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Effectiveness of pH and Butylated Hydroxyanisole (BHA) on Fungicidal Activity of Certain Fungicides for Controlling Green and Blue Mold Diseases on Orange Fruits

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ABSTRACT

Green and blue molds caused by *Penicillium digitatum* and *P. italicum*, respectively, are the major postharvest cause of orange fruit decay, resulting in economic losses in the citrus industry. Four fungicides (i.e. boscalid, kresoxim-methyl, boscalid+kresoxim-methyl and cyprodinil+ fludioxonil) and the antioxidant butylated hydroxyanisole (BHA) were evaluated separately and in mixtures against *P. digitatum* and *P. italicum* causing green and blue mold diseases of navel orange fruits, respectively. The *in vitro* studies showed that higher (8-11) and lower (3-5) pH values reduced the growth of *P. digitatum* and *P. italicum*. Also, the results showed that the premixed fungicide formulations were more efficient in inhibiting the mycelial growth of fungi than individual forms. Both fungi tolerated high concentrations of BHA. The potency of fungicides and BHA against fungi growth was interestingly increased at pH 3 and 9. Also, the fungitoxic activities of fungicides were increased by adding BHA to fungicide-amended medium. The *in vivo* studies illustrated that all the separated fungicide treatments, particularly the mixtures of them, effectively controlled the diseases, while the separate BHA treatments slightly reduced the incidence of diseases. Also, the addition of BHA to the fungicides greatly increased their efficiencies against the incidence of diseases. Moreover, the potency of fungicide and BHA was considerably increased at pH values of 3 and 9.

INTRODUCTION

Citrus fruits, either used freshly or as juice, have essential factors responsible for their nutritional and health benefits such as amino acids, organic acids, sugars, phenolics, vitamins, carotenoids and volatiles (Cerdan-Calero *et al*., 2012). However, these fruits during postharvest handling and storage, are susceptible to some pathogens including *Penicillium digitatum* (Pers.: Fr.) Sacc. and *Penicillium italicum* Wehmer, which are the causal green and blue mold diseases, respectively, and thus caused considerable economic losses (Palou, 2014). During storage, the disease may result in complete damage collapse and liquefaction of infected fruit. Juices dripping from infected fruit can readily spread the pathogen to healthy fruit (Eckert and Eaks, 1989). Good practices such as avoiding injury during harvest and transportation as well as sanitation of packing- and store- houses, can reduce postharvest decay.

For postharvest pathogenic fungi, Prusky *et al*. (2004) stated that pathogens may enhance their virulence by locally modulating the ambient pH of host either up or down. Pitt and Hocking (1997) illustrated that most molds can grow in a wide range of pH (3-8), but most of them prefer acidic pH. Also, Smilanick *et al*. (2005) reported that the pH above 8 inhibits *P. digitatum* growth.

Several fungicides either separately as thiabendazole and imazalil (Smilanick *et al*., 2008 and Sanchez-Torres and Tuset, 2011), boscalid (Serey *et al*., 2007 and Malandrakis *et al*., 2017) and kresoxim–methyl (Jae-Wook *et al*., 2001 and Malandrakis *et al*., 2017) or premixed as boscalid + kresoxim–methyl (Serey *et al*., 2007; Zhang *et al*., 2007 and 2008 and Malandrakis *et al*., 2017) and cyprodinil + fludioxonil (Errampalli and Crnko, 2004; Serey *et al*., 2007 and Sallato *et al*., 2007) have been used to control postharvest diseases.

Owing to their efficacy and low cost, antioxidants including phenolic derivatives such as butylated hydroxyanisole (BHA) are extensively used to control a wide range of plant diseases (Giridhar and Reddy, 2001; Rajkumar *et al*., 2008 and Abdel-Monaim and Ismail, 2010).

The effect of pH on the antimicrobial activity of fungicides or BHA is highly dependent upon the tested fungi. Smilanick *et al*. (2008) found that pH 8-9 enhanced the fungitoxic activity of fludioxonil against *P. digitatum*. The antifungal effect of BHA on *Penicillium* spp. at pH 3.5 was greater than at pH 7 or pH 5.5 (Ghadimipour and Sedigh-Eteghad, 2015).

On the other hand, the addition of BHA improved the fungitoxic activity of imazalil against *Colletotrichum musae* (Khan *et al*., 2001), fluconazole (Simonetti *et al*., 2002), ticonazole (Simonetti *et al*., 2003) against *Candida albicans* and *Escherichia coli*, iprodion, myclobutanil, prochloraz, tetraconazole and trifloxystrobin against *P. digitatum* (Khalifa and Sameer, 2014).

The aim of this study was to evaluate the fungicidal activity of certain fungicides as affected by definite pH values and the antioxidant butylated hydroxyanisole (BHA) in controlling the green and blue mold diseases on orange fruits.

MATERIALS AND METHODS

The present work was carried out in the laboratory of Plant Protection Dept., Faculty of Agriculture, AL-Azhar University, Cairo, Egypt during 2018.

Fungi: Identified isolates of *Penicillium digitatum* and *P. italicum*, the causal agents of green and blue molds citrus fruits, respectively, were obtained from Plant Disease Institute, Agricultural Research Center, Giza.

Fungicides: Four commercial fungicides, represent different chemical groups were selected for the present work. These fungicides are: boscalid (Cantus 50 % WG), kresoxim–methyl (Sevron 50 % WG), the premixed formulation of 20 % boscalid + 10 % kresoxim–methyl (Collis 30 % SC) and the premixed formulation of 37.5% cyprodinil + 25 % fludioxonil (Switch 62.5 % WG).

Antioxidant: Butylated hydroxyanisole (BHA) $C_{11}H_{16}O_2$ (99 % w/w).

***In vitro* fungitoxicity test**

A study was conducted to estimate the fungicidal activity of the tested fungicides and antioxidant either separately under defined pH values or in mixtures under native pH medium. The effect of pH on mycelial growth of fungi was examined by adjusting (PDA) at 3.0 to 11.0 using 1.0 N NaOH or HCl prior to autoclaving, the degree of pH was determined by the pH meter (Turkkan and Erper, 2014). The fungicides were diluted in sterile distilled water, then added to cooled PDA medium at concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 µg a.i. / ml for each, whereas antioxidant was evaluated at concentrations of 10.0, 25.0, 50.0, 75.0, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg a.i. / ml. The influence of pH on the fungicidal activity of the tested fungicides and BHA was evaluated in these concentrations in buffered PDA at pH 3 or 9. In other trials, native PDA media amended with different concentrations of each fungicide were further amended with 25 or 50 µg / ml antioxidant. The poisoned media were poured in plates (9 cm diameter), whereas plates of control treatment contained compounds – free medium. All plates were inoculated by 0.4 cm diameter disk, removed from 7 day-old culture of the fungus, and incubated for 7 days at 25°C. Each treatment was replicated four times. Growth on the each treatment amended medium was determined by measuring the colony diameter (cm). The percentage of growth inhibition was calculated relative to the control treatment. The effective concentration giving 50 % linear growth inhibition (EC_{50}) was determined by regression analysis of the log probit transformed data (Finney, 1971).

***In vivo* test**

This trial was conducted to investigate the fungicidal activity of the tested fungicides and antioxidant either separately under defined pH values or in mixtures under native pH for controlling the incidence of artificial infection with green and blue molds on navel orange under laboratory conditions. Healthy uniform navel oranges were used in this trial. The fruits were washed with soap, rinsed with fresh water, and washed again with 70 % ethanol for surface sterilization. After drying, the fruits were inoculated artificially with *P. digitatum* or *P. italicum*. Inoculation was performed according to Eckert and Kolbezen (1977) by making a scratch 1.0 cm long and 0.1 mm deep in the rind on both sides of each fruit and then applying dry spores dust to the scratches with a small brush. Twenty four hours after inoculation, orange fruits were treated with the tested fungicides and BHA and their mixtures by using dipping method in the solutions for 30 sec. To determine the influence of pH on the fungicidal activity of fungicides or BHA against incidence of green and blue molds on fruits, these compounds were evaluated separately at concentrations (1000, 1500 and 2000 µg a.i. / ml) at 3 or 9 pH. In other trial, fungicides and BHA were evaluated either separately or in mixtures at the same concentrations in native pH. All treatments were replicated 3 times and each replicate contained 8 oranges. Other fruits were dipped in water only as control treatments. The treated fruits were air–dried, and inspected for decay15 days after storage in plastic bags at 25°C. The efficacy of each treatment was determined according to the equation described by Samoucha and Cohen (1989) as follows:

Percentage of control efficacy (PCE) = 100 (1- x / y),

Where, x = number of decayed fruits in treatment and y = number of decayed fruits in control treatment. The results were statistically analyzed according to Snedecor and Cochran (1969).

RESULTS AND DISCUSSION

***In vitro* fungitoxicity tests**

Effect of pH on mycelial growth of the tested fungi:

The results in Table (1) show that both *P. digitatum* and *P. italicum* may differentially grow under acidic and basic pH values. Although pH 6 and 7 did not negatively affect the mycelial growth of these fungi, higher (8-11) and lower (3-5) values considerably reduced this growth. The higher pH 8, 9, 10 and 11 reduced the growth of *P. digitatum* and *P. italicum* to 7.7, 4.8, 4.0 and 3.5 cm and to 7.2, 5.0, 3.8 and 3.5 cm, respectively, On the other hand, the lower pH 5, 4 and 3 reduced the growth of fungi to 7.8, 7.5 and 4.6 cm and to 7.9, 7.8 and 5.0 cm, respectively. The results are compatible with those of Eckert and Eaks (1989) who found that pH values above 8.5 inhibit the germination and growth of *P. digitatum*. Similarly, Smilanick *et al*. (2006 and 2008) found that germination of conidia of *P. digitatum* was more than 95 % at pH 4-7, but at pH 8, germination reduced to 50 % and completely inhibited at pH 9.

Table 1. The effect of pH on mycelial growth of *Penicillium digitatum* and *P. italicum* on PDA for 7 day at 25° C.

pH	Radial growth of fungi (cm)	
	Penicillium digitatum	***Penicillium italicum***
3	4.6	5.0
4	7.5	7.8
5	7.8	7.9
6	9.0	9.0
7	9.0	9.0
8	7.7	7.2
9	4.8	5.0
10	4.0	3.8
11	3.5	3.5

Sensitivity of the tested fungi to the fungicides and BHA

Results in Table (2) show the efficiency of the tested fungicides and BHA against growth of *P. digitatum* and *P. italicum*. Based on EC_{50} values, mixtures of cyprodinil + fludioxonil and boscalid + kresoxim-methyl fungicides are more effective in inhibiting the mycelial growth of *P. digitatum* (EC_{50} values were 0.058 and 0.086 µg a.i. / ml, respectively) and *P. italicum* (EC_{50} values were 0.053 and 0.064 µg a.i. / ml, respectively) than the single fungicides kresoxim-methyl and boscalid (EC_{50} against *P. digitatum* were 0.594 and 1.32 µg a.i. / ml, respectively, and against *P. italicum* were 0.419 and 1.18 µg a.i. / ml, respectively). These results are in agreement with those of Jae-Wook *et al*. (2001) who found that the EC_{50} values of kresoxim-methyl were 0.04-0.16 and 0.08-0.16 µg /ml against mycelial growth of *P. digitatum* and *P. italicum*, respectively. Moreover, Karaoglanidis *et al*. (2011) showed that *P. expansum* was sensitive to cyprodinil and fludioxonil with mean EC_{50} values of 0.55 and 0.08 µg / ml, respectively. Similarly, Helalia and Sameer (2014) indicated that the EC_{50} value of fludioxonil to *P. digitatum* was 0.06 µg / ml. Our results indicated that the EC_{50} values reduced by mixtures of fungicides comparing with the fungicides alone. These findings are in agreement with other previous studies (Errampalli and Crnko, 2004; Serey *et al*., 2007 and Zhang *et al*., 2007 and 2008).

The different modes of action of the tested fungicides (boscalid inhibits succinate dehydrogenase in the cell respiration process, kresoxim-methyl acts at the quinone binding site of the cytochrome bc1 complex in the mitochondrial cell membrane, cyprodinil inhibits excretion of hydrolytic enzymes and methionine biosynthesis in fungal cells, fludioxonil inhibits transport-associated phosphorylation of glucose as well as prevents glycerol synthesis) and their different chemical structures as well as the nature of the target fungus may be contributory factors affecting their fungicidal activities.

The data represented in Table (2) also show that the tested fungi capable to tolerate high concentrations of BHA, whereas its EC_{50} values for *P. digitatum* and *P. italicum* were 281.73 and 267.14 µg / ml, respectively. In this respect, Thompson (1997) reported that the effective concentrations of BHA that reduced radial growth of *Penicillium* spp. by 50 % (EC_{50}) were 100-275 µg /ml. Khalifa and Sameer (2014) found that the EC_{50} value of BHA was 256.21 µg / ml for *P. digitatum*. Ghadimipour and Sedigh-Eteghad (2015) demonstrated that the minimum inhibitory concentration (MIC) of BHA against *Penicillium* spp. was 320 µg /ml.

Effect of pH on fungicidal activity of the tested fungicides and BHA:

Data tabulated in Table (2) clearly indicate that the EC_{50} values either of fungicides or BHA in buffered PDA at pH 3 or 9 were lower than those determined in native PDA indicating that such pH values enhanced the potency of fungicides and BHA against growth of *P. digitatum* and *P. italicum*. This enhancement effect differed according to compound concentration and pH value.

Concerning fungicides, their efficiencies increased in buffered PDA at pH 9 more than pH 3. For example, efficiency of boscalid against *P. digitatum* and *P. italicum* increased by 2.13- and 2.88- fold at pH 9 comparing to 1.67- and 2.41- fold at pH 3, respectively. The premixed fungicide, boscalid + kresoxim-methyl alone had EC_{50} values of 0.086 and 0.064 µg /ml for *P. digitatum* and *P. italicum*, respectively, which decreased to 0.039 and 0.034 and to 0.046 and 0.041 µg / ml in buffered PDA at pH 9 and 3, respectively, indicating that the fungitoxic activity of this fungicide increased by 2.21- and 1.88-fold and by 1.87- and 1.56-fold, respectively. The results obtained are in agreement with those obtained by other investigators. Holmes and Eckert (1999) found that the EC_{50} value for imazalil against *P. digitatum* was 4.66 and 0.88 µg / ml at pH 5.1 and 5.7, respectively. Imazalil effectiveness against *P. digitatum* was significantly better at pH 7.5 compared at pH 4 (Smilanick *et al*., 2005). Also, the EC_{95} value for fludioxonil against *P. digitatum* was 0.028 mg /L at pH 9, however, it was 0.251 mg / L at pH 4 (Smilanick *et al*., 2008).

Table 2. EC_{50}* values (µg a.i. / ml) of the tested fungicides separately and under definite pH values and the antioxidant BHA against mycelial growth of the two fungi.

Compounds	*Penicillium digitatum*									*Penicillium italicum*								
	EC_{50} of the compounds separately (µg / ml)	EC_{50} (µg / ml) of compounds under definite pH				EC_{50} (µg / ml) of fungicides + BHA at				EC_{50} of the compounds separately (µg / ml)	EC_{50} (µg / ml) of compounds under definite pH				EC_{50} (µg / ml) of fungicides + BHA at			
		3		9		25 µg / ml		50 µg / ml			3		9		25 µg / ml		50 µg / ml	
		EC_{50}	SE**	EC_{50}	SE	EC_{50}	SE	EC_{50}	SE		EC_{50}	SE	EC_{50}	SE	EC_{50}	SE	EC_{50}	SE
Boscalid	1.32	0.79	1.67	0.62	2.13	0.59	2.24	0.33	4.0	1.18	0.49	2.41	0.41	2.88	0.37	3.19	0.24	4.92
Kresoxim-methyl	0.594	0.24	2.48	0.171	3.47	0.147	4.04	0.089	6.67	0.419	0.28	1.50	0.22	1.90	0.20	2.10	0.072	5.82
Boscalid + kresoxim-methyl	0.086	0.046	1.87	0.039	2.21	0.028	3.07	0.018	4.78	0.064	0.041	1.56	0.034	1.88	0.023	2.78	0.015	4.27
Cyprodinil + fludioxonil	0.058	0,029	2.0	0.022	2.64	0.020	2.90	0.011	5.27	0.053	0.027	1.96	0.019	2.79	0.016	3.31	0.009	5.89
BHA	281.73	86.46	3.26	114.89	2.45	-	-	-	-	267.14	74.62	3.58	101.86	2.62	-	-	-	-

*** EC_{50}: Effective concentration (as µg a.i / ml) that give 50 % inhibition of the fungal growth.**
**** S.E.: Synergistic Effect = EC_{50} of the fungicide alone / EC_{50} of the mixture**

In view of BHA efficiency, the results showed that its potency against *P. digitatum* and *P. italicum* increased at pH 3 more than pH 9. For example, BHA efficiency increased by 3.26- and 2.45- and by 3.58- and 2.62- fold at pH 3 and 9, respectively. These data are in agreement with other previous studies. Conidial germination for *Penicillium* species was significantly reduced in the presence of 100µg / ml of BHA at 4, 6, 8 and 10pH (Thompson *et al*., 1993). Arroyo (2003) reported that the *in vitro* antifungal activity of BHA against *Penicillium* spp. was increased as pH value decreased. Ghadimipour and Sedigh-Eteghad (2015) found that the MIC values of BHA for *Penicillium* sp. at pH 5.5 reduced to half and at pH 3.5 reduced to one-sixth.

Effect of BHA on fungicidal activity of the tested fungicides:

The results in Table (2) clearly indicate that the fungitoxic activities of fungicides greatly increased by adding BHA to fungicide–amended medium and this observation was BHA concentration and type of the examined fungicide dependent.

For example, boscalid efficiency against both fungi markedly increased as affected by BHA addition. Such increment reached 2.24- and 4.0- fold with *P. digitatum* and reached 3.19- and 4.92- fold with *P. italicum* when BHA was added at 25 and 50 µg / ml, respectively. Concerning the boscalid + kresoxim-methyl efficiency, the results showed that this compound had EC_{50} 0.086 and

0.064 µg / ml against *P. digitatum* and *P. italicum*, respectively. Interestingly, its fungicidal activity against both fungi was increased by 3.07- and 4.78- fold and by 2.78- and 4.27- fold, respectively, when 25 and 50 µg / ml of BHA were added, respectively, to fungicide-amended medium. The EC_{50} values of (cyprodinil + fludioxonil) alone for *P. digitatum* and *P. italicum* were 0.058 and 0.053 µg / ml, respectively, and became 0.011 and 0.009 µg / ml, respectively, when 50 µg / ml of BHA was added to the medium, indicating that its fungitoxic action increased by 5.27- and 5.89- fold, respectively. These findings are in agreement with other previous studies (Khan *et al*., 2001; Simonetti *et al*., 2002 and 2003; Ali, 2008 and Khalifa and Sameer, 2014).

***In vivo* studies**

Effect of pH on green and blue mold diseases:

The results in Table (3) show that pH 3 and 9 slightly controlled the incidence of green and blue molds on orange fruits by 20.83 % and 29.17 % and by 25.0 % and 37.5 %, respectively, and basic pH value was more effective than acidic pH. These findings are in agreement with other previous studies (Eckert and Eaks, 1989; Pitt and Hocking, 1997; Smilanick *et al*., 2005, 2006 and 2008; Guo *et al*., 2014; Turkkan and Erper, 2014 and Ghadimipour and Sedigh-Eteghad, 2015).

Effect of fungicides and BHA on green and blue mold diseases:

Fungicidal efficacy of the tested fungicides and BHA at 1000, 1500 and 2000 µg / ml were evaluated separately for controlling green and blue molds on orange fruits. Results in Table (3) reveal that the green and blue mold diseases are sufficiently controlled only at 1500 and 2000 µg / ml of the tested fungicides. Cyprodinil + fludioxonil, boscalid + kresoxim-methyl, kresoxim-methyl and boscalid all at 2000 µg / ml caused the corresponding controlling rates of 95.83, 91.67, 75.00 and 66.67 % for green mold and of 95.83, 95.83, 75.00 and 70.83 % for blue mold. The results indicated that the PCE values for green and blue mold diseases on orange fruits were highly significant with fungicide mixtures comparing with separated fungicides. The above results confirmed the *in vitro* experiments and are in agreement with those previously reported (Jae-Wook *et al*., 2001; Errampalli and Crnko, 2004; Sallato *et al*., 2007; Serey *et al*., 2007; Zhang *et al*., 2007 and 2008 and Malandrakis *et al*., 2017). The mixture of boscalid + kresoxim-methyl showed significant control efficacy against strawberry grey mold caused by *Botrytis cinerea* than boscalid or kresoxim-methyl separately (Zhang *et al*., 2008). Also, the maximum curative action against *P. expansum* was obtained when both cyprodinil and fludioxonil were applied in combination (Serey *et al*., 2007).

Results in Table (3) also show that BHA slightly controlled the incidence of green and blue mold diseases on orange fruits as PCE values for both diseases did not exceed 25 % at the high concentration (2000 µg / ml). Such findings are in agreement with those previously reported. Ali (2008) found that BHA at 5 and 10 µg / ml had no fungicidal activity against fusarium wilt, damping–off and early blight diseases on tomato plants comparing with the control treatment. Khalifa and Sameer (2014) found that BHA slightly reduced the incidence of green mold on orange fruits.

Synergistic effect of pH to fungicides and BHA against green and blue mold diseases on orange fruits:

Data tabulated in Table (3) clearly indicate that the tested pH values (3 and 9) markedly enhanced the potency of fungicides or BHA for controlling green and blue mold diseases on orange fruits and such fungicidal effect of fungicides was obviously observed at pH 9. For example, PCE values of boscalid and cyprodinil + fludioxonil at 1000 µg / ml for green mold disease were 25.00 and 54.17% which increased to 54.17 and 66.67 % at pH 3 and to70.83 and 83.33 % at pH 9. In the case of blue mold disease, the corresponding PCE values of the two fungicides at the same concentration were 33.33 and 62.50 % which increased to 54.17 and 70.83 % at pH 3 and to 66.67 and 83.33 % at pH 9. These results mean that basic or acidic pH increased the efficiencies of fungicides against the incidence of green and blue mold on orange fruits and basic pH was more effective than the acidic pH. These results are compatible with the *in vitro* tests and are in agreement with those previously reported (Holmes and Eckert, 1999 and Smilanik *et al*., 2005 and 2008). Smilanik *et al*. (2008) suggested that the fungicidal activity of fludioxonil against *P. digitatum* increased at pH 8-9.

On the other side, the results in Table (3) indicate that the efficiency of BHA for controlling green and blue mold diseases increased at pH 3 more than at pH 9. For example, 1000, 1500 and 2000 µg / ml of BHA alone exhibited 8.33, 12.50 and 25.00 PCE, respectively, for green mold disease which became 41.67, 50.00 and 58.33 % at pH 3 and 33.33, 37.50 and 54.17 % at pH 9, respectively. For blue mold disease, BHA at the same concentrations resulted in PCE values of 12.50, 20.83 and 25.00 % which recorded 45.83, 50.00 and 54.17 % at pH 3 and 45.83, 45.83 and 50.00 % at pH 9, respectively. These results confirmed the *in vitro* tests and are in agreement with those previously reported (Thompson *et al*., 1993; Arroyo, 2003 and Ghadimipour and Sedigh-Eteghad, 2015).

Regarding to this finding, Griffin (1994) stated that the influence of pH on fungal growth is complex and dependent upon the ionization of acids or bases in the medium in which the fungus resides, and that pH can alter membrane potentials that change the permeability of fungal membranes to many substances, including fungicides and BHA. Hwang and Klotz (1938) stated that hydrogen and hydroxyl ion concentrations were important factors in the inhibitory or lethal activity of many compounds to fungal spores.

Synergistic effect of BHA on fungicides against green and blue mold diseases on orange fruits

Results in Table (3) indicate that the addition of BHA to the fungicides greatly increased their efficiencies against the incidence of green and blue mold diseases. The addition of 1500µg /ml of BHA to the tested fungicidal treatments, boscalid, kresoxim-methyl, boscalid + kresoxim-methyl and cyprodinil + fludioxonil (each at 1000 µg / ml), raised their fungicidal activities against green mold disease from 25.00, 37.50, 45.83 and 54.17 % to 70.83, 75.00, 79.17 and 83.33 %, respectively, and raised their fungicidal activities against blue mold disease from 33.33, 41.67, 50.00 and 62.50 % to 75.00, 79.17, 91.67 and 91.67 %, respectively. Moreover, the fungicidal activities of the tested fungicides, when applied at 2000 µg / ml, ranged from 66.67 to 95.83 % and from 70.83 to 95.83 % against green and blue mold diseases, respectively, which raised to 91.67-100 % for both diseases. These data are in agreement with other previous studies.

Table 3. Effect of definite pH values and the antioxidant BHA on PCE of certain fungicides against incidence of green and blue mold diseases on orange fruits.

Treatments	Conc. µg/ml	Percentage of control efficiency (PCE)													
		Green mold							Blue mold						
		Separated treatment	Candidate pH degrees		Candidate BHA concentrations			L.S.D. at 5 %	Separated treatment	Candidate pH degrees		Candidate BHA concentrations			L.S.D. at 5 %
			3	9	1000 µg /ml	1500 µg /ml	2000 µg /ml			3	9	1000 µg /ml	1500 µg /ml	2000 µg /ml	
pH 3		20.83	-	-	-	-	-	-	25.00	-	-	-	-	-	-
pH 9		29.17	-	-	-	-	-	-	37.50	-	-	-	-	-	-
Boscalid	1000	25.00	54.17	70.83	58.33	70.83	83.33	10.36	33.33	54.17	66.67	66.67	75.00	79.17	7.33
	1500	50.00	62.50	79.17	66.67	83.33	91.67	8.33	62.50	66.67	75.00	70.83	87.50	95.83	7.58
	2000	66.67	70.83	87.50	79.17	91.67	95.83	8.24	70.83	79.17	91.67	79.17	91.67	95.83	8.03
Kresoxim-methyl	1000	37.50	50.00	75.00	58.33	75.00	79.17	7.62	41.67	54.17	79.17	70.83	79.17	87.50	7.49
	1500	66.67	75.00	83.33	75.00	87.50	91.67	8.05	70.83	79.17	83.33	83.33	87.50	95.83	8.09
	2000	75.00	83.33	91.67	83.33	91.67	95.83	7.78	75.00	79.17	87.50	87.50	91.67	95.83	8.52
Boscalid + kresoxim-methyl	1000	45.83	58.33	83.33	70.83	79.17	87.50	8.27	50.00	62.50	83.33	75.00	91.67	95.83	8.15
	1500	75.00	79.17	87.50	87.50	91.67	100.0	6.15	87.50	87.50	95.83	91.67	91.67	95.83	7.03
	2000	91.67	95.83	95.83	95.83	100.0	100.0	7.91	95.83	100.0	100.0	100.0	100.0	100.0	7.96
Cyprodinil + fludioxonil	1000	54.17	66.67	83.33	75.00	83.33	95.83	8.05	62.50	70.83	83.33	75.00	91.67	100.0	8.18
	1500	83.33	87.50	91.67	87.50	91.67	100.0	7.33	91.67	91.67	95.83	91.67	95.83	100.0	7.05
	2000	95.83	100.0	100.0	95.83	100.0	100.0	6.25	95.83	100.0	100.0	100.0	100.0	100.0	7.36
BHA	1000	8.33	41.67	33.33	-	-	-	7.93	12.50	45.83	45.83	-	-	-	6.26
	1500	12.50	50.00	37.50	-	-	-	8.18	20.83	50.00	45.83	-	-	-	7.42
	2000	25.00	58.33	54.17	-	-	-	6.23	25.00	54.17	50.00	-	-	-	7.63
L.S.D. at 5 %		7.88	7.93	8.05	8.13	8.22	10.14	-	7.56	7.56	8.25	7.95	6.43	9.22	-

Aldunate *et al*. (1992) reported that the synergistic effect of BHA is not fully clear, however, it has been shown to have a direct effect on the mitochondrial electron chain of trypanosomes, thus inhibiting respiration. BHA increases leakage of sugars, amino acids and proteins from *Fusarium* sp. The leakage of sugars, amino acids and proteins may be an indication of mycelial membrane disruption by the BHA as an initial step in inhibition of the tested fungi *in vitro*. This effect may increase the fungitoxic action of the fungicides against the fungal growth (Thompson, 1996). Additionally, Khan *et al*. (2001) cited that BHA may make membranes of *Colletotrichum musae* more leaky and allowing more fungicide into the fungal cells. Simonetti *et al*. (2002 and 2003) suggested that antioxidants such as BHA appear to promote fluconazole activity by increasing cell membrane permeability leading to the leakage of cellular enzymes. The protective properties of antioxidants are probably due to their ability to act as superoxide anion scavengers, thereby protecting cell membranes from mycotoxin-induced damage (Atroshi *et al*., 2002). Baider and Cohen (2003) reported that antioxidants may enhance host resistance to fungal infections. Also, the synergistic action of BHA may be attributed to the prevention or delaying the oxidation of the fungicides within the fungal cells which may reduce their fungitoxic action (Ali, 2008). It is known that the synergistic action is more pronounced when components of the mixture had different modes of action (Gisi, 1996).

Generally, addition of BHA to low concentrations of fungicides may increase their fungitoxic action. Also, the fungicidal activity of fungicides and BHA may be significantly increased when applied under definite pH values. The purpose to use BHA-fungicides mixtures is to reduce the fungicide concentrations and hence minimize residues on fruits as well as the chemical costs.

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تأثير رقم الحموضة و مضاد الاكسدة (بيوتيلاتيد هيدروكسي انيسول) على الكفاءة الابادية لبعض مبيدات الفطريات في مكافحة مرضي العفن الاخضر و الازرق على ثمار البرتقال

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يعتبر مرضي العفن الاخضر و الازرق المتسببين عن فطري بنسليوم ديجيتاتم و بنسليوم اتاليكام من الامراض الرئيسية التي تسبب عفن ثمار البرتقال بعد الحصاد مسببة خسائر اقتصادية في صناعة الموالح. تم تقييم كفاءة بعض مبيدات الفطريات (بوسكاليد و كريسوكسيم-ميثيل و بوسكاليد+ كريسوكسيم-ميثيل و سبرودانيل + فلودايوكسونيل) الى جانب احدى المواد المانعة للاكسدة (بيوتيلاتيد هيدروكسي انيسول) في مكافحة فطري بنسليوم ديجيتاتم و بنسليوم اتاليكام على ثمار البرتقال أبو سرة تحت ظروف حموضة محددة (رقمي حموضة 3 و 9). أوضحت الدراسات المعملية أن أرقام الحموضة المرتفعة (8-11) و المنخفضة (3-5) سببت نقصا ملحوظا في نمو الفطرين المستهدفين، و أن المعاملات في صورة مخاليط كانت أكثر فعالية منها منفردة، و أن الفطرين قد تحملا تركيزات عالية من المادة المانعة للاكسدة، و ان كفاءة مبيدات الفطريات ضد الفطرين ازدادت باضافة المادة المانعة للاكسدة للبيئة المحتوية على تلك المبيدات، و ان رقمي الحموضة 3 و 9 أحدثا تحسنا في فعالية كلا من مبيدات الفطريات و كذا المادة المانعة للاكسدة. و اوضحت الدراسات التي اجريت على العائل أن معاملات مبيدات الفطريات خاصة المخاليط منها قد أسفرت عن مكافحة المرضين بشكل ملحوظ وأن مستويات المكافحة هذه قد ازدادت بشكل ملحوظ عند اضافة المادة المانعة للاكسدة الى تلك المبيدات أو عند اجراء المكافحة في ظل رقمي الحموضة 3 و 9.