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Induced Systemic Resistance in Tomato Plants against *Meloidogyne* spp by Seed Treatment with β, Amino Butyric Acid and Benzothiadiazol

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Abstract
Treatments of tomato seeds with BABA or BTH significantly (p=0.05) reduced nematode infestation of tomato plants. BABA treatment produced significantly the lowest average root gall index (RGI), 2.44 followed by BTH, 3.22 and 3.55 for the untreated nematode infested control plants 15 days after nematode inoculation(ANI).Treatments with BABA and BTH for 30, 60 and 120 min. also caused significantly (p=0.05) less nematode infestation compared with the untreated control plants. BABA caused significantly the lowest average RGI, 1.77 compared with 3.66 and 4.55 for BTH and control respectively. The 120 min BABA treatment recorded significantly the lowest average number or J2 in the roots of tomato plants compared with 75.55 and 116.66 J2 in the roots BTH and control plants respectively. When seeds were socked for 120 min, root average fresh weight (RFW) and root dry weight (RDW) were significantly less, 2.58 and 0.14g in BTH treatments of seeds compared with 1.86, 0.10g for BABA and 5.01 and 0.29g for control respectively 50 days ANI. When seeds were socked for 120 min, the highest average SFW and SDW were, 8.05, 0.68g in BABA treatments, followed by 3.29, 0.22 and 2.43, 0.12g in BTH and control treatments respectively. The highest average SFW and SFW were also recorded for BABA treatments, 2.43, 0.12g followed by 1.5, 0,093 and 1.59, 0.092g in BTH and control respectively 15 days ANI. Similarly, BABA caused the highest average shoot weights 30 and 50 days ANI followed by BTH and control treatments.
Keywords: Induced resistance, *Meloidogyne* spp, β,amino butyric acid, Benzothaiadiazole, Seed treatment, Tomato.

1.Introduction

Tomato *Solanum lycopersicum* L. formerly known as *Lycopersicon esculentum* Mill. is the most important vegetable crop second to potato with annual world production of about 152.9 million ton in 2009 (Anonymus,2009). Annual tomato production in Iraq was estimated at 830.000 ton in 2008 (FAOSTAT, 2008). Tomato plants are subjected to infection by many important plant pathogens including the root knot nematodes, *Meloidogyne* spp. These nematodes are considered as the most important nematode species worldwide and in Iraq. Many effective control measures were used to manage these pathogens such as soil solarization chemical and biological control. Induced systemic resistance to plant pathogen provides an ideal control measures against plant pathogens (Kumagai, 1988). Chemical, physical and biological inducers to control the root knot nematodes were used (Sahebani et al., 2011). Chemical inducers of systemic resistance provides a practical method of plant protection against plant pathogens. Induction of systemic resistance via seed priming is an attractive, simple and cost effective strategy to control plant diseases (Manjunatha et al., 2013). In a recent review many chemicals have been reported to induce systemic resistance (ISR) in various plant species against different plant pathogens such as Oomycetes, fungi, bacteria, viruses and nematodes (Jacob et al., 2013) . Two of these inducers are β-1,3 amino butyric acid (BABA) and . Benzol [1,2,3] thaiadiazole-7-carbothionic acid S-methyl ester (BTH) have been reported to confer ISR in various plant pathogen interactions (Jacob et al., 2013). However, reports on ISR against plant parasitic nematodes are scares. Foliar and soil application of BABA was reported to reduce damage by root knot nematode on tomato (Oka et al., 1999) and *M. javanica* and *Rotylenchulus reniformis* on pineapple (Chinnasri et al., 2006) and induce resistance against *M .javanica* on cucumber (Sahebani et al., 2011). To date, the only published work on ISR to *M .javanica* in tomato reported that soaking of tomato seeds in 25mg/ml of BABA for 24h render tomato plants more resistant to the nematodes (Fatemy et al., 2012). However, socking seeds of soybean BTH protect plants against fusarium damping- off and wilt diseases under greenhouse and field conditions (Abdel-Monaim et al., 2011).

This study was done to assess the possibility of the induction of systemic resistance to the root knot nematodes by pretreatments of tomato seeds with β-1,3 amino butyric acid (BABA) and Benzol [1,2,3] thaiadiazole-7-carbothionic acid S-methyl ester (BTH).

2.Materials and Methods

Experiments were performed in a greenhouse (27±5 C) the Department of Plant Protection, College of Agriculture, Baghdad University and tomato, *Solanum lycopersicum* L. "Supper Regina" highly susceptible to *Meloidogyne* spp plants were used. Plants were grown and maintained in1kg plastic pots throughout the experiments.

2.1Nematode inoculation

Soil from cucumber grown plastic house heavily infested with *Meloidogyne* spp (predominantly *M. javanica*) was collected and stored at 4C in polyethylene pages until use to inoculate tomato seedlings. The nematode infested soil was mixed with peat moss in 1:1 ratio and used as nematode inoculums.

2.2 Tomato plants

Tomato, *Solanum lycopersicum* L. cv. "super Regina" (Genetics International Inc, Modesto, California, USA) susceptible to *Meloidogyne* spp was used.

2.3 Treatment with β, amino butyric acid (BABA) and Benzothaiadiazole (BTH)

Tomato seeds were socked in 40 mM solution of BABA (Sigma Aldrich, St Louis, Missouri, USA) or 50 mg solution of BTH (BION 500 FS, Syngenta Crop Protection Inc.) or distilled water (control) for 30, 60, and 120 minutes. The seeds were individually sown in seedling tray containing sterilized soil and maintained in the greenhouse (27±5C) until they reach 4-5 true leaves age. Seedlings, then were transplanted to 1kg plastic pots containing *Meloidogyne* spp infested soil and peat moss in a 1:1ratio and maintained for 15, 30 and 50 days after nematode inoculation. Treatments were replicated 4 times and arranged in randomized complete blocks in the greenhouse.

2.4 Evaluation of Induced Resistance by BABA and BTH

2.4.1 Effect of BABA and BTH on Rate of Gall Index

The rate of infestation (RGI) of *Meloidogyne* spp was determined 30 days after nematode inoculation (ANI) using a 1-5 level scale (Dube and Smart, 1987): 1= no galls on roots, 2= galls on 25% or the root, 3= galls on 50% or the root, 4= galls on 75% of the root, and 5, galls on 100% of the root.

2.4.2 Effect of BABA and BTH on Nematode Penetration

To determine the effect BABA and BTH treatment on nematode penetration of tomato roots 1 wk ANI. Roots were carefully washed with tab water, stained with acid fuchsine (Byrd et al., 1984), and at least 3 samples of 1g of each root was individually examined under a compound microscope to count nematodes inside the roots. The experiment was replicated 4 times.

2.4.3 Effect of BABA and BTH on Fresh and Dry Weight of Shoot and Root Systems

Plants were carefully uprooted and roots were washed under tap water to remove adhering soil. To determine shoots and roots dry and wet weight, shoots and roots were separately weight and dried at 70C for 48h or until weight is fixed.

3. Statistical Analysis

The data were subjected to analysis of variance and means were separated by the least significant method at (p=0.05) using SAS, 2004.

4. Results

4.1 Effect of BABA and BTH on Rate of Root Gall Index (RGI)

Treatments of tomato seeds with BABA or BTH significantly (p=0.05) reduced nematode infestation of tomato plants (Table 1). BABA treatment produced significantly the lowest average RGI, 2.44 followed by BTH, 3.22 and 3.55 for the untreated nematode infested control plants 15 days after nematode inoculation (ANI). At 30 and

50 days ANI, BABA was superior in reducing RGI over BTH and control plants with averages of 2.88, 3.55, 5, 3.11, 4.55 and 5 respectively. Treatment durations with BABA and BTH (30, 60 and 120 min.) also caused significantly (p=0.05) less nematode infestation compared with the untreated control plants. BABA caused significantly the lowest average RGI, 1.77 compared with 3.66 and 4.55 for BTH and control respectively for the 120 min seed treatment. The untreated nematode infected control recorded significantly the highest RGI, 5 compared with 1.33 for the 120 min BABA treatment.

4.2 Effect of BABA and BTH on Nematode Penetration

Treatments of tomato seeds with BABA and BTH significantly (p=0.05) reduced the number of J2 which enter the roots (Table 2). BABA recorded significantly the lowest average number or J2, 41.11 in the roots of tomato plants compared with 75.55 and 116.66 J2 in BTH treated and control plants respectively. The number of J2 in roots was also significantly affected by duration of socking of seeds in the inducing agents (Table 2).The lowest average number of J2, 52.66 compared with 82.77 and 97.88 in 60 and 30 min treatments respectively. Treatments of seeds with BABA for 120 min caused the lowest penetration of roots by *Meloidogyne* spp J2, 5 compared with 36.33 and 116.66 in BTH and control respectively.

4.3 Effect of BABA and BTH on Fresh and Dry Weight of Shoot and Root Systems

4.3.1 Root Weights

Pretreatments of tomato seeds with BABA and BTH significantly (p=0.05) affected root weights compared to untreated nematode inoculated control plants (Table 3). When seeds were socked for 120 min, average root fresh weight (RFW) and root dry weight (RDW) were significantly less, 2.58 and 0.14g in BTH treatments of seeds compared with 1.86, 0.10g for BABA and 5.01 and 0.29g for control plants respectively 50 days ANI. The lowest average RFW and RDW 15 days ANI were 0.79 and 0.03g for BABA treatment compared with 0.87, 0.038 and 1.02, 0.077g for BTH and control treatments respectively. However, the average RFW and RDW were the highest in control plants, 10.32, 0.42g in the nematode infested control treatment compared with 4.62, 0.26 and 3.69, 0.21g in BTH and BABA treatments 50 days ANI respectively. The lowest root weights were recorded for BABA treated seeds for 120 min compared to 0.81, 0.028 and 0.96, 0.051g in BTH and controls respectively.

4.3.2 Shoot weights

Pretreatments of tomato seeds with BABA and BTH caused significant (p=0.05) increase in shoot weights compared with untreated nematode inoculated control plants (Table 4). When seeds were socked for 120 min, the highest average SFW and SDW were, 8.05, 0.68g in BABA treatments, followed by 3.29, 0.22 and 2.43, 0.12g in BTH and control treatments respectively. The highest average SFW and SFW were also recorded for BABA treatments, 2.43, 0.12g followed by 1.5, 0,093 and 1.59, 0.092g in BTH and control respectively 15 days ANI. Similarly, BABA caused the highest average shoot weights 30 and 50 days ANI followed by BTH and control treatments. The highest average SFW and SDW were recorded for BABA treatments, 11.41and 1.28g 50 days ANI.

5. Discussion

Induction of resistance against plant pathogens by seed treatment is simple, cost effective and an efficient strategy for disease management (Mangumatha el al., 2013). BABA and BTH are potent inducers of many plant pathogen interactions (Thakur and Sohal, 2013; Ostendrop et al., 2001). More research work on the mechanisms of action of these and other chemical inducers and the genes involved in this plant resistance may provide the knowledge needed to develop new strategies against various plant pathogens. Results of this work indicated that application of BABA and BTH as seed treatments (socking) induced systemic resistance against *Meloigogyne* spp in nematode susceptible tomato plants. Such pre infection treatment of the seeds was effective because lag time is required for the activation of plant defense system. It was reported that effective BTH has to be applied as protective or at early stage of the disease (Ruess et al., 1995; Tally, et al., 2000).The induced resistance in this study was manifested by the reduction of galls on roots and numbers of J2 penetrating the roots of BABA and BTH treated plants compared with those in inducers untreated but nematode infected plants (Tables, 1and 2). This was reflected on the growth of the nematode infected plants by increasing root and shoot weights of BABA and BTH treated tomato plants (Tables, 3 and 4). These findings also support previous reports indicating that treatments with β, amino butyric acid reduced root knot disease through decreased root penetration of J2, gall number on roots and nematode development (Oka et al., 1999; Chinnasri, et al., 2006; Sahebani et al., 2011).

Recent work also indicated that number of galls and egg masses of *M. javanica* on tomato were significantly reduced by overnight priming of seeds in 25mgL^{-1} of BABA (Fatemy et al. 2012). In this study, treatments with BABA and BTH reduced the weight of nematode infected roots compared to root weight in nematode infected control plants (Table, 3). Increased root weigh due to *Meloidogyne* spp infection was previously reported and thought to be caused due to the biomass accumulations in infected roots (Fortnum et al., 1994). *Meloidogyne* spp infection is known to have negative effects on water and nutrient elements uptake by infected roots as well as photosynthesis (Melakeberhan et al., 2004). It was reported that *M. incognita* infection caused biomass accumulation in roots and it is probably is controlled by the efficiency of the pathogen in capturing the energy produced by photosynthesis and directing it in favor of the pathogen or the infected host (Melakeberhan et al., 1988). Because of the relative large size of females of *Meloigogyne* spp and its ability to produce large number of eggs, it requires large amount of energy. Beside this energy requirement, these pathogens caused obvious distortion in xylem vessels, swellings of root cells and formation of giant feeding cells which alter root normal functions. Treatments of nematode infected plants with BABA and BTH produced more shoots compared with untreated nematode infected plant. This is mainly due to the fact that *Meloidogyne* infections embed photosynthesis, and chlorophyll synthesis which is negatively influence plant growth (Melakeberhan et al., 2004). The mechanism of induce resistance to *Meloidogyne* in tomato by BABA is not fully understood. It was believed that treatments with this inducer render roots less attractive to J2 through altered plant nutrient assimilation or render plant cell walls harder to penetrate by J2 or that it caused the formation of smaller giant cells which are not able to provide enough nutrients for the developing nematodes (Oka et al., 1999). Treatments with BABA was reported to increase levels of salicylic acid (SA) and pathogenesis related proteins (PRP) (Hwang et al.,1997), and enzymes like catalase (CAT), polyphenoloxidase (PPO) and guaiacol peroxidase (GPOX) (Sahibani et al., 2009; Sahibani et al., 2011) and phenolic compounds (Mpiga et al., 1997). BABA was also reported to induce the accumulations of PPO, GPOX, H_2 0_2, CAT and phenols in *M. javanica* infected cucumber roots (Siegrist et al., 2000). Results clearly showed that as the time of socking of the seeds in the inducing agents increased the degree of induced resistance was increased. This was indicated by significantly less gall number on roots and number of *Meloidogyne* J2 entering the roots of tomato plants as time of treatments increased (Table 1and 2), These results support previous results indicating that seed treatment with BABA induced resistance to *M. javanica* in tomato plants (Fatemy et al., 2012). Although BABA induced resistance to root knot nematode was previously reported, BTH induce resistance to this nematode species was not attempted before**.** A sophisticated relationship had evolved between certain nematode species like *Meloidogyne* and their host plants, therefore, even a settle biochemical changes in host cells may render these hosts un favorable for the nematodes. This may explain the long-lasting resistance to *Meloidogyne* spp which lasted for at least 50 days. It was previously reported that BABA seed treatment provided a log-lasting resistance against powdery mildew disease caused by *Oidium neolycopersici*, which lasted for at least 8 weeks and was related to enhanced expression of host defense genes (Worrall et al., 2011). More knowledge about the resistance induction mechanism involved in inducer treatments of tomato seeds to acquire systemic resistance to *Meloidogyne* spp could enhance the development of a new biologically and environmentally safe method for the sustainable management of these important plant pathogens. Furthermore, research including the assessment of induced resistance by seed treatments with various chemical inducers under field conditions is needed before practical and efficient managements of the root knot nematodes can be developed.

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Table 1. Effect of duration of seed treatments with 40mM of BABA and 50mgL^{-1} of BTH on root gall index of tomato, *Solanum lycopersicum* L. infected with *Meloidogyne* spp

Days ANI	Chemical Inducer	Root gall index Treatment Duration (min)			Mean
		30	60	120	
15	BABA	3	3	1.33	2.44
	BTH	3.33	3.33	3	3.22
	Water	3.33	3.66	3.66	3.55
30	BABA	3.66	3	2	2.88
	BTH	3.33	4	3.33	3.55
	Water	5	5	5	5
50	BABA	4	2.33	2	3.11
	BTH	4.33	4.66	4.66	4.55
	Water	5	5	5	5
Mean	BABA	3.55	3.11	1.77	
	BTH	3.66	4	3.66	
	Water	4.44	4.55	4.55	
LSD (P=0.05) BABA Trea.= 0.30*,Inter. = 0.57* LSD (P=0.05) BTH Trea.= 0.50*,Inter.= 0.87* LSD (P=0.05) only water Trea.= 0.33*, Inter.= 0.57*					

Each number is a mean of three replicates and two plant each.* indicate significant difference. Nematode inoculums (nematode infested soil + peat moss, 1:1) were added when plants were 4-5 true leaves. Gall index was according to 1-5 level scale : 1= no galls on the roots , 2= galls on 1- 25% of the root , 3= galls on 26- 50%

of the root , 4= galls on 51- 75% of the root, and 5= galls on 76-100% of roots . BABA= β amino butyric acid, BTH=Bezothaiadaiazol, ANI= after nematode inoculation.

Table 2. . Effect of duration of seed treatments with BABA and BTH on root penetration of tomato, *Solanum lycopersicum* L. by *Meloidogyne* spp J2 1wk after nematode inoculation

Treatment	No. of J2 in Roots / Duration of Seed Treatment(min)			Mean
	30	60	120	
BABA (40 mM)	76.66	41.66	5.00	41.11
BTH (50 $mgL^{-1)}$	100.33	90.00	36.33	75.55
Water	116.66	116.66	116.66	116.66
Mean	97.88	82.77	52.66	
LSD(P=0.05) Conc.= 5.66*,Trea.= 5.66*,Inter.= 9.80*				

Each number is a mean of three replicates and two plant each.* indicate significant deferens. Nematode inoculums (nematode infested soil + peat moss, 1:1) were added when plants were 4-5 true leaves. ABA= β, amino butyric acid, BTH=Bezothaiadaiazol.

Table 3. Effect of duration of seed treatments with 40mM of BABA and 50mgL^{-1} of BTH on root weights of tomato, *Solanum lycopersicum* L. infected with *Meloidogyne* spp

Days ANI	Conc. (mgL^{-1})	Duration of Seed Treatment with BTH (min)						Weight Mean	
		30		60		120			
		Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
15	50	0.045	0.86	0.042	0.94	0.028	0.81	0.038	0.87
30	50	0.18	3.20	0.15	2.62	0.14	2.57	0.16	2.79
50	50	0.25	4.51	0.29	5.00	0.26	4.36	0.26	4.62
	Mean	0.16	2.85	0.16	2.85	0.14	2.58		
LSD (P=0.05) Durations = 0.21*, Days = 0.21*, Inter.= 0.37* Fresh LSD (P=0.05) Durations = 0.016*, Days = 0.016*, Inter.= 0.029* Dry									
Days ANI	Conc. (mM)	Duration of Seed Treatment with BABA (min)						Weight Mean	
		30		60		120			
		Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
15	40	0.053	0.96	0.041	0.87	0.019	0.53	0.037	0.79
30	40	0.019	3.39	0.21	3.17	0.13	2.06	0.12	2.87
50	40	0.26	4.20	0.20	3.89	0.17	3.00	0.21	3.69
	Mean	0.11	2.85	0.15	2.64	0.10	1.86		
LSD (P=0.05) Durations = 0.15*, Days = 0.15*, Inter. = 0.26* Fresh LSD (P=0.05) Duration = 0.012*,Days = 0.012*, Inter.= 0.021* Dry									
Days ANI	Distilled water	Duration of Seed Treatment with Water (min)						Weight Mean	
		30		60		120			
		Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
15	Water	0.088	1.01	0.051	0.96	0.092	1.09	0.077	1.02
30	Water	0.30	5.00	0.23	3.90	0.38	5.44	0.30	4.78
50	Water	0.50	10.70	0.36	10.18	0.40	10.09	0.42	10.32
	Mean	0.29	5.57	0.12	5.54	0.29	5.01		
LSD (P=0.05) Durations = 0.34*, Days = 0.34 *, Inter. = 0.59 * Fresh LSD (P=0.05) Durations = 0.15*,Days = 0.15*, Inter.= 0.26 * Dry									

Each number is a mean of three replicates and two plant each.* indicate significant difference. Nematode inoculums (nematode infested soil+ peat moss, 1:1) were added when plants were 4-5 true leaves. BABA= β, amino butyric acid, BTH= Bezothaiadaiazol, ANI=after nematode inoculation.

Table 4. Effect of duration of seed treatments with of BABA and BTH on shoot weights of tomato, *Solanum lycopersicum* L. infected with *Meloidogyne* spp

Days ANI	Conc. (mgL-1)	Duration of Seed Treatment with BTH (min)						Weight Mean	
		30		60		120			
		Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
15	50	0.094	1.51	0.087	1.55	0.097	1.46	0.093	1.50
30	50	0.21	4.04	0.24	4.76	0.30	3.70	0.22	4.16
50	50	0.30	5.87	0.37	6.00	0.29	4.70	0.32	5.52
	Mean	0.20	3.81	0.23	4.10	0.22	3.29		
LSD (P=0.05) Periods=0.24*, Days= 0.24*, Inter. =0.41* Fresh LSD (P=0.05) Periods=0.12*, Days= 0.12*, Inter. =0.21* Dry									
Days ANI	Conc. (mM)	Duration of Seed Treatment with BABA (min)						Weight Mean	
		30		60		120			
		Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
15	40	0.26	3.03	0.28	1.86	0.21	2.42	0.12	2.43
30	40	0.49	8.20	0.55	8.04	0.78	9.61	0.60	8.62
50	40	1.55	10.08	1.22	12.01	1.07	12.13	1.28	11.41
	Mean	0.76	7.10	0.62	7.30	0.68	8.05		
LSD (P=0.05) Periods=0.32*,Days= 0.32*, Inter.=0.56* Fresh LSD (P=0.05) Periods=0.010*,Days= 0.010*, Inter.= 0.018 * Dry									
Days ANI	Distilled water	Duration of Seed Treatment with Water (min)						Weight Mean	
		30		60		120			
		Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh
15	Water	0.087	1.22	0.097	1.85	0.092	1.71	0.092	1.59
30	Water	0.21	3.93	0.20	3.77	0.25	4.12	0.22	3.94
50	Water	0.22	4.85	0.37	5.04	0.31	5.55	0.30	5.15
	Mean	0.17	3.33	0.22	3.55	0.21	3.79		
LSD (P=0.05) Periods=0.53^{ns}, Days= 0.53^{ns}, Inter.=0.92^{ns} Fresh LSD (P=0.05) Periods=0.23*,Days= 0.23*, Inter.= 0.39 * Dry									

Each number is a mean of three replicates and two plant each.* indicate significant difference. Nematode inoculums (nematode infested soil + peat moss, 1:1) were added when plants were 4-5 true leaves. BABA= β, amino butyric acid, A, BTH= Bezothaiadaiazol, ANI= after nematode inoculation.