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Effect of chemicals against bacterial soft rot of fruits

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ABSTRACT

An experiment was conducted at microbiology laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University to control the bacterial soft rot of fruits with chemicals in vitro and in vivo condition in 2013. Soft rotted fruit samples of mango, apple, banana, papaya and pineapple were collected from different areas of Bangladesh based on characteristic soft rot symptom. After performing biochemical and physiological tests for characterization of bacterial isolates isolated from fruit samples, three bacteria species namely, Erwinia carotovora subsp. carotovora, Dickeya dadantii, Pseudomonas marginalis were identified. Antibacterial activity of vinegar, boric acid, calcium chloride and potassium sulfate were evaluated against soft rot bacteria. Among these chemicals, vinegar and boric acid showed antibacterial activity against soft rot bacteria in vitro. Based on the results of the in vitro experiment, vinegar and boric acid were used to control soft rot disease of papaya and apple in storage. Vinegar was found more effective than boric acid in controlling the soft rot disease of fruits in storage.

Key Words: *Soft rot, Fruit, Bacteria, Vinegar and Antibacterial activity*

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I. Introduction

Among the causes of post-harvest losses of fruits, bacterial soft rot is one of the major cause of post-harvest losses of fruits. Different pectolytic bacteria cause the soft rot of different fruits (Anon. 2013). The bacterial soft rot disease is commonly found in pineapple, banana, mango, grape, apple, jackfruit, avocado, papaya, citrus etc. In Bangladesh, fruit loss due to soft rot attack is approximately 25-50% (Miaruddin and Shahjahan, 2008). Bacterial soft rot is causing severe economic losses by reducing quantities of produces available for sale and by reducing the quality thus decreases the market value of the crops. Extent of losses varies widely from country to country and is greatly influenced by the climate as well as the conditions of growth and storage. In Bangladesh, annual loss of fruits based on harvest price is about Taka 1780.1 crore and annual loss based on retail price is about Taka 3037.53 crore (Hasan, 2012). As per Farrar *et al.* (2000) there are no effective chemical controls for any of the soft-rot Erwinias. However, many scientists tested various chemicals including bactericides and

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microbial pesticides to control the soft rot bacteria (Chen and Lin, 2000; Wright *et al.* 2005). Several salt compounds inhibited *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* causing bacterial soft rot of potatoes (Mills *et al.* 2006). They also reported that as preventive disease control measures, tubers treated only with alum, aluminum acetate, calcium propionate, sodium bicarbonate, sodium hypochlorite or copper sulfate pentahydrate resulted in less soft rot than untreated control, while as for curative disease control measures tubers treated with only alum, aluminum acetate, sodium hypochlorite or copper sulfate pentahydrate resulted in significantly less soft rot than untreated control. Salts including calcium propionate and calcium chloride reduce tissue maceration of different fruits and vegetables caused by *E. carotovora* (McGuire and Kelman, 1986). Saleh and Huang (1997) reported that benzoic acid and sodium benzoate inhibited soft rot bacterial growth. Considering the above fact, the present study was undertaken to find out the effective management practices of bacterial soft rot of fruits with some chemicals.

II. Materials and Methods

In vitro control of soft rot bacteria with chemicals

An *in vitro* test experiment was conducted to evaluate four chemicals for their antibacterial activity against soft rot bacteria *E. carotovora* subsp. *carotovora* and *P. marginalis*. Chemicals were vinegar, boric acid, calcium chloride and potassium sulfate. Boric acid, calcium chloride and potassium sulfate were used at three concentrations viz. 0.05%, 0.1% and 0.5%. Vinegar was used at three concentrations such as 0.5%, 1.0% and 2.0% (w/w) (Rahman, 2010; Akter, 2012). A standard bacterial growth medium Yeast Peptone Dextrose Agar (YPDA) was prepared. After cooking, the medium was amended with each chemical. Required amount of each chemical was added to YPDA and mixed thoroughly. The amended medium autoclaved for 20 min at 121° C under 1.1 kg/cm^2 pressures. The medium was poured in petridishes at 20 ml/plate and allowed to solidify. To prepare the inoculum of soft rot bacteria *E. carotovora* subsp. *carotovora* and *P. marginalis* were grown on YPDA at 28° C for 24 hr. Bacterial cells were collected from the culture and suspended in sterilized distilled water to a concentration of 10 cfu/ml. After solidification of YPDA with the chemicals, the plates were spot inoculated with bacterial inoculum and incubated at 30° C in an incubator. Plates were inoculated with three replications for each concentration of each chemical. Three additional plates without any chemical were maintained, which served as control. Growth of the test bacteria was observed up to 14 days of inoculation and antibacterial activity of the chemicals was determined. Effect of chemicals on bacterial growth was recorded in terms of inhibition of radial colony diameter. Results were expressed in 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:

$$\% \text{ Inhibition} = X - Y / X$$

Where,
X = Growth of control plate, Y= Growth of treated plate

Efficacy of chemicals to control soft rot disease of fruits in storage

Based on the results of the *in vitro* test, vinegar and boric acid was used at conc. of 2.0% and 0.5% to control soft rot disease of papaya and apple under inoculation condition. Apple and papaya were dipped in 2.0% and 0.5% solution of vinegar and boric acid individually for 30 min prior to inoculation with soft rot bacteria *E. carotovora* subsp. *carotovora*. After dipping, fruits were air dried at room temperature and punctured with sharp needle. Vinegar and boric acid treated fruits were inoculated by spraying of bacterial inocula. Bacterial inoculum was prepared from fresh cultures of soft rot bacteria grown on YPDA at 28^o C for 24 hr suspended in sterilized distilled water at 10^9 cfu. Inoculated fruits were stored at room temperature for 21 days. Untreated control was maintained for each fruits, which was inoculated with the bacterial inocula but not treated with vinegar and boric acid. Data were recorded at 7, 14 and 21 days of inoculation. The severity was measured in terms of percentage of surface disease area infected of the fruits by soft rot disease.

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III. Results and Discussion

Among the four chemicals viz. vinegar, boric acid, calcium chloride and potassium sulfate tested in the experiment, vinegar and boric acid showed antibacterial activity against soft rot bacteria *E. carotovora* subsp. *carotovora* and *P. marginalis*. Growth of these two test bacteria completely inhibited in case of vinegar and boric acid at their higher concentration 2.0% and 0.5%, respectively. The lower concentrations of vinegar (0.5%) and boric acid (0.05%) were failed to inhibit the growth of two test bacteria. While at moderate concentration of vinegar (1.0%) inhibits completely the growth of *E. carotovora* subsp. *carotovora* but failed to inhibit the growth of *P. marginalis.* On the other hand, boric acid at 0.1% concentration inhibits the growth of both the bacterial pathogens. Other two chemicals calcium chloride and potassium sulfate did not show any antibacterial activity against *E. carotovora* subsp. *carotovora* and *P. marginalis.* Laboratory evaluation of these two chemicals has given promising results, indicating their potential in the control of soft rot bacterial disease of fruits.

Table 01. Antibacterial activity of vinegar, boric acid, potassium sulfate,calcium chloride against *E. carotovora* subsp. *carotovora in vitro*

Chemicals	Concentrations	Growth of *E. carotovora* subsp. *carotovora* and *P. marginalis*
Vinegar	0.5%	+
	1.0%	+
	2.0%	-
Boric acid	0.05%	+
	0.1%	+
	0.5%	-
Potassium sulfate	0.05%	+
	0.1%	+
	0.5%	+
Calcium chloride	0.05%	+
	0.1%	+
	0.5%	+

(+) denotes positive growth and (-) denotes no growth of bacteria

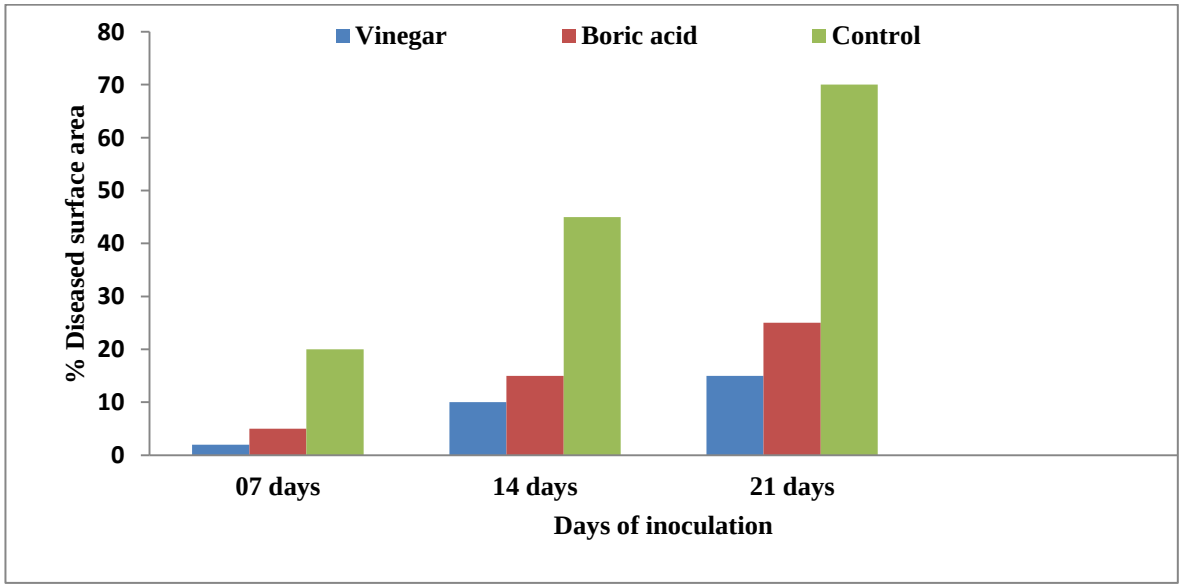

Figure 01. Effect of vinegar and boric acid treatment on the soft rot disease severity of papaya after 7, 14 and 21 days of inoculation.

Efficacy of chemicals to control soft rot disease of papaya and apple in storage

Based on the result of *in vitro* test, vinegar (2%) and boric acid (0.5%) was used to treat papaya and apple for controlling soft rot disease. Treatment of papaya and apple with vinegar and boric acid

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effectively decreased the percentage of infection. After seven days of treatment, in case of papaya, disease area was only 2% and 5% in case of application of vinegar and boric acid, while it was near about 20% under control. After 14 & 21 days treatment, disease area increased to 10%,15%, 45% and 15%, 25%,70% in case of vinegar, boric acid and control treatment respectively (Figure 01). Similarly in case of apple, after seven days of treatment disease area was 2% and 3% for application of vinegar and boric acid, where on the contrary it was near about 10% under control. After 14 & 21 days of treatment, disease area was increased to 5%, 10%, 30% and 10%, 18%, 55% in case of vinegar, boric acid and control treatment respectively (Figure 02).

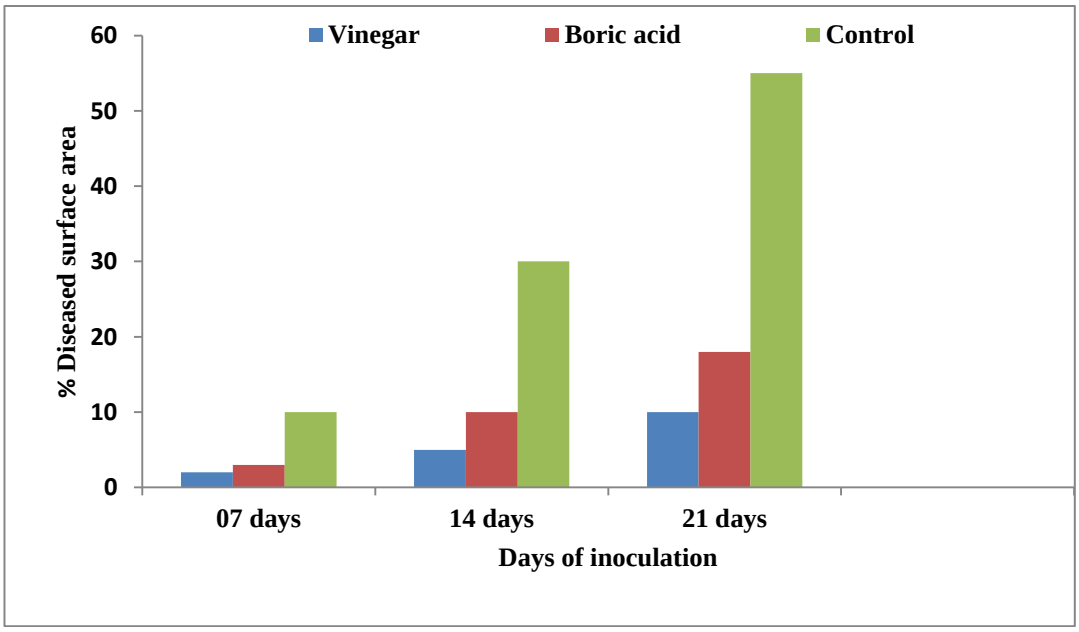

Figure 02. Effect of vinegar and boric acid treatment on the soft rot disease severity of apple after 7, 14 and 21 days of inoculation.

Results suggested that vinegar can control soft rot disease of apple and papaya to some extent at storage condition. Roberts and Dunegan (1932) first documented the use of vapour of acetic acid for controlling of peach rot. Sholberg *et al.* (2001) found large quantities of apple were effectively controlled by fumigation of acetic acid from post-harvest soft rot of apple. Farrar *et al.* (2000) reported peroxy–acetic acid and hydrogen peroxide are effective in reducing the potato tuber surface populations of soft rot organisms resulting less post-harvest loss. Thus results of this present study partially agreed with the findings of Sholberg *et al.* (2001) and Farrar *et al.* (2000). Though, vinegar is generally used as preservatives of different types of pickles, however, use of vinegar in treating fruits needs further study.

IV. Conclusion

Vinegar and boric acid showed antibacterial activity against soft rot bacteria *E. carotovora* subsp. *carotovora and P. marginalis in vitro.* Vinegar may be useful in controlling fruit soft rot disease in storage condition. Further study is necessary for confirmation and recommendation of the results.

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