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Hydroquinone, A Promising Antioxidant for Managing Seed-borne Pathogenic Fungi of Peanut

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Abstract: Six antioxidants were tested *in vitro* and *in vivo* against seed-borne fungi of peanut. Hydroquinone showed a highly significant effect on the growth of the following fungi: *Cephalosporium* sp., *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotium bataticola* and *Verticillium* sp. Soaking peanut seeds in a 20 mM water solution of hydroquinone for 12 hours before planting significantly decreased pre- and post-emergence damping off incited by all tested fungi. Seedling survival and plant vigor in all treatments, including the check, was increased.

Key words: Hydroquinone, antioxidants, seed-borne fungi, peanut

Introduction

Because of the hazards associated with using fungicides for controlling seed-borne fungi, especially in edible seeds, less toxic compounds are being evaluated as potential substitutes. Antioxidants have been used to control a number of pathogens, including *Phytophthora infestans* on potato (Arnoldi *et al.*, 1989), *Colletotrichum gloeosporioides* on avocado (Prusky, 1988) and *Rhizopus stolonifer* on grape berries, *Sclerotinia sclerotiorum* on lettuce and *Botrytis cinerea* on tomato and pepper (Elad, 1992). Galal and Abdou (1996) evaluated sixteen antioxidants *in vitro* against three *Fusarium* species: *F. moniliforme*, *F. oxysporum* and *F. solani*. Most of the antioxidants reduced linear growth of the fungi on Czapek's agar medium at a concentration of 1.0 mM and they were significantly effective at 10 mM. Adding antioxidants directly to the soil was more effective in controlling fusarium diseases than using them as seed treatments. The inhibitory effect of antioxidants increased with increasing concentration. No increase in effect was observed when antioxidants were combined.

The present investigation studied the effects of some common antioxidants on reducing or at least retarding the development of a number of seed-borne fungi isolated from peanut seeds.

Materials and Methods

Fungi: The following pathogenic fungi were isolated from peanut seeds using the seed health testing methods recommended by the Rules of the International Seed Testing Association (ISTA, 1993): *Cephalosporium* sp., *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotium bataticola* and *Verticillium* sp.

Antioxidants: Benzoic acid, citric acid, salicylic acid, sodium benzoate, sodium citrate and hydroquinone (ADWIC Co., Egypt) were the antioxidants selected for this investigation. Each antioxidant was dissolved in water or ethanol to obtain the desired concentrations (10, 15 and 20 mM).

Blotter test: Ten samples from previously examined seed lots colonized by the above mentioned fungi were used for seed health testing. Each replicate consisted of ten seeds plated in an 11 cm diameter Petri-dish containing three layers of blotters soaked in the target solution (water solutions of the tested antioxidant at different concentrations). Blotters soaked in tap water were used as a check. The plates were incubated at $24 \pm 2^\circ\text{C}$ for 7 days under 12 hour alternating cycles of cool white fluorescent light and darkness. The incubated seeds were then examined under a stereoscopic binocular microscope (6-50 X) for the presence and identification of fungi. Whenever necessary, a compound microscope was used to confirm the identification. The most promising antioxidant in controlling seed-borne fungi,

hydroquinone, was further tested to determine its effects on the fungi *in vivo* and *in vitro*.

Linear growth test (*in vitro* studies): Mycelial disks (0.9 mm diameter) cut from 7 day old cultures of each fungus were placed in the center of Petri-dishes containing Czapek's agar media supplemented with different concentrations of hydroquinone to determine its inhibitory effect on the fungus. All cultures were incubated at $20 \pm 1^\circ\text{C}$ for 7 days under 12 hour alternating cycles of cool white fluorescent light and darkness. The linear growth of each fungus was recorded.

Greenhouse experiments (*in vivo* studies): The soil in 10 cm diameter pots was infested with fungal inocula prepared by growing each fungus on peanut pod shells (1% w/w). Pots containing soil mixed with the same amounts of noninfested medium served as checks. Replicates of twenty-five pots were used per treatment. The pots were kept in a greenhouse for 7 days at temperatures of $25 \pm 2^\circ\text{C}$ to allow the fungi to adapt before planting seeds. During that period, the soil was moistened when necessary. Seeds were soaked (50 seeds per treatment) in 100 ml antioxidant solution (20 mM) for 12 hours, then planted in the infested soil (2 seeds/pot). Daily observations were made for germination and for pre- and post-emergence damping off.

Results

Blotter Test: Data presented in Table 1 show that the most effective antioxidant against the following fungi: *Cephalosporium* sp., *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotium bataticola*, and *Verticillium* sp. was hydroquinone, which inhibited the growth of all tested fungi at a concentration of 20 mM. The least effective antioxidant was citric acid. However, the pH value of the tested hydroquinone did not show a significant difference in the concentrations used (Max. 6.7 and Min. 6.53).

Linear Growth: Since hydroquinone was the most affective antioxidant in the blotter test, additional tests were run to study its effect on linear growth of the target fungi. Data in Table 2 show that hydroquinone, even at low concentrations (10 and 15 mM) prevented the mycelial growth of *Cephalosporium* sp. and *S. bataticola*. No growth was observed in the other fungi at a concentration of 20 mM.

Effect of hydroquinone on seed-borne fungi of peanut under greenhouse conditions: Soaking seeds in a 20 mM hydroquinone solution for 12 hours before planting in pots of infested soil decreased pre- and post-emergence damping off incited by all tested fungi. There was an increase in seedling survival with all antioxidant treatments compared to the check treatments (Table 3). Seedling vigor was significantly increased with hydroquinone

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Table 1: Fungal detection percent on peanut seeds grown on blotter treated with different antioxidant at concentrations from 10-20 mM

Fungi Compounds	Check (Tap Water)	Salicylic acid mM			Citric acid mM			Sodium benzoate mM			Benzoic acid mM			Sodium citrate mM			Hydroquinone mM		
		10	15	20	10	15	20	10	15	20	10	15	20	10	15	20	10	15	20
<i>Cephalosporium</i> sp.	3	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0
<i>Fusarium moniliforme</i>	14	10	6	2	10	10	6	6	2	2	4	4	2	2	0	0	0	0	0
<i>Fusarium oxysporum</i>	8	4	4	2	2	2	0	0	0	0	2	2	2	0	0	0	0	0	0
<i>Fusarium solani</i>	28	12	9	6	24	18	12	18	16	12	18	18	12	32	22	12	10	8	0
<i>Rhizoctonia solani</i>	4	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
<i>Sclerotium bataticola</i>	8	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
<i>Verticillium</i> sp.	4	4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

pH value of the tested antioxidants

pH	6.22	2.0	1.69	1.4	2.1	1.88	1.72	6.45	6.53	6.65	2.69	2.56	2.38	8.38	8.47	8.6	6.53	6.51
6.7																		

Table 2: Effect of hydroquinone (20 mM) on linear growth (cm) of the tested fungi 7 days after inoculation

Fungi	Check (cm)	Hydroquinone (cm)		
		10 mM	15 mM	20 mM
<i>Cephalosporium</i> sp.	2.1	NG	NG	NG
<i>Fusarium moniliforme</i>	9.7	3.6	1.7	NG
<i>Fusarium oxysporum</i>	10.5	4	2	NG
<i>Fusarium solani</i>	7.5	3.5	2.1	NG
<i>Rhizoctonia solani</i>	11.0	5.3	2.5	NG
<i>Sclerotium bataticola</i>	11.0	NG	NG	NG
<i>Verticillium</i> sp.	3.7	2.3	1.7	NG

NG: No Growth

Table 3: Effect of hydroquinone treatment on seedling survival of peanut seeds grown in pathogen-infested soil under greenhouse conditions

		Pre-emergence	Post-emergence	Seedling
		damping off	damping off	survival
Check	N	0	0	100
	H	0	0	100
<i>Cephalosporim</i> sp.	F	60	10	30
	F+H	20	0	80
<i>F. moniliforme</i>	F	40	30	30
	F+H	0	0	100
<i>F. oxysporum</i>	F	50	10	40
	F+H	10	0	90
<i>F. solani</i>	F	40	20	40
	F+H	0	0	100
<i>R. solani</i>	F	70	20	10
	F+H	10	0	90
<i>Sclerotium bataticola</i>	F	50	30	20
	F+H	10	0	90
<i>Verticillium</i> sp.	F	60	20	20
	F+H	30	0	70

N: Healthy seeds planted in autoclaved soil. F: Healthy seeds planted in infested soil. H: Hydroquinone soaked seeds planted in autoclaved soil. F+H: Hydroquinone soaked seeds planted in infested soil.

treated seeds as compared to the untreated and the check seedlings.

Discussion

The hazards associated with fungicide treatments, especially when seeds are grown in soil contaminated with fungicides, initiated the search for a safer seed or soil treatment to control

fungi attacking peanut. Consequent research indicated that hydroquinone is a relatively safe and effective antioxidant for controlling the important seed-borne fungi of peanut. It prevents all mycelial growth at a concentration of 20 mM. Pre- and post-emergence damping off were significantly reduced when seeds were soaked in a 20 mM hydroquinone solution for 12 hours before planting. Determination of hydroquinone pH showed that it has no role in inhibiting the fungal growth, but the inhibition may be due to properties of the chemical itself. These results are in agreement with the findings of Galal and Abdou (1996) who showed that hydroquinone inhibited the growth of *Fusarium* spp. in cowpeas *in vitro*.

No other safe, acceptable alternative to conventional fungicides has been found for the control of seed-borne fungi. Due to the effects of hydroquinone on fungal growth and plant vigor, it appears that the antioxidant may be a promising low-toxicity compound for controlling seed-borne fungi of peanut. Its effects appear to be due to the specific and well known antioxidant properties of hydroquinone (Windholz, 1976).

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