



Full Length Article

The Efficacy of Nature-Friendly Chemicals Eugenol and Sodium Bicarbonate against Post-Harvest *Botrytis cinerea* in Two Pepper Cultivars

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Abstract

Grey mold, caused by *Botrytis cinerea*, is the most important pre-harvest and post-harvest disease of pepper. The disease leads to huge losses in quality and yield of pepper. Synthetic fungicides should not be used because of their harmful residues in the fruits postharvest. Hence, alternative chemicals have gained more importance for human health now-a-days. In this study, the postharvest effectiveness of eugenol and sodium bicarbonate (NaHCO₃) to *B. cinerea* was determined on two pepper varieties, 'Demre' and 'Charleston' obtained from pepper fields in Çanakkale. In essay the Eugenol was used at dosages of 0.75 and 1.5%, while sodium bicarbonate was used at dosages of 0.5 and 1.0%. Pepper varieties 'Demre' and 'Charleston' showed significantly different sensitivities against grey mold disease ($P < 0.01$), and this case reflected also to the efficacies of alternative chemicals. During eight days of storage, Charleston was more sensitive to *B. cinerea* than 'Demre' peppers. NaHCO₃ at high dosage (1.0%) completely inhibited the disease on 'Charleston' and 'Demre' peppers. The lower dose of NaHCO₃ (i.e., 0.5%) was also highly effective in 'Demre' (providing 95% reduction in the disease) but its efficacy was a little lower for 'Charleston' variety (84%). Efficacy (83.7%) of eugenol at dosage of 1.5% to *B. cinerea* on 'Demre' was found to be like NaHCO₃ at dosage 0.5% on 'Charleston' peppers. Alternative chemicals had no adverse effects on pepper varieties. It is concluded that both tested nature-friendly chemicals (Eugenol and sodium bicarbonate) could be used (postharvest) against to *B. cinerea* on peppers. © 2021 Friends Science Publishers

Keywords: Grey mold; Alternative control; Postharvest; Pepper; Chemicals

Introduction

Postharvest diseases are one of the most important problems of crops during storage period. If postharvest diseases are not controlled sufficiently, they may cause huge losses in the quality and yield of crops. The losses caused by postharvest diseases are estimated between 20–50% per year (Narayanasamy 2006). *B. cinerea*, causing pre- and postharvest losses, is probably one of the most common and widely distributed diseases of vegetables, fruits, and even field crops throughout the world (Elad 1994).

If grey mold disease is not prevented adequately, it may cause important losses in yield and quality (Coley-Smith 1980; Baraldi *et al.* 2002). In Turkey, grey mold and black rot (*Alternaria alternata*) are the most economically important postharvest diseases of pepper. Grey mold and other postharvest diseases may be controlled by classical fungicides; however, most of the traditional fungicides embody the risk of leaving remnants on products and have negative effects on humans, animals and environment

(Agrios 2005; Wightwick and Allinson 2007). Furthermore, *B. cinerea* may easily develop races with high resistance against many synthetic fungicides (Abou-Jawdah and Itani 1995; Archbold *et al.* 1997).

In this context, recent studies have focused on alternative chemicals, biological agents and plant extracts for controlling the pathogens (Skirdal and Eklund 1993; Farooq *et al.* 2011). Plant extracts, sodium and potassium salts and some antimicrobial food additives have gained importance in the control of pathogens. Nowadays, especially, salts, like sodium and potassium, and antimicrobial food additives have attracted attention in the control of pathogens. In the recent studies against postharvest diseases with alternative substances, sodium bicarbonate and eugenol are remarkable. In these studies, both alternative chemicals have been found effective against various phytopathogens (Yildirim and Yapici 2007; Wang *et al.* 2010; Romanazzi *et al.* 2012). The objective of the present work was to determine the effectiveness of sodium bicarbonate and eugenol against grey mold disease on peppers after harvest.

Materials and Methods

Plant material

Peppers (*Capsicum annuum* L.) cvs. Charleston F1 and Demre F1 were selected manually from commercial orchards in Çanakkale (Turkey) after harvest and used as plant material. The study was carried out between May–July 2014 in Çanakkale Onsekiz Mart University Agriculture Faculty Plant Protection Department Laboratory and Cold Storage Facilities.

Origin of *Botrytis cinerea* isolate and inoculum

B. cinerea isolate was obtained from diseased pepper plant in Çanakkale-Turkey. Isolate which was on Potato Dextrose Agar (PDA), were developed at 21°C in the incubator and to get the stock cultures, it was transferred to tubes which included PDA. Conidia were harvested two-weeks-old cultures that were growing on PDA at 21°C under 12 h light/dark periods. Conidia dislodged by rubbing the surface area with glass rod. Fungal suspension was filtered through two layers of fine sterile muslin to remove the mycelium and other fragments. Spore suspension was then counted with a haemocytometer and adjusted to 10^6 spores mL⁻¹. For inoculation, the pepper fruits were wounded in the stem and end region by means of a sterile pipette tip. They were inoculated with 60 µL spore suspension from both the wounded sides and incubated in dark storage at 20–22°C for 12 h.

Treatments and storage conditions

Eugenol and sodium bicarbonate (NaHCO₃) were used as nature-friendly alternative chemicals to the use of fungicides for controlling postharvest disease *B. cinerea* in peppers. Some properties of both chemicals are given in Table 1. After incubation, peppers were treated with different doses of eugenol and sodium bicarbonate. Eugenol was applied in warm water at $47 \pm 1^\circ\text{C}$. The treatment doses are given in Table 1. Besides, control fruits were only treated with warm water. Then all the fruits were kept on air dry at naturally room temperature for 1 hour. Peppers were stored at 9–10°C and 85–90% relative humidity conditions after inoculations for 8 days, and the daily improvement of grey mold was measured both sides of the peppers with digital calipers.

Experiment design and statistical analysis

The experiment was arranged in completely randomized design with three replications. Ten pepper fruits were assessed for each replication. Analysis of variance was conducted on the efficacy of the test chemicals by using 'Minitab 16 Software' and means were compared by using 'Tukey's method.

Results

According to results, lesion diameter showed an increase especially on untreated pepper fruits during the storage duration. Infected area developed significantly after five days after inoculation. Susceptibilities of artificially inoculated places (tip and middle) of fruits against *B. cinerea* were not different, but both pepper varieties showed different response against pathogen. 'Charleston' was more sensitive against fungus than 'Demre' ($P < 0.01$). Meantime, lesion development was significantly different according to varieties at 6th, 7th and 8th days ($P < 0.05$, Fig. 1).

According to last 3th days measurements after inoculation, the mean of lesion diameters on 'Charleston' was found bigger than on 'Demre' (Fig. 1). Besides significant difference between the mean diameters of lesions developed on 'Demre' was not observed, whereas the lesion development on 'Charleston' continued to increase until 8th day ($P = 0.05$).

Lesion diameter on untreated (control) and treated pepper fruits significantly showed diversity according to varieties ($P < 0.01$; Fig. 2). In Fig. 3 (a, b, c), the development of *B. cinerea* lesions in untreated (Control), sodium bicarbonate and eugenol treated pepper fruits (cv. 'Demre') were fixed.

The lesion diameters of control fruits were 43.2 mm on 'Charleston' and 26.8 mm on 'Demre' peppers, and these were significantly reduced on fruits treated with test chemicals with the increasing dose levels (Fig. 2). The highest lesion development was observed on pepper varieties treated with eugenol at 0.75% dosage, 23.7 mm (Charleston), 12.7 mm (Demre), and lesion diameter of eugenol (1.5%) treated fruits was not significantly different than that of sodium bicarbonate (0.5%) treated ones ($P < 0.01$). However, in fruits of both pepper varieties treated with 1.0% dosage of sodium bicarbonate, no lesion development was observed (Fig. 2).

Chemical applications at postharvest had high efficacy against *B. cinerea*, and effectiveness of chemical applications showed significant differences ($P < 0.01$) according to treatment dosages eight days after of inoculation (Fig. 4).

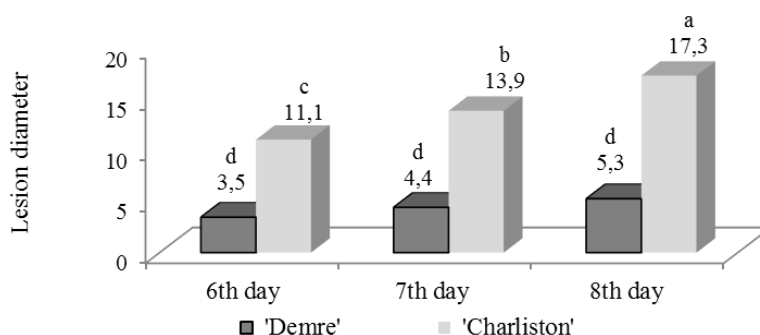
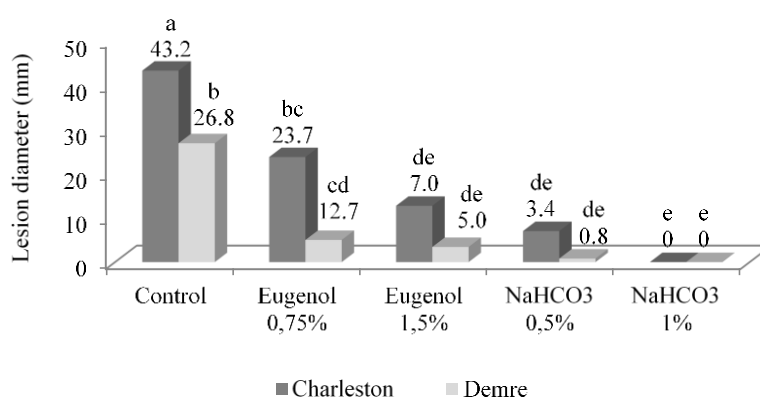
Sodium bicarbonate at 1.0% completely inhibited gray mold caused by *B. cinerea* (100%) in both pepper cultivars and at half dosage (0.5%) exhibited similar effect on 'Demre' (Fig. 3). Sodium bicarbonate at 0.5% on 'Charleston' and eugenol at 1.5% on 'Demre' showed effectiveness close to each other. At half dosage (0.75%), eugenol on 'Demre' showed similar effect at dosage of 1.5% on 'Charleston'; however, it had at least efficacy with 45.6% at dosage of 0.75% on 'Charleston' (Fig. 4).

Discussion

Nowadays, the alternative control of plant diseases has

Table 1: Some properties of the test chemicals

| Trade Names of Chemicals | Active ingredients | Formulation and rate of active ingredient (%) | Dosage (%) |
|--------------------------|-----------------------------------|---|------------|
| Bioxeda | Eugenol (4-allyl-2-methoxyphenol) | Liquid, 18 | 0.75; 1.5 |
| Baking powder | NaHCO ₃ | WP, 99 | 0.5; 1.0 |

**Fig. 1:** Development of disease on pepper varieties**Fig. 2:** Effect of chemicals on the size of lesion on pepper fruit caused by *Botrytis cinerea*

become more important. Especially, plant extracts, sodium and potassium salts have gained importance in the control of pathogens. In this study, the effectiveness of sodium bicarbonate and eugenol against *B. cinerea* (Grey mold disease) on two pepper varieties 'Demre' and 'Charleston' were studied during at storage conditions. 'Demre' and 'Charleston' showed different sensitivities against grey mold disease, and this case reflected also to the effectiveness of alternative chemicals. 'Charleston' was found more sensitive to *B. cinerea* during eight days of storage period than 'Demre' (Fig. 1 and Fig. 2). Difference of sensitivity of both pepper varieties to disease maybe related with ethylene production of plants in different concentration during ripening postharvest (Woltering and Doom 1988). Ethylene causes increase of the tissue sensitivity to diseases in plants, at the same time it stimulates the conidial germination and hyphal growth of *B. cinerea* (Elad 1988; Elad and Evensen 1995). In addition, pepper varieties as well as other plant varieties may have difference defense system against *B. cinerea*. Dix and Webster (1995) reported that some workers determined that the waxes extracts obtained from various plant leaves (broad

bean, sugar beet, red beet, birch, lettuce, tomato, black current and chrysanthemum) had inhibitory effect on spore germination of *B. cinerea*, and this was caused by the inhibitory substances in waxes (Blakeman and Sztejnberg 1973).

Both treatment doses (0.5 and 1%) of sodium bicarbonate showed strong inhibition on the lesion development of *B. cinerea* in both the pepper varieties. However, its effectiveness against grey mold disease at the dosage of 0.5% on 'Charleston' was a little lower than that of on 'Demre'. Disease severity on the pepper fruits especially on 'Charleston' treated with eugenol was higher than that of sodium bicarbonate. Thus, sodium bicarbonate was more successful for the control of *B. cinerea* according to eugenol. In contrast to eugenol, sodium bicarbonate at all doses had a higher effectiveness against grey mold on both the pepper varieties. It completely inhibited the disease at 1% dose and showed similar effect at 'Demre' at 0.5% treatment dose. However, this effect was observed only at high dose (1%) of the chemicals on 'Charleston'. That could be considered as a collective effect of the host resistance and effectiveness of the chemical. Sodium bicarbonate is one of



Fig. 3: In 'Demre' pepper variety, lesion development of *Botrytis cinerea* in untreated pepper fruits (a) and peppers treated with (b) sodium bicarbonate at 1% dosage and (c) eugenol 1.5% dosage

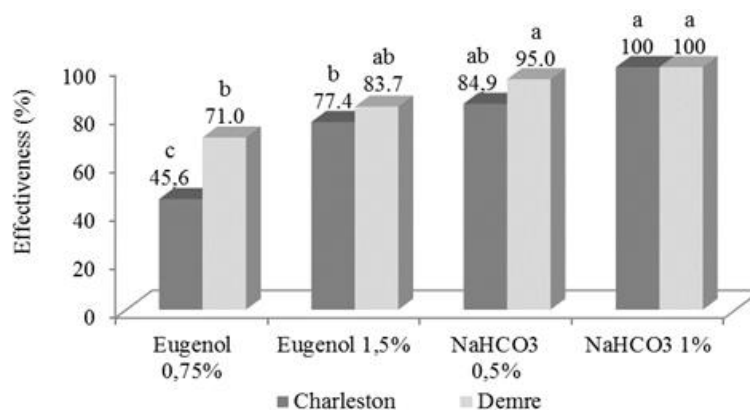


Fig. 4: Effectiveness of chemicals to grey mold caused by *Botrytis cinerea* on pepper fruit

the best-known alternative substances, and previously research works conducted with alternative chemicals had shown that sodium bicarbonate alone or in combination with other antifungal agents could be highly effective against numerous pre- and postharvest diseases (Palmer *et al.* 1997; Zaker 2014; Mlikota and Smilanick 2001).

Contrary to results of this study, it is reported that salts also including sodium bicarbonate applied to table grape after harvest did not show suppressive effect to *B. cinerea* and the preharvest-treatment on grape was more effective to mold than postharvest immersion (Youssefa and Roberto 2014). However, this study on pepper fruits artificially inoculated with *B. cinerea* after harvest demonstrated that sodium bicarbonate had strong inhibitory effect to grey mold at 0.5 and 1.0% doses on both pepper varieties 'Demre' and 'Charleston'. This difference between two studies may be due to the different sensitivities of the plants to the pathogen or due to the buffering response of *B. cinerea* to high pH caused by sodium bicarbonate.

Bicarbonate ion (HCO_3^-) having a buffering property in aqueous solution causes alkalization by elevating the pH of environment, and this results in inhibition of the mycelial growth and conidia germination of *B. cinerea* as well as the polygalacturonase activity (Palmer *et al.* 1997; Fagundes *et al.* 2013). However, high pH may not be sufficient to explain the mode of action of salts in all circumstances; thus, it is reported that high pH of salt solution was not enough for the inhibition of *B. cinerea* on grape berries (Nigro *et al.* 2006). Thus, the production of extracellular enzymes (polygalacturonases, pectin

methylesterases, proteases and laccases) playing important role on pathogenicity show diversity according to host and environmental factors. However, high pH may not be sufficient to explain the mode of action of salts in all circumstances (Wang *et al.* 2010; Shah *et al.* 2012; Fagundes *et al.* 2013). It is reported that the hydrogen ion concentration existing in environment controlled the ionization of salts and hence the availability of ions to the fungus, and the permeability of the plasmalemma (Dix and Webster 1995). Thus, ion uptake, enzyme activity and extracellular enzyme activity is influenced depending on pH values. Besides, one of the mode actions of salts is the reduction of the turgor pressures of fungal structures, and this results in the collapse and shrinkage of fungal hyphae as well (Fallik *et al.* 1997).

Effect of the sensitivity difference between pepper varieties was clearly observed in application of eugenol. At the low dosage (0.75%) of eugenol, the effectiveness against the disease was found more about at 50% ratio on Demre than that of on Charleston (Fig. 2). Eugenol at high dose (1.5%) was as effective as NaHCO_3 at low dose (0.5%). It showed high effectiveness against for eight days. It may be accepted to be successful for the control of gray mold on peppers.

Eugenol has antimicrobial activity against various fungal pathogens (Rhayour *et al.* 2003). In some studies, it has been observed that eugenol could inhibit hyphal growth of *B. cinerea* but had no biological activity on conidial germination. Eugenol triggers H_2O_2 production in the cytoplasm, leading to increasing free Ca^{2+} , that lead to

membrane binding and permeability changes and consequently destabilization and disruption of the plasma membrane (Wang *et al.* 2010).

As a result, it was determined that each chemical, eugenol and NaHCO₃ had strong postharvest effectiveness against *B. cinerea* on peppers. In addition, the effectiveness of applied alternative chemicals was variable depending on the pepper variety of dose of the chemicals. Eugenol applied at higher dosage to *B. cinerea* on ‘Demre’ was found as effective as that of NaHCO₃ applied at lower dosage to the pathogen on ‘Charleston’. These findings mean that the resistance of plants against pathogens is an important aspect regarding the effectiveness of chemicals.

Conclusion

The eugenol and sodium bicarbonate can be used against post-harvest diseases in organic and traditional agriculture. The results of Eugenol and sodium bicarbonate against lead mold disease show difference according to sensitivities of plant varieties to pathogen. In application doses, it is necessary to pay attention to the susceptibility of plant varieties. In general, sodium bicarbonate at 0.5% dose and eugenol 1.5% can be applied by spraying at the surface of the fruit or by dipping the fruits into water containing chemical.

Author Contributions

Yildirim made the inoculum, materialized the inoculation and found literatures, Sakaldas made the measurements and assessed the statistical datas and found the plant material.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Data Availability

The data will be made available on acceptable requests to the corresponding author.

Ethics Approval

Not applicable.

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