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POST-HARVEST CONTROL OF APPLE BLUE MOLD UNDER COLD STORAGE CONDITIONS

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ABSTRACT

To control blue mold of apples caused by *Penicillium expansum* after harvest and during commercial cold storage, microwave exposure was investigated as a physical measure and compared with the use of calcium chloride and the systemic fungicide, carbendazime. Microwave exposure of Golden Delicious and Royal Gala apples at 2450 Mhz for 10, 30 and 45s was effective as 4% $CaCl_2$ and carbendazime on significantly reducing the disease incidence %. Calcium chloride was more effective at 8% than 4% or at 1% and was as effective as carbendazime in reducing the mold severity and incidence. Microwave exposure for 10, 30 and 45s was as effective as 8% $CaCl_2$ and carbendazime on controlling blue mold caused by *P. expansum* stored under the conditions of commercial cold storage. Different apple cultivars have different degrees of susceptibility to the blue mold. Granny Smith and Red Delicious were relatively more tolerant than Golden Delicious, Starking or Royal Gala. However, sight variations were found in their responses to different treatments. Therefore, 10-45s microwave exposure of apple fruits before cold storage alone or in a combination with other methods can be an environmental safe physical alternative to chemical fungicides for controlling *P. expansum* infections during cold storage.

Keywords: Calcium Chloride, Control, *Penicillium Expansum*, Microwave

1. INTRODUCTION

The domesticated apple (Malus×domestica Borkh.) is a main and common fruit crop in the world (Velasco *et al*., 2010). Consumers are increasingly demanding fruit of consistent high quality (Zeebroeck *et al*., 2007). Long-term cold storage of apples without significant loss of quality is greatly important (Deng *et al*., 2013). Post-harvest losses of fruits and vegetables are high, ranging from 10 and 40% depending on the species and technologies used in the packinghouses (Arras and Arru, 1999; Wilson and Wisniewski, 1994). Higher risk of bacterial and fungal contamination could lower the shelf-life of apple fruits during transportation and storage. Some qualitative and quantitative fruit losses are mainly due to pathogenic fungi which usually infect the host through wounds made during harvest, handling and processing (Wilson and Wisniewski, 1994). In apples, post-harvest losses are mainly due to blue mold caused by *Penicillium expansum* Link and grey mold caused by *Botrytis cinerea* Pers.: Fr. *Penicillium expansum* is a highly destructive pathogen that causes production losses during handling of the fruit (Sanzani *et al*., 2010) in form of post-harvest rots of pome fruits (Rosenberger *et al*., 1991). This pathogen is also a major producer of patulin, a mycotoxin which can reach high concentrations in infected apples and pears (Battilani *et al*., 2008) and has cytotoxic, genotoxic and immunosuppressive activities (Wouters and Speijers, 1996).

Despite the wide-spread use of modern storage facilities and techniques, synthetic chemical fungicides such as carbendazime, benomyl, iprodione, pyrimethanil

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and fludioxonil are frequently used immediately before or after harvest to control post-harvest molds of fruit (Eckert and Ogawa, 1988; Li and Xiao, 2008; Xiao *et al*., 2011). However, chemical control is increasingly limited because of environmental and toxicological risks as well as the onset of fungicide-resistant strains of fungal pathogens (Spotts and Cervantes, 1986; Delp, 1988; Holmes and Eckert, 1999). Moreover, the legal limits of chemical pesticide residues in imported fruit are much narrow in some countries, thus discouraging the use of pesticides. In the absence of safely effective post-harvest fungicides, alternative or integrative measures are becoming increasingly important in controlling post-harvest diseases. To solve the problem of fungicide resistance and to fight against certain physiological disorders of apples and pears linked to calcium deficiency such as bitter pit, calcium chloride was used (Mason and Welsh, 1970; Perring, 1986; Raese and Stahly, 1988). Post-harvest calcium treatments can aid in helping the fruit to become more resistant to decay and were effectively used against blue mold of apples (Moline and Locke, 1993; Holmes and Eckert, 1999; Maouni *et al*., 2007). Biological control by antagonistic microorganisms is a promising tool for preventing post-harvest fungal rots and minimizing the use of fungicides (Janisiewicz and Korsten, 2002; Ippolito *et al*., 2004). However, biocontrol agents are sometimes not sufficient to control fungal infections when applied alone under practical conditions and their use should be integrated with other methods (Janisiewicz *et al*., 2003).

High frequency microwaves, when hit objects, can create heat (Wang and Tang, 2001). Microwave oven is widely used in food industry (Ikediala *et al*., 1999). Microwave exposure can directly affect living organisms due to its thermal effect on living tissue (Ondracek *et al*., 1976). Microwave treatments are widely used for drying, disinfesting and pasteurizing agricultural products while maintaining product quality and as a physical measure previously used effectively to control pests (Fanslow *et al*., 1975; Cunningham, 1980; Ikediala *et al*., 1999; Bol'shakov *et al*., 2001; Ernieenor and Ho, 2010). There are few reports (Karabulut and Baykal, 2002; Zhang *et al*., 2006) on the effect of microwaves as a post-harvest treatment for controlling blue mold of apples or even other diseases of post-harvest fruits. Therefore, this study aimed at investigating the utility of microwaves as a post-harvest treatment on blue mold of apples and comparing its effects with calcium chloride and carbendazime on different apple cultivars under cold storage conditions.

2. MATERIALS AND METHODS

2.1. Pathogen Culture and Fruit Inoculation

Penicillium expansum were isolated from decayed apples showing typical symptoms of blue mold. Spores of *P. expansum* were harvested with 10 mL^{-1} of sterile distilled water containing a drop of Tween 20. Spore counts were determined with a hemacytometer and adjusted with sterile distilled water to a 36000 spores/mL concentration.

2.2. First Experiment

During the 2011 and 2012 growing seasons, apples (about 7 cm-in-diameter) of two cultivars; Golden delicious was harvested at commercial maturity stage from an orchard conducted by the traditional crop management and kept at a refrigerator prior to use. Prior to treatment, apples were rinsed in tap water for 4 min. Three fruits were placed in a clear and ventilated storage plastic container (20×30 cm^2). Five containers were set for each treatment thus each treatment was replicated five times. The following treatments were achieved; microwave exposure through placing one container containing three *P. expansum*-inoculated apple fruits in a 2450 Mhz microwave oven (R-480J, SHARP® Sensor, Sharp corporation, Thailand) providing 1200 watt microwave power with a rotating platform (to insure uniform microwave exposure) for 10 and 30s or dipping the fruits of each container into a solution of calcium chloride at 10 g L^{-1} (1%) or 40 g L^{-1} (4%), carbendazime 50% WP at 0.6 Ml/L, or tap water (as untreated control). Blue mold disease incidence and severity % were assessed after cold storage for 4 weeks. Disease incidence was assessed as the number of decay spots of blue mold per apple fruit. Disease severity was measured as the proportion of decay lesion in the fruit and the following 0-5 scale was used; 0: no lesions, 1: 1-10, 2: 11-25, 3: 26-50, 4: 51-75, 5: 76-100%, entire fruit decayed) and calculated as the sum of all numerical ratings per a treatment multiplied with 100% and divided on the multiplication of their total number with the maximum disease category (5).

2.3. Second Experiment

Three fruits of each apple cultivars; Golden Delicious, Red Delicious, Starking, Granny Smith and Royal Gala were placed in a clear and ventilated storage plastic container (20×30 cm^2). Five containers were set for each treatment thus each treatment was replicated five times. The following treatments were achieved; microwave exposure through placing one

container containing three *P. expansum*-inoculated apple fruits in a 2450 Mhz microwave oven for 10, 30 and 45s or dipping the fruits of each container into a solution of calcium chloride at 40g L^{-1} (4%) and 80 g L^{-1} (8%), carbendazime 50% WP at 0.6 mL L^{-1}, or tap water (as untreated control). Blue mold disease incidence and severity % were assessed after cold storage for 4 weeks. Disease severity and incidence were assessed as previously mentioned.

2.4. Statistical Analysis

Data were analyzed statistically using General Linear Model (GLM) procedure (SPSS software version 11.5; SPSS Inc., Chicago, USA). Least Significance Difference (LSD) test was used for mean separation at the 0.05 probability level. Significance of main factors and interactions was tested at the 0.05 probability level.

3. RESULTS

Results of the first experiment revealed a significant reduction of blue mold incidence on Golden Delicious fruit after 30s microwave exposure and carbendazime treatments followed by 10s microwave exposure and 4% calcium chloride treatments used when compared with the untreated check while 1% calcium chloride treatment did obviously reduced the disease incidence (**Fig. 1A**). On other hand, all treatments except % calcium chloride were able to reduce the disease severity significantly (**Fig. 1B**).

Results of the second experiment revealed that the 10s exposure to microwave significantly gave the lowest reduction in blue mold disease severity and incidence in a similar level to the fungicide, Carbendazime. The other exposure times were also effective in reducing the disease amount (**Table 1**). Calcium chloride at 8% was obviously more effective than at 4% in suppressing the disease severity but with similar incidence on the different apple cultivars (**Table 1 and 2**). Among the apple cultivars, Granny Smith and Red Delicious had a significantly the lowest number of disease spots (**Table 1**) and the lowest disease severity % (**Table 2**).

Both treatment and cultivar as main factors had significantly (Probability values ≤0.050) affected the disease incidence and severity (**Table 3**). The treatment X cultivar interaction of them was significantly affected the severity of the blue mold (**Table 3**).

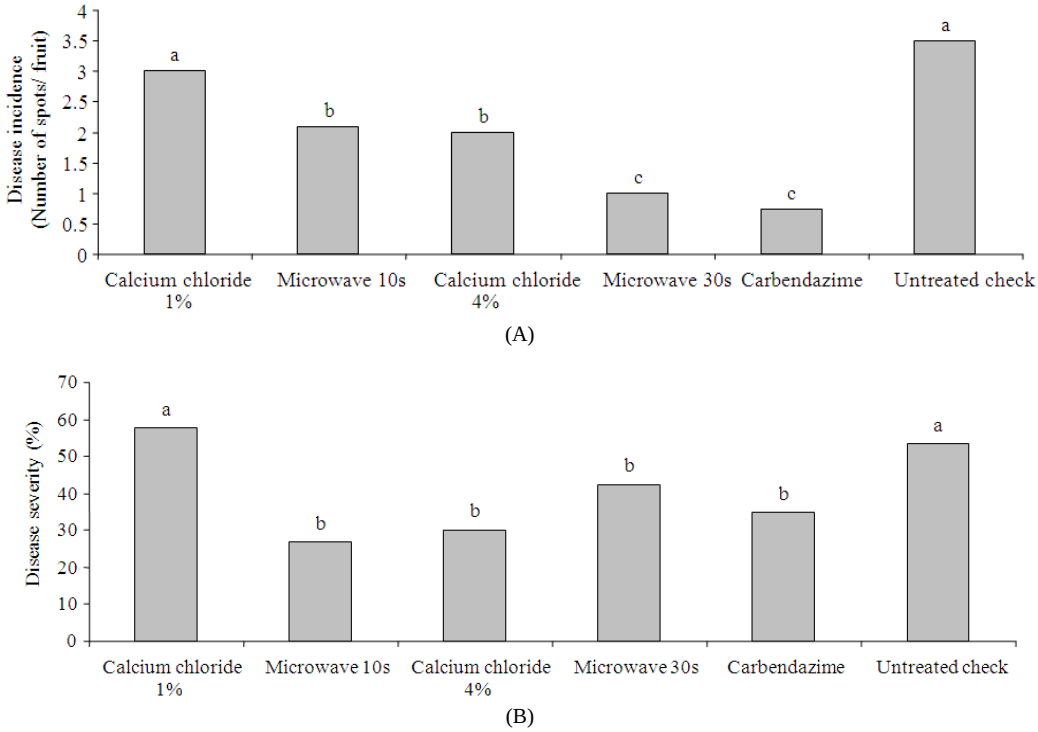

Fig. 1. Effects of different treatments used in the second experiment on blue mold disease incidence (A) and severity (B) on apple cv. Golden Delicious under cold storage conditions

Table 1. Effects of different treatments on disease incidence% of blue mold on different apple cultivars

	Cultivar					
Treatment	Golden delicious	Red delicious	Starking	Granny smith	Royal gala	Means
10s microwave	0.3[1]	1.0	0.0	0.3	1.0	0.5 b[2]
30s microwave	3.30	0.3	3.0	1.3	0.7	1.7 a
45s microwave	1.70	0.3	0.5	0.3	1.0	0.8 b
4% $CaCl_2$	1.60	1.0	2.3	1.0	0.0	1.2 ab
8% $CaCl_2$	1.70	1.0	1.3	0.3	0.7	1.0 ab
Carbendazeme	1.00	0.3	0.9	1.0	1.0	0.8 b
Control	2.80	1.0	1.0	0.3	2.0	1.4 ab
Means	1.8 a[2]	0.7 b	1.3 ab	0.6 b	0.9 ab	

[1]Means of five replicates (2 fruits/container)
[2]Means within columns or rows followed by the same letter are significantly different at 0.05 probability level

Table 2. Effects of different treatments on disease severity % of blue mold on different apple cultivars

	Cultivar					
Treatment	Golden delicious	Red delicious	Starking	Granny smith	Royal gala	Means
10s microwave	1.71	10.0	0.0	3.3	16.7	6.3 cd[2]
30s microwave	33.30	20.0	18.3	3.3	48.3	24.6 b
45s microwave	23.30	3.3	50.0	16.7	7.8	20.2 b
4% $CaCl_2$	45.30	32.1	53.3	13.3	25.3	33.9 a
8% $CaCl_2$	5.00	13.3	10.0	16.7	24.2	13.8 c
Carbendazeme	3.30	5.0	0.0	10.0	13.3	6.3 d
Control	50.00	20.0	66.7	23.3	38.3	39.7 a
Means	23.1 a[2]	14.8 b	28.3 a	12.4 b	24.8 a	

[1]Means of five replicates (2 fruits/container)
[2]Means within columns or rows followed by the same letter are significantly different at 0.05 probability level

Table 3. Main and interaction effects of treatments and cultivar type on blue mold of apple under cold storage conditions

Source	Disease incidence	Disease severity
Treatment	0.003*	0.008
Cultivar	0.000	0.002
Treatment X cultivar	0.156	0.028

*Probability values ≤0.050 are significant

4. DISCUSSION

Fungal spores and latent infections of *P. expansum* are found either on the surface or in the first few cell layers under the peel of the fruit (Lurie, 1998). Post-harvest treatment is very important since it can eliminate blue mold infections through removing spores from the fruit surface and acting directly on their viability and/or induce fruit defense mechanisms in the outer layers of the epicarp which reduce pathogen growth and development (Schirra *et al*., 2000).

Calcium chloride was more effective at 8 than 4 or at 1% and was as effective as carbendazime in reducing the mold severity and incidence. *In vitro*, $CaCl_2$ had significantly little efficacy against *P. expansum*, even at 6% while in vivo and at low temperature, it was effective at 4% (Maouni *et al*., 2007). Compared to benomyl, 2% $CaCl_2$ was not effective in controlling *P. expansum* (Moline and Locke, 1993). As a mode of action, calcium ions might bind with intercellular pectic constituents of fruit tissue; which becomes resistant to the fungal pectolytic enzyme, polygalactouronase (Bateman, 1964; Conway *et al*., 1999). As a core ion of plant cell wall, calcium might increase the firmness of the treated fruit which may a benefit for permitting an extension of the fruit shelf life (Margosan *et al*., 1997; Sams *et al*., 1993).

In the present study, 2450 Mhz microwave exposure for 10, 30 and 45s was as effective as 8% $CaCl_2$ and carbendazime on controlling blue mold caused by *P. expansum* in apples stored under the conditions of commercial cold storage. The mode of action of microwave on the blue mold may be due its direct effect on the conidia of *P. expansum* and indirectly by heating the surface of treated apple fruit to a temperature detrimental for *P. expansum* growth and development. A 2450 MHz and 450 watt microwave exposure for 2 or 3 min of *P. expansum*-inoculated pear fruits was able

effectively to reduce blue mold of pear without affecting fruit quality (Zhang *et al*., 2006).

The effectiveness of microwaves is exposure-time dependent. About 60% of the larvae of blow fly species, Chrysomya megacephala lived after 7 sec of microwave exposure, most larvae died by 15 sec and all larvae were dead at 30 and 60 sec (Sripakdee *et al*., 2005). Fanslow *et al*. (1975) demonstrated that microwaves at 2450 MHz destroy the eggs of the Southern corn rootworm, Diabrotica undecimpunctata. Microwaves at 460 MHz can delay larval development in some insects, such as Drosophila melanogaster (Bol'shakov *et al*., 2001). Therefore, microwave exposure for certain duration time e.g., 10-45 sec may sufficiently be used as a post-harvest physical method of treating apple fruits of some tolerant cultivars e.g., Golden Delicious against the blue mold which is the most important post-harvest disease of apples.

Different apple cultivars have different degrees of susceptibility to the blue mold. Granny Smith and Red Delicious were relatively more tolerant than Golden Delicious, Starking or Royal Gala. However, sight variations were found in their responses to different treatments. Therefore, the use of microwave has feasible applicability for controlling apple blue mold.

Practically, as an instrument in commercial apple packing and cold storage houses, a 2450 Mhz microwave oven can be mechanically set over a driving belt that usually used for grading apple fruits before packaging to provide at least 10s microwave exposure of apple fruits against post-harvest diseases e.g., blue mold and and can be thermostatically controlled using computer-based program (Mota *et al*., 2011; Srinivasan and Krishnan, 2012). Interestingly, the use of microwave is expected to be environmentally sound, durable, non-residual, easy to apply, cost and time effective non-chemical and non-biological method when compared with the use of chemical fungicides, bioagents and mineral salts.

A combination of post-harvest methods could be more effective and consistent than the use of one method alone (Janisiewicz *et al*., 2003; Spadaro *et al*., 2004). Various alternative methods to fungicide application developed during the past decades were not commercially implemented or were implemented on a small scale because of insufficient mold control (Conway *et al*., 1999; Leverentz *et al*., 2000; Janisiewicz *et al*., 2003; Mclaughlin *et al*., 1990; Wisniewski *et al*., 1995; El-Ghaouth *et al*., 1994; Smilanick *et al*., 1999; Davila-Avina *et al*., 2011). Therefore, the use of microwaves could be combined with the application of other method e.g., $CaCl_2$ to provide sufficient control of apple blue mold.

5. CONCLUSION

In a conclusion, 10-45s microwave exposure of apple fruits before cold storage alone or in a combination with other methods can be an environmental safe physical alternative to chemical fungicides for controlling blue mold of apple caused by *P. expansum*.

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