In-Vitro Management of *Erwinia carotovora* the Causal Organism of Potato Soft Rot Disease

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Abstract*—In vitro efficacy six chemical substance were evaluated against Erwinia carotovora the causal organism of potato soft rot disease. E. carotovora was isolated from diseased potato tubers by dilution plate technique and different biochemical and pathogenicity test were performed to confirm the bacterial species. Six chemicals viz. Copper Oxychloride @ 0.2%, Mancozeb @ 0.2%, Boric acid @ 0.1%, Kasugamycin @ 0.02%, Carbendazim @ 0.3% and Sodium Hypochlorite @ 0.2% were tested against E. carotovora subsp. carotovora by well diffusion method. For each treatment there were four replications and tested chemical volume was 100 µl. Data were recorded up to five days of incubation. Maximum zone of inhibition (mm) was obtained after 48 hours of incubation with Copper Oxychloride (30.35 mm), followed by Mancozeb (20.15 mm), Boric acid (19.15 mm) and Kasugamycin (16.28mm). Copper Oxychloride produced the maximum growth inhibition (33.72%) of the pathogen, on the other hand Sodium Hypochlorite (2.68%) did not efficiently inhibit the growth of E. carotovora. Copper Oxychloride proved to be the best chemical followed by Mancozeb under in-vitro management against E. carotovora subsp. carotovora.*

***Keywords*—Soft rot, in-vitro, chemicals, zone of inhibition.**

I. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a starchy tuber crop belongs to the family Solanaceae. Potato possess number four position food crop after wheat, maize and rice in the world (Douches *et al.*, 1996) [1]. In Bangladesh the average yield of potato has been estimated 82, 05, 470 metric tons in the year 2011-2012 (BBS, 2012) [2]. Still potato production is quite low in comparison to that of the leading potato growing countries of the world.

Among many pathogenic bacteria *E. carotovora* subsp. carotovora causing potato soft rot disease is considered as most important disease (Akbar *et al.*, 2014) [3]. It is an important post-harvest disease which cause huge losses in stored potatoes if not properly managed. It has been estimated that every year 22% of potatoes are lost due to fungal, viral and bacterial diseases and pests, which is comparable to a yearly loss of more than 65 million tones and bacterial soft rot contributes it as much as 50% alone of the total potato production (Czajkowski *et al.*, 2011) [4]. The effect of soft rot disease is more prominent in the countries where suitable storage facilities are insufficient.

Generally chemical substances are not prescribed for the management of bacterial disease because it has high chance of lingering issue on human wellbeing and negative impression on the environment. However, many researchers evaluated various chemicals in order to control the soft rot bacteria. Copper-based compounds were found more effective against *E.carotovora* among numerous chemicals *in-vitro* (Rashid *et al.*, 2013) [5]. Acetic acid, boric acid and bleaching powder considerably reduced the infection rate, loss in weight and percentage of disease reduction against *E. carotovora* subsp. *carotovora* (Rahman *et al.*, 2017) [6]. The antibiotics had a substantial effect on plant pathogenic microorganisms which formed cell wall degrading enzymes (Alice and Sivaprakasam, 1995) [7].

Considering the great economic losses, the present investigation was undertaken to isolate and identify of causal organism and to evaluate the efficacy of six different chemicals against *E. carotovora* subsp. *carotovora* under *in-vitro* condition.

II. MATERIAL AND METHOD

The experiment was done in the Molecular Plant Pathology Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University. The experiment comprised of the purification and identification of the bacteria of potato soft rot disease and evaluation of efficacy of six chemicals against the causal organism under *in-vitro* condition.

2.1 Purification of the bacteria from diseased tubers

The potato tubers were collected from various places in Dhaka district. The pathogen was isolated by dilution plate method. Diseased tubers were surface sterilized with 95% ethanol for 3 mins, rinsed entirely with sterile water. The diseased parts of potato tuber were separated and macerated in sterile water to make a bacterial suspension. Tenfold serial dilution was made from the stock solution. 0.1 ml of each dilution was put on a nutrient agar (NA) plate and distributed using glass rod. Similar procedure was done three times (Goszczynska and Serfontein, 1998) [8] and were incubated at 30±1 ^{o}C for two days. A part of a well isolated typical colony was taken using a sterile wire loop and streaked on fresh NA plate to get pure culture.

2.2 The bacteria identification

The bacteria were identified by the following tests:

2.2.1 Grams staining reaction

A well isolated young colony was smeared on a glass slide followed by heat fixation. After a series of Grams staining reaction, described by Gerhardt (1981) [9] at 100x magnification the slide was viewed.

2.2.2 Biochemical Tests

In KOH solubility test, the bacterial colony was mixed with 3% KOH solution and any alteration in the consistency was recorded (Suslow *et al*., 1982) [10]. During catalase test 2-3 drops of afresh ready 3% H_2O_2 (Hydrogen peroxide) was placed on two days old pure bacterium culture on nutrient agar plate and observed whether the bacteria formed bubbles within a few seconds or not (Schaad, 1988) [11]. In oxidase test the bacteria colony was smeared on filter paper containing NNN'Ntetramethyl-p-phenylene-diamine-dihydrochloride and color changes was recorded (Kovacs, 1956) [12]. During gelatin liquefaction test nutrient broth containing 12% gelatin with bacterial *E. carotovora* culture incubated at 30 ^{o}C for 1-2 days followed by 5 ^{o}C in refrigerator for 15 minutes and it was observed whether the bacteria liquefied gelatin or not (Salle, 1961) [13]. In starch hydrolysis test, bacterium pure culture was streaked on the central of NA plate containing 2% soluble starch followed by incubation then the plate was awash with lugol's iodine solution. The existence or non- existence of clear zones in stained media was noted. (Cowan, 1974) [14].

2.2.3 Pathogenicity test:

Bacterial culture was suspended into sterile distilled water to make bacterial cell suspension. 0.5 ml suspension was placed into a hollow cut in the healthy potato tuber. Growth of rot on the tuber was viewed for 1-2 days after incubation at 28±2 °C (Prashant B Sandipan, 2014) [15]. Bacteria were re- isolated from macerated tissue and compared with the original isolate of inoculated pathogen (Shashirekha *et al*., 1987) [16].

2.3 *In-vitro* management of *Erwinia carotovora* subsp. *carotovora*

Six selected chemicals viz. Copper oxychloride, Mancozeb, Boric acid, Kasugamycin, Carbendazim and Sodium hypochlorite were tested against the test bacterium *E. carotovora* subsp. *carotovora* by well diffusion method measuring the zone of inhibition. Two wells were made with a cork borer of 5 mm in diameter in the individual NA plate and the pure culture of *E. carotovora* subsp. *carotovora* was streaked thoroughly on it with a sterile loop. One well was filled with definite concentration chemical suspension with 100 µl volume and other well was filled with sterile water. Each combination of pathogen, chemical and sterile water was replicated four times and plates were incubated at 30±1ºC. Inhibition zone around the wells was measured by observing the growth of bacterial and noted each day for 5 days.

Growth inhibition percentage (%) was determined using the formula modified by Amadioha (2004) [17] as

$$\text{inhibition} = \frac{dc - dt}{dc} x100\% \quad (1)$$

Here,

dc = Diameter of colony in control

dt = Diameter of colony in treatment

2.4 Data analysis

Collected data during experiment period were tabulated and analyzed using computer software MSTAT-C.

III. RESULTS AND DISCUSSION

3.1 Identification of bacteria from colony morphology

The colonies of bacteria *Erwinia carotovora* subsp. *carotovora* were found creamy white, round, slightly raised, smooth with entire edges, small to moderate large on NA media (Figure 1). Corresponding types of colonies were found by Opara and Agugo, 2014 [18].

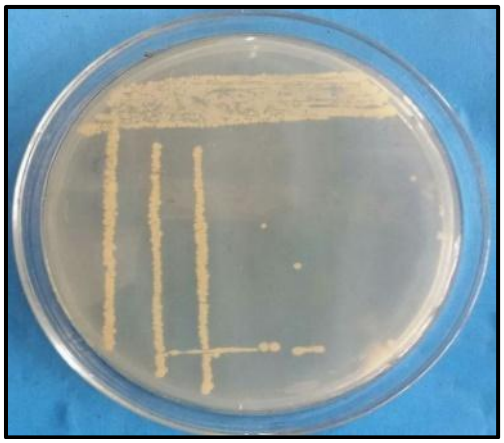


FIGURE 1. Pure culture of bacteria *Erwinia carotovora* subsp. *carotovora*

3.2 Identification of bacteria from biochemical characters

The isolated bacteria, *Erwinia carotovora* subsp. *carotovora* was confirmed by different biochemical tests (table 1). The bacteria were gram negative as they resulting red color after a series of Gram reaction test. In Gram differentiation test or KOH solubility test, the bacteria formed a mucoid strand when lifted with the help of toothpick. The bacteria formed bubbles resulting positive catalase test. The bacteria formed dark purple color in oxidase test. In gelatin liquefaction test the bacteria liquefied gelatin. A clear zone appeared around the colony, in starch hydrolysis test.

3.3 Pathogenicity test

Artificially inoculated potato tubers yielded the bacterial colonies alike to the genuine ones resulting positive pathogenicity test. Based on the morphological, biochemical and pathogenicity test, the pathogen was identified as *E. carotovora* subsp. *carotovora*.

TABLE 1
CHARACTERISTICS OF ISOLATED BACTERIA *E. CAROTOVORA* SUBSP. *CAROTOVORA* TO DIFFERENT TESTS ARE LISTED BELOW

Name of tests	Reaction
Gram staining	-
Gram differentiation test (KOH solubility test)	+
Gelatinliquification test	+
Starch hydrolysis test	+
Catalase test	+
Oxidase test	+
Pathogenicity test	+

3.4 Management of *E. carotovora* subsp. *carotovora* under *in-vitro* condition

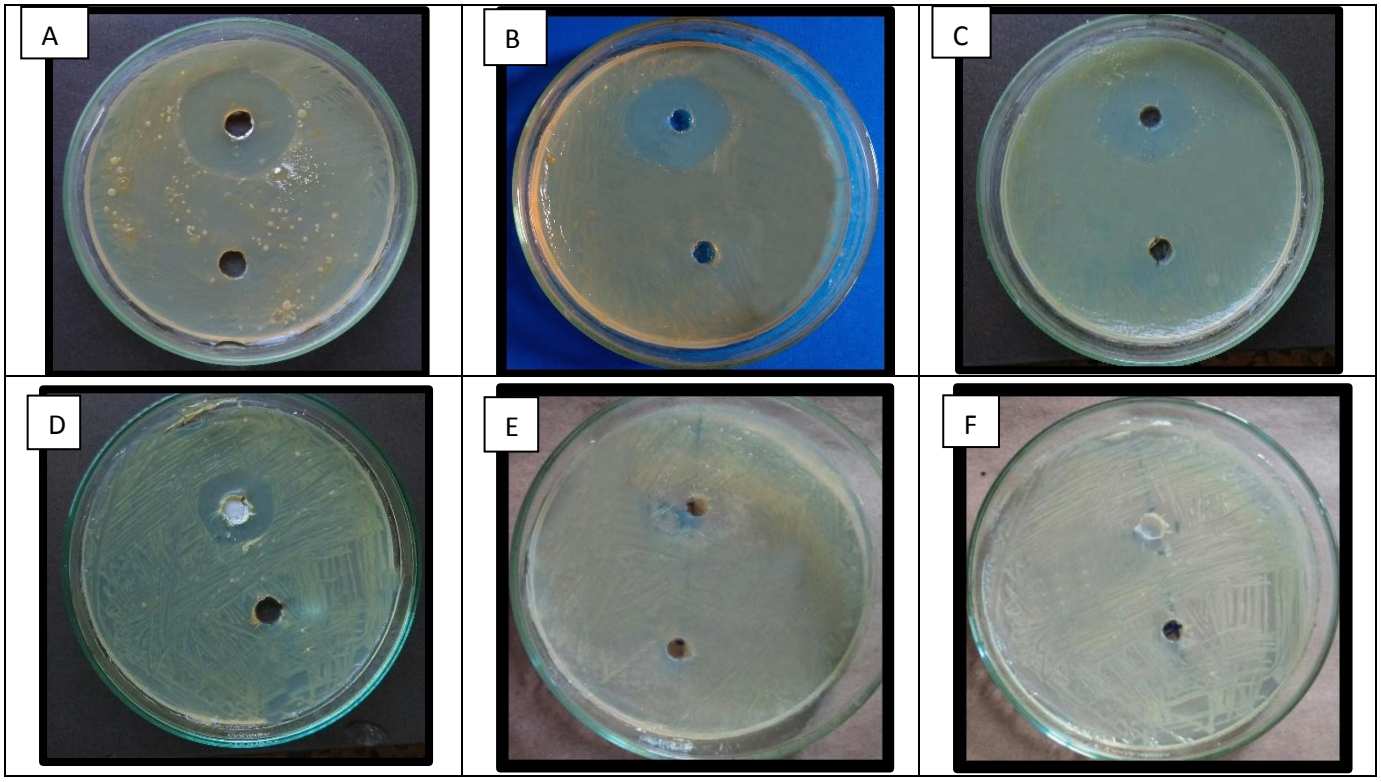
In-vitro evaluation of six different chemical substances were studied against *Erwinia carotovora* subsp. *carotovora* and found significant variations in terms of inhibition zone of isolated bacteria (Table 2 and Figure 2). Among the six chemicals, Copper oxychloride at 0.2% showed the maximum inhibition zone (30.35 mm) after two days of incubation followed by Mancozeb (20.15mm). Boric acid at 0.1% and Kasugamycin at 0.02% showed moderate inhibition zone 19.15mm and 15.08mm, respectively. Sodium hypochlorite at 0.2% showed the minimum inhibition zone 2.42mm.

TABLE 2

ACTIVITY OF SIX CHEMICALS AGAINST *ERWINIA CAROTOVORA* SUBSP. *CAROTOVORA* THE RESPONSIBLE ORGANISM OF POTATO SOFT ROT DISEASE *IN VITRO*

Chemicals	Con. (%)	Volume µl	Inhibition Zone (mm)				
			24h	48h	72h	96h	120h
Copper oxychloride	0.2	100	27	30.35	28.25	26.05	22.28
Mancozeb	0.2	100	18.4	20.15	18.48	16.18	13.65
Boric Acid	0.1	100	16.83	19.15	17.63	15.83	12.83
Kasugamycin	0.02	100	14.00	15.08	13.88	11.98	9.68
Carbendazim	0.3	100	2.39	3.83	1.28	0	0
Sodium hypochlorite	0.2	100	1.15	2.42	0.675	0	0

Note: Each data represents the mean of four replications



Clear zone indicates the inhibition zone

FIGURE 2: Screening of six chemical substance against *Erwinia carotovora* subsp. *carotovora* (A) Copper oxychloride (B) Mancozeb (C) Boric Acid (D) Kasugamycin (E) Carbendazim and (F) Sodium hypochlorite after 48 hours of incubation.

Efficiency of chemical substance used for this experiment was studied. Different chemicals showed different effects on growth inhibition of *E. carotovora* subsp. *carotovora*.

TABLE 3

EFFICACY OF SIX CHEMICALS IN INHIBITION OF GROWTH OF *E. CAROTOVORA* SUBSP. *CAROTOVORA*

SI No.	Chemical substance	Growth inhibition (%) of *E. carotovora* at 48 hours after incubation
1.	Copper oxychloride	33.72
2	Mancozeb	22.38
3	Boric Acid	21.28
4	Kasugamycin	16.76
5	Carbendazim	4.26
6	Sodium hypochlorite	2.68
7	Control	0.00

The results presented in Table 3 revealed that Copper oxychloride produced the maximum growth inhibition (33.72%) of the pathogen after two days of incubation and was statistically superior over rest of the chemicals tested. Other chemicals viz., Sodium hypochlorite (2.68%) did not effectively inhibit the growth of *E. carotovora* subsp. *carotovora.*

IV. CONCLUSION

Bacteria can multiply very fast and produce disease in favorable condition and the most serious aspect is that there are hardly any prospects to manage bacterial pathogens on potato. Employ of chemical substance free from health risk can be conducive and suitable way to manage potato soft rot disease. Among the chemicals tested in this experiment Copper oxychloride @ 0.2% was found most effective against the bacteria, other chemicals also had moderate effect against this bacterium *in vitro*. A through and large-scale research is required to find out an effective method to control soft rot of potato.

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