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Effects of Combination of Diniconazole Fungicide with Antioxidants on Vital Ingredients of Certain Fungi

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Abstract

The main objective of this study is to evaluate addition of some antioxidants to diniconazole fungicide on the three isolated fungi, i.e., *Alternaria alternata*, *Fusarium graminearum* and *Pyrenophora tritici-repentis*. Three antioxidants, i.e., selenium at 5ppm, butylated hydroxyanisole at 50ppm and ascorbic acid at 100ppm were combined with different concentrations of diniconazole, 0.1, 0.5, 1, 3 and 5ppm, in Potato Dextrose Broth (PDB) medium. The vital ingredients, i.e., fresh weight, total carbohydrates, lipids and proteins of the fungi were determined. The results clearly indicated that fungicide-antioxidant mixtures decreased the vital ingredients of the tested fungi more effectively than the fungicide or antioxidants applied alone. Regardless the isolated fungi, the percent reduction of any of the vital ingredients followed the order; Se > BHA > AsA. These effects increased with increasing the concentration of the fungicide in culture medium. This means that antioxidants, especially selenium, enhanced the fungitoxic activity of diniconazole against the isolated fungi.

Keywords: diniconazole, antioxidants, *Alternaria alternata*, *Fusarium graminearum*, *Pyrenophora tritici-repentis*.

Introduction

Fungi, which may attack the seed, root, crown and foliage, cause reduction in crop production. Seed and soil-borne fungi are the most important wheat diseases. Several fungi were recorded as pathogens of seed and root-rot diseases on wheat such as *Alternaria alternata*, *Fusarium* spp. (*F. culmorum F. graminearum*, *F. equiseti* and *F. solani*) *Pyrenophora tritici-repentis* and *Rhizoctonia solani* (El-Kholy, 1999, Hashem and Hamada-Afaf, 2002; Atef-Nagwa, 2008; Asran and El-Eraky-Amal, 2011; Harvey *et al*, 2015 and El-Ballat, 2017).

Several reports illustrated that, the addition of antioxidants to fungicide enhance the activity of fungicide. Butylated hydroxyanisole enhanced the efficacy of difenoconazole, flutolanil, metiram, tetraconazole and trifloxystrobin against *F. oxysporum*, *lycopersici*, *R. solani* and *A. solani*. As well as, selenium enhanced the efficacy of azoxystrobin, difenoconazole, metalaxyl and trifloxystrobin against *A. solani*. Additionally, ascorbic acid and BHA increased the fungitoxic activity of flutolanil, metalaxyl and tetraconazole against *A. niger*, *F. oxysporum* and *P. chrysogenum* (Ali, 2008; Ali, 2013 and Mahmoud-Amira 2016)

The aim of this work is to evaluate addition of certain antioxidants to diniconazole on the fresh weight, total carbohydrates, proteins and lipids of the tested fungi.

Materials and methods

The present study was carried out in the laboratory of Plant Protection Department, Faculty of Agriculture, Cairo, AL-Azhar University, Egypt during 2016.

Fungicide and antioxidants

Diniconazole (Sumi eight 2 % WP) was produced by Kafr El-Zayat for Chemicals and Pesticides Co. This fungicide is systemic, belonging to triazole group. The chemical name is (*E*)-(*RS*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pent-1-en-3-ol.

The antioxidants were ascorbic acid, AsA, ($C_6H_8O_6$, 100% w/w) provided by Biotech for laboratory chemical Co., butylated hydroxyl-anisole, BHA, ($C_{11}H_{16}O_2$, 100% w/w) and selenium, Se, (sodium selenite, Na_2SeO_3 98.8%) were obtained from El-Goumhouria Co.

Fungi

The fungi were isolated from seeds and roots of wheat according to the method described by Moubarak and Abdel Monaim (2011). These fungi were *Alternaria alternata*, *Fusarium graminerum* and *Pyrenophora tritici-repentis*. Identification of fungal isolates was confirmed by Plant Pathology Dept. Faculty of Agriculture, Cairo, AL-Azhar University, Egypt.

Effect of the antioxidants on fungicide activity

Fungi were cultured into 250 ml conical flask capacity, containing 100 ml of liquid Potato Dextrose Broth (PDB) medium amended with different concentrations of fungicide alone, separately antioxidants or a mixture of both. The concentrations of fungicide were 0.1, 0.5, 1, 3 and 5 ppm. Ascorbic acid was used at 100 ppm (AsA 100ppm), butylated hydroxyl-anisole at 50 ppm (BHA 50ppm), however selenium at 5 ppm (Se 5ppm). The flasks were incubated at 25±2°C for 10 days. The cultures of fungi were harvested by filtration using Buchner funnel and washed thoroughly with distilled water Patil *et al.* (2011). The fresh weights of the mycelia were determined as milligram. Free cell extracts were obtained by grounding the mycelial matrix with an approximately equal weight of clean sand in mortars under cold condition and extracted with 70 % (v/v) ethyl alcohol in case determination of total proteins and total carbohydrates while using chloroform-methanol 3:1(v/v) in case total lipids David and Van Etten (1966). The obtained slurry was centrifuged at 6000 rpm for 20 minutes. The supernatant was used to determinate the total carbohydrates, lipids and proteins according to **Dubois *et al*. (1956)**, **Zollner and Kirsch („„„„„ 1962)** and **Doumas *et al.* (1981)**, respectively.

Results and Discussion

The results in Table (1) show the effect of tested antioxidants, fungicide used alone and in combinations on fresh weight of *Alternaria alternata, Fusarium graminerum* and *Pyrenophora tritici-repentis*. Generally, the fresh weight of fungi decreased by increasing the concentration of diniconazole in culture medium. The effect of diniconazole on *F. graminerum* was higher than *P. tritici-repentis* flowed by *A. alternata*. Fungicide-antioxidant mixtures decreased fresh weight of fungi more effectively than the fungicide or antioxidant applied separately. For example, diniconazole alone at 3 ppm reduced fresh weight of *A. alternata* by 9.98 %, interestingly the fresh weight reduced by 13.65% when AsA 100ppm was added to the fungicide-amended medium, by 15.69% with addition BHA 50ppm and 20.31 % with Se 5ppm. Diniconazole alone at 5 ppm decreased fresh weight of *F. graminerum* by 32.84%, interestingly it reduced by 34.55, 38.79 and 44.28 % of fresh weight when AsA 100ppm, BHA 50ppm and Se 5ppm were added to the fungicide-amended medium, respectively. The above trend agreed with **Ali (2008)** who mentioned that mixed difenoconazole at 10ppm with 5 ppm of BHA decreased fresh weight of *F. graminerum* more effectively than the fungicide or antioxidant applied alone.

Table 1. Effect of diniconazole applied alone or in a mixture with antioxidants on the percent reduction of fresh weight of the tested fungi.

Fungi	Antioxidants (ppm)	Fungicide concentrations (ppm)					
		Control*	0.1	0.5	1	3	5
		Reduction % of fungi fresh weight					
Alternaria altrnata	Control**	0.00	5.20	6.91	8.45	9.98	13.31
	AsA100	1.62	7.74	9.13	11.52	13.65	16.13
	BHA50	4.69	8.28	11.43	14.08	15.69	20.48
	Se5	6.48	10.32	13.05	17.15	20.31	24.32
Fusarium graminerum	Control**	0.00	21.85	24.71	27.80	30.55	32.84
	AsA100	8.01	24.59	26.20	30.09	32.15	34.55
	BHA50	10.53	27.69	29.63	32.61	35.47	38.79
	Se5	15.45	31.12	34.44	38.90	41.88	44.28
Pyrenophora tritici-repentis	Control**	0.00	9.28	11.62	14.14	17.03	21.17
	AsA100	0.63	11.08	14.05	16.39	18.11	22.79
	BHA50	1.84	13.42	16.76	21.26	25.13	29.09
	Se5	4.14	15.58	17.93	22.16	25.86	32.34

*control: medium without fungicide. **control: medium without antioxidant.

Generally, the highest percentage reduction of fresh weight was 44.28% for fungicide at 5ppm mixed with Se 5ppm on *F. graminerum*. It was noted that, addition of Se 5ppm to diniconazole at all concentrations was better than BHA 50ppm flowed by AsA 100ppm.

Results illustrated in Table (2) indicate that increasing the concentration of tested fungicide in culture medium resulted in great reduction of total carbohydrates content of fungi. Also, the potency of fungicide to reduce total carbohydrates content in fungi increased by adding antioxidants to fungicide-amended media. The highest effect was at concentration 5 ppm of Se mixed with 5 ppm of diniconazole against *F. graminerum*, whereas, the carbohydrates reduction percent was 97.36%. These results are in agreement with that obtained by **Gowily-Ahlam *et al*. (1996),** who found that thiabendazole- selenium mixtures reduced carbohydrates in *A. tenus* and *F. oxysporum* rather effectively than thiabendazole alone. **El-Khawaga-Maie (2006)** mentioned that the addition of antioxidants (AsA, BHA and Se) to tetraconazole, penconazole and fenarimol fungicides increased the reductive potency of fungicides to reduce carbohydrates, lipids and proteins of *F. oxysporum, P. tritici-repentis* and *R. solani*.

Table 2. Effect of diniconazole applied alone or in a mixture with antioxidants on the percent reduction of total carbohydrates of the tested fungi.

Fungi	Antioxidants (ppm)	Fungicide concentrations (ppm)					
		Control*	0.1	0.5	1	3	5
		Reduction % of fungi total carbohydrates					
Alternaria altrnata	Control**	0.00	17.92	23.99	28.29	33.94	40.20
	AsA100	8.08	21.69	27.41	35.48	43.15	47.25
	BHA50	9.99	35.01	41.89	46.25	48.99	53.01
	Se5	13.25	50.76	58.11	64.89	70.64	77.48
Fusarium graminerum	Control**	0.00	14.16	16.21	27.27	38.71	44.02
	AsA100	5.56	42.18	50.77	59.21	64.57	71.76
	BHA50	7.54	69.25	73.88	67.61	82.64	87.82
	Se5	9.69	87.56	91.21	92.71	95.48	97.36
Pyrenophora tritici-repentis	Control**	0.00	14.61	17.38	19.06	26.97	36.04
	AsA100	4.29	30.40	36.68	44.68	53.65	59.89
	BHA50	7.36	53.19	63.82	68.25	75.16	80.97
	Se5	10.09	79.30	85.61	87.21	92.63	96.38

*control: medium without fungicide. **control: medium without antioxidant.

Results in the Table (3) indicate that the fungicide-antioxidant mixtures reduced total lipids of *A. alternata*, *F. graminerum* and *P. tritici-repentis* rather effectively than the fungicide or antioxidants used alone. It was noted that, effect of diniconazole used alone on *A. alternata* was more effectively than *P. tritici-repentis* and *F. graminerum* at all concentrations. Se at 5ppm affects all fungi more than BHA at 100ppm and AsA at 50ppm.

Table 3. Effect of diniconazole applied alone or mixed with antioxidants on the percent reduction of total lipids of the tested fungi.

Fungi	Antioxidants (ppm)	Fungicide concentrations (ppm)					
		Control*	0.1	0.5	1	3	5
		Reduction % of fungi total lipids					
Alternaria altrnata	control**	0.00	37.37	41.56	53.38	59.57	64.48
	AsA100	2,08	60.43	64.16	66.08	71.74	77.54
	BHA50	6.67	76.97	80.85	84.34	86.44	87.37
	Se5	9.50	89.68	90.03	92.53	93.45	94.48
Fusarium graminerum	Control**	0.00	9.09	9.49	10.50	15.94	25.48
	AsA100	6.39	13.45	19.65	26.17	33.85	40.05
	BHA50	8.53	38.49	43.56	46.25	49.77	55.20
	Se5	8.81	62.05	71.64	84.15	87.39	91.14
Pyrenophora tritici-repentis	Control**	0.00	22.50	26.29	29.17	35.58	37.79
	AsA100	2.08	27.83	33.00	35.42	47.33	50.67
	BHA50	6.67	59.83	62.17	71.01	83.92	86.92
	Se5	9.50	88.50	94.33	95.83	96.46	97.87

*control: medium without fungicide. **control: medium without antioxidant.

Generally, increasing the concentration of tested fungicide, especial with antioxidants, resulted in increasing the reduction of total lipids in isolated fungi. Diniconazole at 3 ppm caused 59.57% reduction of lipids in the mycelial matrix of *A. alternata*, interestingly, the same concentration reduced lipids by 71.74% as a result of addition of AsA 100ppm, 86.44% with BHA 50ppm and 93.45% by Se 5ppm. As well as, diniconazole alone at 5 ppm caused 25.48% lipids reduction of *F. graminerum*, but lipids reduction percent increased to 40.05% by adding AsA 100ppm, 55.20% by BHA 50ppm and 91.14% with addition of Se 5ppm to diniconazole-amended medium. The obtained results illustrated that a mixture of 5 ppm Se and 5 ppm diniconazole caused highest reduction of lipids (97.87%) in the mycelial matrix of *P. tritici-repentis*. Generally, the effect of diniconazole-antioxidants mixture on lipids of *A. alternata* was higher than *F. graminerum* and *P. tritici-repentis*.

Results in Table (4) indicate that the reduction of total proteins in mycelia of the tested fungi was positively correlated with increasing the concentration of tested fungicide in culture medium. Concerning the fungicide-antioxidant mixtures, the results showed that the addition of antioxidants to fungicide-amended media increased the potency of the reduction of proteins of the fungal matrix compared to fungicide alone. The least reduction percent of proteins was 6.24% at AsA 100ppm of *P. tritici-repentis*. On the other hand, the highest reduction percent of proteins was 94.43% at Se 5ppm mixed with 5 ppm of diniconazole of *A. alternata*. The above data are in agreement with **Ali (2008)** who demonstrated that the addition of BHA to difenoconazole and tetraconazole fungicides increased the efficiency of these fungicides to reduce fresh weight, total carbohydrates, lipids and proteins of *A. solani*, *F. oxysporum* and *R. solani*. **Mahmoud-Amira (2016)** cited that tetraconazole

Table 4. Effect of diniconazole applied alone or mixed with antioxidants on the precent reduction of total proteins of the tested fungi.

Fungi	Antioxidants (ppm)	Fungicide concentrations (ppm)					
		control*	0.1	0.5	1	3	5
		Reduction % of fungi total lipids					
Alternaria altrnata	control**	0.00	22.64	26.14	41.51	56.24	76.01
	AsA100	9.63	34.32	42.32	55.88	85.89	91.77
	BHA50	12.67	32.88	52.20	72.06	90.39	92.21
	Se5	15.36	42.32	62.89	77.45	92.08	94.43
Fusarium graminerum	Control**	0.00	16.77	32.18	55.65	68.52	80.95
	AsA100	11.99	32.84	55.95	73.90	81.39	91.11
	BHA50	20.66	23.55	51.05	64.89	77.71	84.06
	Se5	21.45	19.18	44.00	61.95	73.12	78.68
Pyrenophora tritici-repentis	Control**	0.00	13.97	35.54	73.77	78.43	88.19
	AsA100	6.24	16.18	38.60	75.98	85.17	89.26
	BHA50	9.94	24.88	43.38	78.43	87.01	90.98
	Se5	11.56	28.43	52.57	75.98	90.38	93.61

*control: medium without fungicide. **control: medium without antioxidant.

decreased total carbohydrates, lipids and proteins of *A. solani*, *F. oxysporum* and *P. chrysogenum.* The results also showed the appearance of antagonistic effect when Se 5ppm was added to the media containing diniconazole at concentrations 5ppm, where reduction percent of protein content in *F. graminerum* was 80.95% with diniconazole alone while it was 78.68% with diniconazole-Se mixtures. The antagonistic effect is not clear, so it need further studies.

The obtained results clearly indicated that a combination of antioxidants with diniconazole increased the efficiency of fungicide against the isolated fungi more effectively than the fungicide or antioxidants applied alone. Increasing the efficiency of fungicide may be attributing to disorder in the mycelial membrane; this may be make the membrane allows by increasing concentration of fungicide into the fungal cells. The mechanism of the synergistic effect of the tested antioxidants is not clearly explained. However, Khan *et al*., (2001); Atroshi *et al*., (2002) and Simonetti *et al*. (2003) reported that antioxidant may make the fungal membrane more leaky and allowing more fungicide to enter into cell of the fungus and leading to leakage of cellular enzymes. Another explanation was reported by Ali (2008) who suggested that antioxidants may be reducing the oxidation of the fungicides which might increase their fungitoxicity.

Generally, the results showed that the addition of antioxidants to diniconazole increased the potency of fungicide to reduce fresh weight, total carbohydrates, lipids and proteins of the isolated fungi which may be explained its mechanism of action.

Finally, this study suggests that the tested antioxidants could be added to diniconazole fungicide to increase the effectively, additionally to reduce environmental pollution.

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تأثير خلط مبيد الفطر دينكونازول مع مضادات الأكسدة على المكونات الحيوية لبعض الفطريات

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تهدف هذه الدراسة إلى تقييم تأثير إضافة بعض مضادات الأكسدة إلى مبيد الفطر دينكونازول على الفطريات الثلاثة *Alternaria alternata*, *Fusarium graminearum* and *Pyrenophora tritici–repentis.* تم خلط ثلاثة مضادات أكسدة، وهى السيلينيوم بتركيز 5 جزء في المليون، بيوتيل هيدروكسي أنسول بتركيز 50 جزء في المليون وحامض الأسكوربيك عند 100 جزء في المليون (كلٍ على حدى) مع تركيزات مختلفة من الدينكونازول (0.5 ، 1 ، 3 ، 5 جزء في المليون) بإستخدام بيئة سائلة عبارة عن مستخلص البطاطس مع الدكستروز. تم تقدير المكونات الحيوية، الوزن الغض والمحتوى الكلى للكربوهيدرات والبروتينات والدهون للفطريات المعزولة. أشارت النتائج بوضوح إلى أن إستخدام مخاليط مضادات الأكسدة مع مبيد الفطر قد خفضت المكونات الحيوية للفطريات المعزولة بشكل أكثر فاعلية من إستخدام مبيد الفطر أو مضادات الأكسدة بشكل منفرد. وبغض النظر عن الفطريات المعزولة، فإن انخفاض نسبة المكونات الحيوية كان أكبر فى حالة إستخدام السيلينيوم يليه بيوتيل هيدروكسي أنسول ثم حامض الأسكوربيك (Se > BHA > AsA) وهذا يعني أن مضادات الأكسدة، وخاصة السيلينيوم ، عززت من فعالية الدينكونازول ضد الفطريات المعزولة.