CHEMICAL CONTROL OF SOFT ROT OF VEGETABLES CAUSED BY *ERWINIA CAROTOVORA* IN STORAGE

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ABSTRACT

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Antibacterial activity of five chemicals namely vinegar, copper compound (Cupravit), potassium chloride, calcium chloride and boric acid was evaluated *in vitro* against the soft rot causing bacterial pathogen, *Erwinia carotovora* subsp. *carotovora*. Among them vinegar, Cupravit and boric acid showed highly antibacterial activity. Complete inhibition (100%) of the growth of test pathogen was observed in vinegar at 1.0 and 2.0%, Cupravit at 0.05 and 0.1% and boric acid at 0.1% concentrations of the formulated product. These three chemicals were tested before storage to control soft rot of tomato, cucumber and okra. Each of the fresh vegetables were treated by dipping in 0.5% solution of vinegar, 0.1% solution of Cupravit and 0.1% solution of boric acid individually for 30 min and inoculated with the soft rot bacterial suspension by single needle puncture method. All the three vegetables under untreated control were completely damaged within 9 days of inoculation. Vinegar was found more effective in controlling soft rot of the three vegetables compared to Cupravit and boric acid. On 3rd day of treatment with chemicals soft rotted area ranged from 1.2 to 2.5%, while in control it was 3.0, 7.5 and 13.4% for tomato, cucumber and okra, respectively. On 6th day of treatment soft rotted area ranged from 3.33 to 7.66%, 3.5 to 7.5% and 6.0 to 11.6% in tomato, cucumber and okra, respectively. Soft rotted area under control was 33.33, 27.5 and 33.0% for tomato, cucumber and okra, respectively. On 9th day of treatment soft rotted area in tomato was 40.0, 71.66 and 80.0% with vinegar, Cupravit and boric acid, respectively. Soft rotted area in cucumber treated with vinegar, Cupravit and boric acid was 22.5, 76.0 and 80.0%, respectively. The soft rotted area of okra was 70.0, 90.0 and 98.0% with vinegar, Cupravit and boric acid, respectively. Among the three chemicals vinegar showed the best performance to control soft rot of tomato, cucumber and okra caused by *E. carotovora* subsp. *carotovora*.

Key words: Bacterial soft rot, *Erwinia carotovora*, vegetables, chemical control

INTRODUCTION

Generally, shelf-life of vegetables is maximum of 7-14 days at 5C depending on crop species and quality. The shelf-life is reduced due to endogenous biochemical and physiological changes in the produces as well as the attack of spoilage microorganisms (Garcia Gimeno and Zurera Cosano 1997, Heard 1999, 2002). Vegetables contains high quantity of water resulting in a high water activity (>99%). Tomato, cucumber and okra are common fruit vegetables in Bangladesh. They are grown in the country almost throughout the year. During plant growth, harvest, transportation, handling and post harvest processing, the produce may be physically damaged. Such damage may be important avenues for penetration of bacterial pathogens, which are the causes of post harvest bacterial soft rot of vegetables. The disease may cause 15-30% loss of harvested crops. Due to lack of proper management practices during harvesting, storage and marketing, the damage may be greatly extended. Wounded areas on plant tissues provide a suitable substrate for microbial growth providing sufficient nutrients availability (King *et al*. 1991, Zagory 1999).

Bacterial soft rot causing bacteria have high potentiality for spoilage of vegetables because they produce pectolytic enzymes, which have capacity to degrade pectin, a major constituent of the primary plant cell wall and middle lamella (Brummell 2006). Species of *Erwinia* belonging to the carotovora group are usually referred to as the soft rot bacteria (Lelliot and Dickey 1984). Many scientists recommended various chemicals to control the soft rot bacteria (Chen and Lin 2000, Khair 2004, Wright *et al.* 2005). Dipping potato tubers in acetyl-salicylic acid (ASA) increases resistance against *E. carotovora* subsp. *carotovora* (Bokshi *et al.* 2003). Salt treatments can inhibit plant pathogens or suppress toxin production by pathogens (McGuire and Kelman 1986, Olivier *et al.*1998). Saleh and Huang (1997) reported that benzoic acid and sodium benzoate at 1, 5 and 10 mM inhibited soft rot bacterial growth. Effectiveness of acetic acid, boric acid and bleaching powder against soft rot bacteria of onion *Burkholderia cepacia* was reported by Rahman *et al*. (2013). In view of the above research findings. the present investigation was carried out to find out effective chemicals to control bacterial soft rot disease of fruit vegetables in storage.

MATERIALS AND METHODS

In vitro evaluation of chemicals

A preliminary experiment was conducted to evaluate five chemicals namely vinegar, boric acid, calcium chloride, potassium chloride and Cupravit (a copper compound) for their bactericidal activity against a standard isolate P 138 of soft rot bacteria, *Erwinia carotovora* subsp. *carotovora* under *in vitro* conditions. Boric acid, calcium chloride, potassium chloride and Cupravit were tested at 0.02, 0.05 and 0.10% (w/v), and vinegar was tested at 0.5, 1.0 and 2.0% concentrations. A standard bacterial growth medium, yeast extract peptone dextrose agar (YPDA) was used as basic medium. After cooking, the YPDA medium was amended with each chemical at the selected concentrations and mixed thoroughly. The amended medium was autoclaved for 20 min at 121C under 1.1 kg/cm² pressure. The sterilized medium was poured into sterilized Petri dishes at 20 ml/plate, five plates were used for each treatment and allowed to solidify under sterile condition.

After solidification, the amended YPDA in Petri plates were spot inoculated with *E. carotovora* subsp. *carotovora* P 138 suspension (ca. 10^8 cfu/ml) and incubated at 30 C in an incubator. To prepare the inocula of *E. carotovora* subsp. *carotovora* P 138 causing soft rot in vegetables, bacterial cells were collected from 24 hr old fresh culture on YPDA and suspended in sterilized distilled water. Additional plates without any chemical were maintained, which served as control. Growth of the test bacterium was observed up to 14 days of inoculation and antibacterial activity of the chemical was determined.

Effect of the chemicals on bacterial colony growth was recorded in terms of percent inhibition of radial colony diameter based on colony growth on control plates. The percent inhibition was computed using a standard formula (Sundar *et al*. 1995) as shown below:

$$\%\text{inhibition} = \frac{X - Y}{X} \times 100$$

Where, X= Growth in control plate and Y= Growth in treated plate

Efficacy of the chemicals to control soft rot

Based on the results of the *in vitro* test, vinegar, Cupravit and boric acid were selected to evaluate their efficacy to control soft rot of tomato, cucumber and okra in storage. Each of the fresh vegetables were dipped in 0.5% suspension of vinegar, 0.1% suspension of Cupravit and 0.1% suspension of boric acid individually for 30 min prior to inoculation with soft rot bacterial (*E. carotovora* subsp. *carotovora*) suspension. After dipping in chemical suspensions, the vegetables were air dried at room temperature. Chemical treated vegetables were inoculated with the bacterial inocula by single needle puncture method. Fresh cultures of soft rot bacteria grown on YPDA at 28C for 24 hr were used as inocula. Inoculated vegetables were kept in plastic basket and stored at room temperature for 9 days. Untreated control was maintained for each vegetable, which was inoculated with the pathogen but not treated with any chemical. Incidence of soft rot was recorded on 3rd, 6th and 9th day of inoculation. The severity was measured in terms of percentage of surface area diseased (SAD).

RESULTS AND DISCUSSION

In vitro evaluation of chemicals

Among five chemicals tested *in-vitro*, calcium chloride and potassium chloride did not show any antibacterial activity against *E. carotovora* subsp. *Carotovora*. Out of three effective chemicals, vinegar was found most effective. Complete inhibition (100%) of colony growth was observed at two higher concentrations of vinegar (1.0 and 2.0%), while 90% growth inhibition was recorded at 0.5% concentration. Complete inhibition (100%) of colony growth was also found with two higher concentrations (0.05 and 0.1%) of Cupravit. At 0.02% of Cupravit, inhibition was 70%. In case of boric acid, complete inhibition (100%) of the bacterial colony was recorded only from the highest concentration of 0.1%. Growth inhibition with boric acid at 0.02 and 0.05% concentration was 50 and 80%, respectively (Table 1).

Table 1. Antibacterial activity of five chemicals against soft rot bacterium *E. carotovora* subsp. *carotovora* P 138 *in vitro*

Chemical	Concen-tration (%)	Inhibition (%)
Vinegar	0.50	90.0
	1.00	100.0
	2.00	100.0
Cupravit	0.02	70.0
	0.05	100.0
	0.10	100.0
Boric acid	0.02	50.0
	0.05	80.0
	0.10	100.0
Calcium Chloride	0.02	0.0
	0.05	0.0
	0.10	0.0
Potassium chloride	0.02	0.0
	0.05	0.0
	0.10	0.0
Control	0.00	0.0

Laboratory evaluation of these three chemicals has given encouraging results, indicating their potential for control of bacterial soft rot disease of vegetables. Based on the results of the *in vitro* test these three chemicals were selected to evaluate their effectiveness to control soft rot disease of vegetables in storage.

Efficacy of selected chemicals to control soft rot

Pre-storage treatment of tomato, cucumber and okra with the suspensions of vinegar, Cupravit and boric acid at different concentrations reduced the severity of soft rot disease in storage. Mean percentage of surface area diseased (SAD) of the three vegetables was the lowest in case of vinegar treatment, which was followed by Cupravit and boric acid. In tomato, on 3rd day of treatment surface diseased area ranged from 1.33 to 2.0%, while it was 3.0% under control. On 6th day of treatment, soft rot area increased to 33.33% under control, while it was 3.33, 7.66 and 6.66%, respectively in vinegar, Cupravit and boric acid treatment. On 9th day of treatment, tomato was destroyed completely under control, while SAD was 40.0, 71.66 and 73.0% under vinegar, Cupravit and boric acid, respectively (Fig. 1).

In cucumber, on 3rd day of treatment SAD in different treatments ranged from 1.5 to 2.5% but in control it was 7.5%. On 6th day, SAD ranged from 3.5 to 7.5%, while it was 27.5% under control. On 9th day SAD of cucumber increased drastically with 100% under control, while it was 22.5, 76.0 and 80.0% under vinegar, Cupravit and boric acid treatment, respectively (Fig. 2).

In okra, on 3rd day of treatment SAD ranged from 1.2 to 1.8% under different treatments but it was 13.4% under control. On 6th day of treatment SAD ranged from 6.0 to 11.6% under three treatments while it was 33.0% under control. On 9th day of treatment the SAD was 70.0, 90.0 and 98.0% under vinegar, Cupravit and boric acid, respectively while it was 100% under control (Fig. 3).

The results suggested that vinegar, Cupravit and boric acid can control soft rot disease of tomato, cucumber and okra to some extent in storage. Other researchers also reported similar results. Mills *et al*. (2006) tested several salt compounds as inhibitors of *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* causing bacterial soft rot of potato. *In-vitro* studies with sodium metabisulphate, propyl paraben, alum, potassium sorbate, calcium propionate and copper sulphate pentahydrate showed that these chemicals were completely inhibitory at lowest concentration (0.002M). Salts including calcium propionate and calcium chloride, potassium sulfate and ammonium phosphate reduce tissue maceration of potato tubers caused by *E. carotovora* (McGuire and Kelman 1986, Hajhamed *et al*. 2007). Farrar *et al*. (2009) reported antimicrobial agents such as peroxy-acetic acid and hydrogen peroxide**,** applied as a final rinse in the packing process, are effective in reducing the potato tuber surface populations of soft-rot organisms, resulting in less post-harvest loss due to *Erwinia* rot. Rahman *et al*. (2013) found bleaching powder, boric acid and acetic acid effective to control the soft rot disease of potato and onion in storage. So, the results of the present study partially agreed to the findings of Farrar *et al.* (2009) and Rahman *et al*. (2013).

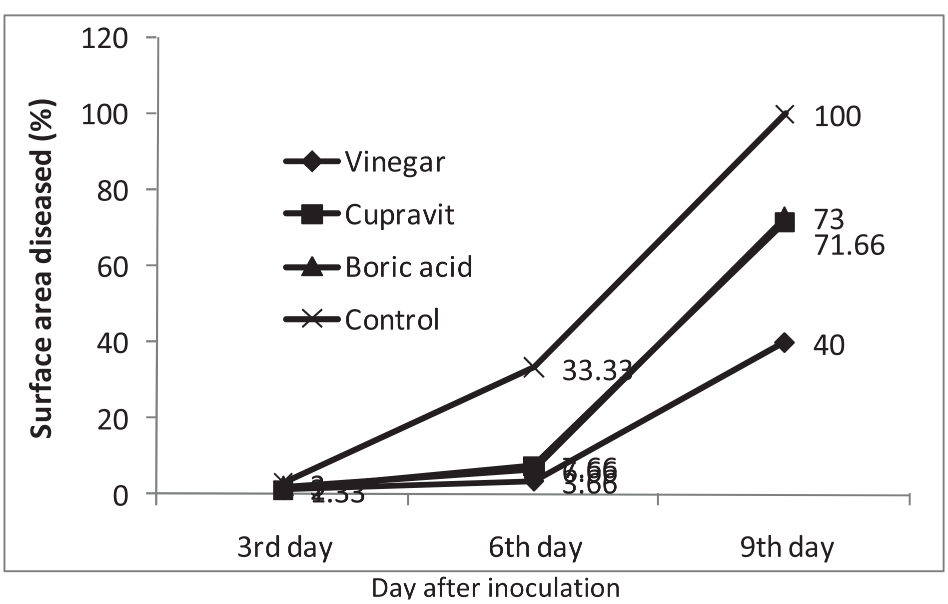

Fig.1. Effect of chemical treatment soft rot disease severity of tomato in storage

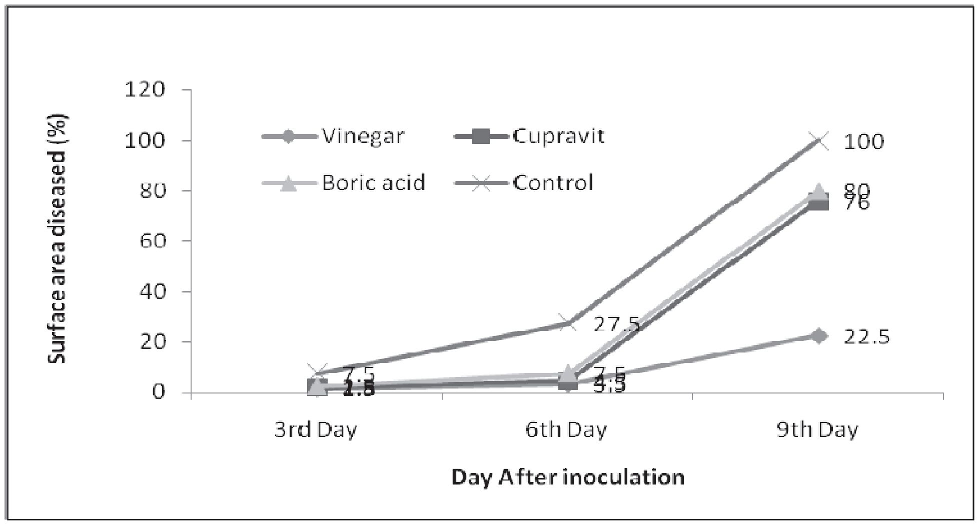

Fig. 2. Effect of vinegar, Cupravit and boric acid treatments on the soft rot disease severity of cucumber up to 9 days of storage

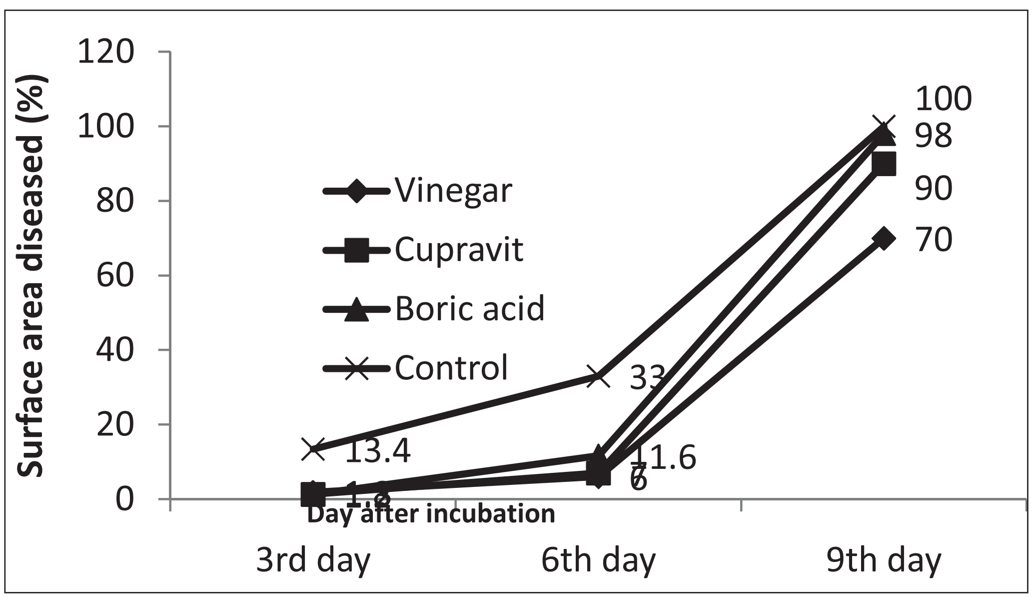

Fig. 3. Effect of vinegar, Cupravit and boric acid treatments on the soft rot disease severity of okra up to 9 days of storage

Among the three effective chemicals, vinegar is generally used as preservative for different types of jam, jelly and pickles. Use of vinegar as chemical treatment of vegetables may not create health hazards. Therefore, vinegar may be used to control soft rot disease of vegetables caused by *E. carotovora*. The residual effect of Cupravit and boric acid in different vegetables is unknown. Therefore, use of Cupravit and boric acid in treating vegetables needs further study to know the residual effect of these chemicals.

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