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Management of Postharvest Decay in Carrot (*Daucus carota* L. *var. sativus*) through Eco-friendly Approaches

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A B S T R A C T

Now a days, vegetables prices are hiked daily due to reduction in area of cultivation, poor storage and low distribution. Carrot (*D. carota* L. *var. sativus*) is one of the most important edible root vegetables around the world and cultivated in temperate zones. Currently, its production and distribution were retarded due to postharvest decay. This is caused by a ubiquitous soil borne bacteria *Erwinia carotovoroa* subsp. *carotovora* (*Ecc*). In this background this study was carried out for management of postharvest decay through eco-friendly approaches. Under this study, botanicals, plant volatiles, organic acids, organic salts and essential oils were used against *E. carotovora* subsp. *carotovora* under *in vitro*. Based on antibacterial efficacy, curative application was formulated against decay in carrot by dipping under laboratory conditions. Out of these all treatments, EC formulated consortia (cinnamon + lemongrass + thyme) oils at 0.1% given the better results against disease reduction (98.0%), weight loss (3.0%), and preserve the edible nature.

Introduction

Carrot (*Daucus carota* L. *var. sativus*) is one of the most important root vegetable crop in the world. Mostly, it was cultivated for their nutritional status of α and β carotene, vitamins (A, B6 and K), minerals (Ca and potassium) with edible fibre (Heinonen, 1990). Naturally, it was grown in the temperate regions with high humidity agro-ecosystems. Under global wide, about 60% of carrot production has shared by Asia alone. In India, carrot was cultivated around 108 thousand ha. with production of 1626 M. tonnes (NHB, 2019). Under Tamil Nadu, carrot cultivation has been done at 3000 ha. with production of 107.3 M. tonnes through productivity of 26.2 tonnes / ha. Normally, all root vegetables were shelf-life oriented and highly perishable nature due to occurrence of fungal and bacterial diseases were accompanied with atmospheric changes and poor storage conditions from field to postharvest. So, it causes severe product and economic loss from harvest to before consumption for farmers, sellers and consumers (Seljasen *et al*., 2013). Especially, during cusine preparations it gave annoying experience to the women in the kitchenary.

In major diseases of carrot *viz*., *Alternaria* blight, cavity spots and damping off (*P. violae*

and *P. sulcatum*), powdery mildew, white mold (*S. sclerotiorum*) bacterial blight (*X. campestris* pv. *carotae*) and soft rots or postharvest decay (*E. carotovora* subsp. *carotovora* and *E. chrysanthemi*) causing severe yield loss 50-100% from field to storage under favourable conditions (Bhat *et al*., 2010). Among them, soft rot causing *E. carotovora* subsp. *carotovora* (*Ecc*) as a ubiquitous seed and soilborne nature, rod shaped, gram negative bacterium and causing complete loss due to their easily survival nature, rapid colonization, prevalence and regeneration of bio-competence ability (Czajkowski *et al*., 2011). Mostly it survived under moist soil conditions >50% and temperature around 27-30˚C with drizzling rainy conditions on temperate regions. This bacterium as a polyphagous nature on wide host specificity in the major food crops. Under pathogenic nature, it was synthesized the pectinolytic and cellulolytic enzymes for rapid infection and to a produce typical watery lesions and rots (Clark *et al*., 1998). Spreading of this disease through wounds and infected seed inoculum during harvest to storage conditions due to improper handling of transit accompanied with imbalance of atmospheric conditions (Barkai-Golan and Phillips, 1991). Using fungicides and chemicals towards against soft rots were provided the on- time relief and makes a slow toxigenic for who intakes their diet regularly (Utama *et al*., 2010).

Using the safest alternative strategies *viz*., botanicals [*Ecc* P-138 / jute and cheerota] – (Rahman *et al*., 2012), [*P. carotovora* subsp. *carotovora* / turmeric 30%] – (Adamu *et al*., 2017); plant volatile compounds such as acetaldehyde, benzaldehyde, cinnamaldehyde, benzyl alcohol, have also been found to have antifungal activity against the fruit and vegetable pathogens *viz*., *A. alternata*, *P. digitatum, R. stolonifer, Colletotrichum musae and Erwinia carotovora* during *in vitro* trials (Almenar *et al*., 2008; Abd Alla *et al*., 2008). Using organic acids such as acetic acid, boric acid and calcium hypochlorite (bleaching powder) were completely retarded the growth and infection of soft rot pathogen *P. carotovoroa* subsp. *carotovora* (P 138) under storage conditions (Rahman *et al*., 2017). The organic salts like aluminium chloride, sodium thiosulfate, sodium benzoate were inhibited the growth *Pectobacterium* sp. in poato at modified atmospheric storage conditions (Betchem *et al*., 2019). An oil of spearmint (*Mentha piperita* L.) at 0.5% controlled the disease severity (>33%) of soft rot in cabbage *cv*. Natsume when during spraying (Guerra *et al*., 2014).

Keep these views, the work was carried out for management of postharvest decaying pathogen in carrot through eco-friendly approaches *viz*., botanicals, plant volatiles, organic acids, organic salts and essential oils under controlled atmospheric conditions.

Materials and Methods

Selection of strain

The virulent strain was selected on the basis of pathogenic nature and it was isolated from infected portion of carrot surface sterilized with 0.1% sodium hypochlorite solution (for 30 seconds) and washed with sterile distilled water, then ground the samples within 0.85% saline solution using sterile mortar and pestle under aseptic conditions. The bacterial suspension was left undisturbed for few minutes and it was vortexed at high speed for 60seconds for uniform homogenization. Aseptically, the bacterial suspension was tenfold diluted in sterile saline solution (0.85%, w/v). A loop ful of diluted suspension was streaked to the plates containing YEPA (yeast extract peptone agar) medium and incubated at 28˚C for 24 hours (Rahman *et al*., 2017).

Antibacterial activity of eco-based properties against to *Ecc*

Botanicals

Preparation of plant extracts

A total of nine plants *viz*., white mustard (*Sinapis alba*), myrobalan (*Terminalia chebula*), Malabar tamarind (*Garcinia gummugutta*), giant milk weed (*Calotropis gigantean*), adathoda (*Adathoda vesical*), ashwagantha *(Withania somnifera*), Indian coleus (*Coleus forskohlii*), clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) were tested against with *E. carotovora* subsp. *carotovora*. Dried leaves were collected and it was grinded with distilled water 1:1 (w/v) by using sterilized pestle and mortar. Then filtered through double layered muslin cloth at least two times. The filtered extract was kept in conical flasks and closed with aluminium foil and preserved at 4°C for further uses.

In vitro assay

A fresh sterilized YPDA medium was amended with plant extracts at (1:10) ratio and it was poured in the Petri dishes and wait few minutes for the solidification. After, a testing virulent strain of *Ecc* was streaked on the Petri dishes and kept under 28°C for 48 hours. An observation was recorded on the basis for the presence of growth nature by positive (+) (present) or negative (-) (absent) in Petri dishes (Rahman *et al*., 2012).

Plant volatiles

Three different synthetic plant volatile compounds such as benzaldehyde, benzyl alcohol and thymol were used as different concentrations *viz*., 10, 15 and 20%. The sterilized volatile compounds were amended with volume of (10 /15 / 20 ml per 100 ml YPDA medium). These volatiles inoculated medium was poured in the Petri dishes and allowed for solidification at few minutes. After a virulent strain of *Ecc* was streaked on the Petri dishes and kept under 28°C for 48 hours. A growth observation was recorded on the basis of positive (present^{+}) or negative (absent^{-}) (Huang *et al*., 2011).

Organic acids

Five different organic acids *viz*., boric acid, lactic acid, acetic acid, propionic acid and citric acid were used in the three different concentration *viz*., 0.5, 0.75 and 1.0%. Before adding the organic acids, the concentrations were prepared and adding in sterilized YPDA (Yeast Peptone Dextrose Agar) medium and it was poured in sterilized Petri plates for allowing solidification for few minutes. After the 48 hrs old *Ecc* strain was taken and make a gentle streak and control was maintained without any organic acids. Inoculated plates were kept under 28°C for 48 hours. A growth observation was recorded on the basis of positive (present^{+}) or negative (absent^{-}) (Rahman *et al*., 2017).

Organic salts

The nine different organic salts such as, EDTA, copper sulphate, sodium molybdate, sodium bicarbonate, sodium carbonate, sodium acetate, potassium chloride, calcium chloride and ammonium acetate were used as different concentration *viz*., 0.5, 1.0, 2.0 and 4.0%. The salts were prepared as stock in 100 ml (w / v) and prepared stock was taken as required volume (v /v) was added in the sterilized tryptic soy agar (TSA) medium. After the organic salts amended medium 15 ml / each was poured in the Petri dishes. A pure culture of *Ecc* has streaked on the plates containing medium and control was kept without salts. These plates were kept under 28°C for 48 hours. The growth observation

was recorded by the descriptions given by Yaganza *et al*. (2014).

Essential oils

The eight different aromatic-medicinal oils *viz*., Lemongrass (*Cymbopogon citratus*), Cinnamon (*Cinnamomum zeylanicum*), Thyme (*Thymus vulgaris*), Wintergreen (*Gaultheria procumbens*), Citronella (*Pelargonium citrosum*) Citriodora (*Backhousia citriodora*), Eucalyptus (*Eucalyptus camaldulensis*) and Clove (*Syzgium aromaticum*) were used as four different concentrations like, 0.1, 0.5, 1.0 and 2.0%. Testing oils were dispersed in distilled water to obtain required concentrations (0.1, 0.5, 1.0 and 2.0%) (v/v) mixed with Tween 20 (1:1) and well agitated. Each oil was amended with sterilized YPDA medium separately and poured in Petri dishes and allowed for solidification. A well grown 48 hrs old *Ecc* strain was streaked on the Petri dishes containing medium and kept in 28°C for 48 hours. Control plate was kept without oil. An observation was recorded on the basis of culture growth (Guerra *et al*., 2014).

Curative efficacy of eco-based consortia against to postharvest decay in carrot

Based on the results of the *in vitro* test, botanical (Cinnamon bark extract – 10%), plant volatiles (Benzaldehyde and Thymol – 10%), organic acid (Acetic acid – 0.5%), organic salt (Calcium chloride 2.0% and EC formulated essential oils like, lemongrass, cinnamon and thyme were used as 0.1% concentrations. A well prepared eco-based consortia (EC formulated solution) was used and the freshly harvested carrots (200g) were dipped in 15 minutes and kept in sterilized plastic trays under 28°C for two weeks (Controlled atmosphere) and maintained with completely randomized design. Healthy control was maintained without treatments. The following treatment details were given in the table 1.

Observations

The treated carrots decay incidence and per cent of weight loss (%) were recorded from 14 days after application from the formula described by Abd-El-Khair and Karima (2007) and Utama *et al*. (2014).

Per cent of decay (%) =

$$\frac{\text{No. of infected Carrots}}{\text{Total No. of Carrots}} \times 100$$

Weight loss (%) =

$$\frac{\text{Initial Weight} - \text{After treated Weight}}{\text{Initial Wight}} \times 100$$

After application of eco-based consortia, the treated carrots were observed from first day to 10 days on the phenomic and sensory nature via., appearance, colour, flavour, texture taste and overall edible acceptance were checked on the basis of scale (9 to 1 – Highly acceptable to Not acceptable) (Hajhamed *et al*., 2007).

Results and Discussion

Antibacterial activity of eco-based properties against to *Ecc*

The antibacterial activity of eco-based properties *viz*., botanicals, plant volatiles, organic acids, organic salts and essential oils were checked against to *Ecc* under *in vitro* conditions.

Botanicals

Totally nine botanicals were for antibacterial efficacy against *Ecc* under *in vitro* among them, cinnamon (*Cinnamomum zeylanicum*)

was completely inhibited the growth of *E. carotovora* subsp. *carotovora* (Table 1; Fig 1). The results revealed that cinnamon had antibacterial compounds of (E) – cinnamaldehyde and proanthocyanidins are against to food borne and multi-drug resistant pathogenic bacteria *viz*., *L. monocytogenes, S. aureus, E. coli, S. anatum* (Liang *et al*., 2019), *B. cereus*, and *P. aeruginosa* under laboratory conditions (Bin Chan *et al*., 2007; El Atki *et al*., 2020).

Plant volatiles

In three plant volatile compounds, benzaldehyde and thymol were significantly inhibited the bacterial growth under least conc. of 10% (Table 1; Fig 2). These results revealed that benzaldehyde completely inhibited the sporogenesis of *B. cinerea* and *M. fructicola* at 25 and 125 µl / L. Under mycelial inhibition, three volatile compounds *viz*., benzaldehyde, methyl salicylate and ethyl benzoate were inhibited the mycelial growth at conc. of 370 µl / L (Wilson and Wisniewski, 1989). Normally benzaldehyde used as protectant for peaches from *Rhizopus* rot under storage godowns (Utama *et al*., 2002).

Organic acids and salts

Out of five organic acids and nine salts, acetic acid, citric acid at 0.5% and copper sulphate and calcium chloride at 2.0% were completely inhibited the growth of *E. carotovora* subsp. *carotovora* (Table 2; Fig 2). The results revealed with chemicals like, acetic acid, boric acid and calcium hypochlorite were inhibited the potato soft rot pathogen *P. carotovora* subsp. *carotovora* at 0.05% conc. under *in vitro* (Rahman *et al*., 2017). Under laboratory conditions, low molecular concentration of salts *viz*., aluminium salts, sodium benzoate, sodium metabisulfite, potassium sorbate and trisodium phosphate were completely inhibited the growth of *F. sambucinum* causing dry rot in potato (Mecteau *et al*., 2002).

Essential oils

Out of these eight oils, lemongrass oil, cinnamon oil and thyme oil were most effectively inhibited the growth of *E. carotovora* subsp. *carotovora* at minimal concentration of 0.1% (Table 3; Fig 3). These results confirmed with Jeong *et al*. (2009) reported that lemongrass oil, inhibited the growth of *P. carotovora* subsp. *carotovora* at 0.5% concentration. Clove oil gives a better growth inhibition against *X. campestris pv. vesicatoria* causing bacterial spot in tomato (Lucas *et al*., 2012).

Curative efficacy of eco-based consortia against to postharvest decay of carrot

During the bioassay, all the eco-based consortia were significantly reduced the incidence of decay in carrot contributed in weight loss also. Among them, treatment like (T2) carrots were dipped in EC formulated three oils (Cinnamon oil + Lemongrass oil + Thyme oil) based consortia at 0.1% was recorded as very least per cent (2.0%) of decay and weight loss (3.0%) with maximum per cent reduction at 98.0% compared than others (Table 4; Table 5; Fig 4). It also recorded better improvements in the preservation and edible qualities *viz*., appearance, colour, flavour, texture, taste with overall acceptability. These results are revealed that Utama *et al*. (2014) reported that oil-water emulsion of sesame (0.5%) and lemongrass oil (0.5%) was reduced the fruit rot and weight loss in tomato under room conditions. This failure of decay incidence due to the presence of antimicrobial compounds like citral and geraniol. Both are inhibited the mycelial growth and sporogenesis of *A. flavus* and *A. fumigatus* on tomato (Karkala and Genjalawa, 2009).

Table.1 Antibacterial activity of different botanicals and plant volatiles against with *E. carotovora* subsp. *carotovora*. under *in vitro*

S. No.	Name of the botanicals	Antibacterial activity (growth)	Name of the Plant Volatiles	Concentrations & Antibacterial activity (growth)		
				10%	15%	20%
1.	*Sinapis alba* (White mustard)	+	Benzaldehyde	-	-	-
2.	*Terminalia chebula* (Myrobalan)	+	Benzyl alcohol	+	+	+
3.	*Garcinia gummugutta* (Malabar tamarind)	+	Thymol	-	-	-
4.	*Calotropis gigantean* (Giant milk weed)	+	Control	+	+	+
5.	*Adathoda vesical* (Adathoda)	+				
6.	*Withania somnifera* (Ashwagantha)	+				
7.	*Coleus forskohlii* (Indian Coleus)	+				
8.	*Syzygium aromaticum* (Clove)	+				
9.	*Cinnamomum zeylanicum* (Cinnamon)	-				
10.	Control	+				

(+: Growth / -: No growth)

Table.2 Antibacterial activity of different organic acids and organic salts against with *E. carotovora* subsp. *carotovora* under *in vitro*

S. No.	Organic acids	Concentrations (%)			Organic salts	Concentrations (%)			
		0.5	0.75	1.0		0.5	1.0	2.0	4.0
1.	Boric acid	+	+	+	EDTA	+	+	+	+
2.	Lactic acid	+	+	+	Copper sulphate	-	-	-	-
3.	Acetic acid	-	-	-	Sodium molybdate	+	+	+	+
4.	Propionic acid	+	+	+	Sodium bicarbonate	+	+	+	+
5.	Citric acid	-	-	-	Sodium carbonate	+	+	+	+
6.	Control	+	+	+	Sodium acetate	+	+	+	+
7.					Potassium chloride	+	+	+	+
8.					Calcium chloride	+	+	-	-
9.					Ammonium acetate	+	+	+	+
10					Control	+	+	+	+

(+: Growth / -: No growth)

Table.3 Antibacterial activity of different essential oils against with *E. carotovora* subsp. *carotovora* under *in vitro*

S. No.	Name of the essential oils used	Concentrations (%) & bacterial growth (+ / -)			
		0.1	0.5	1.0	2.0
1.	Lemongrass oil	-	-	-	-
2.	Cinnamon oil	-	-	-	-
3.	Thyme oil	-	-	-	-
4.	Wintergreen oil	+	+	+	+
5.	Citronella oil	+	+	+	+
6.	Citriodora oil	+	+	+	+
7.	Eucalyptus oil	+	+	+	+
8.	Clove oil	+	+	+	+
8.	Control	+	+	+	+

(+: Growth / -: No growth).

Table.4 Management of postharvest decay in carrot by eco-based consortia under *in vitro*

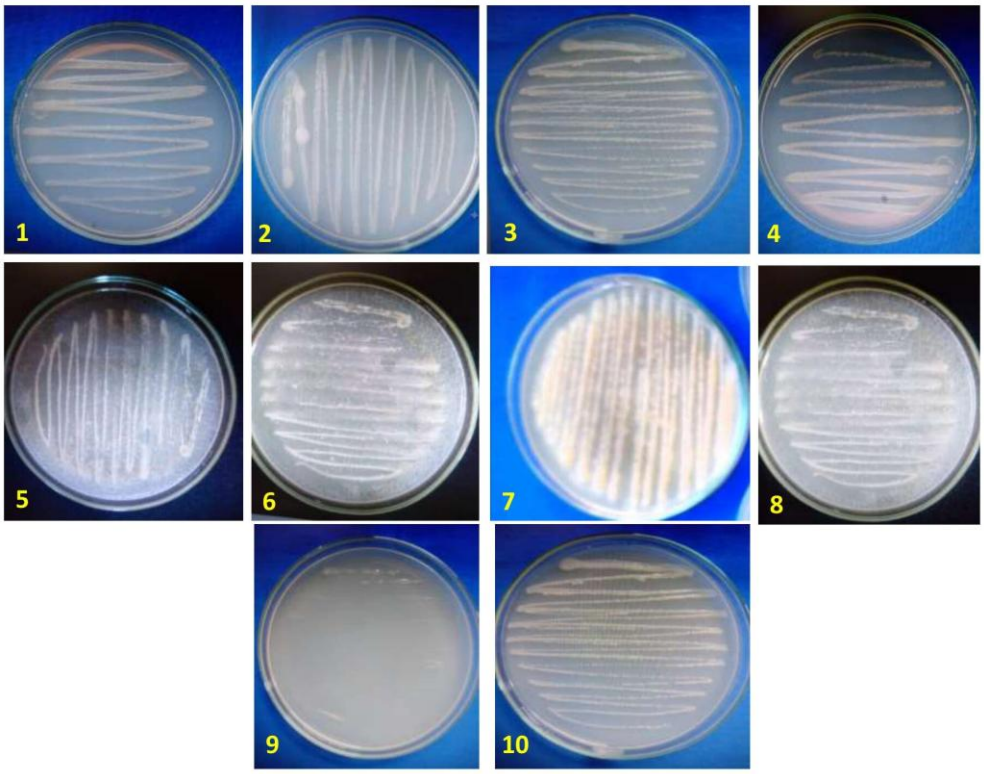
T. No.	Treatment details	Decay (%)	Per cent decay over control	Weight loss (%)
T1.	Dipping freshly harvested carrot with boiled bark extract of Cinnamon (10%)	9.0 [b]	91.0	11.0
T2.	Dipping freshly harvested carrot with EC formulated essential oils (Cinnamon oil, Lemon grass oil and Thyme oil) @ 0.1% conc.	2.0 [a]	98.0	3.0
T3.	Dipping freshly harvested with carrot organic Acetic acid (0.5%)	34.0 [c]	66.0	23.0
T4.	Dipping freshly harvested carrot with organic salts calcium chloride (2.0%)	49.5 [e]	50.5	50.0
T5.	Fumigated with Benzaldehyde (10%) and stored the freshly harvested carrot	54.5 [f]	45.5	58.5
T6.	Fumigated with Thymol (10%) and stored the freshly harvested carrot	59.5 [g]	40.5	61.0
T7.	Healthy Control	38.0 [d]	62.0	43.0
T8.	Inoculated control	100.0 [h]	-	69.0
S Ed.		6.01		
CD (P<0.05)		1.94		

Values are mean of three replications.
Means followed by a common letter are not significantly at 5 % level by DMRT.

Table.5 Phenomic and organo-sensory tests for best eco-based consortia treated carrot against postharvest decay

Organo-sensory charcteristics	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
Appearance **9 8 7 6 5 4 3 2 1** ---------- **Highly acceptable Not acceptable**	9	9	9	9	9	9	9	8	8	8
Colour **9 8 7 6 5 4 3 2 1** ---------- **Highly acceptable Not acceptable**	9	9	9	9	9	9	9	9	8	8
Flavour **9 8 7 6 5 4 3 2 1** ---------- **Highly acceptable Not acceptable**	7	8	9	9	9	9	9	9	9	9
Texture **9 8 7 6 5 4 3 2 1** ---------- **Highly acceptable Not acceptable**	9	9	9	9	9	9	9	9	9	9
Taste **9 8 7 6 5 4 3 2 1** ---------- **Highly acceptable Not acceptable**	9	9	9	9	9	9	9	9	9	9
Overall acceptability **9 8 7 6 5 4 3 2 1** ---------- **Highly acceptable Not acceptable**	9	9	9	9	9	9	9	9	9	9

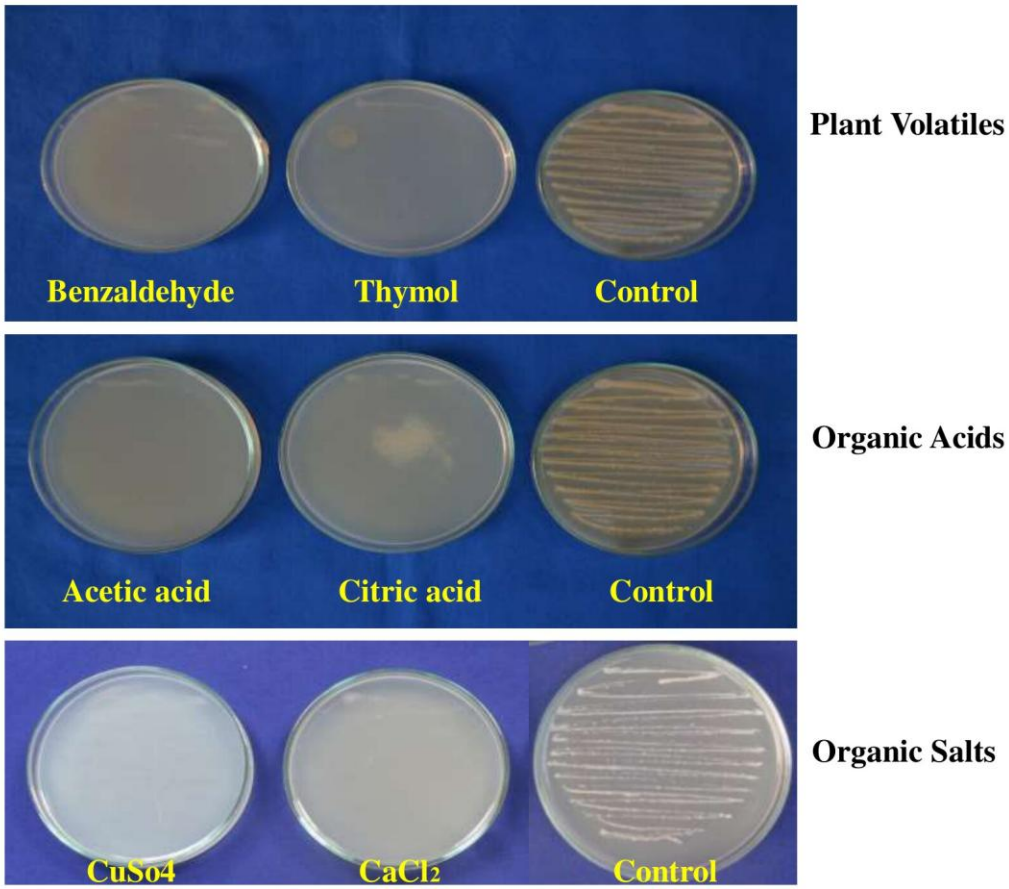
Figure.1 Antibacterial activity of different botanicals against with *E. carotovora* subsp. *carotovora* under *in vitro*



List of botanicals

1. White mustard
2. Myrobalan
3. Malabar tamarind
4. Giant milk weed
5. Adathoda
6. Ashwagantha
7. Indian Coleus
8. Clove
9. Cinnamon
10. Control

Figure.2 Antibacterial activity of different plant volatiles, organic acids and salts against with *E. carotovora* subsp. *carotovora* under *in vitro*

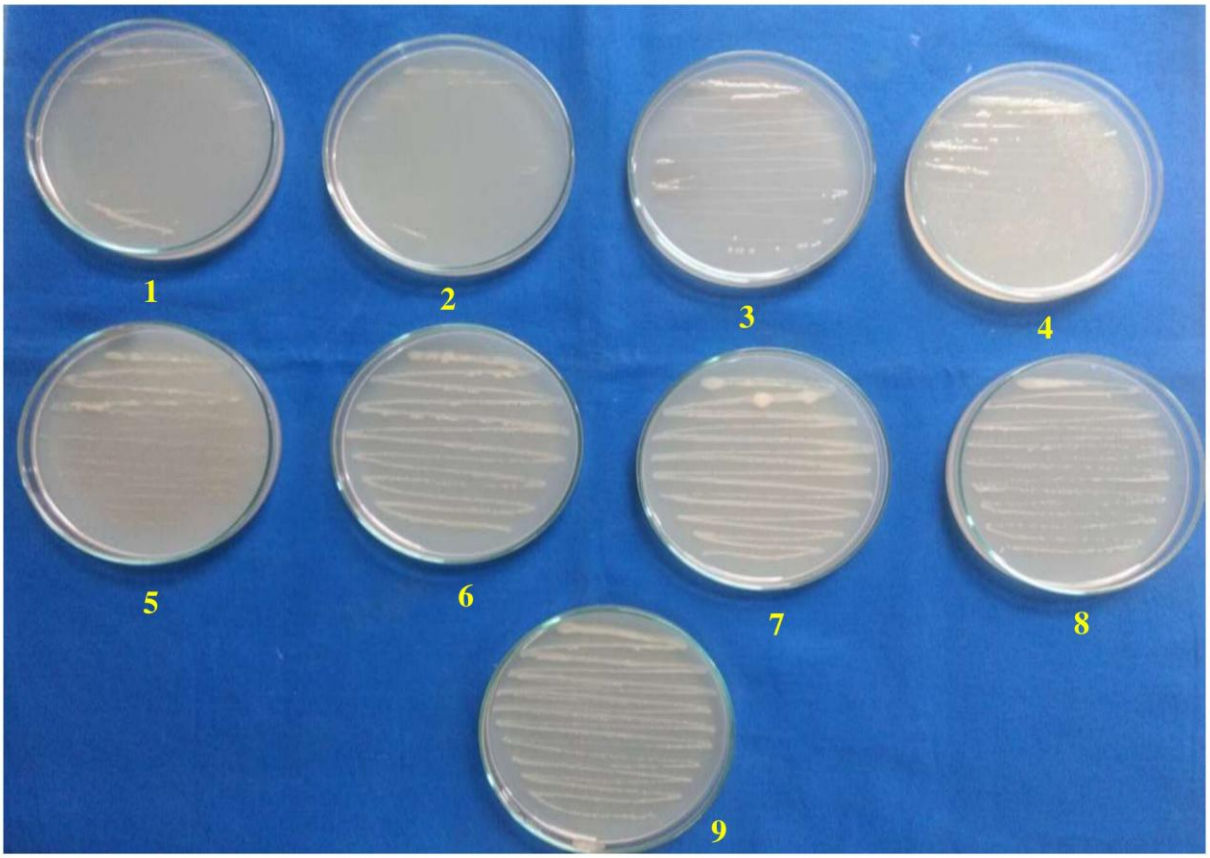


Plant volatiles @ 10% Conc.

Organic acids @ 0.5%

Organic salts @ 0.5% ($CuSo_4$) and 2.0% ($CaCl_2$)

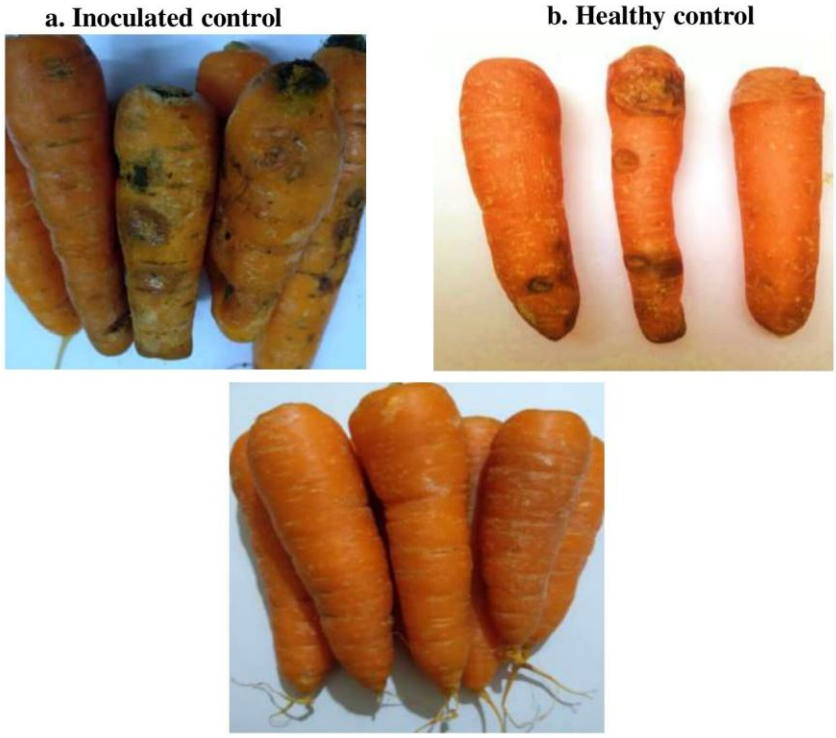
Figure.3 Antibacterial activity of different essential oils against with *E. carotovora* subsp. *carotovora* under *in vitro*



List of essential oils (0.1% conc.)

1. Lemongrass oil
2. Cinnamon oil
3. Thyme oil
4. Wintergreen oil
5. Citronella oil
6. Citriodora oil
7. Eucalyptus oil
8. Clove
9. Control

Figure.4 Management of postharvest decay in carrot through eco-friendly approaches under *in vitro*



During cinnamon oil with combined application at low concentration was given a better result against to postharvest pathogens and causing decay in fruits and vegetables with avoiding perishable nature due to single antimicrobial compound of trans-cinnamaldehyde (Unlu *et al*., 2010).

It is concluded that naturally, edible fruits and vegetables are discarded before on our eating plate due to highly influence of pathogens and causing diseases. It was occurred with association of agro-climatic factors from pre-harvest to consumption with improper harvesting, handling issues, poor storage. Carrot (*Daucus carota* L. *var. sativus*) is one of the most infected root vegetable and occurring by decay causing pathogens after harvest. About these results, the carrot was treated with EC formulation of cinnamon, lemongrass and thyme oil at 0.1% were preserved the vegetable nature and avoid the decay incidence and pathogen's invasion due to the formation of biofilm and exploration of antimicrobial compounds under room conditions.

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