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Control of the Green Mold of Orange Using Fungicides Alone and In Combination with Antioxidants

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ABSTRACT

Five fungicides (iprodione, myclobutanil, prochloraz, tetraconazole and trifloxystrobin) and two antioxidants i.e. selenium (Se) and butylated hydroxyanisole (BHA), were evaluated separately or in mixtures in laboratory against *Penicillium digitatum* causing green mold disease of orange fruit. Depending upon EC_{50} values, the *in vitro* studies showed that tetraconazole recorded the highest fungicidal activity against *P. digitatum* growth followed by prochloraz, myclobutanil, trifloxystrobin and iprodione, while that fungus tolerated high concentration of antioxidants. Interestingly, the fungitoxic activities of the tested fungicides were greatly increased by adding either Se or BHA to the fungicide-amended medium. The *in vivo* studies on Navel oranges showed that all the separated fungicide treatments, particularly tetraconazole and prochloraz effectively controlled the green mold disease, while antioxidant treatments slightly reduced the incidence of such disease. Moreover, the addition of Se and BHA antioxidants improved the performance of the tested fungicides for controlling the green mold decay caused by *P. digitatum*.

Key words: orange, green mould, fungicides, antioxidants, *Penicillium digitatum*.

Introduction

Navel orange (*Citrus sinensis* L. Osbeck) as one of the most important citrus fruits in Egypt, is a subtropical fruit of high commercial value on the international fruit market and is commonly eaten fresh. Under Egyptian conditions, it is a common practice to store mature navel orange fruits until the suitable time for marketing. Injuries sustained to navel orange fruit during harvest allow the entry of wound pathogens, including *Penicillium digitatum*, the causal agent of green mold which commonly responsible for postharvest decay of citrus, contributing up to 90 % of total losses, especially in arid and sub-tropical climates (Eckert & Wild, 1983; Eckert & Eaks, 1989; Zhang & Swingle, 2005 and El-Mougy *et al*., 2008). It causes serious problems for the harvested citrus fruits during handling, transportation, exportation and the storage process (Abdel Wahab – Sahar & Rashid, 2012). In storage, the disease may result in complete collapse and liquefaction of infected fruit. Juices dripping from infected fruit can readily spread the pathogen to healthy fruit (Eckert & 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. However, the application of fungicides after harvest has been shown to provide the most effective control. Thiabendazole and imazalil are the most commonly used fungicides world wide for managing green mold of citrus (Bus, 1992 and Smilanick *et al*., 2005 &2006).

Several fungicides including iprodione (Brown, 1992 and Mahmoud, 1998), myclobutanil, prochloraz, tetraconazole (Mahmoud & Hanafy, 1991; El-Khawaga-Maii, 2006 and Sanchez-Torres & Tuset, 2011) and trifloxystrobin (Margot *et al*., 1998) were evaluated for controlling postharvest diseases by several means of applications.

Antioxidants including Se and BHA which save to human and environment had been used successfully to control some plant diseases such as anthracnose of avocado (Prusky *et al*., 1995), anthracnose of banana (Khan *et al*., 2001), faba bean chocolate spot (Hassan *et al*., 2006), root rot and leaf blight in lupine (Abdel-Monaim, 2008), damping – off in pepper (Rajkumar *et al*., 2008) and root rot and wilt in pepper (Abdel-Monaim & Ismail, 2010). Giridhar & Reddy (2001) found that BHA completely inhibited the growth of *Slachybotrys atra* at 250 µg / ml concentration.

It was suggested that the addition of certain antioxidants to the fungicides increased their fungitoxic activity. Selenium improves the activity of thiabendazole against *P. digitatum* and *P. italicum* (Mahmoud, 1993), thiabendazole or carboxin – captan against *Alternaria lenuis* and *Fusarium oxysporum* (Mahmoud *et al*., 1996 a & b), carbendazim and carboxin-thiram against *Heleminthosporum sativum* (Gwily-Ahlam *et al*., 2002). Sameer (2013) found that selenium enhanced the fungitoxic activity of difenoconazole, azoxystrobin, trifloxystrobin and metalaxyl against *Alternaria solani*. Also, butylated hydroxyanisole (BHA) improves the activity of prochloraz against *Colletotrichum gloeosporioides* (Prusky *et al*., 1995), imazalil against *C. musae* (Khan *et al*., 2001), fluconazole (Simonetti *et al*., 2002), ticonazole (Simonetti *et al*., 2003) against *Candida albicans* and *Escherichia coli*. El-Khawaga-Maii (2006) found that selenium and BHA enhanced the fungitoxic

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activity of tetraconazole, penconazole, fenarimol, trifloxystrobin and flutolanil against *F. oxysporum*, *Aspergillus niger*, *P. digitatum*, *Pyrenophora tritici repentis* and *Rhizoctonia solani*. Ali (2008) found that BHA enhanced the fungitoxic activity of tetraconazole, difenoconazole, trifloxystrobin, pyraclostrobin + metiram, metiram and flutolanil against *F. oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani* and *Alternaria solani*.

Based on these studies, the focus of this study was to evaluate five fungicides (iprodione, myclobutanil, prochloraz, tetraconazole and trifloxystrobin) and two antioxidants (selenium and butylated hydroxyanisole) used alone or in combinations for green mold control on orange fruit.

Materials and Methods

Fungus:

An identified isolate of *Penicillium digitatum*, the causal agent of green mould citrus fruits, was obtained from Plant Disease Institute, Agricultural Research Center, Giza.

Fungicides:

Five fungicides belonging to three fungicidal groups were used, (1) glutathione synthetase inhibitor, iprodione (Rovral, 50 % W.P.), (2) ergosterol biosynthesis inhibitors, myclobutanil (Systhane, 12.5 % E.C.), prochloraz (Master, 25 % E.C.) and tetraconazole (Domark, 10 % E.C.) and (3) quinol-oxidizing inhibitor, trifloxystrobin (Flint, 50 % W.G.).

Antioxidants:

Selenium was used as sodium selenite, (Na_2SeO_3 98 % w/w).
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 antioxidants. 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 antioxidants were evaluated at concentrations of 10.0, 25.0, 50.0, 75.0, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg / ml for each. In other trials, PDA media amended with different fungicide concentrations were further amended with 10 and 15 µg / ml antioxidant. The poisoned media were poured in plates (9 cm diameter), other plates containing compounds-free medium were used as control treatment. The medium was seeded by 0.4 cm diameter disk, removed from 7 day-old culture of the fungus, on solidified medium and incubated for 7 days at 25^{o} C. Each treatment was replicated four times. Growth on the fungicides, antioxidants and their mixtures amended medium was determined by measuring the colony diameter (cm). The percentage of growth inhibition was calculated relative to the control treatment. The concentration giving 50 % linear growth inhibition (EC_{50}) was determined by regression analysis of the log probit transformed data (Finney, 1971).

In vivo studies:

This trial was conducted to investigate the efficiency of fungicides and antioxidants applied alone or in mixtures for controlling the incidence of artificial infection with green mold 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*. Inoculation was performed according to Eckert & 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. The inoculated fruits were treated with the fungicides, antioxidants and their mixtures 24 hrs after inoculation. The treatments were carried out by dip method in the solutions for 30 sec. The concentrations of fungicides were 250, 500, 1000, 1500 and 2000 µg a.i. / ml and those of antioxidants were 500, 1000 and 1500 µg a.i. / ml alone or in mixtures. Each treatment contained 24 oranges in 3 replicates. Other fruits were dipped in water only as control treatments. After drying the treated fruits were stored in plastic bags and inspection for decay was carried out 15 days of storage at 25^{o} C. The efficacy of each treatment was determined according to the equation described by Samoucha & Cohen (1989) as follows: PCE = 100 (1- x / y), where PCE percentage of control efficacy, x = number of decayed fruits in treatment and y = number of decayed fruits in control treatment. The results were statistically analyzed according to Snedecor & Cochran (1969).

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Results and Discussion

Sensitivity of the fungus to the fungicides:

The effect of tested fungicides on growth of *P. digitatum* was determined by measuring the growth of the fungus on PDA media containing different concentrations of each fungicide and the concentration that inhibits 50 % of the fungal growth (EC_{50}) was calculated. Results in Table (1) show that increasing the concentration of iprodione, myclobutanil, prochloraz, tetraconazole and trifloxystrobin decreased gradually the growth of the tested fungus. For example, these fungicides at 0.1 µg / ml reduced the growth of *P. digitatum* by 42.67, 61.11, 63.00, 61.11 and 57.44 %, respectively. In general, myclobutanil, prochloraz and tetraconazole fungicides (ergosterol biosynthesis inhibitors) inhibited the fungal growth more than iprodione and trifloxystrobin. Such inhibition percents, reached 63.00, 68.56, 68.56, 55.56 and 59.33 % at 0.5 µg / ml and recorded 94.44, 96.33, 100.0, 87.00 and 88.89 % at 10 µg / ml.

Table 1: Growth inhibition percent of *Penicillium digitatum* grown on PDA medium amended with candidate concentrations (µg a.i* / ml) of the tested fungicides.

Concentrations µg a.i*/ml	Fungicides				
	Iprodione	Myclobutanil	Prochloraz	Tetraconazole	Trifloxystrobin
0.001	0.00	1.89	5.56	9.22	1.89
0.005	0.00	5.56	7.44	9.22	3.78
0.01	5.56	20.33	20.33	18.56	18.56
0.05	33.33	48.22	50.00	53.78	46.33
0.10	42.67	61.11	63.00	61.11	57.44
0.50	55.56	63.00	68.56	68.56	59.33
1.00	68.56	74.11	77.78	83.33	72.22
2.50	72.22	74.11	79.66	85.22	74.11
5.00	75.89	77.78	83.33	88.89	77.78
10.0	87.00	94.44	96.33	100.0	88.89
EC_{50}**	0.371	0.20	0.119	0.069	0.263

**active ingredient*

***EC_{50} = A concentration as µg a.i / ml that give 50 % inhibition of the fungal growth.*

Based on the EC_{50} values, the isolate of *P. digitatum* is considered to be more sensitive to tetraconazole, prochloraz and myclobutanil (EC_{50} = o.o69, 0.119 and 0.20 µg a.i. / ml, respectively) followed by trifloxystrobin and iprodione (EC_{50} = 0.263 and 0.371 µg a.i. / ml, respectively). Thus, fungicides belonging to ergosterol biosynthesis inhibitors are more potent to *P. digitatum* growth than those belonging to quinol-oxidizing inhibitors (trifloxystrobin) or glutathione synthetase inhibitors (iprodione). This result was confirmed by Mahmoud & Khalifa (2005) who found that tetraconazole was more potent to *P. digitatum* growth than iprodione, where their EC_{50} values were 0.11 and 0.278 µg / ml, respectively. El-Khawaga-Maii (2006) found that the EC_{50} values of tetraconazole and trifloxystrobin on *P. digitatum* were 0.048 and 0.23 µg / ml, respectively. Eckert & Wild (1983) and Mahmoud (1986) suggested that ergosterol biosynthesis inhibitors could be used for controlling fungi-resistant to benzimidazole fungicides.

Effect of antioxidants on fungal growth:.

The data represented in Table (2) show the effect of the tested antioxidants on fungal growth of *P. digitatum*. The results clearly indicated that selenium and butylated hydroxyanisole started to inhibit the fungal growth at 100 and 75 µg / ml, respectively, with 5.56 % inhibition for each. At 350 µg / ml, moderate inhibition rates were recorded being 59.33 % for Se and 61.11 % for BHA. However, at 500 µg / ml, the two antioxidants gave fungal growth inhibition rates of 85.22 and 88.89 %, respectively. Based on the EC_{50} values of Se (295.22 µg / ml) and BHA (256.21), the *in vitro* sensitivity test indicated that the isolated fungus tolerate high concentrations of both antioxidants. In this respect Zohri *et al*. (1997) reported that the fungal biomass of *Aspergillus parasiticus* was slightly decreased at higher concentrations of selenium. El-Khawaga-Maii (2006) indicated that selenium at 25 µg / ml and BHA at 50 µg / ml had no effect on *P. digitatum*. Also, Sameer (2013) found that *Alternaria solani* tolerated high concentrations of selenium according to its EC_{50} value (263.03 µg / ml).

Concerning BHA, Prusky (1988) indicated that low concentration of BHA 0.1 mM had no effect on *Colletotrichum gloeosporioides*, but < 15 mM of BHA had greatest antifungal activity against *Colletotrichum musae* which cause anthracnose of banana. Thompson *et al*. (1993) found that conidial germination in *Penicillium* spp. was partially inhibited in the presence of 100 µg / ml of BHA at all pH levels. The effective concentrations of BHA that reduced radial growth of *Penicillium* spp. by 50 % (EC_{50}) were reported as 100-275 µg / ml (Thompson, 1997). Ali (2008) found that the EC_{50} values of BHA were 95.5, 138.04 and 371.54 µg / ml for *Fusarium. oxysporum* f.sp. *lycopersici*, *Rhizoctonia solani* and *Alternaria solani*, respectively. Torres *et al*. (2003) found that BHA at 500 µg / ml reduced the growth rate of *F. verticillioides* and *F. proliferatum*.

Table 2: Growth inhibition percent of *Penicillium digitatum* grown on PDA medium amended with candidate concentrations (µg a.i* / ml) of the tested antioxidants.

Concentrations µg a.i*/ml	Antioxidants	
	Selenium (Se)	Butylated hydroxyanisole (BHA)
10.00	0.00	0.00
25.00	0.00	0.00
50.00	0.00	0.00
75.00	0.00	5.56
100.0	5.56	7.44
150.0	5.56	22.22
200.0	22.22	31.55
250.0	35.22	46.33
300.0	50.00	57.44
350.0	59.33	61.11
400.0	70.00	74.11
450.0	77.78	83.33
500.0	85.22	88.89
EC_{50}**	295.22	256.21

**active ingredient*
***EC_{50} = A concentration as µg a.i / ml that give 50 % inhibition of the fungal growth.*

Effect of antioxidants on the fungicidal activity:

Data tabulated in Table (3) show the effects of the tested antioxidants on the fungitoxic action of the tested fungicides against *P. digitatum*. The results clearly indicated that the EC_{50} values of fungicide - antioxidant mixtures were lower than those of the fungicide alone. Therefore, the addition of antioxidants enhanced the potency of the fungicides against growth of *P. digitatum*. This enhancement effect is type and concentration of both antioxidant and fungicide dependent.

Table 3: The effect of antioxidants on the activity of different fungicides against *Penicillium digitatum*.

0.1	EC_{50}* of the fungicides (µg / ml)	EC_{50}* of the fungicides + Se				EC_{50}* of the fungicides + BHA			
		10 µg / ml		15 µg / ml		10 µg / ml		15 µg / ml	
		EC_{50}*	S.E. **	EC_{50}*	S.E. **	EC_{50}*	S.E. **	EC_{50}*	S.E. **
Iprodione	0.371	0.137	2.71	0.076	4.88	0.092	4.03	0.049	7.57
Myclobutanil	0.20	0.068	2.94	0.042	4.76	0.049	4.08	0.037	5.41
Prochloraz	0.119	0.036	3.31	0.020	5.95	0.023	5.17	0.013	9.15
Tetraconazole	0.069	0.028	2.46	0.015	4.60	0.020	3.45	0.012	5.75
Trifloxystrobin	0.263	0.057	4.61	0.033	7.97	0.048	5.48	0.019	13.84

**EC_{50} = A 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*

The results indicated that the efficiency of iprodione (a glutathione synthetase inhibitor) against *P. digitatum* markedly increased by adding both antioxidants and particularly with BHA. Such increment reached 4.03 and 7.57 fold and reached 2.71 and 4.88 fold when both BHA and Se were added at 10 and 15 µg / ml, respectively. Similar tendency was observed with ergosterol biosynthesis inhibitors (myclobutanil, prochloraz and tetraconazole) and with respiration inhibitor (trifloxystrobin). In all cases, BHA exhibited a potential effect to these fungicides more than Se specially on the latter one. When trifloxystrobin fungicide was combined with 10 and 15 µg / ml Se and BHA antioxidants, its fungicidal activity against *P. digitatum* was increased by 4.61 and 7.97 fold and by 5.48 and 13.84 fold, respectively.

These findings are in agreement with other previous studies (Mahmoud *et al*., 1996 a & b; Khan *et al*., 2001; Gwily-Ahlam *et al*., 2002; El-Khawaga-Maii, 2006; Ali, 2008 and Sameer, 2013).

In vivo studies:

Effects of fungicides at 250, 500, 1000, 1500 and 2000 µg / ml and antioxidants at 500, 1000 and 1500 µg / ml separately or in mixtures were evaluated for controlling green mold disease on orange fruits. Results in Table (4) reveal that the green mold disease is sufficiently controlled only at 1500 and 2000 µg / ml of the tested fungicides, and tetraconazole, prochloraz and myclobutanil were the best effective ones followed by trifloxystrobin and finally iprodione. Increasing the concentration of fungicides resulted in increment their effectiveness against the pathogen which ranged between 66.63 % for iprodione and 91.63 % for tetraconazole when all fungicides were applied at 2000 µg / ml. The above results confirmed the *in vitro* experiments and are in agreement with other previous studies (Mahmoud & Hanafy, 1991; Brown, 1992; Mahmoud, 1998; El-Khawaga-Maii, 2006 and Sanchez-Torres & Tuset, 2011).

Results in Table (4) also, showed that antioxidants slightly reduced the incidence of green mold on orange fruits as the PCE values of both Se and BHA at their high concentration (1500 µg / ml) were 29.13 and 33.38 %, respectively. Similar results were obtained by Zohri *et al*. (1997) who found that Se at higher concentrations slightly reduced the fungal biomass of *Aspergillus parasiticus*. Ali (2008) found that the usage of BHA alone at 5 and 10 µg / ml did not consistently control the early blight, fusarium wilt and damping-off diseases on tomato plants comparing with the control treatment. Also, Sameer (2013) found that Se slightly reduced the incidence of early blight on tomato plants.

Table 4: Percentage of control efficiency (PCE) of fungicides and antioxidants alone or in mixtures against incidence of green mold on orange fruits caused by *Penicillium digitatum*.

Fungicides	concentrations (µg / ml)	Percentage of control efficiency							LSD at 5%
		Separate treatments	Candidate concentrations of Se			Candidate concentrations of BHA			
			500 µg / ml	1000 µg / ml	1500 µg / ml	500 µg / ml	1000 µg / ml	1500 µg / ml	
		Separate treatments	4.13	12.50	29.13	8.38	16.63	33.38	7.32
Iprodione	250	8.38	16.63	25.00	37.50	20.88	29.13	41.63	7.14
	500	12.50	41.63	50.00	62.50	37.50	50.00	58.38	9.18
	1000	20.88	66.63	79.13	83.38	70.88	83.38	91.63	10.25
	1500	54.13	70.88	87.50	91.63	79.13	87.50	91.63	8.18
	2000	66.63	79.13	91.63	95.88	87.50	95.88	95.88	7.56
Myclobutanil	250	12.50	20.88	37.50	41.63	29.13	45.88	45.88	7.26
	500	16.63	45.88	66.63	66.63	50.00	66.63	70.88	9.27
	1000	37.50	66.63	83.38	87.50	75.00	91.63	91.63	8.24
	1500	66.63	79.13	91.63	91.63	75.00	95.88	95.88	10.14
	2000	79.13	83.38	100.0	100.0	91.63	100.0	100.0	6.15
Prochloraz	250	12.50	29.13	45.88	54.13	33.38	50.00	54.13	7.56
	500	16.63	58.38	66.63	75.00	58.38	75.00	75.00	10.36
	1000	41.63	75.00	91.63	95.88	83.38	87.50	95.88	8.05
	1500	66.63	83.38	95.88	100.0	91.63	95.88	100.0	10.11
	2000	87.50	91.63	100.0	100.0	91.63	100.0	100.0	8.19
Tetraconazole	250	12.50	37.50	50.00	58.38	37.50	54.13	54.13	7.27
	500	25.00	54.13	70.88	87.50	58.38	83.38	87.50	7.93
	1000	50.00	75.00	91.63	95.88	79.13	95.88	95.88	8.25
	1500	79.13	87.50	95.88	100.0	95.88	100.0	100.0	7.56
	2000	91.63	95.88	100.0	100.0	95.88	100.0	100.0	6.14
Trifloxystrobin	250	8.38	20.88	37.50	45.88	16.63	41.63	50.00	7.26
	500	16.63	37.50	58.38	66.63	37.50	66.63	70.88	8.33
	1000	33.38	70.88	87.50	95.88	75.00	91.63	95.88	10.15
	1500	58.38	75.00	91.63	95.88	87.50	95.88	95.88	7.33
	2000	79.13	87.50	100.0	100.0	91.63	100.0	100.0	7.56
LSD at 5%		6.23	7.26	7.58	8.22	8.18	7.93	7.56	-

Results in Table (4) also showed that the addition of antioxidants to the tested fungicides greatly increased their efficiencies against the green mold disease and reduced their concentrations required to give the best control rates. For example, the PCE values of iprodione, myclobutanil, prochloraz, tetraconazole and trifloxystrobin alone at 250 µg / ml were 8.38, 12.5, 12.5, 12.5 and 8.38 %, respectively, which reached 37.5, 41.63, 54.13, 58.38, 45.88 %, and reached 41.63, 45.88, 54.13, 54.13, 50 % when both Se and BHA, respectively, were separately added at 1500 µg / ml. Moreover, 1000 µg / ml of these fungicides alone exhibited 20.88, 37.5, 41.63, 50.00 and 33.38 PCE, respectively, which became 79.13, 83.38, 91.63, 91.63, 87.5 % and became 83.38, 91.63, 87.5, 95.88, 91.63 % when such concentration was combined with Se and BHA at 1000 µg / ml for each.

The results also clearly indicated that when prochloraz and tetraconazole were applied separately at 1500 µg / ml, they exhibited only 66.63 and 79.13 PCE, respectively. However, both fungicides at the same concentration completely prevented the incidence of the disease when combined with 1500 µg / ml Se or BHA. Also, all fungicides, except iprodione, at their higher concentration (2000 µg / ml) did not completely prevent the green mold incidence. Although, the same concentration completely controlled the disease when combined with 1000 µg / ml of Se or BHA (Table 4). Thus, it could be mentioned that addition of antioxidants to fungicides increased their efficiencies against the incidence of green mold on oranges caused by *P. digitatum*. These effects were also reported by (Mahmoud, 1993; Prusky *et al*., 1995; Khan *et al*., 2001; Simonetti *et al*., 2002 & 2003; Ali, 2008 and Sameer, 2013).

The mechanism of the synergistic effect of the tested antioxidants is not clearly explained. However, Khan *et al*. (2001) reported that antioxidant may make the fungal membrane more leaky and allowing more fungicide to enter into the fungal cells. Simonetti *et al*. (2002 & 2003) stated that antioxidants appear to promote fungicide activity by increasing cell membrane permeability leading to the leakage of cellular enzymes. Also, Prusky *et al*. (1995) and Baider & Cohen (2003) reported that antioxidants may enhance host resistance to fungal infections. Another explanation is the antioxidants may play a part in reducing the oxidation of the fungicides in the fungal cells, which may reduce the fungitoxic action, thus increase the fungitoxicity (Ali, 2008). Generally, this work suggests that the tested antioxidants could be used with low concentrations of the fungicides to increase their fungitoxic action as well as to minimize environmental pollution.

References

Abdel-Monaim, M.F., 2008. Pathological studies of foliar and root diseases of lupine with special reference to induced resistance. Ph.D. Thesis, Fac. Agric., Minia Univ.

Abdel-Monaim, M.F. and M.E. Ismail, 2010. The use of antioxidants to control root rot and wilt diseases of pepper. Not Sci. Biol., 2: 46-55.

Abdel Wahab-Sahar, M. and I.A.S. Rashid, 2012. Safe postharvest treatments for controlling *Penicillium* molds and its impact maintaining navel orange fruits quality. American-Eurasian J. Agric. & Environ. Sci. 12: 973-982.

Ali, W.M.S.A., 2008. Integrated control of some tomato diseases. Ph.D. Thesis, Fac. of Agric., Al-Azhar Univ.

A. Baider, and Y. Cohen, 2003. Synergistic interaction between BABA and mancozeb in controlling *Phytophthora infestans* in potato and tomato and *Pseudoperonospora cubensis* in cucumber. Phytoparasitica, 31 (4): 399-409.

Brown, G.E., 1992. Evaluation of iprodione and fosetyl-Al and other fungicides for postharvest citrus decay control. Proc. Fla. State Hort. Soc., 105: 131-134.

Bus, V.G., 1992. ED_{50} levels of *Penicillium digitatum* and *P. italicum* with reduced sensitivity to thiabendazole, benomyl and imazalil. Postharvest Biology and Technology, 1: 305-315.

Eckert, J.W. and M.J. Kolbezen, 1977. Influence of formulation and application method on the effectiveness of benzimidazole fungicides for controlling postharvest diseases of citrus fruits. Neth. J. Pl. Path. 83: 343-352.

Eckert, J.W. and B.L. Wild, 1983. Problems of fungicide resistance in *Penicillium* rot of citrus. Pages 525-555 in Pest Resistance to Pesticides. G.P. Georghion and T. Saites eds. Plenum Publishing Crop, New York.

Eckert, J.W. and I.L. Eaks, 1989. Postharvest disorders and diseases of citrus fruits. Pages 180-260 in: The Citrus Industry: Volume V. W. Reuther, C.E. Calavan, and G.E. Carmen, eds. University of California, Division of Agriculture and Natural Resources, Oakland.

El-Khawaga- Maii, A. 2006. Effect of antioxidants on the efficacy of some fungicides. Ph.D. Thesis, Fac. of Science, Al-Azhar Univ.

El-Mougy, N.S., N.G. El- Gamal and F. Abd-El-Kareem, 2008. Use of organic acids and salts to control postharvest diseases of lemon fruits in Egypt. Arch. Phytopathol. Plant Protect. 41 (7): 467-476.

Finney, D.I., 1971. Probit analysis. Cambridge University Press, London, 450 p.

Giridhar, P. and S.M. Reddy, 2001. Phenolic antioxidants for the control of some mycotoxigenic fungi. Journal of Food Science and Technology Mysore, 38 (4): 397-399.

Gwily-Ahlam, M., M.F. Abdel-Lateef, A.A. Razak and T.E. Ramadan, 2002. Influence of certain fungicides, selenium and their mixtures against growth and morphological feature of *Heleminthosporum sativum*. 2nd International Conference for Plant Protection Research Institute, Cairo, Egypt, December 21-24, 2002, 751-756.

Hassan, M.E.M., S.S. Abdel-Rahman, I.H. El-Abbasi and M.S. Mikhail, 2006. Inducing resistance against faba bean chocolate spot disease. Egypt. J. Phytopathol. 34: 69-79.

Khan, S.H., J. Aked and N. Magan, 2001. Control of the anthracnose pathogen of banana (*Colletotrichum musae*) using antioxidants alone and in combination with thiabendazole or imazalil. Plant Pathology, 50: 601-608.

Mahmoud, M.B., 1986. Resistance of citrus rots to some fungicides. Possible counter measures to fungicides resistance. Ph. D. thesis, Faculty of Agriculture, Al-Azhar University, Egypt.

Mahmoud, M.B., 1993. Effect of selenium on the efficiency of thiabendazole against resistant isolates of *Penicillium digitatum* and *P. italicum*. Afr. J. Mycol. & Biotech., 1: 131-144.

Mahmoud, M.B., 1998. Evaluation of alternate treatments and fungicide mixtures for reducing resistance in *Penicillium expansum* to fungicides. Al-Azhar J. Agric. Res., 28: 145-158.

Mahmoud, M.B. and S.M. Hanafy, 1991. Acquired resistance of *Penicillium digitatum* and *Penicillium italicum* to some postharvest fungicides on navel oranges. Ann. Agric. Sci. Ain Shams Univ., Cairo, 36: 681-691.

Mahmoud, M.B. and H.M.S. Khalifa, 2005. Some biochemical changes correlated with resistance in *Penicillium digitatum* to fungicides with different mode of actions. Egypt. J. Biotechnol., 20: 1-15.

Mahmoud, M.B., M. Ahlam-Gwily, M.F. Abdel-Lateef, A.A. Razak and T.E. Ramadan, 1996a. Influence of selenium on efficiency of TBZ and carboxin-captan against growth, conidiogenesis and morphological features of some fungi. Proc. 1st Int. Confer. On Fungi: Hops & Challenges, Cairo, Egypt, 2-5 Sept., 1: 31-44.

Mahmoud, M.B., M. Ahlam-Gwily, M.F. Abdel-Lateef, A.A. Razak and T.E. Ramadan, 1996b. Influence of selenium on efficiency of TBZ and carboxin-captan to control *Alternaria tenuis* and *Fusarium oxysporum* on broad-bean. Proc. 1st Int. Confer. On Fungi: Hops & Challenges, Cairo, Egypt, 2-5 Sept., 1: 57-73.

Margot, P., F. Huggenberger, J. Amrein and B. Weiss, 1998. CGA 279202, a new broad – spectrum strobilurin fungicide. Proceedings of Brighton Crop Protection Conference on Pests and Diseases, UK, November 16-19, (2): 375-382.

Prusky, D., 1988. The use of antioxidants to delay the onset of anthracnose and stem end decay in avocado fruits after harvest. Plant Dis., 72 (5): 381-384.

Prusky, D., H.D. Ohr, N. Grech, S. Campbell, I. Kobiler, G. Zauberman and Y. Fuchs, 1995. Evaluation of antioxidant butylated hydroxyanisole and fungicide prochloraz for control of postharvest anthracnose of avocado fruit during storage. Plant Dis., 79 (8): 797-800.

Rajkumar, M., K.J. Lee and H. Freitas, 2008. Effects of chitin and salicylic acid on biological control activity of *Pseudomonas* spp. against damping – off of pepper. South African J. of Botany, 74: 268-273.

Sameer,W.M., 2013. Synergistic action of profenofos insecticide and selenium to some fungicides against tomato early blight (*Alternaria solani*). Afr. J. Mycol. & Biotech, 18: 37-53.

Samoucha, Y. and Y. Cohen, 1989. Field control of potato late blight by synergistic fungicidal mixtures. Plant disease 73: 751-753.

Sanchez-Torres, P. and J.J. Tuset, 2011. Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. Postharvest Biology and Technology, 59: 159-165.

Simonetti, G., A. Villa and N. Simonetti, 2002. Enhanced contact activity of fluconazole in association with antioxidants against fluconazole-resistant organisms. Journal of Antimicrobial Chemotherapy, 50(2): 257-259.

Simonetti, G., N. Simonetti and A. Villa, 2003. Increase of activity of tioconazole against resistant microorganisms by the addition of butylated hydroxyanisole. International Journal of Antimicrobial Agents, 22 (4): 439-443.

Smilanick, J.L., M.F. Mansour, D.A. Margosan, F. Mlikota Gabler and W.R. Goodwine, 2005. Influence of pH and NaHCO3 on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. Plant Dis., 89: 640-648.

Smilanick, J.L., M.F. Mansour and D. Sorenson, 2006. Pre- and postharvest treatments to control green mold of citrus fruit during ethylene degreening. Plant Dis., 90: 89-96.

Snedecor, G.W. and W.G. Cochran, 1969. Statistical method. The lowa Status Uni. Press, Ames, IA593pp.

Thompson, D.P., 1997. Effect of phenolic compounds on mycelial growth of Fusarium and Penicillium species. Journal of Food Protection, 60 (10): 1262-1264.

Thompson, D.P., L. Metevia and T. Vessel, 1993. Influence of pH alone and in combination with phenolic antioxidants on growth and germination of mycotoxigenic species of *Fusarium* and *Penicillium.* Journal of Food Protection, 56 (2): 134-138.

Torres, A.M., M.L. Ramirez, M. Arroyo, S.N. Chulze and N. Magan, 2003. Potential use of antioxidants fpr control of growth and fumonisin production by *Fusarium verticillioides* and *Fusarium proliferatum* on whole maize grain. International journal of Food Microbiology, 83 (3): 319-324.

Zhang, J. and P.P. Swingle, 2005. Effects of curing on green mold and stem-end rot of citrus fruit and its potential application under Florida packing system. Plant Dis. 89: 834-840.

Zohri, A.A., M.S. Sabah and M.E. Mostafa, 1997. Effect of selenite and tellurite on the morphological growth and toxin production of *Aspergillus parasiticus* var. *globosus* IMI 120920. Mycopathologia, 139: 51-57.