Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2016; 4(5):413-421 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Antimicrobial and cytotoxicity of the causative organism *Colletotrichum acutatum* isolated from anthracnose disease of chili

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Abstract: *Colletotrichum acutatum* is one of the pathogenic organisms of anthracnose disease of chili fruit, which was isolated from the chili species *Capsicum annuum*. The pathogen was identified from the observation of its colony size, shape, colour, mycelium, conidiophore, conidia, hyaline, spore, acervuli, appressoria, and setae in the PDA culture. The pathogenic organism produced antibacterial activity when cultured in PD liquid broth medium. The highest production of antibiotic from the organism *Colletotrichum acutatum* was found at pH 6.0 and 27^0C. The crude chloroform extracts showed antibacterial activity against a number of Gram positive and Gram negative bacteria. TLC analysis showed the presence of three fractions of crude chloroform extracts from *C. acutatum*. The minimum inhibitory concentration (MIC) of the crude chloroform extract from *C. acutatum* against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Shigella sonnie* were 256 µgm/ml, 128 µgm/ml, 128 µgm/ml and 64 µgm/ml respectively. The LD_{50} (Lethal Dose) values of the cytotoxicity assay over brine shrimp of the crude chloroform extract from *C. acutatum* was found 956.02 µgm/ml.
Keywords: *Colletotrichum acutatum*, Anthracnose disease, $LD_{50,}$ antibacterial activity.

INTRODUCTION:

Chili (*Capsicum annuum*) is an important vegetable and spice crop worldwide that is produced and consumed as fresh or processed [1]. It is one of ingredients in South, South-East Asian food recipe. Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre- and post-harvest fruit decay [2, 3]. Anthracnose causes extensive pre- and postharvest damage to chili fruits causing anthracnose lesions. Even small anthracnose lesions on chili fruits reduce their marketable value [4]. Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. *Colletotrichum* species are the most important pathogens that cause latent infection [5]. In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host [7, 8].

It was reported that different species cause diseases of different organs of the chili plant; for example, *C. acutatum* and *C. gloeosporioides* infect chili fruits at all developmental stages, but usually not the leaves or stems, which are mostly damaged by C. coccodes and C. dematium [9]. C. capsici is widespread in red chili fruits, whereas *C. acutatum* and *C. gloeosporioides* have been reported to be more prevalent on both young and mature green fruits [10], [11]. Anthracnose is a very common disease of chili in Bangladesh. This disease is occurred by the infection of a fungus – *Colletotrichum capsici* and *C. acutatum* [12]. Here the pathogenic organism responsible for the anthracnose disease of chili fruits was isolated, purified and characterized. It was well known that fungus, bacteria, and related organisms produced antibiotic both extracellularly and intracellularly [13], [14]. The antibiotics have wide spread medicinal importance. Scientists continue their effects to isolate and purify new types of antibiotics. In this study, the antibiotic produced from the hypersensitive organism *Colletotrichum* spp. was isolated and its antibacterial activity as well as cytotoxic effect was detected.

MATERIALS AND METHODS

The fungal materials were isolated from the ‘anthracnose’, oval or irregular, grayish brown spots of chili fruits collected from different chili fields of Bangladesh Agricultural Research Institute (BARI), Pahartali, and Hathazari, Chittagong, Bangladesh. The

sample specimens were collected at the peak harvest time May-June.

Samples were collected after proper noting down the symptoms of the disease and placed separately in polyethylene bags which were labeled with date for future reference. After collection, the samples were brought to the laboratory and detail observations on the symptoms of the anthracnose disease infected chili fruits were made.

After proper sterilization, 5-6 diseased chili fruits were incubated in humid chamber for 7-10 days at 28 ± 2^0C. The pathogenicity of the fungus was tested on two individual fruits with the spores. The pathogen was reisolated from the diseased fruits and Koch's postulates were satisfied. The stock cultures were maintained on solidified basal medium (PDA). Sub-cultures were made at intervals of every 15 days.

Examinations were made within 10-15 days under stereoscopic binocular microscope and finally slides were prepared using lactophenol as mounting medium for morphological studies under compound microscope. Whenever necessary, cotton-blue was used to stain the fungal materials.

Isolation of pathogens

Sterilized diseased fruit parts were incubated in humid chamber. The affected parts were incubated for 7 days at 28 ± 2^0C. During this period the fungus was found to sporulate on the host fruit surface. Spores were collected by touching them with the agar block and could be seen lying attached to the transparent surface of agar, which was then transferred to freshly prepared PDA plates, and fungal colonies without contamination were found to develop after incubation.

Single spore isolation

A dilute conidial suspension was prepared by adding sterilized distilled water to a 5-10 days old culture of fungus grown on PDA medium. The organism was diluted in 10 ml sterilized distilled water and shacked well. Then 0.5 ml of it was spread out uniformly on freshly prepared plates of 2% plain agar and the plates were stored at 28 ± 2^0C for 4-6 hours. At the end of this period the conidia were found to form germ tubes in plenty with the aid of a stereoscopic binocular microscope and a spear-headed tungsten needle, these were cut out individually along with a peace of agar and transferred on the freshly prepared slopes of PDA medium in test tubes. The tubes were then incubated at 28 ± 2^0C for 5-7 days. All the pure cultures were preserved at 4^0C in refrigerator and subsequent transfer was made at 2 weeks intervals.

Preparation of slides

A suitable portion of culture of pure organism from PDA plate was selected under a stereoscopic binocular microscope and was taken out with the help of needle and put in 1 or 2 drops of lactophenol on clean slides. It was then gently warmed by heating and cooled over a low flame burner for 6 to 8 times, but was never allowed to boil whenever needed the material was stained with a small quantity of cotton blue. A clean cover glass slip was placed over the material. Excess fluid was removed by soaking with blotting paper and examined under compound microscope.

Pathogenicity test

Healthy chili fruits (host) were collected from different chili trees and brought to the laboratory. The materials were washed thoroughly in running tap water, dipped in one litre of 0.1% mercuric solution. Surface sterilization of the materials was continued for 2-3 minutes by rotating the container frequently.

Preparation of conidial suspension:

Sterilized distilled water was used for the preparation of conidial suspension. Conidia were washed in sterilized distilled water from 7 days old culture of the organism grown on PDA. At first, 20 ml of sterilized distilled water was added to the culture plate was shaken well to remove maximum of conidia in it. Before being used, the density of conidia in water was adjusted to $40x10^3 - 50x10^3$/ml by hemo cytometer count.

Method of isolation:

Previously surface sterilized fruits were incubated by spraying the conidial suspension of the purified organism with automizer. Before spraying the conidial suspension, the surface of the fruits were pierced or wounded numerously by previously sterilized needle. The incubated fruits were transferred into germ free full humidly (98-100%) desiccators and incubated at 28 ± 2^0C for 4-7 days. Observation was made after 5-7 days when fruits had developed characteristic lesions and compared with the naturally developed previously recorded symptoms.

Re-isolation of pathogen:

Reisolation of the pathogen was made from the artificially infected fruits following the usual procedures, which was described before. In this case, pathogen was successfully isolated from the infected fruits on PDA plates. The morphological characters of the reisolated organism were compared with the original isolates by which the fruits were inoculated.

Determination of antibacterial activity

A streak of the antagonistic species (fungus) was made in a plate containing sterile PDA medium (pH 5.5) by means of a sterile loop. The plate was incubated for 7 days to allow sufficient time for growth as well as concomitant production of antibiotic in the medium. Nutrient agar medium was prepared, poured into clean test tubes, plugged with cotton and then autoclaved. After cooling a little bit, the medium in the test tubes were seeded with the test organism by means

of a sterile loop and rotated for uniform distribution. The contents in the test tubes were poured into sterile petridishes and allowed to keep some time for solidify. Then agar medium containing the selected organism was transferred to these plates. The plates were kept overnight in refrigerator ($+4^0$C) for diffusion of antibiotic produced by the organisms into the nutrient agar medium. After overnight refrigeration, the plates were incubated at 37^0C for a day. Clear zones in the petridishes around (organism containing medium) indicated the antagonistic activity of the organism.

Disc diffusion method [15, 16]

Production of antibiotics in liquid medium

It is difficult to extract antibiotics from solid media. So it is necessary to get the active antibiotic principle in liquid media. The antibiotic can easily isolated by solvent extraction or by any other suitable chemical process that does not denature the active form of the antibiotic from liquid culture medium.

Broth culture

The stock culture of the fungus was sub-cultured and organism was incubated in the liquid media and allowed to grow at 28 ± 2^0C with minimal shaking for 10 days. When the organism was grown vastly, fungal materials (mat) were removed from the solution by filtering. The filtrate solution (extracellular) and cell bound organism (intracellular) were then used as the source of antibiotics and crude enzyme extract for the further experimental purposes.

Effect of pH on antibiotic production

PD medium (liquid broth) was prepared and 50 ml of it was transferred to each of eight different 100 ml conical flasks (two flasks for each pH). The pH of the medium were adjusted 2, 4, 6, and 8 by using 2N HCl and 2N NaOH solution. After sterilization and cooling, the media in the flasks were inoculated with spores of the fungus grown in agar slants and incubated at 28 ± 2^0C for 10 days. After incubation, the antibiotic activity of the culture filtrate was determined against *Shigella sonnie* by disc diffusion method.

Effect of temperature on antibiotic production

PD media was prepared and transferred 50 ml of it to each of 8 different 100 ml conical flasks. After sterilization, cooling and inoculation with the spores of fungus, the flasks were incubated at different temperatures 25^0C, 27^0C, 30^0C, & 35^0C respectively (two flasks for each temperature). After 10 days of incubation, the antibiotic activities of the culture filtrates were tested against Shigella spp. by disc diffusion method.

For this purpose the organism was allowed to grow in a number of culture flasks of 1000 ml capacity containing 500 ml PD medium in each flask. These were incubated at 28 ± 2^0C for 10 days. Then the culture filtrate was extracted with chloroform.

Preparation of the inoculums and incubation

PDA slants media were prepared by inoculation with spores of the organism and were allowed to grow until sporulation was occurred. Matured spores were aseptically transferred to conical flasks containing 25 ml of sterilized PD broth medium to make spore suspension. This spore suspension was again aseptically transferred to a number of large culture flasks (500 ml) each containing 300 ml of sterile PD broth medium. These were incubated at 28 ± 2^0C for 10 days.

Isolation of antibiotic from the culture media

After 10 days of incubation, the medium in the flasks turned into yellow coloured with thick, uniform mat on the surface. The liquid was then separated from its mycelial mat and filtered through a fresh piece of cotton, and then Whatman filter paper 2. The culture filtrate thus obtained was preserved at $+4^0$C by adding 2/3 drops of toluene as preservative for the extraction of the antibiotics.

Extraction of antibiotic

Procedure

100 ml of the culture filtrate was taken in a separating funnel. This was shaken for 30 minutes with 30 ml of chloroform for the first time. Then the lower layer having the chloroform extract was separated and kept in a suitable beaker. The remaining medium in the separating funnel was again shaken for 30 minutes with 30 ml of chloroform for the second time and lower layer was collected. This same procedure was done for the third and last time. In this way the total filtrate was extracted with chloroform. The chloroform fraction thus obtained was evaporated under reduced pressure in a rota-evaporator at 45^0C, until a yellowish solid mass was obtained. It was then transferred to a small beaker using chloroform and the weight of the solid extract was measured. The solid extract thus obtained was used as a active principle for experimental purpose.

Antimicrobial activity of the crude chloroform extract

The antibacterial activity of the chloroform extract was done at a concentration of 500 µgm/ disc against some pathogenic organisms by disc diffusion method. The crude extract was dissolved in methanol (as it is nontoxic) in such a concentration that 20 µl of it contains 500 µgm. 20 µl of this solution was placed on sterile filter paper (Whatman or double ring) discs by means of micropipette to obtain the desired concentrations. Then the discs were air dried. One dried filter paper disc and one standard disc (Kanamycin K-30) were then aseptically transferred to each nutrient agar plates with the help of sterile forceps, previously seeded with the test organism (bacteria). The plates were kept at low temperature ($+4^0$C) in refrigerator overnight to allow complete diffusion of the compound in agar media before any growth of the organism. The petridishes were then incubated at 37^0C for overnight.

Presence of clear zone of inhibition around the paper discs indicated the antibacterial activity of the crude chloroform extract. The diameter of the zones was measured with a centimeter scale.

Test organisms used for the study

The bacterial strains used in this sensitivity test are listed in the following table (table-1). The pure culture of the strains was collected form the research laboratory of the Department of Microbiology, University of Chittagong, Bangladesh.

Table 1: List of the test organisms

Gram positive	Gram negative
Bacillus cereus	*Escherichia coli*
Bacillus subtilis	*Salmonella typhae*
Bacillus megaterium	*Vibrio cholerae*
Staphylococcus aureus	*Shigella sonnie*

Discs Preparation

A solution of the crude chloroform extract was prepared in chloroform so that 20 µl contain 500 µgm of the sample. Filter paper (Whatman or Double Ring) discs were made with the help of a paper punch machine. The discs were then taken in a clean, dry petridish and sterilized in an autoclave at 121^0C temperature and 15 lb pressure/Sq. inch for 20 minutes. 20 µl of the crude chloroform sample solution was placed on the discs with the help of a micropipette. Thus discs containing 500 µgm of the sample were prepared. These discs were then air-dried.

Standard discs

Readymade Kanamycin K– 30 discs containing 30 µgm/ disc was used as standard disc. After 24 hours of incubation, the antibacterial activity was determined by measuring the zone of inhibition in millimeters by a transparent plastic millimeter scale. Inhibitory zones obtained by the samples were compared with that of the standard disc.

Determination of Minimum Inhibitory Concentration (MIC) of crude chloroform extract

The lowest concentration of antimicrobial agent required to inhibit the growth of the organism in vitro is the minimum inhibitory concentration (MIC). MIC is the lowest drug concentration at which there is no growth of the subject microorganism. Serial dilution technique or turbidimetric assay was used [17]. In this present study, 'serial dilution technique' was followed using nutrient broth medium. The MIC value of the compound was determined against the following five test organisms – *Bacillus subtilis*, *Bacillus cereus*, *Shigella sonnie*, *Staphylococcus aureus*, *E. coli*.

Cytotoxicity Test

Cytotoxicity was performed by using shrimp lethality assay [18]. Ten nauplii were drawn through a glass capillary and placed in each vial containing 3.0 ml of brine solution. In each experiment, different volume of sample A & B were added to 3.0 ml of brine solution and maintained at room temperature for 24 hour under the light and surviving larvae were counted. Experiments were counted along with control (distilled water treated), different concentrations of the test substances in a set of three tubes per dose.

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LD_{50} value was obtained from the best-fit line plotted concentration verses percentage of lethality.

RESULTS

The causal organism was identified by Dr. Md. Nural Anwar, Professor, Department of Microbiology, Biological Science Faculty, University of Chittagong.

Morphology of *Colletotrichum*

- Distinctive heavily melanized setae
- Asexual form
- Conidia produced in acervuli with setae

Morphology of the casual organism (Fig 1)

Mycelium colour: white to gray
Reverse colony colour: orange pink
Presence of acervuli: present
Conidia shape: fusiform, cylindrical (ellipsoidal), most with acute end**.**
Conidia size: 14.5 x 4.7 µ
Appressoria: round to ovate
Setae: present on PDA media.

Taxonomic position [19]**:**

Kingdom: Plantae
Sub-kingdom: Mycota
Division: Eumycota
Sub-division: Deuteromycota
Class: Coelomycota
Order: Melanconiales
Family: Melanconiaceae
Genus: *Colletotrichum*
Species: ***C. acutatum***

A. **B.**

Fig 1: Growth of *Colletotrichum acutatum* on PDA media; a. front and b. reverse

Production of antibiotics in liquid medium

Different broth media were tried for the successful production of antibiotic and the antibacterial activity of the crude broth was tested against *Bacillus subtilis* and *Shigella sonnie* by disc diffusion method. Among the tested media, the acidic PD broth was found to be the most suitable for abundant production of antibiotic.

Effect of pH on antibiotic production

To determine the pH value at which maximum antibiotic activity was obtained, the organism was cultured at a wide range pH and the activity was determined against *Shigella sonnie*. As shown in Table-2 the maximum amount of antibiotics are produced by the organism at pH 6.0 (Figure-2).

Table 2: Antibiotic production at different pH for *Colletotrichum acutatum*

pH value	Diameter of the zone of inhibition (mm)		
	Flask no. 1	Flask no. 2	Average
2	12	8	10
4	11	13	12
6	18	18	18
8	7	8	7.5

Fig 2: Effect of pH on antibiotic production at pH 6.0.

Effect of temperature on antibiotic production

For rapid and proper growth, every microorganism has an optimum temperature. The organism was cultured at different temperatures covering a wide range and the antibacterial activity was checked against *Shigella sonnie*. As given in Table-3 the maximum amount of antibiotics produced by the organism around 27^0C for *C. acutatum* (Figure-3).

Table 3: Antibiotic production at different temperature for *Colletotrichum acutatum*

Temperature value	Diameter of the zone of inhibition (mm)		
	Flask no. 1	Flask no. 2	Average
25^0C	13	11	12
27^0C	14	17	15.5
30^0C	13	12	12.5
35^0C	10	10	10

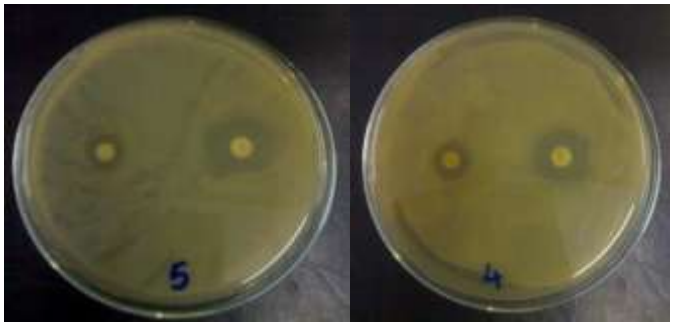
Fig 3: Effect of temperature on antibiotic production at 27^0C.

Antimicrobial activity of the crude chloroform extract of cultural broth

The antibacterial activity of the chloroform extract was tested against a series of Gram positive and Gram negative bacteria at a concentration of 500 µgm/disc and the results were compared with that of a standard antibiotic disc Kanamycin K- 30 (Figure-4).

Table 4: Antibacterial activity of the crude chloroform extract of broth culture for *Colletotrichum acutatum*

Test bacteria	Diameter of zone of inhibition (mm)	
	500 µgm/disc	Kanamycin K- 30
Gram positive		
Bacillus cereus	10	21
Bacillus subtilis	12	21
Bacillus megaterium	16	30
Staphylococcus aureus	12	19
Gram negative		
Escherichia coli	13	22
Salmonella typhae	11	20
Vibrio cholerae	15	29
Shigella sonnie	9	23



A. **B.**

Fig 4: Effects of crude chloroform extracts (500 µgm/disc) form *Colletotrichum acutatum* and Kanamycin K (30 µgm/disc) on a. *Bacillus subtilis* and b. *E. coli*

Minimum inhibitory concentration (MIC) of the crude chloroform extract

The minimum inhibitory concentration (MIC) of the crude chloroform extract was determined against *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, and *Shegella sonnie* by serial dilution method. The concentrations at which first sign of inhibition observed in the experiment against respective test organisms are shown in the following table-5 and Table-6.

Table 5: First sign of inhibition in MIC determination of *Colletotrichum acutatum*

Test organisms	MIC of the crude chloroform extract (µgm/ml)
Bacillus subtilis	256
Staphylococcus aureus	128
Escherichia coli	128
Shigella sonnie	64

No inhibition was observed in test tubes containing compound lower than the above mentioned concentrations against respective test organisms. Growth of the organism was observed in the test tube C_I (Medium + Inoculum) and no growth was observed in test tube C_M (Medium) & C_S (Medium + Sample).

Cytotoxicity test

The results of brine shrimp lethality assay for sample clearly indicated (Table-6 & Figure-5) the toxic effects of the extract. The results showed that the brine shrimp survival is inversely proportional to the amount of the extracts used with LD_{50} values. A test compound in order to be considered highly toxic, it needs to show shrimp death of 50% or more. In this assay dose of crude extract of sample over 250 µgm was found to be highly toxic. However, other doses (15.625 µgm to 250 µgm) of sample displayed some or very little toxicity.

Table 6: Results of brine shrimp lethality bioassay of the chloroform extract from *Colletotrichum acutatum*

Test sample	Conc. of sample (µgm/ml)	Log of Conc.	No. of shrimp taken (each vial)	No. of survival (average)	% of mortality	LD_{50} (µgm/ml)
Control	0		10	10	0	
Chloroform extract	15.625	1.1938	10	10	0	237.62
	31.25	1.4949	10	9	10	
	62.5	1.7959	10	8	20	
	125	2.0969	10	7	30	
	250	2.3979	10	6	40	
	500	2.6990	10	4	60	
	1000	3.0000	10	1	90	
	2000	3.3010	10	0	100	

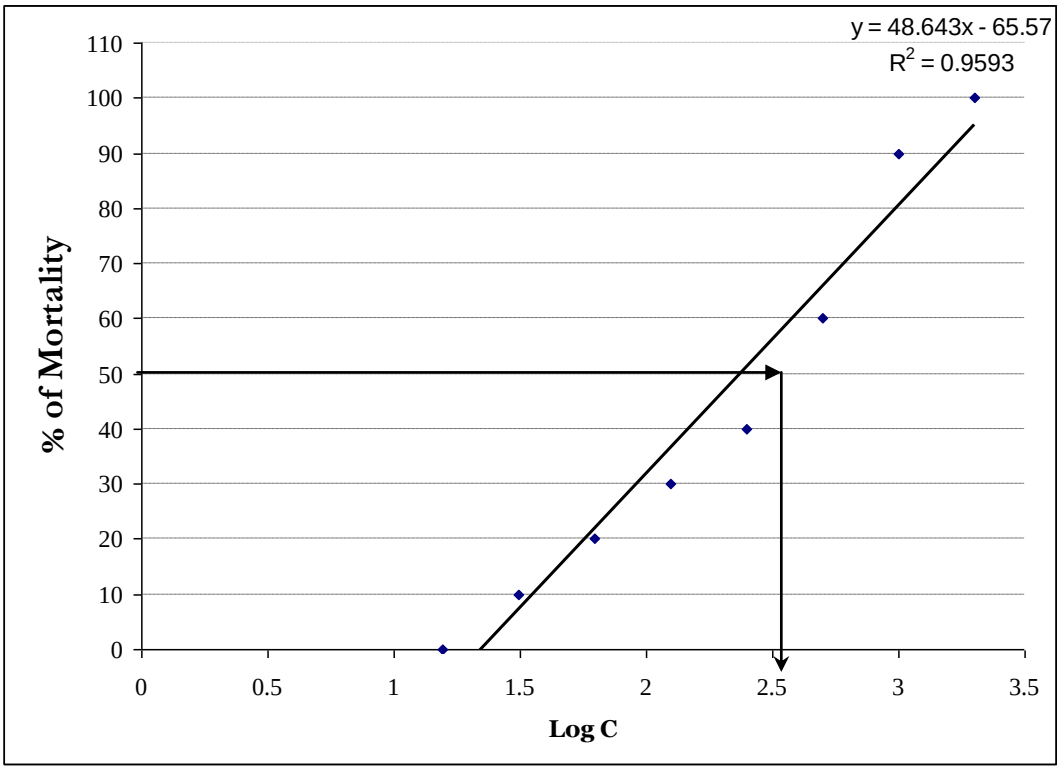


Fig 5: Determination of LD_{50} of the crude chloroform extract from *Colletotrichum acutatum* against brine shrimp larvae

DISCUSSION

Chile is an important vegetable and spice crop worldwide that is produced and consumed as fresh or processed. The pool of all chili cultivars comes from five species of the genus Capsicum: *C. annuum, C. chinense, C. baccatum, C. frutescens*, and *C. pubescens*. Today, chili is found throughout the world and plays a significant socio-economic role. Major producers of chili include Asia, Latin America, Africa, Europe, and North America [20]. The anthracnose has been well

recognized as one of the most devastating diseases wherever peppers (Capsicum spp.) are grown under hot and rainy season [21]. The prevalence of diseases varies geographically. Several microorganisms may be involved in causing diseases in chili. Anthracnose is caused by fungi in the genus *Colletotrichum*, which is a very common group of plant pathogens, and they are responsible for diseases on numerous plant species worldwide. Identification of *Colletotrichum* to species is usually based on more than one characteristic, such as physical appearance and pathogenicity on host(s). Many species of *Colletotrichum* infect more than one host and, to confound identification, more than one *Colletotrichum* sp. may be present on one host. At least three species of *Colletotrichum* (C. gloeosporiodes, C. capsici, and *C. acutatum*) are reported to cause this disease on pepper in Asian region [22].

In this study *C. acutatum* – one of the pathogenic organisms of chili anthracnose disease was collected and cultured in PDA media and single colony was isolated.

The current study showed that the optimum temperature of the fungal growth of *C. acutatum* was 27^0C in the broth media. The antibacterial activity of the crude chloroform extract of the fungal metabolite also found to be optimum at those temperatures respective to the organism. On other hand, the antibacterial activity of the crude chloroform extract of the fungal metabolite found to be optimum at pH 6.0 for the organism. Previously it was found that the maximum amount of antibiotic i.e. active compound was produced at pH 5.6 from *Colletotrichum gloeosporioides* on PD media [23]. It has been also reported that the maximum amounts of antibiotics were produced by *C. gloeosporioides* at 30^0C in PD broth [23].

An important observation was that the activity of the active compound varied depending on their temperature and pH. The crude chloroform extract of different pH gives different activities. This is an important finding in case of antimicrobial activity. A significant observation of this study was that there was direct correlation between the growth of the fungus and its antibiotic activity at given temperature and pH. So, it may be concluded that the temperature and pH would be detrimental to the antibiotic property when it is intended to use against bacteria. These observations should be taken in account when the antibiotic would be produced in large scale.

From this study it is clear that the chloroform extracts showed significant antibacterial activities against the entire test organism irrespective to Gram positive or Gram negative. However, the extract was found to be more sensitive against the Gram positive bacteria than the Gram negative bacteria. Previously it was reported the isolation and identification of ethisolide as an antibiotic product from *Penicillin capsulatum* [14]. The study of antimicrobial activity has been proved to be useful in drug design and their effective treatment policy against the bacteria fungus. The underlying cause and mechanism of these types of properties could be helpful for understanding the nature of their antibiotic activities, which also helps to find out the possibility of their utilization in treatment.

The minimum inhibitory concentrations (MIC) of the crude chloroform extract of the organism was found at moderate concentration for both Gram positive and Gram negative bacteria by serial dilution method. The concentrations at which first sign of inhibition observed in the experiment against respective test organisms indicated that the crude chloroform extract has a moderate antimicrobial property. The moderate concentrations also indicated that at purified condition, it may be a potential antibacterial agent in low amount dose. It was reported that the crude chloroform extract from *C. gloeosporioides* is sensitive against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* [23]. Brine shrimp lethality bioassay is a recent development in the bioassay for bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities eg: anticancer, antiviral etc. bioactive compounds are almost always toxic in high doses. Pharmacology is a simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. In this study in vivo lethality of a simple zoological organism (brine shrimp nauplii) is used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. There is a positive correlation between brine shrimp toxicity and cytotoxicity. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activities of natural products. In the brine shrimp lethality bioassay, the chloroform extract of organism showed positive results, indicating that the extracts are biologically active. The mortality rate of brine shrimp nauplii was found to be increased with the increase of concentration of the sample and a plot of logarithms of concentration verses percent mortality on graph paper gave an almost linear correlation between them.

CONCLUSION:

Present study showed that the crude chloroform extract of the causative organism *Colletotrichum acutatum* has fair amount of antibacterial activity against a number of Gram positive and Gram negative bacteria and cytotoxicity assay over brine shrimp.

ACKNOWLEDGMENT

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REFERENCES

1. Than PP, Prihastuti H, Phoulivong S, Taylor PWJ, Hyde KD; Chili anthracnose disease caused by *Colletotrichum* species. J Zhejiang Univ Sci B. 2008; 9(10): 764-778.
2. Hadden JF, Black LL; Anthracnose of Pepper Caused by *Colletotrichum* spp. Proceeding of the International Symposium on Integrated Management Practices: Tomato and Pepper Production in the Tropics. Asian Vegetable Research and Development Centre, Taiwan, 1989; 189-199.
3. Bosland PW, Votava EJ; Peppers: Vegetable and Spice Capsicums. England: CAB International, 2003; 233.
4. Manandhar JB, Hartman GL, Wang TC; Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. Plant Disease, 1995; 79: 380-383.
5. Jeffries P, Dodd JC, Jegeran, MJ, Plumbley RA; The biology and control of *Colletotrichum* species on tropical fruit crops. Plant Pathology, 1990; 39(3): 343-366.
6. Simmonds JH; A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. Queensland Journal Agriculture and Animal Science, 1965; 22: 437-459.
7. Freeman S, Katan T, Shabi E; Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Disease, 1998; 82(6): 596-605.
8. Cannon PF, Bridge PD, Monte E; (Eds.); Linking the Past, Present, and Future of *Colletotrichum* Systematics. In Host specificity, Pathology, and Host-pathogen Interaction. 1st edition, APS Press, St. Paul, Minnesota, 2000; 1-20.
9. Kim KK, Yoon JB, Park HG, Park EW, Kim YH; Structural modifications and programmed cell death of chilli pepper fruits related to resistance responses to *Colletotrichum gloeosporioides* infection. Genetics and Resistance, 2004; 94: 1295-1304.
10. Hong JK, Hwang BK; Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of pepper plants by *Colletotrichum* cocodes. Plant Disease, 1998; 82(10): 1079-1083.
11. Kim KD, Oh BJ, Yang J; Differential interactions of a *Colletotrichum gloeosporioides* isolate with green and red pepper fruits. Phytoparasitica, 1999; 27: 1-10.
12. Elias SM, Hossain MI; Chili cultivation in Bangladesh: Agro economic survey and constraints to its higher production at farm level. Research Report, AED, BARI, Joydebpur, Gazipur, Bangladesh, 1984; 72.
13. Addy SK, Goodman RK; Polyphenol oxidase and peroxidase activity in apple leaves inoculated with a virulent strain of Erwinia amylovona. Indian Phytopath. 1972; 25: 575-9.
14. Atienza J, Enrique H, Jaime P; Isolation and identification of ethisolide as an antibiotic product from Penicillium capsulatum. Appl. Microbiol. Biotechnol, 1992; 37 (3): 279-300.
15. Bauer AW, Kirby MM, Sherris JC, Truck M; Antibiotic susceptibility testing by a standardizing single disk method. Am J Clin Pathol, 1966; 45: 493-496.
16. Barry AL; The antimicrobic susceptibility test: principles and practices. 1st edition, Lea & Febiger, Philadelphia, 1976; 3-11.
17. Reiner R; Antibiotics, Chemotherapeutic agent and development of cgemotherapy: Antibiotics - An Introduction. Roche scientific service, Switzerland, 1982; 21-25.
18. Mayer BN, Ferrigni NR, Putanam JE, Jacobsen LB, Nichols DE, McLaughlin JL; Brine shrimp: a convenient general bioassay for active plant constituents. Plant Medica, 1982; 45: 31-34.
19. Asharafuzzaman H; Baboharik Udvid Rogtattya. 1st edition, Bangla Academy, Dhaka, 1993; 8-12.
20. Sanogo S; Chile pepper and the threat of wilt diseases. Plant Health Progress. 2003.
21. Park H.G; Problems of Anthracnose in Pepper and Prospects for its Management. In Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, 2007; 19: 441-853.
22. Roberts PD, Pernezny KL, and Kucharek TA; *Colletotrichum*, Plant Pathology Department, Florida Cooperative Extension Service, Insitute of Food and Agricultural Sciences, University of Florida. 2001. http://edis.ifas.ufl.edu PP104.
23. Kabir, MG, Absar N; Identification of the microorganism responsible for anthracnose disease of mango- leaves and isolation of bio-active compound from pathogen. J.bio.Sci, 2002; (10): 59-63.