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Severity, Incidence and Host Range of *Pectobacterium caratovora* subsp.*caratovora* Causing Bacterial Soft Rot of Carrot in Meghalaya, India

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ABSTRACT

Carrot (*Daucus carota* L.*)* is one of the most important widely grown root vegetables in Meghalaya and ranked among the top-ten important vegetable crops, in terms of both area of production and market value. Bacterial soft rot is the major postharvest disease of vegetables and reported losses up to 15-30%. An investigation was undertaken to study the incidence and severity of soft rot of carrot caused by *Pectobacterium caratovora* subsp.*caratovora* (Pcc) in two districts of Meghalaya, India aswell it host ranges. The wide range of variation was observed in both soft rot incidence (0-50%) and severity (0-17.17%). The highest soft rot incidence (50%) and severity (17.17%) was recorded in Nongpoh during the month of August, whereas the lowest (4.57%) in Laitumukhrah (East Khasi Hills district) in the month of February. The host range of Pcc also studied by inoculating on eight different hosts (tomato, chilli, cabbage, ginger, potato, radish onion and pineapple). Among the tested hosts, radish was found to be most susceptible to the soft rot showing 22.67% of rot intensity and ginger was found to be resistant with no symptom developed on rhizomes at 5 days after inoculation. The present study can concluded that this bacterial soft rot disease is a major factor for post-harvest losses of carrot in two districts of Meghalaya and test pathogen has an also wide host range that causes severe post-harvest losses in many crops.

1. Introduction

Carrot (*Daucus carota* L.) is an important widely grown root vegetable in India and it ranked in the top-ten economically important vegetable crops, in terms of both area of production and market value (Amarasinghe*et al.,* 2014). In India, this crop covers an area of 88000 hectares and with a production of1446000 metric tonnes. In Meghalaya, production of the crop shares 1.68per cent with a production of 22.712Ton though/metric tonnes (Anonymous, 2017-2018). Bacterial soft rot is one of the major post-harvest diseases of vegetables and caused losses up to 15-30% (Agrios, 2005). Post-harvest losses of carrot is mainly due to rot diseases like *Pectobacterium* rot, Sclerotinia rot and Botrytis rot, (Lunt, 2013). Among these, bacterial soft rot *Pectobacterium caratovora* subsp. *caratovoa* is the most common causal organism for the soft rot disease (Mansfield *al.,* 2012). The bacterial soft rot pathogen have very broad host ranges and can attack many commercial vegetables *viz*., carrot, potato, cabbage, and lettuce (Raju et al., 2008). This disease causes heavy losses of carrot and acts as a limiting factor in the pre and post cultivation of carrot. In order to increase in production, it is imperative to avoid losses from diseases in the field as well as prevent losses during storage and transit. Soft rot of carrot is caused by many pathogens, but there is limited information on incidence and severity of bacterial sot rot of carrot in Meghalaya. Therefore, the present investigation was undertaken to study the per cent incidence and severity of soft rot caused by *P. caratovora* subsp. *caratovora* of carrot as well as it host ranges under *in vitro* condition.

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2. Materials and Methods

Market survey

The survey was conducted in the months of July-September and November – February during 2018-2019 in two districts (Ribhoi and East Khasi Hills) of Meghalaya, India selecting four major market locations (Fig. 1) in each district i.e., Ri-Bhoi (Byrnihat, Nongpoh, Bhoirymbong and Umroi) and East Khasi Hills (Bara Bazaar, Jhalupara Laitumkhrah and Polo Lawmaking). From each market, four vegetable shops were selected to record soft rot incidence and severity of carrot. The samples were collected randomly from the four shops of each market. One hundred carrots were randomly selected from each shops and number of diseased carrots were counted to record disease incidence and severity

Percent of incidence was calculated by using the formula:

$$\text{Disease incidence (\%)} = \frac{n}{N} \times 100$$ (Bhat *et al.*, 2010)

Where, n=No of carrots showing soft rot symptom

N=Total number of carrots observed

Per cent disease severity was calculated using the formula:

$$\text{Disease severity (\%)} = \frac{\text{Sum of the scores recorded}}{\text{Total carrots observed} \times \text{highest rating}} \times 100$$

And disease severity was recorded by using fallowing scale (0-5) given by Liao and Wells (1987): where, 0 = No maceration;1 = 1-20% maceration;2 = 21-40% maceration; 3 = 41-60% maceration;4 = 61-80% maceration and5 = 81-100% maceration.

Pathogen isolation

Carrot showing the typical symptoms of soft rot were collected from markets and brought to laboratory for pathogen isolation. Small pieces of infected carrot were cut aseptically using sterile blade along with little portion of healthy tissues. Excised samples were surface sterilized using 1% of sodium hypochlorite and washed in sterile distilled water for 3 times to remove traces of chemical. The surface sterilized pieces were macerated with 1 or 2ml sterile distilled water under aseptic conditions. A loop full of bacterial suspension was streaked on Petri plates containing nutrient agar medium. The streaked plates were incubated at 28^{o}C for 48 h. The bacterial colonies developed on nutrient agar medium were observed under a microscope for the shape, size and tested gram reaction of the bacterium.

Pathogenicity test

The healthy carrots were surface sterilized using 1% of sodium hypochlorite and washed thrice with sterile distilled water before inoculation of the test pathogen. The cotton stabs are dipped in the bacterial suspension of 10^{9}cfu/ml and inoculated to carrots using pin pricking method (Janse 2005) and incubated for 3 to 4 days, sterile water is sprinkled to maintain humidity. Inoculated carrots were observed for symptom development and severity at 5 days after inoculation. The test bacterium was re-isolated and confirmed pathogenicity by following Koch's postulates and colony characters were assessed at 48 hr after of incubation. The morphological characteristics such as shape, size, elevation, surface, edge, colour, opacity and solubility in water were studied following the guidelines described Holt *et al.* (2000).

Biochemical analysis

Various biochemical tests like starch hydrolysis, oxygen requirement, gelatine liquefactions, catalase, oxidase, nitrate reduction, potato soft rot growth at37^{o}C and sensitivity test to erythromycin, were conducted by following techniques described in the "Laboratory Manual of Plant Bacteriology" by Thind (1987) and "Laboratory Guide for Identification of Plant Pathogenic Bacteria"(Schaad *et al.*,2001).

Host range

The host range of the *Pcc* was studied on different hosts (tomato, chilli, cabbage, ginger, potato, radish, onion and pineapple) using technique of DeBoer*et al.*, (1978) with slight modification. A drop of an aqueous bacterial cell suspension (10^{10}cfu/ml) of the *Pcc* was applied with the help of a sterile pipette on each vegetable part and a flamed straight pin inserted through the drop into the centre of vegetable part and then withdrawn. Inoculated hosts were kept in sterile plastic bags, and incubated at temperature of 27±1^{0}C. The inoculated vegetables were recorded for symptom development after every 24 hours and the observations were recorded on days taken to express first symptom development and intensity of soft rot was measured after five days of Inoculation.

Statistical analysis

The experiments were carried out in a complete randomized design (CRD) with three replications. The validity of the experiment is obtained through one way ANOVA and means were separated by Duncan's multiple range tests at $p \leq 0.05$ (Gomez and Gomez, 1984).

3. Results and discussions

Soft rot incidence and severity

The wide range of incidence and severity of bacterial soft rot recorded in all the locations and period of surveyed. Among the locations, the highest incidence (50.00%) was recorded in Nongpoh (Ri-Bhoi district) market, in August 2018, whereas the lowest (4.50%) in Laitumukrah (East Khasi Hills district), during February, 2019. The highest soft rot severity (17.70%) recorded in Nongpoh (Ri-bhoi district), in July 2018, whereas

it was lowest (3.47%) in Jhalupara (East Khasi Hills district) in the month of February 2019. The incidence and severity of soft rot of carrot was recorded the maximum during peak rainy season (July-August) and the lowest during winter season (December-January). This could be due to congenial weather for the growth and development of bacterial pathogen during rainy season. Bacterial soft rots tend to be more of a problem during hot and wet weather with plenty of rainfall trigger the disease to occur and can be more severe when plants lack sufficient calcium (Lunt, 2013). Our results also supported by Bhat *et al* (2010) reported during 2008-2009that bacterial soft rot pathogen (*P. caratovora* subsp. *caratovora*) was more severed during summer than the cooler months in the three districts of Kashmir.

Pathogenicity test

Pathogenicity test was carried out in carrot by using pin pricking method. The carrots were inoculated with the bacterial suspension of 48h old culture. Small water soaked lesions developed on inoculated region of carrots after 2-3 days. Symptoms of affected area became watery, soft, fleshy and oozing of the tissue was observed in the region of the inoculated area (Fig 2.). To prove the Koch's postulates re-isolation of the pathogen was carried out on NA media from artificially inoculated carrot. Cultural colony characters were compared with the original culture and were found similar. Similar symptoms were also described by Coplin (1980) and Walker (2006). Association of the same bacterium was found from inoculated carrots upon re-isolation. Hence, the study revealed that the bacteria isolated from infected carrots are responsible for the disease. It proved Koch's postulate.

Biochemical assay

A total of ten biochemical tests were carried out on *Pcc* and the results obtained were presented inTable 3.The *Pcc* showed +ve reaction for Oxygen requirement, Nitrate reduction, Potato soft rot, H_2S from cystein, Growth at 37^{o}C while –ve reaction to Starch hydrolysis, Oxidase test, Catalase test and sensitive to erythromycin. Similar results were also reported by Dickey (1978), Lelliot and Dickey (1984), Dickey and Kelman (1988) and Kim *et al.* (2007).

Host range

The host range study was carried out on 8 different hosts namely tomato, chilli, cabbage, ginger, potato, radish onion and pineapple by inoculating *Pcc* under artificial conditions. The days taken to express first soft rot symptoms and intensity at 5 days after inoculation revealed that radish and chilli expressed first symptom within 1 day (24 h) with severity of 22.67% and 13.67% followed by cabbage (17.00%), tomato (12.00%), pineapple (11.90%), onion (9.67%) and potato (6.0%) which expressed first symptom on 2 and 3 days after inoculation whereas no soft rot symptom developed on ginger rhizomes. Similar findings were also reported by Bradbury (1986) and Bhat*et al.* (2010). Walker (1998) reported that if not all but most of the crucifers were susceptible to bacterial soft rot including cabbage, cauliflower, brussel sprouts, kohlrabi, turnip, radish, rape, horse radish, and rutabaga.Ginger (*Zingiber officinale*) was found resistant to *Pectobacterium* spp might be due to presence of volatile essential oil (Opara, 2013).

4. Conclusion

From the present investigation it canbe concluded that *P. caratovora* subsp. *caratovora* was the pathogen associated bacterial soft rot of carrot causing post-harvest disease Soft rot of carrot is more severed during hot humid weather conditions and rainy season but less in winter months. It was found that *P. caratovora* subsp. *caratovora* is having broad host range and can causes severe pre and post-harvest losses up to 15 to 50%. Therefore, it requires further studies on formulating effective management practices for preventing the pre and post-harvest losses due to soft rot disease and increase the income of farmers.

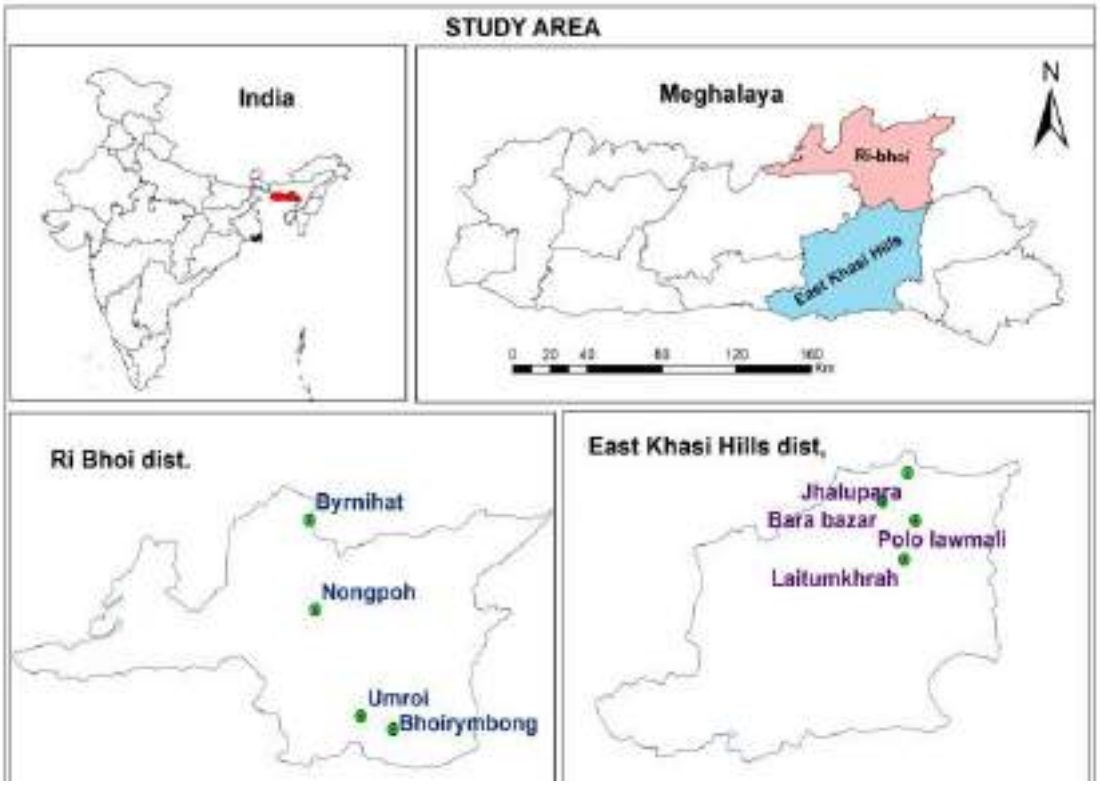


Fig 1. Different locations of markets survey area in the Ri Bhoi and East Khasi Hill district of Meghalaya, India

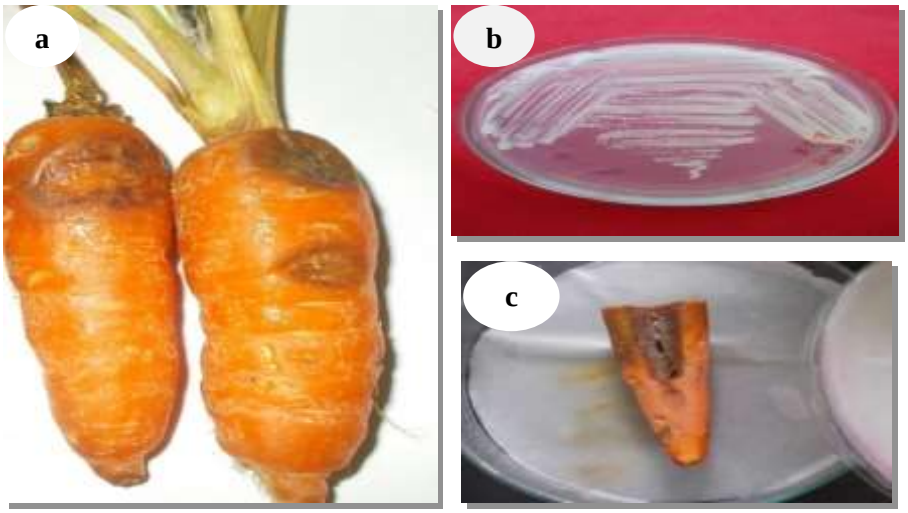


Fig 2 Pathogenicity test(a)Soft rot symptoms on carrot caused by *P. caratovora*subsp*caratovora (Pcc);* (b) Pure culture of the *P*cc NA medium; (c) soft rot symptom on carrot at 15 days after inoculation

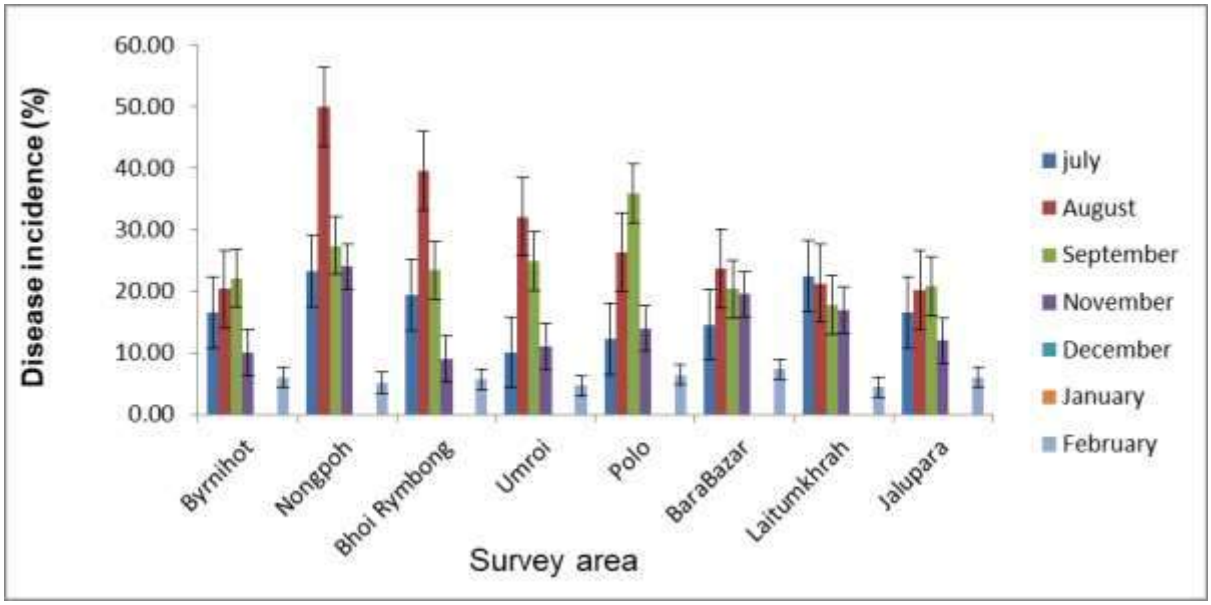


Fig. Bar diagram representing the disease incidence of bacterial soft rot ofcarrot in different market places of Ri-Bhoi and East- Khasi Hills district Meghalaya. The error bars indicate standard error of three independent replications

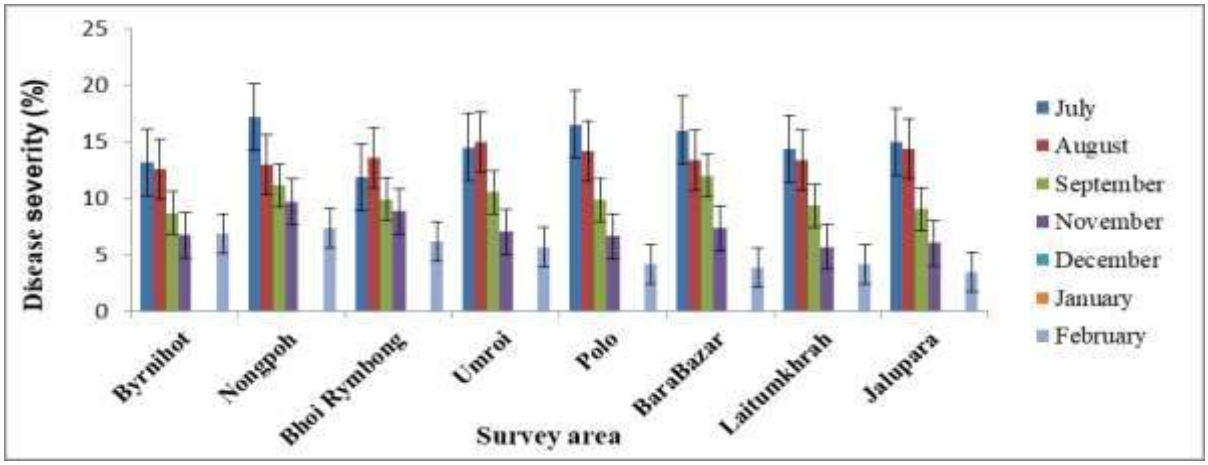


Fig. Bar diagram representing the disease severity of bacterial soft rot of carrot in different market places of Ri-Bhoi and East-Khasi Hills districtMeghalaya. The error bars indicate standard error of three independent replications

Table 3. Biochemical characterization of the *P. caratovora* subsp *caratovora*

Sl.No	Test	Results
1	Starch hydrolysis	-ve
2	Oxygen requirement	+ve*
3	Gelatin liquefactions	+ve
4	Catalase	+ve
5	Oxidase	-ve
6	Nitrate reduction	+ve
7	Potato soft rot	+ve
8	Growth at 37^{o}C	+ve
9	Sensitive to erythromycin	-ve

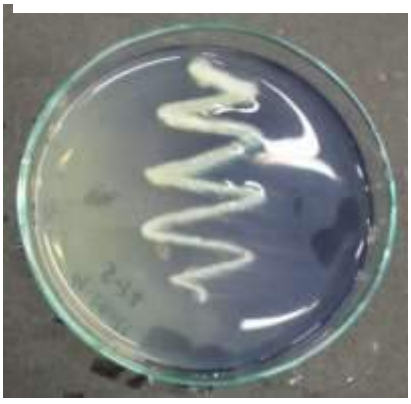
*Facultative anaerobe

Test pathogen growth at 37° C

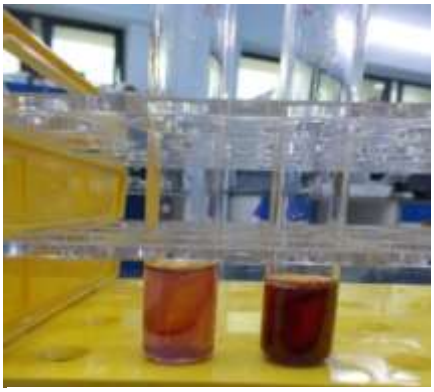
Insensitive to Erythromycin

Starch hydrolysis

Gelatin liquefaction



Oxidase test



Nitrate reduction C

Fig Biochemical characterization of the test pathogen, *P. caratovora* subsp. *caratovora*

Table 4. Host range of soft rot pathogen, *P. caratovora* subsp. *caratovora* under artificial inoculation condition

Host	Part used	Infectivity	Days taken to express first symptom	Soft rot intensity at 5days after inoculation
Tomato	Fruit	+	2	12.00*(3.535)[d]
Chilly	Fruit	+	1	13.67(3.764)[c]
Cabbage	Leaf	+	2	17.00(4.183)[b]
Ginger	Rhizome	-	-	0.00(0.707)[g]
Potato	Tuber	+	3	6.00(2.548)[f]
Radish	Root	+	1	22.67(4.813)[a]
Onion	Bulb	+	2	9.67(3.188)[e]
Pineapple	Fruit	+	3	11.90(2.11)[g]

$CD_{(0.05)} = 0.109$; *= Mean of 3 replications**;** += Symptom development **and;** -= No symptom

Radish (22.60%)

Ginger (0.00%)

Tomato (12.00%)

Chilli (13.67%)

Cabbage (17.00%)

Potato (6.00%)

Onion (9.67%)

Pineapple (11.90%

Fig Host range of *P. caratovora* subsp. *caratovora* causing soft rot on different hosts

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