

USE OF BENZOTHIADIAZOLE (BTH) FOR INDUCING SYSTEMIC RESISTANCE IN COTTON SEEDLINGS AGAINST SOME SOIL-BORNE PATHOGENIC FUNGI

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ABSTRACT

The resistance elicitor Benzothiadiazole (BTH) has no significant inhibitory effects on seed germination of cotton cultivars Giza 86 and Giza 90 or on mycelial growth of the tested fungi, *i.e.* *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Pythium* sp. and *Rhizoctonia solani*. BTH seed treatment (seed soaking) resulted in resistant cotton plants against infection by all pathogenic fungi tested. Efficiency of BTH to induce resistance in cotton seedlings varied with BTH concentration and fungi tested. Increasing BTH concentration enhanced resistance capacity of cotton cultivars. In addition. When the concentration was increased to 100 ppm, BTH significantly increased plant height from 8.36 to 26.07 % and from 8.93 to 28.82 % for cvs. Giza 86 and Giza 90, respectively. Also, it increased dry weight from 2.87 to 53.26 % and from 9.85 to 31.16 % for cvs. Giza 86 and Giza 90, respectively. The highest efficiency value (60%) was achieved by BTH against *F. oxysporum* on Giza 86 and *F. moniliforme* on Giza 90 while the least efficiency was observed by BTH seed treated against *Rhizoctonia solani* infection. Since 50 ppm of BTH seed treatment caused 18.18 and 21.49% protection in cotton seedlings of cvs. Giza 86 and Giza 90, respectively. Using 100ppm of BTH gave cotton seedlings with 31.81 and 25.62 % protection for Giza 86 and Giza 90, respectively.

INTRODUCTION

Seed- and soil-borne organisms, acting singly or in combination, produce the seedling disease complex of cotton. These organisms are found in all cotton-producing areas of Egypt. The disease occurs as pergermination decay of the seed, decay of the seedling on the way to the soil surface (pre-emergency damping-off) partial or complete girdling of the emerged seedling at or near the soil surface (sore shin or post-emergency damping-off) and seedling root rot (Watkins, 1981). Several of the fungi involved in seedling disease complex are also implicated in root rot of older plants. The pathogens most commonly involved in the disease complex are *Rhizoctonia solani* (El-Akkad, 1997 and El-Samawaty, 1990), *Fusarium* spp. (Aly *et al.*, 1996 and El-Samawaty., 2004), *Macrophomina phaseolina* (Aly *et al.*, 1998; Abdel-Latif *et al.*, 2000 and Omar, 2005), *Pythium* spp. (Eisa, 1983) and *Sclerotium rolfsii* (Khashaba, 1972).

Upon infection with necrotizing pathogens, many plants develop an enhanced broad spectrum resistance to pathogens in the area of primary infection and in the distal, uninoculated organs (Sticher *et al.*, 1997). This so-called systemic acquired resistance (SAR) requires the endogenous

accumulation of salicylic acid (SA) (Dempsey *et al.*, 1999) and can also be induced by exogenous application of SA or its synthetic analogs 2,6-dichloroisonicotinic acid (Me´traux *et al.*, 1991; Elad, 1992; Galal and Abdou, 1996 and Galal *et al.*, 2001) and benzothiadiazole (BTH) (Friedrich *et al.*, 1996; Ismail *et al.*, 2006). SAR is associated with the activation of genes encoding pathogenesis-related (PR) proteins, some with antimicrobial activity (Van Loon and Van Strien, 1999), and with the ability to induce cellular defense responses more rapidly and to a greater degree than in noninduced plants (Mur *et al.*, 1996). Thus, inducing systemic resistance in the host plant becomes a good method for minimizing disease incidence/severity with least cost and without environmental pollution (Elad, 1992 and Galal and Abdou, 1996 Galal *et al.*, 2001). Over the past decade, a parsley cell culture/*Phytophthora sojae* cell wall elicitor model system has proven useful in studying cell priming and the resulting potentiation of cellular plant defense responses (Conrath *et al.*, 2001): Preincubation with SA, 2,6-dichloroisonicotinic acid, or BTH, in a strictly time-dependent process, primed parsley cells for stronger low-dose elicitation of various of cellular defense responses (Kohler, 2002).

Acibenzolar-*S methyl* (ABM), or benzothiadiazole (BTH), has been released in Europe as BION (Syngenta Ltd., Basel, Switzerland) and in the United States as Actigard (Syngenta Crop Protection Inc., Greensboro, North Carolina). ABM has been reported to induce resistance in wheat against fungal pathogens (Görlach *et al.*, 1996 and Morris *et al.*, 1998), in bean against fungal infections (Siegrist *et al.*, 1997.), and in tobacco and *Arabidopsis* spp. against fungal, bacterial, and viral infections (Cole, 1999 and Lawton *et al.*, 1996). ABM complies with the definition of a systemic acquired resistance (SAR) inducers: it gives protection to the same spectrum of pathogens, causes the expression of the same molecular and biochemical markers (*e.g.*, PR proteins) as biological inducers, and does not have direct antimicrobial activity (Kessmann *et al.*, 1994). Resistance inducers are not necessarily a replacement for traditional fungicides and bactericides. Its use in conjunction with or alternated with these pesticides may lead to a reduction of the number of applications and perhaps dose rate (Lyon and Newton, 1999 and Ortega *et al.*, 1998). It may also help to extend the durability of resistance in cultivars with genes for resistance to specific pathogen races (Romero *et al.*, 1998).

The objective of this study was to determine the response of cotton seedlings to seed treatment by resistance elicitor BTH for controlling infection with various soil-borne fungi pathogenic to cotton.

MATERIALS AND METHODS

Pathogens

Seven fungi pathogenic to cotton seedlings, *i.e.* *Fusarium solani*; *F. oxysporum*, *F. moniliforme*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Pythium* sp. and *Rhizoctonia solani*, were used in the present study. The fungi were isolated from seedlings infected with a variety of damping off symptoms or rooted rots of cotton plants that obtained from several cotton-

producing areas in Egypt. Pure culture of the tested fungi were identified according to Gilman (1966) or Barnet and Hunter (1979) at cotton pathology lab., Plant Pathology Res. Inst., Agric. Res. Center, Giza.

Seed treatment

Seed of cotton cultivars Giza 86 and Giza 90 were treated with benzothiodiazole (BTH) at the rate of 50 and 100 ppm. Seeds of cotton were soaked in BTH concentrations for 12h, before planting in soils infested with the tested fungi.

Effect of resistance elicitor BTH on cotton seed germination

BTH was dissolved in deionized distilled water (DDW) at 50 and 100 ppm and DDW was served as control. Seeds of cotton cultivars Giza 86 and Giza 90 were delented using 1.0 % sulfuric acid then washed thoroughly by DDW and blotted dried immediately before soaking in the test solution. Sterilized seeds were soaked in the test solutions for 12 hrs before planting. Treated seeds were distributed onto sterilized filter papers saturated with sterilized distilled water in sterilized Petri plates at the rate of 10 seeds per plate. Seeded plates were incubated at 25°C for 10 days. Germination rate (%) was measured as described by Strandberg and White (1989). Three plates were used per treatment and the experiment was repeated twice.

Effect of resistance elicitor BTH on growth of the tested fungi

BTH was prepared in 50 ml of autoclaved distilled water, then added after cooling at room temperature to nutrient broth liquid medium before solidify to obtain the concentrations of 50 and 100 ppm, shaken gently, then poured into sterilized Petri dishes. Petri dishes were individually inoculated with one disk (5-mm diam.) of 10-day-old cultures of the tested fungi and incubated at 27°C for 14 days. Three replicates were used for each treatment and linear growth (mm) was measured. For mycelial dry weight, concentrations of BTH compound (i.e. 50 and 100 ppm) were prepared in 50 ml of autoclaved nutrient broth liquid medium. Flasks were individually inoculated with one disk (5-mm diam.) of 10-day-old cultures of the tested fungi and incubated at 27°C for 14 days. Three flasks were used for each treatment. The mycelial fungi were filtered through filter paper Whitman No.1, dried at 70°C for 24 h. then dry weight was recorded.

Effect of BTH on susceptibility of cotton seedlings to the tested fungi

Substrate for growth of the tested isolates of fungi was prepared in 500 ml glass bottles; each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of each bottle were autoclaved for 30 minutes. Isolate inoculum, taken from one – week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonized sorghum for 3 weeks. Batches of autoclaved clay loam soil were infested separately with inoculum of each isolate of *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Pythium* sp., and 3 *Fusarium* spp. At the rates of 0.5, 5, 10, 30 and 50g/kg soil, respectively. Infested soil was dispensed in 10 cm diameter clay pots and these were planted with 10 BTH-treated seeds per pot. In the control treatment, seeds were treated with DDW and planted in infested soil. Pots were randomly distributed on a greenhouse bench. The experimental design was a randomized complete block with five replications. The prevailing temperature during study was 21.5±6.5 (minimum) and 26.5±6.5 (maximum).

Infection (%) plant height (cm/plat), and dry weight (mg/plant) were recorded 45 days after planting.

Statistical analysis of the data

Analysis of variance (ANOVA) of the data was performed with MSTAT-C statistical package (A micro-computer program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Least significant difference (LSD) and standard error for means (SEM) were used to compare treatments means.

RESULTS AND DISCUSSION

Effect of BTH on cotton seed germination

Data summarized in Table (1) showed that the resistance elicitor BTH has no significant inhibitory effects on seed germination of cotton cultivars Giza 86 and Giza 90. Data are consistent with the effects resistance elicitors on sunflower seed germination (Ismail *et al.*, 2006).

Table 1. Effect of (BTH) on seed germination of cultivars Giza 86 and Giza 90.

Cotton cultivars	Conc. (ppm)	% Germination rate
Giza 86	0.0	88 ± 3
	50	85 ± 6
	100	80 ± 5
Giza 90	0.0	86 ± 4
	50	84 ± 6
	100	82 ± 4

Data are means of 2 experiments (each experiment included 3 replicates (plates), ± Standard error for means (SEM).

Effect of BTH on growth of the tested fungi

BTH has no significant antifungal effects on mycelial dry weight or linear growth (Table 2). These results were recorded previously (Ismail *et al.*, 2006).

Efficiency of BTH on susceptibility of cotton seedlings to the tested fungi

Generally, BTH seed treatment (seed soaking) resulted in resistant cotton plants against infection by all pathogenic fungi tested, *i.e.* *F. solani* (Table 3), *F. oxysporum* (Table 4), *F. moniliforme* (Table 5), *Sclerotium rolfsii* (Table 6), *Macrophomina phaseolina* (Table 7), *Pythium* sp. (Table 8) and *Rhizoctonia solani* (Table 9). Efficiency of BTH to induce resistance in cotton plants varied with BTH concentration and fungi tested. Increasing BTH concentration enhanced resistance capacity of cotton cultivars. In addition, BTH seed treatment raised plant heights and weights of 45-day old plants.

Under *F. solani* infection, cotton cv. Giza 86 showed 35.7 % protection upon seed soaking in 50ppm of BTH while cotton cv. Giza 90 gave 50% protection (Table 3). Increasing BTH concentration to 100ppm enhanced resistance of cotton plants cv. Giza 86 to 50% while resistance of cotton plants cv. Giza 90 remained unchanged (50% protection).

Table 2. Effect of BTH on mycelial dry weight and linear growth of tested fungi.

Fungi	Conc. (ppm)	Mycelial dry weight (mg/50 ml liquid medium)	Linear growth (mm)
<i>Fusarium solani</i>	0.0	246 ± 4	83 ± 2
	50	248 ± 8	80 ± 5
	100	242 ± 6	82 ± 3
<i>Fusarium oxysporum</i>	0.0	266 ± 4	84 ± 1
	50	258 ± 8	83 ± 2
	100	255 ± 5	82 ± 3
<i>Fusarium moniliforme</i>	0.0	325 ± 6	82 ± 3
	50	320 ± 5	84 ± 1
	100	325 ± 8	83 ± 2
<i>Sclerotium rolfsii</i>	0.0	286 ± 5	85
	50	282 ± 8	85
	100	278 ± 6	85
<i>Macrophomina phaseolina</i>	0.0	256 ± 5	85
	50	258 ± 8	83 ± 2
	100	254 ± 4	82 ± 3
<i>Pythium sp.</i>	0.0	245 ± 5	83 ± 1
	50	246 ± 48	82 ± 3
	100	241 ± 6	84 ± 2
<i>Rhizoctonia solani</i>	0.0	336 ± 5	85
	50	334 ± 8	85
	100	328 ± 4	85

Data are means of 2 experiments (each experiment included 3 replicates, (plates or flasks), ± SEM.

Table 3. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Fusarium solani* and on plant height (cm/plant) and dry weight (mg/plant) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	35	40	0.0	0.0	20.13	19.5	207.5	185.1
50	22.5	20	35.7	50	21.88	21.58	208.6	202.2
100	17.5	20	50	50	24.17	23.98	233.6	217.4
LSD for Conc.	6.82	6.52			1.85	2.10	16.15	17.21

Regarding *F. oxysporum* infection, cotton resistance increased by increasing BTH concentration for both cotton cultivars tested. Since 50ppm of BTH exhibited 40 and 23.1% protection in cotton seedlings of cvs. Giza 86 and Giza 90, respectively (Table 4). Using 100 ppm of BTH induced resistance by 60 and 53.85 % in cvs. Giza 86 and Giza 90, respectively.

As for *F. moniliforme* infection (Table 5), cotton plants of cv. Giza 90 were more responsive to BTH seed treatment than cv. Giza 86. However, plants of cv. Giza 90 showed the highest value of protection (60%) at both

BTH concentrations tested. Plants of cv. Giza 86 showed 36.25% and 50% protection upon seed treatment with 50 and 100 ppm of BTH, respectively.

Planting BTH-seed treated in *Sclerotium rolfsii*-infested soil resulted in resistant cotton seedlings. Protection of cotton plants increased with increasing BTH concentrations (Table 6). Using 50 ppm of BTH induced resistance by 43.75 and 41.67 % to cotton seedlings of cvs. Giza 86 and Giza 90, respectively. Increasing BTH to 100 ppm enhanced cotton protection to 56.25 and 58.33% for Giza 86 and Giza 90, respectively.

Table 4. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Fusarium oxysporum* and on plant height (cm) and dry weight (mg) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	37.5	32.5	0.0	0.0	19.2	18.75	205.3	184.5
50	22.5	25	40	23.1	21.33	19.35	223.1	187.5
100	15	15	60	53.8	22.75	22.48	237.4	225.1
LSD for Conc.	6.82	6.52			1.85	2.10	16.15	17.21

Table 5. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Fusarium moniliforme* and on plant height (cm) and dry weight (mg) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	40	37.5	0.0	0.0	20.58	17.82	207.5	185.1
50	25.5	15	36.25	60	21.15	18.90	208.6	202.2
100	20.0	15	50	60	22.30	22.0	233.6	217.4
LSD for Conc.	6.82	6.52			1.85	2.10	16.15	17.21

Table 6. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Sclerotium rolfsii* and on plant height (cm) and dry weight (mg) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	40	30	0.0	0.0	19.10	19.92	209.4	185.7
50	22.5	17.5	43.75	41.67	20.13	20.33	211.6	212.4
100	17.5	12.5	56.25	58.33	24.08	21.71	238.5	227.3
LSD for Conc.	6.82	6.52			1.85	2.10	16.15	17.21

Concerning *Macrophomina phaseolina* infection (Table 7), BTH seed treatment significantly increased resistance in cotton seedlings where cv. Giza 90 plants were more protected than plants of cv. Giza 86. Since 35.71 and 41.67% protection were expressed to cvs. Giza 86 and Giza 90, respectively, upon 50ppm of BTH treatment. Protection values were increased with increasing BTH concentration and 100ppm of BTH achieved

41.43 and 58.33% protection to cvs. Giza 86 and Giza 90, respectively. AS for *Pythium* sp. Infection (Table 8), both concentration of BTH gave same protection for cotton plants of cv. Giza 90, while protection of cotton seedlings of cv. Giza 86 increased with increasing BTH concentration.

Table 7. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Macrophomina phaseolina* and on plant height (cm) and dry weight (mg) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	35	30	0.0	0.0	19.55	19.92	171.7	185.7
50	22.5	17.5	35.71	41.67	22.0	20.33	193.6	212.4
100	20.5	12.5	41.43	58.33	23.70	21.70	207.8	227.3
LSD for Conc.	6.82	6.52			1.85	2.10	16.15	17.21

Table 8. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Pythium* sp. and on plant height (cm) and dry weight (mg) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	42.50	37.50	0.0	0.0	20.18	18.63	167.4	176.5
50	32.50	27.50	23.52	26.67	21.23	20.35	193.0	210.5
100	27.50	27.50	35.29	26.67	24.23	24.0	256.6	231.5
LSD for Conc.	6.82	6.52			1.85	2.10	16.15	17.21

However, least protection values were observed by BTH seed treated when they were planted in *Rhizoctonia solani*-infested soil (Table 9). Fifty part per million of BTH seed treatment caused 18.18 and 21.49% protection in cotton plants of cvs. Giza 86 and Giza 90, respectively. Using 100ppm of BTH gave cotton seedlings with 31.81 and 25.62 % protection for Giza 86 and Giza 90, respectively.

Systemic resistance in plants can be induced by exogenous application of different chemicals, for instance salicylic acid (SA), and functional analog of SA such as 2-6-dichloro iso nicotinic acid (INA) and benzo (1,2,3) thiadiazole -7-carbothionic acid S-methyl ester (BTH) as reported previously (Elad, 1992; Kesmann *et al.*, 1994; Galal and Abdou, 1996; Friedrich *et al.*, 1996 and Ismail *et al.*, 2006).

Over the last 2 decades research on systemic acquired resistance (SAR) or induced systemic resistance (ISR) using model system has improved our understanding of the molecular basis of induced resistance and promoted the development of the synthetic elicitors. The future use of SAR or ISR to control crop pests in conventional agriculture seems promising because they provide good tools to control disease without causing direct selective pressure on pathogen population (Vallad and Goodman, 2004 and Mandal *et al.*, 2008).

Table 9. Effect of seed soaking in solutions of BTH on the infection of two cotton cultivars by *Rhizoctonia solani* and on plant height (cm) and dry weight (mg) at 45 days after planting.

Conc. (ppm) of BTH	Infection (%)		Protection (%)		Plant Height cm/plant		Dry weight mg/plant	
	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90	Giza 86	Giza 90
0.0	55	60.5	0.0	0.0	18.6	18.32	□□□□□	213.2
50	45	47.5	18.18	21.49	19.9	20.87	□	216.3
100	37.5	45	31.81	25.62	21.7	22.67	□	234.2
LSD for Conc.	6.82	6.52			1.85	2.10		17.21

The application of resistance elicitors also seems to be environmentally benign relative to currently used pesticides. The obtained results and the previous studies on SAR suggest that using resistance elicitors could be an attractive approach for managing crop diseases in a sustainable manner within the scope of a conventional agriculture system.

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استخدام البنزوثياديازول لتحفيز المقاومة الجهازية في بادرات القطن ضد بعض
فطريات التربة الممرضة

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تبين أن محفز المقاومة بنزوثياديازول ليس له تأثير مثبت على إنبات بذور نباتات القطن
صنفي جيزة ٨٦ وجيزة ٩٠. أيضاً ليس له تأثير مثبت على النمو الميسليومي للفطريات الممرضة
المختبرة *Fusarium solani*; *F. oxysporum*, *F. moniliforme*, *Sclerotium*
rolfsii, *Macrophomina phaseolina*, *Pythium* sp. and *Rhizoctonia solani*,
نتج عن معاملة بذور نباتات القطن بمحفز المقاومة زيادة في مقاومة النباتات ضد الإصابة
بالفطريات المرضية المختبرة. إن كفاءة مركب BTH تباينت باختلاف التركيز المستخدم
والفطريات المختبرة، إن زيادة التركيز للمركب أدى إلى زيادة مقاومة النباتات للإصابة بالإضافة
لذلك فإن التركيز الأعلى ١٠٠ جزء في المليون من المركب أعطى زيادة في طول النباتات بنسبة
من ٨,٣٦% إلى ٢٦,٠٧% لصنف جيزة ٨٦ و بنسبة من ٨,٩٣% إلى ٢٨,٠٢% لصنف
جيزة ٩٠ وكذلك زيادة وزن النباتات الجاف بنسبة من ٢,٨٧% إلى ٥٣,٢٨% لصنف جيزة ٨٦
وبنسبة من ٩,٨٥% إلى ٣١,١٦% لصنف جيزة ٩٠، إن أعلى كفاءة لمركب BTH كانت ٦٠
% ضد الإصابة بالفطر *F. oxysporum* على الصنف جيزة ٨٦ وضد الإصابة بالفطر *F.*
moniliforme على الصنف جيزة ٩٠ أما أقل كفاءة كانت ضد الإصابة بالفطر *R. solani* على
كلا الصنفين.