.1.2 Sequence analysis and BLAST: The data analysis revealed that the 18S of rDNA sequence of both fungal isolate (Isolate-1 and Isolate-2) showed 99% similarity with the original sequence of *Colletotrichum musae and Lasiodiplodiatheobromae,* respectively. The 16S of rDNA sequence of soil bacteria had 99% identity with *Bacillus cereus*. The sequence data of isolate *C. musae* strain, *L*. *theobromae* strain and *B. cereus* isolate was deposited to the GenBank directly with access code of MH071339, MH084941 and MH119128 respectively (available to ENA in Europe and the DNA Data Bank of Japan).

3.1.5Pathogenicity tests: Inoculated banana, ladies' finger and apple fruits showed typical crown rot symptoms, which were sunken, circular, necrotic, and dark-brown lesions indicating that fungal isolates were highly pathogenic. Later, whitish mycelia developed on the lesions (Figure 6).

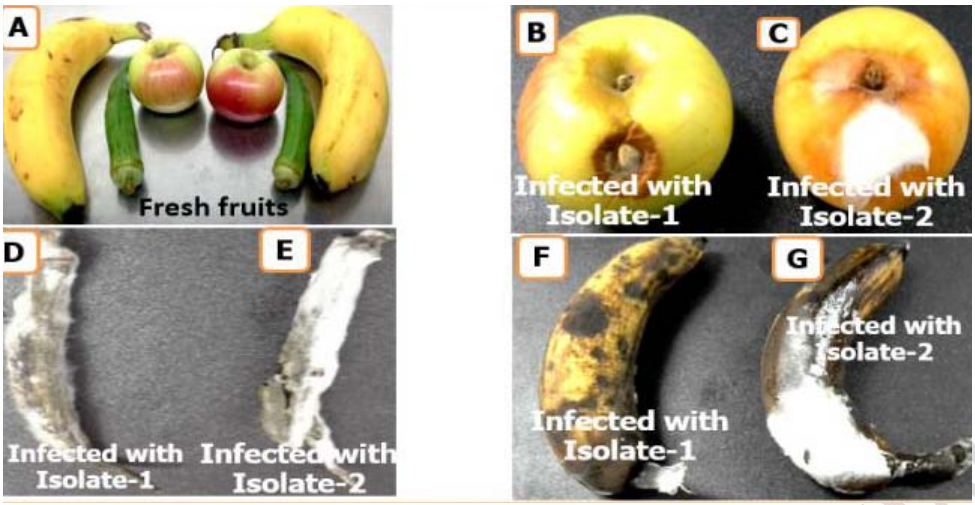


Figure-6: Showing pathogenic behavior of Isolate-1 and 2.

3.1.6Effects of commercial fungicide: Carbendazim inhibitedradial growth of mycelium the most at 54%compared to positive control against Isolate-1 while kanamycin inhibits nearly the same at 51% after 7 days of experiments(Figure 7: A-D). Carbendazim changed the normal color of the isolate-2 fungus but had little inhibitory effect on the growth of the mycelium while kanamycin inhibits by 11% (Figure 8: A-D).

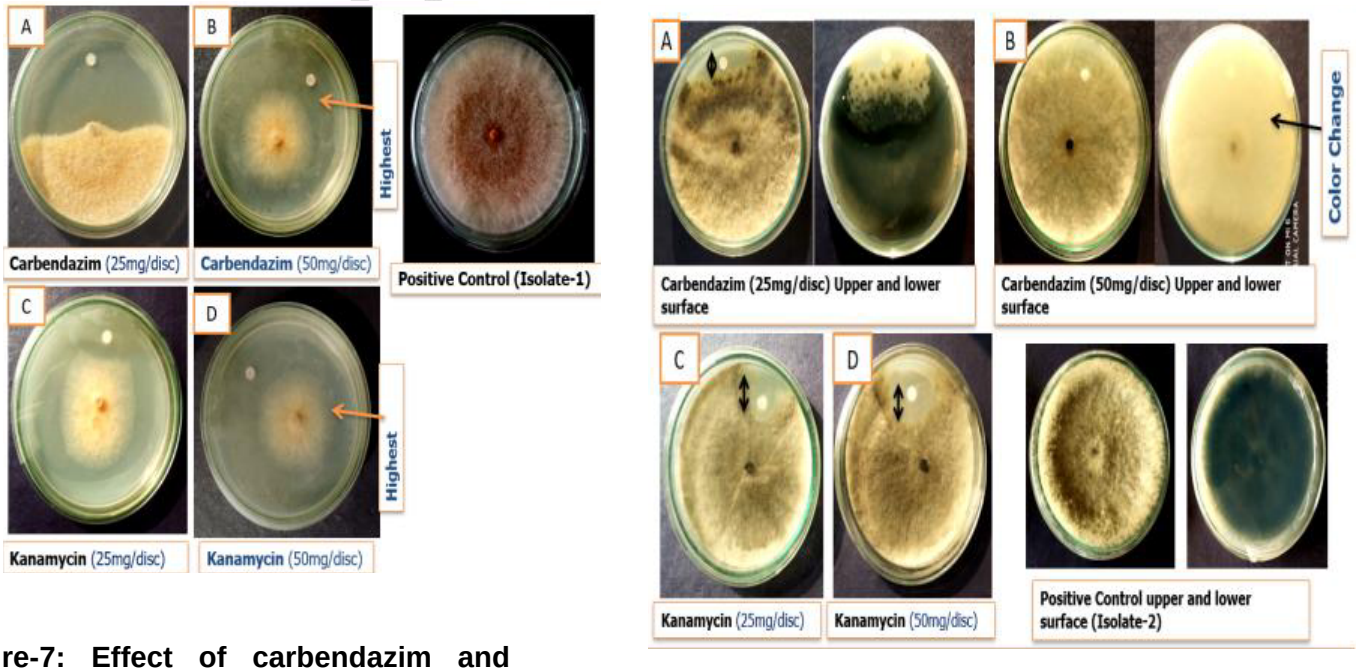
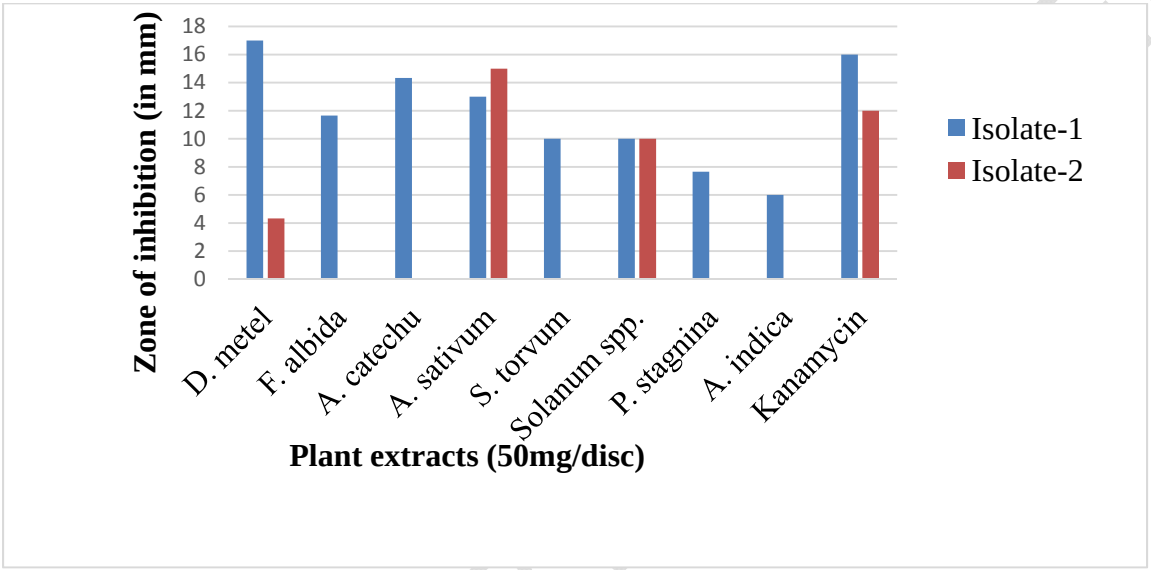


Figure-7: Effect of carbendazim and

kanamycin against isolate-1

Figure-8: Effect of carbendazim and kanamycin against isolate-2

3.1.7Antifungal activity screening of plant extracts: In the experiment, methanolic extracts of different plants showed inhibition at different levels against the aerial growth of two fungal isolates (Graph-H). The results revealed that the MeOH extracts of *D. metel, A. catechu, A. sativum* showed prominent inhibitory effects against Isolate-1 while only *Allium sativum* showed promising antifungal effect against Isolate-2 compare to standard kanamycin (positive control).



Graph-H: Showing antifungal activity of eight different plant extracts.

3.1.8*In vitro* effect of *Allium sativum* extract against conidial suspension: *Allium sativum* extract showed satisfactory antifungal activity against conidial suspensions of both of the fungal isolates (Figure9).

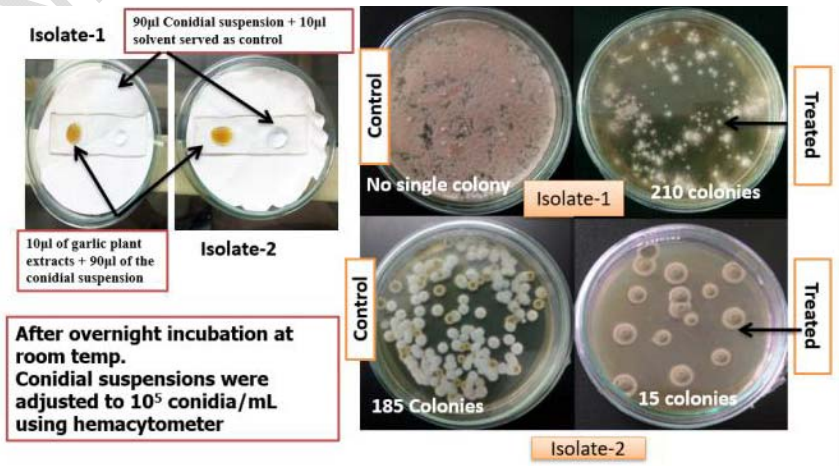


Figure-9: *In vitro* effect of garlic extract against conidial suspension of both the isolates.

3.1.9Determination of different quality parameters after *in vivo* application: The severity of fruit rot disease was equivalent to less than 1% fruit area affected in fruits treated with *Allium sativum* extract. Fruits in the untreated control ripened quickly and this led to the reduction of all the estimated overall quality of banana fruits (Figure10).

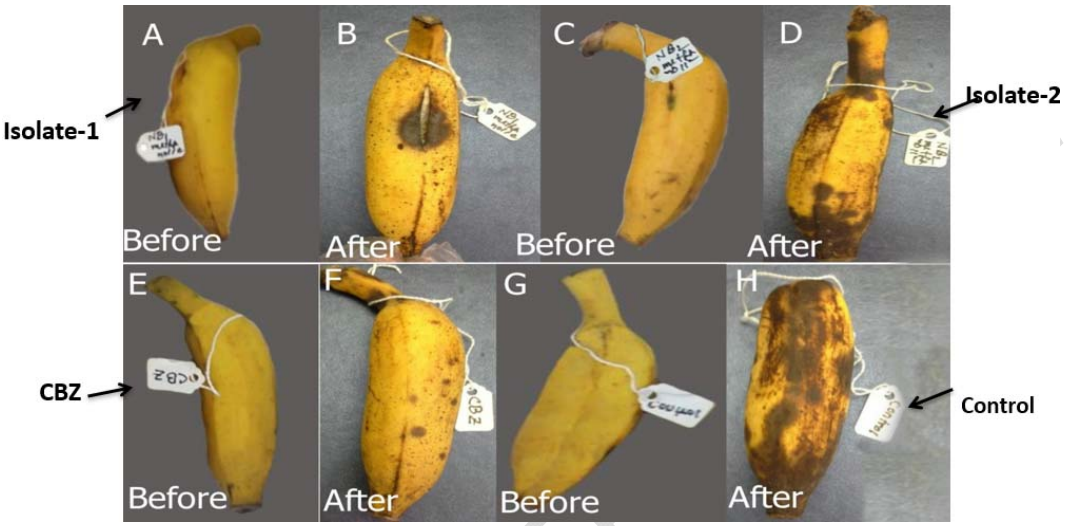


Figure-10: Results of *in vivo* evaluation of garlic extract and carbendazim.

Legend:(A-B) artificially inoculated with isolate-1, (C-D) artificially inoculated with isolate-2, (E-F) artificially inoculated with carbendazim (G-H) Control (dipped in to methanol).

However, 25% methanol extract of *A. sativum* resulted in TTA values comparable to that of fruits treated with carbendazim. Quality analysis after storage for total soluble solids, pH, total titratable acid and ascorbic acid for banana showed minor measurable differences among treatments (Table 2). This implies all the treatments, which increased fruit shelf-life and retain comparable color class of banana fruits, also preserved fruit internal quality. **Table-2: Quality parameters of banana after application of treatments**

Treatments		**Disease severity (M±SE)**	**Diseases incidence (M±SE)**	**Results (Quality Parameters) (M±SE)**			
				p^H	**TSS**	**TTA**	**AA**
A. sativum (25% w/v)	Isolate-1	11.18±0.90	49.66±1.24	5.30±0.41	18.83±0.62	0.43±0.40	5.03±0.73
	Isolate-2	25.09±1.38	79.9±1.593	5.03±0.02	17.23±0.40	0.35±0.44	4.40±0.41

Carbendazim	3.62±0.41	50.66±0.942	5.10±0.07	17.46±0.44	0.16±0.13	5.87±0.17
Control (Methanol)	15.47±0.54	98.9±0.941	4.72±0.52	16.23±0.88	0.05±0.04	5.71±0.40

Legend: TSS= Total Soluble Solids (°Brix**)**; TTA= Total Titratable Acidity (%); AA= Ascorbic Acid (%);±= Plus/minus.

3.1.10Antagonistic activity: The most promising antagonistic activity was found when the isolates were co-cultivated with *T.harzianum*with at least seven days.On the other hand,soil bacteria (*Bacillus cereus*) showed some minor antagonistic activity against the tested fungi (Table3).

Table-3: Antagonistic activity against the isolated fungi

Antagonistic agent	**Target fungus**	**Results (Zone of inhibition in mm) (M±SE)**	
		4 days	**7 days**
T. harzianum	Isolate-1	69.66±1.24	84.33±0.47
	Isolate-2	45.33±1.24	70.66±0.81
B. cereus	Isolate-1	10.0±1.63	15.0±2.55
	Isolate-2	5.33±1.24	5.33±1.24

Legend:mm= Millimeter; M±SE= Mean Plus/minus Standard Error, Isolate-1: *C. musae***,** Isolate-2: *L. theobromae***.**

3.2 Discussion

Two types of fungi were obtained from infected tissues isolation technique, and later identified as *C. musae*and*L. theobromae* according to the precise results of morphological and molecular approaches[25]. The optimum temperature was 25±1ºC and pH of isolate-1 and isolate-2 was 5.0 - 7.0 respectively[26]. Molecular analysis using ITS5F and ITS4R primer indicates approximately 99% similarity with the fungus *C. musa*e (isolate-1) and *L. theobromae*(isolate-2) responsible for post-harvest crown rot of banana [27]. Morphological tests of isolated soil bacteria indicated that it was gram positive and rod shaped. Molecular detection using 27F and 1492R primer and sequence (16S rRNA gene sequence) analysis of the isolated soil bacterium revealed it was *Bacillus cereus* (99% similarity)[28]and [29]. Both the isolated fungishowed high infection ability on fresh banana, apple and ladies finger fruits [30]. Mycelial growth of Isolate-1 was significantly inhibited by methanol

extracts (50mg/disc) of all the eight plant extracts while isolate-2 showed high sensitivity against *A. sativum*extracts compared to commercial fungicide and standard kanamycin B[9]and [25]. From the results of commercial fungicide and standard kanamycin B tests, it can be concluded that kanamycin B had an inhibition activity (inhibit 51% radial growth of isolate-1) which was similar to the inhibition activity of 54% radial growth of isolate-1for carbendazim. Complete control of crown rot pathogen is possible through application of benomyl, carbendazim and mancozeb[31]and [32]. Satisfactory *in vitro* antifungal activity of MeOH garlic clove extracts was observed against conidial suspension of both the isolates in the present investigation. *In vivo* evaluation of *A. sativum* treated fruit exhibit lowest disease severity and disease incidence compare to control. Fruit pH decreased in all treatments and storage temperatures during the storage period. A similar reduction in fruit pH in banana was reported previously [33]. On the other hand, contrasting result for mango was reported [34]. Irregular changes of banana fruit pH during ripening were also reported [35]. The values of the quality parameters of the *A. sativum* extract treated fruits showed some minor difference compare to fungicide treated banana fruits and sterile water treated banana fruits. The most promising antagonistic activity was found when the isolates were co-cultivated with *T.harzianum*with at least seven days. On the other hand, soil bacteria (*Bacillus cereus*) showed some minor antagonistic activity against the tested fungi. The effectiveness of *T. harzianum* and *Bacillus* spp. against mycelia growth of *L. theobromae* and *C. musae*was also reported [36], [37], [38] and [39]. The antifungal activity of *A. sativum* extract, and kanamycin B 25% (w/v) was moderately comparable to antifungal activity of commercial fungicide which simply increase banana fruit shelf-life and maintain fruit quality. It also concluded that *D. metel* and *B. cereus* also showed some minor inhibition activity against Isolate-1.Establishment of biopesticides to prevent banana crown rot post-harvest disease, from the active antifungal component of effective plant extracts, antagonistic agents and kanamycin Bis one of the major future perspective of the present investigation.

4. Conclusions:

Advanced molecular technique-sequencing revealed the identity of fungal isolates as *C. musae*and *L. theobromae,* respectively which are the causal agents of crown rot diseases of banana in Bangladesh. The findings from the present study also suggest that *L. theobromae* was more prevalent than *C. musae*. This study suggests that *A. sativum, D. metel*extracts and kanamycin B 25%w/v (weight per volume) might be used as alternative quality improvement agent in the post-harvest stage. This study will help the researchers to

uncover the critical areas of the inhibition mechanism of these bioagent that many researchers were not able to explore. Thus, a new theory on eco-friendly quality improvement approach of harvested banana may be arrived at.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Ethical Issue

Authors have declared that, there was no ethical issues regarding this manuscript.

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