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Eco-friendly Post Harvest Management 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 and widely grown root vegetables in Meghalaya and ranks 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%. It is having broad host range causes severe post-harvest loss during transits and storage. An eco-friendly post-harvest management was undertaken to manage the bacterial soft rot with the combination of potential *Bacillus subtilis* isolates, botanicals, post harvest salts and packaging materials on severity of bacterial soft rot. Eight potential *Bacillus subtilis* isolates (Bs110, Bs216, Bs167, Bs190, Bs174, Bs217, Bs219 and COB5Y1) were tested against soft rot pathogen by paper diffusion disc method. Among them the isolate B216 measured with highest (16.3mm) zone of inhibition, followed by Bs190 (15.1mm). *In-vitro* evaluation of botanicals (neem, beetlevine, garlic, vetiver and aloe vera) at three different concentration (3%, 5% and 10%) was evaluated. Among them garlic at 10% was showed most effective botanical with 10.8mm inhibition zone, followed by neem (8.83mm) at 10%. Among the tested postharvest salts (sodium bicarbonate, ammonium bicarbonate sodium hypochlorite and acetic acid) at different concentration, sodium hypochlorite at 5% concentration was showed maximum zone of inhibition (12.17mm) followed by ammonium bicarbonate (11.7mm) at 3%. Among the packaging material (net bag, gunny bag, perforated polythene bag and bamboo box), net bag was found most effective with less severity of (8.51%), followed by gunny bag with (10.52%) of severity. Among the integrated treatments (sodium hypochlorite and net bag combination) was found effective in reducing soft rot severity of 6.80%, followed by garlic and net bag combination with 7.7% severity. Garlic, Sodium hypochlorite and Net bag in alone and in combination were found most effective and promising in preventing the post harvest losses caused by *Pectobacterium caratovora* subsp. *caratovora* in both transits and storage.

Keywords: Botanicals, Carrot, Meghalaya Post harvest salts and *Pectobacterium caratovora* subsp. *Caratovora*.

INTRODUCTION

Carrot (*Daucus carota* L.) is an important widely grown root vegetable in India and it ranks as an important crop among 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 of 1446000 metric tonnes. In Meghalaya, production of the crop shares 1.68per cent with a production of 22.712 ton per hectare (Anonymous, 2017-18). Bacterial soft rot is one of the major postharvest diseases of vegetables and causes losses up to 15-30% (Agrios, 2005). Postharvest losses of carrot is mainly due to rot diseases like *Pectobacterium* rot, *Sclerotinia* rot and *Botrytis* rot, (Lunt, 2013). Among these, bacterial soft rot, caused by *Pectobacterium caratovora* subsp. *caratovoa* reported that the most common causal organism for the soft rot disease (Mansfield *et al*., 2012). The bacterial soft rot pathogen has very broad host ranges and can attack many vegetables *viz*., carrot, potato, cabbage, and lettuce (Walker, 1998). Bacterial soft rot of vegetables are controlled earlier by using a number of measures, which is fumigation of storage building with formaldehyde (Walker, 1998), and cleaning of storage house with Sodium hypochlorite, Calcium hypochlorite or formaldehyde solution (Anonymous, 1990), treatment with some salts like alum, copper sulphate pent hydrates, aluminum acetate (Mills *et al.*, 2006). This disease causes heavy losses to the carrot and acts as a limiting factor in the pre and post cultivation of

carrot. In order to obtain increase in production, it is imperative to avoid losses from diseases in the field and prevent losses during storage and transit. Soft rot of carrot is caused by many pathogens, but there is limited information on effective Eco friendly management practices and uses of storage structures against *P.c*.subsp *c* to prevent losses of carrot in transits and storage.

MATERIALS AND METHODS

Pathogen isolation. Carrot showing the typical symptoms of soft rot were collected from market 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±1°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 10^6cfu/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 carrots were observed for symptom development and severity at 5 days after inoculation. The test bacterium were re-isolated by following similar standard methods i.e. Koch's postulates and colony characters were observed at 48 hr after of incubation.

In-vitro* evaluation of *Bacillus subtilis. The eight potential *Bacillus subtilis* (Bs110, Bs216, Bs167, Bs190, Bs174, Bs217, Bs219 and COB5Y1) were collected from Plant Pathology laboratory of CPGS; to evaluate against the *Pectobacterium carotavora* ssp *caratovora*. Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer *et al.* (1966) to assess the presence of antibacterial activities of the potential *B. subtilis*. A tested bacteria culture (*P. carotavora* ssp *caratovora*), was used to prepare lawn on agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of potential *B. subtilis* placed on agar surface with test pathogen. Each test plate comprises of one disc. Disc soaked in sterile distilled water serve as a control. The plate was then incubated at 27°C for 24 to 48 hours. After the incubation, the plates were examined for inhibition zone. The zone of inhibition was recorded using calipers.

***In-vitro* evaluation of botanicals.** Five botanicals *viz,* neem, beetlevine, garlic, vetiver and aloe vera were evaluated against *P. carotavora* ssp *caratovora* by using standard paper disc method of Thornberry (1950). Nutrient agar medium seeded with the pathogen was immediately poured in 10 cm Petri dishes and allowed to solidify. Filter paper discs, 7 mm in diameter, were soaked aseptically for 5 minutes in aqueous solution of different botanicals and at different concentrations (3, 5 and 10%), separately. The botanical extracts were prepared by adding 100 ml distilled water to 100 g of botanicals gives 100% of concentration of botanicals (1:1 w/v). Aqueous solutions of different treatments were prepared by diluting the extracts to 3, 5 and 10% using distilled water (Malik *et al.,* 2016). For each botanical three concentrations were tested and for each concentration three Petri plates were used. In the centre of each Petri dish, one impregnated paper disc was placed. In case of control, paper discs soaked in sterile distilled water were used. The Petri plates were incubated for 72h at 27±1°C. The efficacy of the various botanicals was assessed by measuring the zone of inhibition surrounding the filter paper disc after the incubation period.

***In- vitro* evaluation of postharvest salts.** The efficacy of four postharvest salts at different concentrations sodium hypochlorite 2%, 3% and 5% (Amarasinghe *et al*., 2014). Sodium bicarbonate and ammonium bicarbonate at 1%,2% and3% (Aslam *et al.*, 2012) and acetic acid at 500, 1000 and 1500ppm (Anonymous, 1990) were tested against *P. carotavora* ssp *caratovora* by using standard paper disc method of Thornberry (1950).

Evaluation of packaging materials. Four packaging materials (net bag, gunny bag, perforated polythene bag and, bamboo box) were used for this experiment. The fresh carrots without infection were surface sterilized with 0.1% mercuric chloride. The bacterial suspension prepared from 24-48 h old culture, inoculated into fresh carrots by using pin pricking method (Janse, 2005). Carrot kept in bamboo box serve as Control. Inoculated carrots were put in packaging materials and incubated 27±1°C for 72h (Bhat *et al.*, 2010). Observation recorded for soft rot severity. Based on observation best packaging material was selected for further study.

Integration of treatments for effective management of bacterial soft rot of carrot. In this experiment the botanical, postharvest salt and packaging material which was shown effective against bacterial soft rot pathogen in *in-vitro* was tested alone as well as in integration. Integration of treatments was described below:

T_1 - Best botanical alone
T_2 - Best post harvest salt alone
T_3 - Washing with water + best packaging material
T_4 – Best botanical +Best packaging material

T_5 -Best post harvest salt + Best packaging material
T_6 - Control

RESULTS AND DISCUSSIONS

Isolation and identification of pathogen. The colonies were studied for their shape, size and color the colonies were found to produce light yellow to cremish yellow colonies during the isolation procedure. Based on the morphological, colony a character, the causal agent was identified as gram –ve bacteria (table 1). The colony characters of the isolated pathogen were matching with those of the genus *Pectobacterium* which was described by Dickey and Victoria (1980). The similar finds (Dickey and Kelman, 1988; Holt *et al*., 2000; Ullah *et al*., 2011).

Pathogenicity test. 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 (Plate 1). 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. Similar symptoms were also described by Coplin (1980); Walker (2006).

In-vitro evaluation of Potential isolates of *Bacillus subtilis.* Among the tested potential *B. subtilis* isolates, Bs216 was recorded for maximum zone of inhibition (16.3mm) followed by Bs190 with (15.1mm) .The lowest zone of inhibition was recorded in Bs110 with (12.6mm) zone of inhibition. *Bacillus subtilis* had showing stronger antagonistic effect against *Pectobacterium caratovora* sub sp *caratovora* (Table 2). The results were agreement with the findings of Rahaman *et al.* (2012); Sowmya *et al.* (2012). Applications of *B. cerus*, *B. subtilis* and *B. megaterium* showed good antagonistic activity against *Pectobacterium caratovora* sub sp *caratovora* (Issazadeh *et al.*, 2012).

***In-vitro*- evaluation of botanicals.** Among five evaluated botanicals, garlic was found most effective at all the tested concentrations (3%, 5% and 10%) and differs significantly from the other tested botanicals. Maximum zone of inhibition (mm) was recorded at 10% garlic concentration i.e. 11.1 mm followed by neem (9.6mm), vetiver (7.8mm) and betlevine (6.3mm) which exhibited less significant difference with each other and aloe vera was found least effective against the bacterial soft rot pathogen at same concentration with 4.3mm inhibition zone (Table 3). Antimicrobial properties of garlic might be due to the presence of an essential oil that contains allyl disulphide (C6H2 S2) diallyl disulphide (C6 H10 S2), and two or more sulphur-containing compounds. Bakht *et al.* (2011) reported that butanol extracted sample of garlic was found to be more effective in inhibiting the growth of *Erwinia carotovora* i.e. 75% at 2mg/disc. The potential of garlic on bacterial soft rot pathogen was in agreement with the findings of (Ahmed and Agnihotri, 1977; Agrawal, 1978 and Simeon and Abubakar, 2014). Probably neem plant contains some compounds, which have bactericidal effects on the bacterial soft rot pathogen. Emechebe (1996) also reported that foliar application of the aqueous neem seed extract controlled bacterial blight of cowpea in Nigeria, further buttressing the bactericidal properties of neem products. Neem leaf and seed aqueous extracts significantly reduces the incidence and severity of potato tuber soft rot (Bdliya and Dahiru 2006).

***In-vitro*- evaluation of Post harvest salts.** Among the tested four postharvest salts sodium hypochlorite was found most in inhibiting the bacterial growth at all tested concentrations (2, 3 and 5%) and the zone of inhibition was recorded was (10mm, 12mm and 13mm), followed by Ammonium bicarbonate, (1, 2 and 3%) was showing 4.1mm, 10.5mm and 11.8mm, sodium bicarbonate (1, 2 and 3%) was showing 2.9mm, 8.9mm and 10.20. lowest inhibition was recorded for acetic acid 6.00mm, 3.30mm and 7mm at concentrations 500ppm, 1000ppm and 1500ppm. Immersion of freshly inoculated potato tubers in sodium hypochlorite for 5 min reduces 99% of soft rot symptoms on the potato tubers and immersion of potato tubers in citric, acetic, ascorbic and malonic acid also reduced rotting caused by *Erwinia caratovora in-vitro* conditions (Table 4). The present findings correlate with the findings of (Bartz and Kelman 1986). The effecacies of sodium hypochlorite and ammonium bicarbonate was reported by Mills *et al*. (2006); Aslam *et al.* (2012), Amarasinghe *et al.* (2014); Himel *et al*. (2017).

***In-vitro*- evaluation of Packaging materials.** Among four evaluated packaging materials (net bag, gunny bag, perforated polythene and bamboo box) less severity of 8.51% was recorded in net bag and it is significantly differ from the other packaging materials, followed by gunny bags (10.5%), bamboo boxes (13.23%) and highest severity was recorded in perforated polythene bags (17.20%). The packing materials play a vital role in maintaining different temperature and humidity during transit and storage (Fig. 1). The severity of bacterial soft rot pathogen is more severe during high humidity favorable with moderate temperature. The study is, more or less supported by previous studies of Bhatacharya and Mukarjee (1986); Raju *et al.* (2008) who as a result of their studies concluded that increased RH enhanced soft rot in storage. Net bag was found as good storage structure because it does not provide conditions favorable for the soft rot pathogen. The similar results were also recorded by Bhat *et al.* (2010).

Integrated disease management of soft rot of carrot. Among the tested best botanicals, post harvest salts and packaging materials, Sodium hypochlorite was recorded for less soft rot (12.33%) severity than the garlic (15.83%), when tested alone aginst bacterial soft

rot of carrot and sodium hypochlorite differes signifcantly from garlic. Among the integration less severity was observed in sodium hypochlorite and net bag combination (6.80%), followed by Garlic and net bag (7.7%), washing with water and net bag (8.6%), sodium hypochlorite (12.3%) and more severity (21.63%) was recorded in control Efficacy of botanical and postharvest salt has been reported (Table 6). previously by several workers (Obagwu, 1997; Bdliya and Dahiru, 2006; Amarasinghe *et al.*, 2014; Himel *et al.*, 2017). Sodium hypochlorite and net bag are more effective because the integration is not much favourable for the growth of the pathogen; hence the severity was found less. The integration results were found similar with the findings of Bhat *et al*. (2010).

Table 1: Morphological and cultural characters of the bacterial soft pathogen Isolated from infected carrot tissue.

Character	Description
Shape	Straight rod
Length	1-2.5µm
Width	0.7-1.0 µm
Average size	0.75×2 µm
Gram reaction	-ve
Pigmentation	Non-pigmented
Colony colour	Cremish to yellow
Colony shape	Round, mucoid

Table 2: In-vitro evaluation of Potential *B. subtilis* isolates against *P. caratovora* sub sp. *Caratovora.*

***Bacillus subtilis* isolates**	**Zone of inhibition**
Bs110	12.41 (0.49)
Bs216	16.32 (0.66)
Bs167	12.61 (0.55)
Bs190	15.37 (0.54)
Bs174	13.48 (0.52)
Bs217	14.57 (0.37)
Bs219	12.45 (0.59)
COB5Y1	13.65 (0.602)
Control	0.00 (0.00)
C.D	1.52
S.E. (m)	0.50
S.E (d)	0.71
C.V.	7.14

*****Mean of the three replication
Figures in parentheses are square root transformed values
F- test at 5%level

Table 3: *In-vitro* Evaluation of Botanicals against soft rot pathogen of carrot.

Botanicals	**Inhibition zone (%) at different concentration ***		
	3%	**5%**	**10%**
Neem	6.63* ± 0.08[b] (2.671)	7.80 ±0.15[b] (2.88)	8.83 ±0.12[b] (3.05)
Beetle vine	3.43 ±0.12[d] (1.983)	4.77 ±0.08[d] (2.29)	5.87 ±0.06[d] (2.52)
Garlic	8.70 ±0.05[a] (3.03)	9.60±0.05[a] (3.17)	10.80 ±0.17[a] (3.36)
Vetiver	5.57 ±0.17[c] (2.463)	6.90 ±0.23[c] (2.72)	7.50 ±0.20[c] (2.28)
Alovera	2.33 ± 0.12[e] (1.68)	3.37 ±0.08[e] (1.96)	4.20 ±0.05[e] (2.16)
Control	0.00 ±0[f] (0.707)	0.00 ±0[f] (0.70)	0.00 ±0[f] (0.70)
CD (p=0.05)	0.32	0.327	0.28

*****Mean of the three replication
Means having common letter are not significantly different
Figures in parentheses are square root transformed values
F- test at 5%level

Table 4: *In-vitro* Evaluation of Postharvest salts against soft rot pathogen of carrot.

Postharvest salt	Zone of inhibition (%) at different concentration*		
	Conc 1	Conc 2	Conc 3
Sodium hyphochlorite	10.97* ±0.29[a] (3.38)	11.27 ±0.67[a] (3.42)	12.17 ±0.44[a] (3.55)
Amonium bicarbonate	3.93 ±0.08[c] (2.10)	9.87 ±0.34[b] (3.21)	11.17 ± 0.49[a] (3.41)
Sodium bicarbonate	2.50 ±0.23[d] (1.72)	8.10 ±0.40[c] (2.93)[c]	9.57 ±0.34[b] (3.17)
Acetic acid	5.83 ±0.12[b] (2.51)	5.87 ±0.23[d] (2.52)	6.70 ±0.25[c] (2.68)
Control	0.00 ±0[e] (0.70)	0.00 ±0[e] (0.70)	0.00 ±0[d] (0.70)
CD (p=0.05)	**0.12**	**0.19**	**0.16**

***Mean of the three replication
Figures in parentheses are square root transformed values
Means having common letter are not significantly different
F- test at 5%level

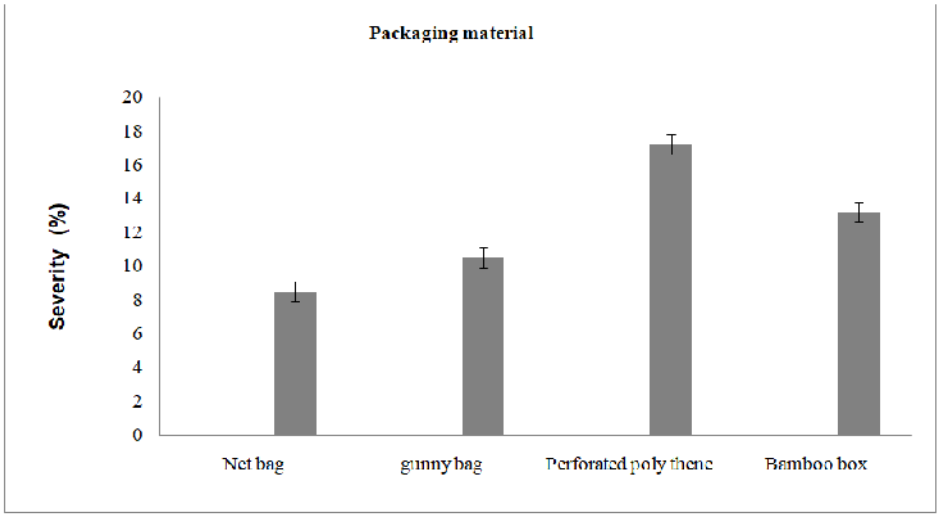


Fig. 1. Bar diagram showing effect of different packaging materials against *P. caratovora* subsp. *caratovora* of carrot. The error bars indicate standard error of three independent replicates.

Integrated disease management of soft rot of carrot

Treatement	Severity(%)*
Garlic	15.83 ±0.44[b] (3.97)
Sodium hypochlorite	12.33 ±0.60[c] (3.51)
Washing with water+Net bag	8.60 ± 0.17[d] (2.93)
Garlic+ Net bag	7.77±0.14[d] (2.78)
Sodium hypochlorite+Net bag	6.80 ± 0.15[e] (2.60)
Control	21.63 ±0.86 [a] (4.64)
CD (p=0.05)	**1.16**

*Mean of the three replications
Means having common letter are not significantly different
Figures in parentheses are square root transformed values
F- test at 5%level

Plate 1. Bacterial soft rot symptom on carrot sub sp *caratovora* (Pathogenicity test).

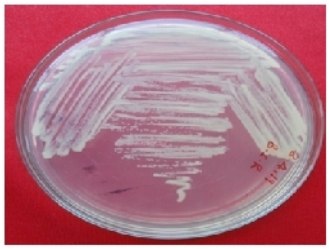


Plate 2. Pure culture of *P. caratovora.*

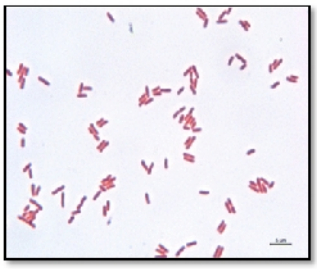


Plate 3. Gram sating reaction of *P. caratovora* sub sp *caratovora.*

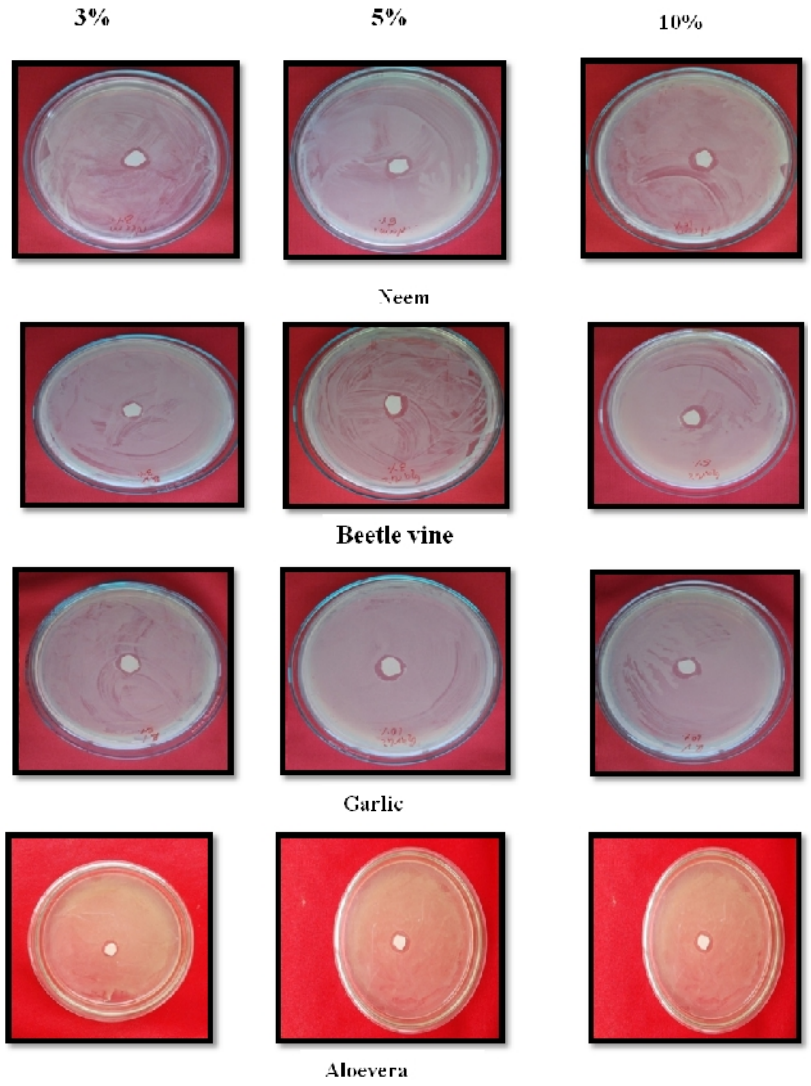


Plate 4. *In-vitro* evaluation of botanicals against bacterial soft rot Pathogen of carrot at different concentrations.

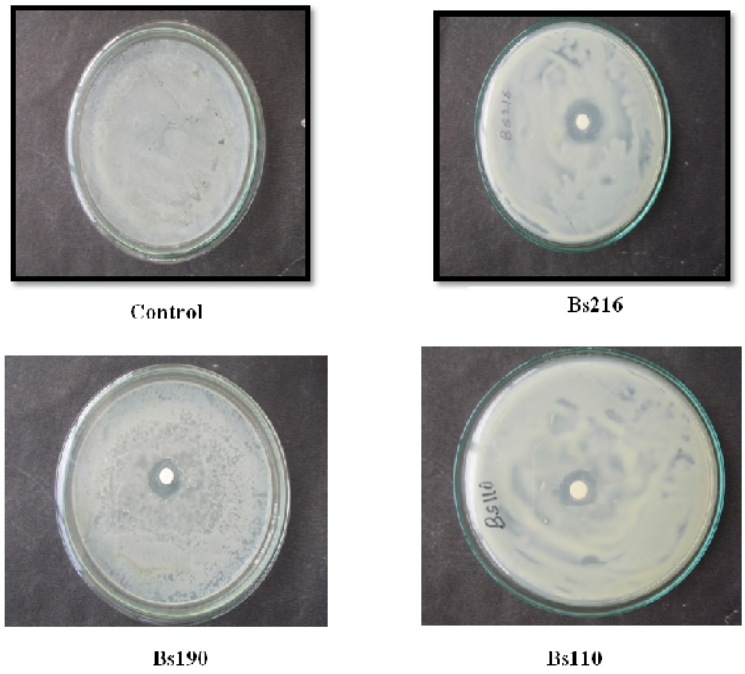
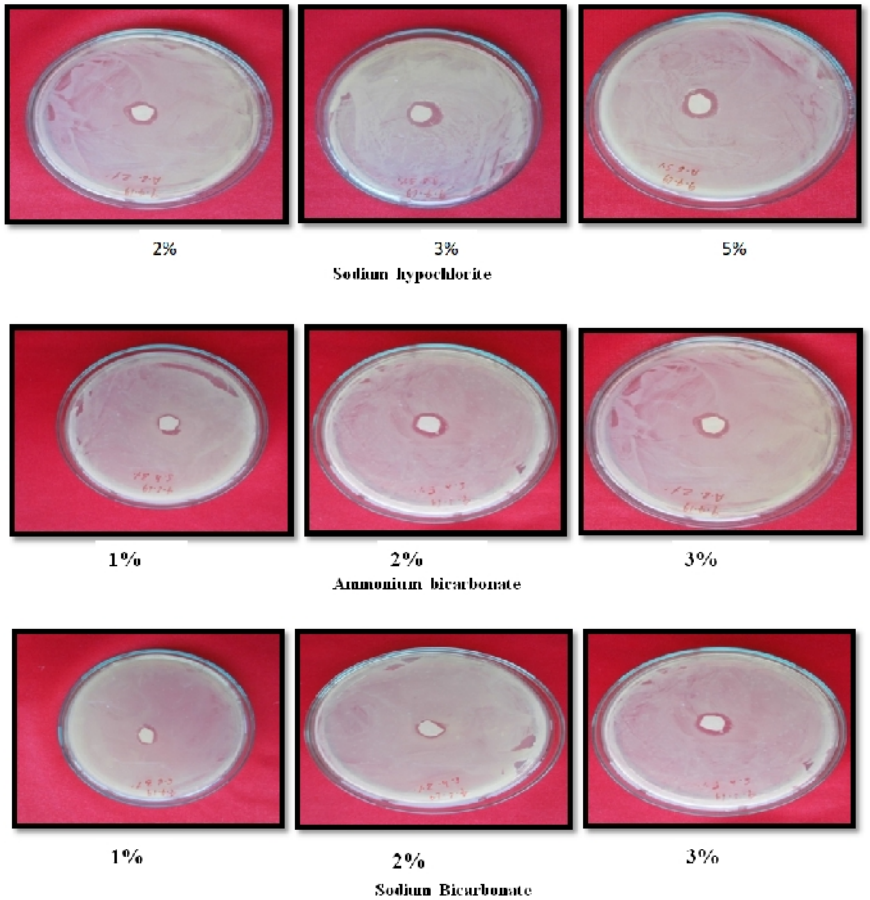


Plate 5. *In-vitro* evaluation of *Bacillus subtilis* against bacterial soft rot pathogen of carrot at different concentrations.



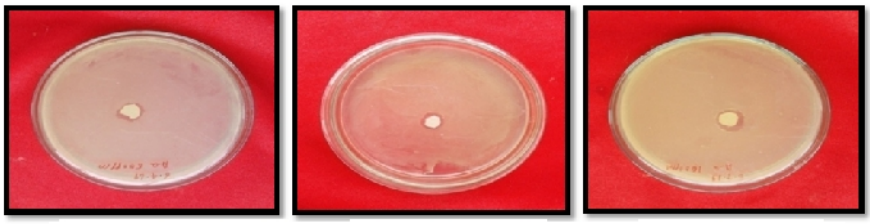


Plate 6. *In-vitro* evaluation of post harvest salts against bacterial soft rot pathogen of carrot at different concentrations.

Plate 7. Bacterial soft rot Evaluation of packaging materilas against pathogen.

CONCLUSION AND FUTURE SCOPE

From the present investigation it could be concluded that *P. caratovora* subsp. *caratovora* was the major pathogen associated with postharvest bacterial soft rot of carrot. It was found that *Bacillus subtilis*, Garlic, Sodium hypochlorite and Net bag at different concentration and combination could be the promising treatments against *P. caratovora* subsp. *caratovora* causing bacterial soft rot of carrot. Garlic, Sodium hypochlorite and net bag are good components in integrated postharvest disease management of carrot in Meghalaya. Further studies is needed to be done in *in vivo* conditions to see the results which found effective in *in vitro* conditions for adoption of best management practice for preventing the pre and post harvest loss of carrot in field and storage and to increase the income of farmers. This eco-friendly management practices can be widely adopted in larger area for the prevention of post-harvest losses in storage.

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