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Phytophthora Blight (*Phytophthora capsici* Leonian) Control in Pepper by Salicylic Acid and Beta Amino Butyric Acid and Disease Resistance Mechanism

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ABSTRACT

In this study, the effects of Salicylic Acid (SA) and DL-β-amino-n-butyric Acid (BABA) on Phytophthora blight caused by *Phytophthora capsici* were investigated. SA completely inhibited the mycelial growth of *P. capsici* at 250ppm concentration in vitro. In the pot experiments, the applications of soil and leaves of SA reduced the disease severity of *P. capsici* with 75.1-92.2% and 87.2-95.0%, respectively. In the both greenhouse and field conditions, the effects of SA on the disease severity of *P. capsici* were 68.9 and 62.0% at 1g/m^2 dose of soil drench, 61.6% at 500ppm and 50.2% at 1000ppm of leaves sprays, respectively. BABA had no effect on mycelial growth of *P. capsici* up to 1000ppm concentrations in vitro. However, in the pot experiments, the applications of soil and leaves of BABA reduced the disease severity of *P. capsici* with 60.1-84.3% and 83.6-97.2%, respectively. In the both greenhouse and field conditions, the effects of BABA on the disease severity of *P. capsici* were 70.5 and 49.0% at 1g/m^2 dose of soil drench, 63.4% at 500ppm and 46.4% at 2000ppm of leaves sprays, respectively. The applications of leaves with 1000ppm of SA and 2000ppm of BABA increased the capsidiol level in pepper plants.

Key words: Beta Amino Butyric Acid (BABA), capsidiol, induced resistance · pepper salicylic acid (SA)

INTRODUCTION

The most important phytopathological problem of pepper is Phytophthora blight caused by *Phytophthora capsici* Leonian. This disease is soil-borne and mostly

observed on poor soil drainage areas where water has ponded and causes random singular or group deaths. Symptom are water soaked appearance on the stem in the beginning and later these spots are converted to brown necrosis. The plant detoriates and dies rapidly. (Black et al. 1991). The disease could appear at any stage of plant development which makes this disease very critical. Furthermore fungus could stay dormant in soil for several years. Sensitivity of pepper variety, irrigation frequency and amount, soil composition and drainage caharactersitics are crucial factors influencing development of the disease. Considering afore mentioned factors yielded the development of new cultivation practice called inclined plane planting which has helped to contain the disease in a great extent in recent years. Another suggested practice is the use of fungicides containing metalaxyl as active ingredient. Though the results are not satisfactorily efficacious. Furthermore, the difficulty in struggle against soil-borne diseases, high costs in application on large extent and most importantly the risk of development of resistance to the certain chemical by patogen renders the chemical use very difficult if not impossible. Moreover the limited success rate of present cultural practices which causes diminished production in pepper cultivation requires the immediate development of new cultivation methods and appplication strategies to be formulated.

Different approaches have been considered and performed by plant pathologist through biotechnology or induced resistance in plants against diseases. One of these approaches is to apply non spesific elicitors to increase rapid response against infections by phytopathogenic microorganisms. In collaboration with the suggestion of the phenomenon of “Induced Systemic Resistance” or “Systemic Acquired Resistance”, it made progress related with the approaches from past to present. Systemic acquired resistance is providing resistance against diseases by local infections, microbial components or products or groups of organic and inorganic compounds systemically and resulting protection against diseases (Kuc, 1995; Kuc, 2000; Heil and Bostock, 2002). In studies related with the induced resistance in plants, abiotic inducers such as UV, ethylene, salicylic acid and isomers of the butyric acids and biotic inducers such as non pathogenic strains, cell wall fragments and application of weak or non viable fungal spores were used and attained succesful results under controlled conditions (Benhamou and Theiault, 1992; Dempsey and Klessig, 1995; Durner et al. 1997). Among abiotic inducers, salicylic acid and butyric acids isomers have important roles in inducing systemic resistance by means of providing accumulation of salicylic acid endogenously in plants (Palva et al. 1994; Zimmerli et al. 2001; Nakashita et al. 2002). These chemicals were used for inducing resistance as pure or mixed form in laboratory and greenhouse conditions. Researches conducted with these elicitors considered to possess potantial theoretically and practically resulted to carry today the phenomenon by improving synthetic elicitors and available to use under field conditions practically (Lyon and Newton, 1997). Eventhough control methods using synthetic elicitors appeared to be phantesy in the past, it conferred new and interesting scope in food

safety and sustainable diseases control nowadays. The effects were higher in plant protection systems with elicitors under laboratory, controlled pot and greenhouse conditions. Sometimes, researchers confront some problems during establishment of systems because of environmental interactions under field conditions. However, promising results have obtained in conducted field studies against diseases up to now and it appeared that application of inducers have advantages, economically costly and environmentally desirable. In addition, the protective effects are higher compared to some fungicides when applicated before pathogen infections and also inhibition or curative effects against infections (Siegrist et al. 1997; Kim et al. 2001; Ziadi et al. 2001). Induced resistance has stabile feature in plants because of being applicable against fungal, bacterial and viral pathogens without spesific activitiy on any diseases and acquired resistance is causal of activation of various mechanisms in plants. Besides that, pathogens have not developed resistance against inducers as is systemic fungicides. Although acquired resistance is systemic character, the activation of resistance mechanism is local generally thus it arised when needed.

In the view of such information, the possibility of using chemical inducers – salicylic acid and DL-β-amino-n-butyric acid against Phytophthora blight caused by *Phytophtora capsici*, one of the most important problem pepper growing under pot, greenhouse and field conditions and the role of capsidiol, a phytoalexine, in disease resistance were investigated in this study.

MATERIALS and METHODS

Plant, Pathogen and chemical inducers

Pepper (*Capsicum annuum* L.) cv Charliston Bagci was used in the pot, greenhouse and field studies. *Phytophthora capsici* Leonian was isolated from diseased tissue of naturally infected pepper plants on corn meal agar. To induce resistance against pathogen, salicylic acid (SA) (Sigma, S-0875) and DL-ß-amino-n-butyric acid (BABA) (Sigma, A-2004) were used at different concentrations.

In vitro studies

Determination of the effects of SA and BABA on mycelial growth of *Phytophthora capsici*

The stock solutions of SA and BABA in steriled distilled water and 10ml PDA in glass tube were prepared. Media autoclaved at 121ºC 20 min. Media cooled to 50°C in water bath and concentrations between 0-350 ppm and 0-1000 ppm were adjusted from SA and BABA stock solutions respectively, added into media and poured into petri dishes. Media without addition of SA and BABA were prepared for comparison, also. Six milimeter diameter discs were transferred to media with or without chemicals from the fresh culture of *P. capsici* growed on PDA and incubated at 25°C.

Experiment designed as completely randomized block with 5 replications. During incubation, colony diameters were measured daily until 5 days. Data were subjected to analysis of variance and means were compared using Fisher's least significant difference test at P = 0.05 (Gomez and Gomez, 1983). ED_{50} value was calculated with regression analysis using mycelial colony diameter for SA effectiveness.

Determination of the effects of SA and BABA on mycelial dry weight of *Phytophthora capsici*

The stock solutions of SA and BABA in steriled distilled water. 100ml of PD (Potato Dextrose) liquid media were prepared in 250ml erlenmayer flask and autocklaved at 121ºC, 20 min. Media were cooled and added concentations between 0-250ppm and 0-1000ppm from stock solutions of SA and BABA, respectively. Media were inoculated with one 6mm diameter disc of *P. capsici* and incubated at 25°C and dark conditions on horizontal shaker at 120 rpm during 2 weeks. Experiment designed as completely randomized block with 3 replications. At the end of the experiment, media were drained using Buhner funnel contained Whatman No.1 filter paper. After filtering, fresh and dry mycelial (dried at 75°C, 1 day) weight were measured. Data were subjected to analysis of variance.

Seedling production and plant growth conditions

Pepper seeds were surface disinfested in 2% of NaOCl solutions for 5 min and thoroughly washed twice with sterile distilled water. Pepper seedlings were produced in plastic containers (30x40cm). The mixture of soil, sand and pumice (1/1/1, v/v/v) was autoclaved at 121°C and 100 kPa twice for each time and used as growth medium. The plastic containers were placed in a growth room at 25±ºC temperatures. During seedlings production until 3-4 leaf stage cultural practices were performed.

Determination of the effects of SA and BABA on *Phytophthora capsici* under pot conditions

For determination of the effects of SA and BABA on *P. capsici*, soil and upper part applications of both chemicals at three different concentrations were performed. Plants with 3-4 leaves were transplanted into 15cmdiameter pots containing autoclaved soil. Plants maintained in a growth room at 25±ºC temperatures with 8.000 lux illumination for 12 h a day in a completely randomized block experimental design.

Stock solutions were prepared for SA and BABA which concentration of soil applications of SA and BABA were 25, 50 and 100mg/kg soil; leaf applications were 500, 1000 and 1500ppm for SA and 1000, 2000 and 3000ppm for BABA.

Soil and leaf applications were performed at 7-8 leaf stages. Soil applications of SA and BABA were done before pathogen inoculations as 50 ml for each concentration around rhizosphere. Leaf applications were performed as spraying using hand atomiser for each concentration two times 3 day interval.

Three days after second applications, plants were inoculated with the pathogen, *P. capsici*. The fungus was grown on oatmeal agar plates at 28°C for 7 days and placed under fluorescent light for sporulation. Culture plates were incubated in sterile distilled water for 40 min at 4°C and then for 30 min at room temperature. Zoospore released from sporangia of *P. capsici* were collected by filtering through two-layers of cheesecloth and zoospore concentrations was adjusted to $2x10^6$ zoospore ml^{-1} using haemocytometer (Sunwoo et al., 1996). A 10 ml spore suspension was applied to soil around the root of plant in each pot. One week after inoculation, symptoms were evaluated based on a 0-5 scale: where 0 = no visible disease syptoms; 1 = leaves slightly wilted with brownish lesions beginning to appear on stems; 2 = 30-50% of entire plant diseased; 3 = 50-70% of entire plant diseased; 4 = 70-90% of entire plant diseased and 5 = plant dead (Sunwoo et al. 1996). Lesion length formed by *P. capsici* on stems was measured for treatment. Disease index were calculated using scale values.

Determination of the effect of SA and BABA on disease severity of *Phytophthora capsici* under greenhouse and field conditions

Experiments were conducted under greenhouse and field conditions. For each treatment, 2 m^2 plots were prepared and 70cm safety zone provided between plots for both experiment. In each plot, 20 seedlings with 3-4 leaves were transplanted into the greenhouse and field. Experiments were designed as a completely randomized block design in the greenhouse and a randomized complete block design in the field with four replications.

Soil and leaf applications of SA and BABA were performed at 7-8 leaves stage. Concentrations of soil applications of SA and BABA were determined for both chemicals as $1g/m^2$ under greenhouse and field conditions. Leaf applications of SA and BABA determined as 250 and 500ppm under greenhouse conditions and; 500 and 1000ppm for SA and 1000 and 2000ppm for BABA under field conditions. Soil application was done before pathogen inoculation one time by draining around root at 100ml for each concentration. Leaf applications were performed before inoculation of pathogen two times 3 days interval and one time after pathogen inoculation by spraying using hand atomiser.

Four week after transplanting, plants were inoculated artifically with *P. capsici* ($2x10^6$ zoospores ml^{-1}). Plants were evaluated 2 weeks after inoculation according to Sunwoo et al. 1996.

Capsidiol analyses

A capsidiol standard was obtained according to Egea et al. (1996a) and Ustun (1995). To obtain capsidiol in sufficient quantities, 100ml of 1% $CuSO_4$ suspension was used as the elicitor in fruits. Fifty semi-ripe pepper fruits were injected with elicitor and incubated in sterilized, covered glass tray at 15°C for 3 days. Seeds were then removed

and the liquid within the fruits was collected and poured into a container. Chloroform-soluble substances were isolated from the diffusate by three extractions and separated by thin layer chromatography at Rf 0.2.

For the determination of quantitiy of capsidiol in treatments, 2-3 leafed-stage and unapplicated plants were transferred to 15cm-diameter pots which were placed in growth room at 25±ºC and illuminated by 8.000 lux for 12h a day. Treatments were as follows: SA soil (50 mg kg^{-1}), BABA soil (50 mg kg^{-1}), SA Leaf (1000ppm), BABA Leaf (2000ppm), *P. capsici* (PC), uninoculated control

Treatments were repeated three times. Plants were inoculated with *P. capsici* at a concentration of $2x10^6$ zoospores ml^{-1}. Six days after inoculation, capsidiol was extracted 1g of stem of plants with $CHCl_3$:MeOH (2;1, v/v) separated by thin layer chromotography, and identified and quantified by gas chromatography (Egea et al. 1996b).

Quantification was made by (ATI UNICAM 610 Series) equipped with flame ionisation detector and Schimadzu CPBS-S25-050 30m capillary column. Column, injector and dedector were kept at 150, 240 ve 300°C, respectively. Capsidiol amount was determined with ATI UNICAM integrator system assembled to the gas chromatograph.

Statistical analysis

The data were subjected to analysis of variance (*F*-test). Means were compared using Fisher's least significance difference (LSD) test at $P = 0.05$ (Gomez and Gomez, 1983).

RESULTS

The effects of SA and BABA on mycelial growth of *Phytophthora capsici* in vitro

SA reduced the mycelial colony diameter depending increasing concentration on solid medium. The colony diameter was found as 60mm at 200 ppm in control while it decreased 6 mm at 200 ppm at fifth day. SA was completely inhibited the mycelial growth of *P. capsici* at 250ppm concentration (Table 1).

Depending on SA concentrations, the data related with colony growth of *P. capsici* were subjected to linear regression analysis and r^2 was found as 0.93. ED_{50} value of SA was calculated using regression equation as 145.4 ppm against *P. capsici* (Figure 1).

BABA did not show any inhibition effect on mycelial growth of *P. capsisi* between 0 and 1000 ppm concentrations (Table 2). Colony diameter was 65.2 mm in control while it was found as 64.2 mm even at 1000ppm.

Table 1 The effect of different concentrations of SA on mycelial growth of *P. capsici*

Concentrations (ppm)	Colony diameter (mm)	Effect (%)
0	60,0 **ı***	
10	55,0 **g**	8,3
20	52,0 **f**	13,3
30	52,0 **f**	13,3
40	50,7 **f**	15,5
50	49,0 **f**	18,3
60	47,7 **e**	20,5
70	46,3 **e**	22,8
80	46,0 **e**	23,3
90	41,0 **d**	31,7
100	38,7 **c**	35,5
150	37,7 **c**	37,2
200	6,0 **b**	90
250	0,0 **a**	100
300	0,0 **a**	100
350	0,0 **a**	100

*Means within column followed by different letters are significantly different (P=0.05) according to Fisher's LSD test.

Table 2 The effect of different concentrations of BABA on mycelial growth of *P. capsici*

Concentrations (ppm)	Colony diameter (mm)	Effect (%)
0	65,2**a***	
10	66,0**a**	-1,2
100	65,1**a**	0,2
200	64,8**a**	0,5
300	64,3**a**	1,4
400	64,1**a**	1,7
500	64,2**a**	1,5
600	62,3**a**	4,4
700	63,6**a**	2,5
800	64,2**a**	1,5
900	64,6**a**	0,9
1000	64,2**a**	1,5

ᵏMeans within column followed by different letters are significantly different P=0.05) according to Fisher's LSD test.

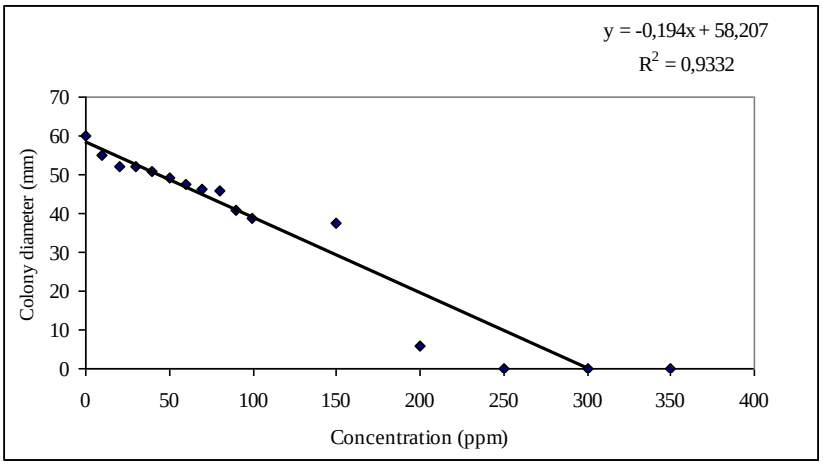

Fig.1. ED_{50} value with regression analysis using mycelial colony diameter for SA effectiveness

The effects of SA and BABA on mycelial fresh and dry weight of *Phytophthora capsici* in vitro

SA reduced the fresh and dry weight of *P. capsici* depending increasing concentration in liquid medium (Table 3). After 2 weeks incubation period, mycelial fresh and dry weight were found as 5.853g and 0.943g in control, while they were 1.001g and 0.110g at 200 ppm concentration, respectively. SA at 250 ppm concentration completely inhibited the mycelial development of *P. capsici*.

BABA has similar effects on mycelial growth of *P. capsici* in liquid medium also (Table 4). The values of fresh and dry weight were found as 6.806g and 0.975g in control and 6.726g and 0.872g at 1000ppm, respectively.

Table 3 The effect of different concentrations of SA on mycelial growth of *P. capsici*

Concentrations (ppm)	Fresh weight (g)	Prevention Ratio (%)	Dry weight (g)	Prevention Ratio (%)
0	5,853 **f***		0,943 **f**	
50	4,340 **e**	25,8	0,758 **e**	19,6
100	3,539 **d**	39,5	0,641 **d**	32,0
150	2,512 **c**	57,1	0,456 **c**	51,6
175	2,450 **c**	58,1	0,380 **c**	59,7
200	1,001 **b**	82,9	0,110 **b**	88,3
250	0,000 **a**	100	0,000 **a**	100

*Means within column followed by different letters are significantly different (P=0.05) according to Fisher's LSD test.

Table 4 The effect of different concentrations of BABA on mycelial growth of *P. capsici*

Concentrations (ppm)	Fresh weight (g)	Prevention Ratio (%)	Dry weight (g)	Prevention Ratio (%)
0	6,806 **a***		0,975 **a**	
100	6,566 **a**	3,5	0,906 **a**	7,1
200	6,767 **a**	0,6	0,998 **a**	-2,4
300	6,800 **a**	0,1	0,972 **a**	0,3
400	6,476 **a**	4,8	0,931 **a**	4,5
500	6,567 **a**	3,5	0,915 **a**	6,2
600	6,800 **a**	0,1	0,951 **a**	2,5
700	6,692 **a**	1,7	0,906 **a**	7,1
800	6,948 **a**	-2,1	0,890 **a**	8,7
900	6,681 **a**	1,8	0,921 **a**	5,5
1000	6,726 **a**	1,2	0,872 **a**	10,6

*Means within column followed by different letters are significantly different (P=0.05) according to Fisher's LSD test.

The effect of SA and BABA on *Phytophthora capsici* in pot conditions

The effects of three different concentrations of soil and leaf applications of SA and BABA on *P. capsici* were determined. Increasing concentration of soil and leaf applications of SA showed increasing effect against *P. capsici* (Table 5). The highest effect soil applications of SA were obtained from 50 and 100mg and leaf applications of 1000 and 1500ppm. Disease severity of pathogen inoculated plants only was 78.0%, while disease severity of 50 and 100mg soil applications of SA were 8.3% and 6.1%, respectively. Thus, reduced disease severity of these concentrations were 89.3% and 92.2%, respectively. Leaf applications of SA with 1000 and 1500ppm concentrations provided 89.3% and 95% effects. The lowest concentrations 25mg and 500ppm soil and leaf applications were reduced the diseases severity by 75.1% and 87.2%, respectively. Stem lesion formation of by *P. capsici* was reduced or delayed by soil and leaf applications of SA. In control plants (+Pc), the mean lesion length of stem was 7.8cm, whereas in the 100mg soil and 1500ppm leaf applications it decreased to 0.2cm. Other concentrations applied to plants reduced the lesion length compared to control and it changed between 0.3 and 1.3cm. SA with 1000 and 1500ppm leaf applications caused some phytotoxcity on pepper plants but this situation did not show any negative effect and plant recompensed it.

BABA showed similar effect as SA on *P. capsici* in pot conditions. The effects of soil and leaf applications of BABA on disease severity of *P. capsici* changed between 60.8 and 97.2% (Table 6). BABA showed similar effects on *P. capsici* except 25mg soil application. Disease severity was 78.0% in control plant whereas it was 11.7% and 12.2% at soil applications of 50 and 100mg, respectively. In leaf applications of BABA, showed growing effect proportionally depending on the increasing concentrations. Leaf

applications of BABA reduced disease severity of *P. capsici* compared to control. Disease severity reduction changed between 83.6 and 97.2% in leaf applications of BABA. Lesion lenght of stem caused by pathogen was reduced by BABA, also. Lesion length was 7.8cm in control whereas it was reduced to 0.9 and 0.3cm in 2000 and 3000ppm leaf applications of BABA and it changed between 1.0 and 2.7cm in other applications.

Table 5 The effect of soil and leaf applications of SA on disease severity of *P.capsici* at pot condition

Treatments	Disease Index	Disease Severity (%)	% Effect	Lesion Lenght (cm)
Control	3,90	78,0 **c***		7,8 **c**
SA-Soil 25mg	0,97	19,4 **b**	75,1	1,3 **b**
SA-Soil 50mg	0,42	8,3 **a**	89,3	0,3 **ab**
SA-Soil 100mg	0,31	6,1 **a**	92,2	0,2 **a**
SA-Leaf 500ppm	0,50	10,0 **ab**	87,2	1,1 **ab**
SA-Leaf 1000ppm	0,42	8,3 **a**	89,3	1,1 **ab**
SA-Leaf 1500ppm	0,19	3,9 **a**	95,0	0,2 **a**

*Means within column followed by different letters are significantly different (P=0.05) according to Fisher's LSD test.

Table 6 The effect of soil and leaf applications of BABA on disease severity of *P. capsici* at pot condition

Treatments	Disease Index	Disease Severity (%)	% Effect	Lesion Length (cm)
Control	3,90	78,0 **c***		7,8 **c**
BABA-Soil 25mg	1,53	30,6 **b**	60,8	2,7 **b**
BABA-Soil 50mg	0,58	11,7 **a**	85,0	1,2 **ab**
BABA-Soil 100mg	0,61	12,2 **a**	84,3	1,2 **ab**
BABA-Leaf 1000ppm	0,64	12,8 **a**	83,6	1,0 **ab**
BABA-Leaf 2000ppm	0,22	4,4 **a**	94,3	0,9 **a**
BABA-Leaf 3000ppm	0,11	2,2 **a**	97,2	0,3 **a**

* Means within column followed by different letters are significantly different (P=0.05) according to Fisher's LSD test

The effects of SA and BABA on *Phytophthora capsici* under greenhouse and field conditions

Effectiveness of soil and leaf applications of SA and BABA on *P. capsici* were determined under greenhouse and field conditions. Soil application with 1g/m^2 and leaf applications with 250 and 500ppm of SA and BABA were treated to plants and determined the effectiveness against *P. capsici* under greenhouse condition (Table 7). All treated concentrations showed reduced effect compared to control and it changed

between 51.3 and 70.5%. Soil application of SA and BABA (1g/l) reduced disease severity by 68.9% and 70.5% SA with 250 and 500ppm and BABA with 250 and 500ppm leaf treatments reduced disease severity by 69.0-61.6% and 51.3-63.4%, respectively.

Soil application with 1g/m^2 and leaf applications with 500 and 1000ppm of SA and 1000 and 2000 of BABA were treated to plants and determined the effectiveness against *P. capsici* under field conditions (Table 8). Disease severity was 66.7% in control whereas it reduced to 25.3% and 49.0% in soil applications of SA and BABA, respectively. Leaf applications of BABA with 1000 and 2000ppm reduced disease severity by 43.5 and 46.2%, respectively. However, SA with 500 and 1000ppm leaf applications reduced disease severity by 36.1% and 50.2%, respectively, depending on the increasing concentrations

Table 7 The effect of soil and leaf applications of SA and BABA on disease severity of *P. capsici* under greenhouse condition

Treatments	Disease Index	Disease Severity (%)	% Effect
Control	1,74	34,8 **b***	
SA-Soil (1g/m2)	0,54	10,8 **a**	68,9
SA-Leaf 250ppm	0,54	10,8 **a**	69,0
SA-Leaf 500ppm	0,67	13,4 **a**	61,6
BABA-Soil (1g/m2)	0,51	10,3 **a**	70,5
BABA-Leaf 250ppm	0,85	16,9 **a**	51,3
BABA-Leaf 500ppm	0,64	12,8 **a**	63,4

*Means within column followed by different letters are significantly different (P=0.05) according to Fisher's LSD test.

Table 8 The effect of soil and leaf applications of SA and BABA on disease severity of *P. capsici* under field condition

Treatments	Disease Index	Disease Severity (%)	% Effect
Control	3,33	66,7 **c***	
SA-Soil (1g/m2)	1,27	25,3 **a**	62,0
SA-Leaf 500ppm	2,13	42,6 **b**	36,1
SA-Leaf 1000ppm	1,66	33,2 **ab**	50,2
BABA-Soil (1g/m2)	1,70	34,0 **ab**	49,0
BABA-Leaf 1000ppm	1,88	37,7 **ab**	43,5
BABA-Leaf 2000ppm	1,79	35,8 **ab**	46,4

* Means within column followed by different letters are significantly different(P=0.05) according to Fisher's LSD test

Capsidiol accumulation

Applications of SA and BABA increased the amount of capsidiol in pepper plant compared to control (Table 9).

The amount of capsidiol in control plants was 12.5µg g^{-1} fresh weight whereas the leaf applications of BABA and SA have the highest level of capsidiol as 56.8 and 52.3µg g^{-1} fresh weight, respectively. After *P. capsici* inoculation, capsidiol accumulation was observed in necrosis of stems (21.8µg g^{-1} fresh weight). In soil applications of SA and BABA treatments, capsidiol levels were lower at 15.0 and 18.7µg g^{-1} fresh weight, respectively.

Table 9 Accumulation of capsidiol in stems of pepper plants in different concentrations

Treatments	Capsidiol amount µg/g fresh weight	Standard Deviation	% Standartd Deviation
Control	12,5	1,4	10,8
P. capsici	21,8	0,6	2,7
SA Soil	15,0	1,6	11,0
SA Leaf	52,3	0,2	0,5
BABA Soil	18,7	2,4	12,6
BABA Leaf	56,8	4,5	7,9

The concentration of capsidiol are the means of three extraction

DISCUSSION

SA reduced the mycelial development of *P. capsici* depending increasing concentration on solid and in liquid medium. In some studies reported that, SA had inhibitory effects on mycelial growth of some fungal pathogens in artificial medium. Bayraktar and Dolar (2002) reported that mycelial growth of *Ascochyta rabiei* was reduced by SA at increasing concentration on solid medium which was between 0-15mM concentrations and completely inhibited at 7.5mM. In some cases, SA did not any inhibitory effect on some bacterial pathogen like bacterial speck caused by *Pseudomonas* syringae pv.*tomato* in vitro (Çökmüş and Sayar, 1991). Bayraktar and Dolar (2002) indicated that concentrations between 0-15mM SA suspensions had inhibitory effect on spore germination of *Ascochyta rabiei*, also; similarly 7.5mM of SA reduced the germination of spore by 97.8% however concentration greater than 7.5mM completely inhibited it. Besides the inhibitory effects on mycelial growth in solid and liquid medium, researchers notified the increasing effects on fresh and dry mycelial weigth in liquid medium as in reported by Küçükkömürcü et al. (2002). As mentioned study indicated that SA had increasing effect on fresh and dry mycelial growth of *Fusarium oxysporum* f.sp *melongena* in liquid medium.

In the present study, BABA had no inhibitory effect on mycelial growth of *P. capsici*. Previous reports indicated that BABA had no inhibitory effect on mycelial growth of pathogen in vitro, also however systemic resistance was enhanced when applied to plant and provided protection against pathogen. As a matter of fact, isomers of aminobutyric acid did not inhibit mycelial growth and spore germination of *P. capsici* even at 1000 ppm concentration in vitro (Sunwoo et al. 1996). Janjun et al (1996) reported that 3-aminobutyric acid (3-ABA) had no inhibitory effect on mycelial development of *Verticillium dahliae* at 50 and 100ppm on V8 agar in vitro. In malt agar medium, 3-ABA had no effect on mycelial development of *Fusarium oxysporum* f.sp. *lycopersici* even at 1000ppm.

Tosi et al. (1998) reported that BABA solutions including 100, 200 and 300µg ml-1 concentrations did not inhibited germination of zoosporangium of downy mildew of sunflower caused *Plasmopara helianthi* in vitro. Accordingly, Cohen (1994a) pointed out that DL-3-amino-n-butanoic acid between 0 and 1000ppm concentrations showed any effect on spore germination and mycelial growth of *Phytophthora infestans*.

In this study, increasing concentration of soil and leaf applications of SA showed increasing effect against *P. capsici.* SA synthesized hormon in plant constitution has an important role in plant disease resistance directly or indirectly as well as applications of synthetic SA compounds to plants have fungistatic and fungitoxic activity against pathogen (Raskin, 1992; Delaney et al. 1994). Mills and Wood (1984), SA injection (%0.02) to cotyledon of cucumber inhibited development of *Colletotrichum lagenarium*. Bayraktar and Dolar (2002) reported that seed application of SA at 1mM was the most effective against the anthracnose of chickpea caused by *Ascochyta rabiei* and reduced by 23%. SA application of upper part of the plant reduced the disease by 48.15% when treated one time before inoculation at 0.8mM.

The highest level of protection of leaf application of BABA was by the reason of penetration and transportation systemically after taken in plants. Cohen and Gisi (1994) revealed that the effect of different application methods of BABA on *Phytophthora infestans*. Indicating that leaf spraying of BABA penetrated to leaf and transported acropetally in plant and localized in young leaves. In addition, BABA could be obtaine by roots when applied to soil as irrigation and transported to leaves of tomato plants. Depending on the mode of transport, resistance mechanism was enhanced and protected against *P. infestans*.

BABA has broad spectrum activitiy on causal organisms such as viral, bacterial, oomycet and other fungal species in plants (Jakab et al. 2001). Hwang et al. (1997) revealed that spraying of BABA with 1000µg ml^{-1} at the stage of first true leaf of pepper reduced the Phytophthora blight by 75%.

Leaf spraying of BABA with 2000 and 3000ppm caused very little necrotic lesions on leaves; however plants maintained the growth after response without any

effect. Cohen (1994b) reported that among the isomers of butyric acid, BABA (β-aminobutyric asid) and AABA (α-aminobutyric acid) phytotoxicity when sprayed to leaves of tobacco plants at 100μgmL^{-1} after two days.

Aly et al (1998) revealed that Acetyl salicylic acid (ASA) and Sallicyclic acid (SA) induced systemic resistance against *Pseudoperonospora cubensis* in cucumber. ASA and SA at 5mM sprayed to seedlings was the most effective in pot conditions; under ASA and SA at 5mM reduced the disease severity by 77% and 68% greenhouse conditions continuing effect after sowing during 70 days. Dann et al (1998) reported that different concentration of 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) (35, 45 and 65mg a.i l^{-1}) sprayed to plants to protection of white rot caused by *Sclerotium rolfsii* in soybean under greenhouse and field conditions. INA with 3-4 applications reduced disease severity by 20-70% in Elgin 87 and Williams 82 cultivars known being sensitive to disease under natural conditions. BTH with 2-4 times applications reduced disease severity by 20-60% in sensitive cultivars.

Manandhar et al. (1998) reported that soil application of salicyclic acid (SA) at 1mM to different cultivars under greenhouse conditions reduced rice blight caused by *Pyricularia oryzae* by 47 to 66% inducing systemic resistance. Similarly, SA at 10mM leaf sprayind reduced disease severity. SA at 10mM leaf spraying two times reduced disease severity by 38% under field conditions.

Acibenzolar-S-methyl (ASM) belong to important chemical class as synthetic analog of SA which has an important role in induced systemic resistance used against some fungal, bacterial and viral pathogens succesfully. Cole (1999) reported that ASM with 2-3 times applications reduced disease severity of *Pseudomonas syringae* pv. *tabaci*, *Thanathephorus cucumeris* and *Cercospora nicotianae* in tobacco. ASM at 25 and 37.5g a.m/ha could be reduced *Peronospora hyoscyami* f.sp. *tabacina* when applicated 10 days interval (Perez et al. 2003).

In present study, results showed that capsidiol has an important role in inducing resistance againt *P. capsici*. Any doubt physical and biochemical factors act a part together in plants in resistance mechanism against pathogen. It's known that phytoalexine level increase depending on some stres factors in plants. Likewise some researches revealed that capsidiol accumulation were induced by biotic and abiotic elicitors in pepper (Üstün and Ercoskun, 1994; Garcia-Perez et al. 1998). In this study, capsidiol amounts were higher in some treatments such as leaf applications of SA and BABA especially compared to control and decided could be a part of biochemical resistance against *P. capsici* in pepper. Beside that soil applications of SA and BABA had also higher level effect when considered the results related with pot experiment, it thought that other biochemical factor except capsidiol also could be take part in resistance.

Phytoalexine accumulation causes resistance following pathogen infections. In previous studies, capsidiol had an important role in plants showed resistance depending on age or metalaxyl-applicated pepper plants in Phytophthora blight control (Hwang and Sung, 1989; Hwang and Kim, 1990). Lotan and Fluhr (1990) reported that DL-alpha-n-amino-butyric acid applications induced capsidiol accumulation in tobacco leaves. Capsidiol accumulation was enhanced in stem when BABA sprayed at 1000µg ml^{-1} to plants resulting synthesized SA endogenously as a signal (Hwang et al 1997).

In this study, capsidiol observed in necrotic zones after *P. capsici* inoculation. Some authors also reported that increased phytoalexine level, soluble phenolic acid and pathogenesis related proteins observed as a response to pathogens formed necrosis (Mauch et al. 1988b; Kim and Hwang, 1994; Candela et al 1995). Capsidiol level was found as 19.9µg and 32.68μg^{-1} fresh weight in control and *P. capsici* inoculated plants after six day (Ahmed et al 2000). Egea et al (1996b) reported that high level of capsidiol was observed after stem inoculation of resistant Smith-5 pepper cultivar in necrotic areas after six day resulting inhibition of pathogen. Capsidiol was fungistatic at 3.75mM and fungitoxic at 5mM in vitro and had an important role in disease resistance.

ÖZET

SALİSİLİK ASİT VE BETA AMİNO BUTİRİK ASİT İLE BİBERDE KÖK-KÖKBOĞAZI ÇÜRÜKLÜĞÜ (*Phytophthora capsici* Leonian) KONTROLÜ VE HASTALIĞA DAYANIKLILIK MEKANİZMASI

Bu çalışmada, salisilik asit (SA) ve DL-β-amino-n-butirik asit (BABA)'in *Phytophthora capsici* tarafından neden olunan kök kökboğazı çürüklüğüne etkisi araştırılmıştır. SA, in vitro'da *P. capsici*'nin miseliyal gelişmesini 250 ppm konsantrasyonda tamamen inhibe etmiştir. Saksı çalışmalarında, SA'in toprak ve yaprak uygulamaları *P. capsici*'nin hastalık şiddetini sırasıyla %75.1-92.2 ve %87.2-95.0 oranlarında azaltmıştır. Sera ve tarla koşullarında, SA'in *P. capsici* üzerine etkisi 1g/m^2 dozda toprak uygulamasında sırasıyla %68.9 ve 62.0 oranında iken, 500ppm ve 1000ppm yaprak uygulamalarında sırasıyla %61.6 ve %50.2 oranında olmuştur. BABA, in vitro'da 1000ppm konsantrasyona kadar *P. capsici*'nin miseliyal gelişmesi üzerie etkisi olmamıştır. Ancak, saksı çalışmalarında, BABA'in toprak ve yaprak uygulamaları *P. capsici*'nin hastalık şiddetini sırasıyla %60.1-84.3 ve %83.6-97.2 oranlarında azaltmıştır. Sera ve tarla koşullarında, BABA'in *P. capsici*'nin hastalık şiddetini 1g/m^2 toprak uygulamasında sırasıyla %70.5 ve %49.0% oranlarında azaltırken, 500ppm ve 2000ppm yaprak uygulamalarında sırasıyla %63.4 ve %46.4 oranlarında azaltmıştır. SA'in 1000ppm ve BABA'in 2000ppm BABA yaprak uygulamaları biber bitkilerinde kapsidiol düzeyini arttırmıştır.

Anahtar Kelimeler: Beta amino butirik asit (BABA), kapsidiol, dayanıklılığın teşviki biber, salisilik asit (SA)

LITERATURE CITED

AHMED, AS., CP. SANCHEZ, ME. CANDELA, 2000. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. **European Journal of Plant Pathology,** 106, 817-824.

ALY, MM., WE, ASHOUR, F. ABD EL-KAREEM, MM. DIAB, 1998. Systemic acquired resistance in greenhouse cucumber for controlling downy mildew using salicyclic acid and acetylsalicyclic acid. 7th International Congress of Plant Pathology. 16th August 1998, Edinburg. Paper Number:1.4.30 (http://www.bspp.org.uk/icpp98/1.4/30.html).

BAYRAKTAR, H., FS. DOLAR, 2002. Induction of resistance in chickpea to Ascochyta blight (*Ascochyta rabiei* (Pass.) Labr.) by salicyclic acid. **The Journal of Turkish Phytopathology**, 31(1), 49-61.

BENHAMOU, N., G. THEIAULT, 1992. Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici*. **Physiological and Molecular Plant Pathology**, 41, 33-52.

BLACK, LL, SK. GREEN, GL. HARTMAN, JM. POULOS, 1991. Phytophthora Blight. Pepper Disease: A Field Guide. 45p.

CANDELA, ME., MD. ALCAZAR, A. ESPIN, C. EGEA, L. ALMELA, 1995. Soluble phenolic acids in *Capsicum annuum* stems infected with *Phytophthora capsici*. **Plant Pathology**. 44, 116-123.

COHEN, Y., U. GISI, 1994. Systemic translocation of ^{14}C-DL-3-aminobutyric acid in tomato plants in relation to induced resistance against *Phytophthora infestans*. **Physiological and Molecular Plant Pathology**, 45, 441-446.

COHEN, Y. 1994a. 3-Amino butyric acid induces systemic resistance against *Peronospora tabacina*. **Physiological and Molecular Plant Pathology**, 44, 273-288.

COHEN, Y. 1994b. Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-amino-n-butanoic acids. **Phytopathology**, 84, 55-59.

COLE, DL. 1999. The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. **Crop Protection**, 18, 267-273.

ÇÖKMÜŞ, C., AH. SAYAR, 1991. Effect of Salicyclic Acid on the Control of Bacterial Speck of Tomato Caused by *Pseudomonas syringae* pv. *tomato*. **Journal of Turkish Phytopathology**, 20(1), 27-32.

DANN, E., B. DIERS, J. BYRUM, R. HAMMERSCHIMDT, 1998. Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. **European Journal of Plant Pathology**, 104, 271-278.

DELANEY, TP., S. UKNES, B. VERNOOJI, H. FRIEDRICH, K. WEYMAN, D. NEGROTTO, T. GAFFNEY, M. GUT-RELLA, H. KESSMAN, E. WARD, J. RYALS, 1994. A central role of salicyclic acid in plant disease resistance. **Science**, 266, 1247-1250.

DEMPSEY, DA, DF. KLESSIG, 1995. Signals in plant disease resistance. **Bull. Inst. Pasteur**., 93, 167-186.

DURNER, J., J. SHAH, DF., KLESSIG, 1997. Salicyclic acid and disease resistance in plants. **Trends in Plant Science**, 2(7), 266-274.

EGEA, C., MD. GARCIA-PEREZ, ME. CANDELA, 1996a. Capsidiol accumulation in *Capsicum annum* stems during the hypersensitive reaction to *Phytophthora capsici*. **Journal of Plant Physiology**,149, 762-764.

EGEA, C., MD. ALCAZAR, ME. CANDELA, 1996b. Capsidiol: Its role in the resistance of *Capsicum annuum* to *Phytophthora capsici*. **Physiologia Plantarum**, 98, 737-742.

GARCIA-PEREZ MD., C. EGEA, ME. CANDELA, 1998. Defence response of pepper (*Capsicum annuum*) suspension cells to *Phytophthora capsici*. **Physiologia Plantarum**, 103, 527-533.

GOMEZ, AK., AA. Gomez, 1983. Statistical Procedures for Agricultural Research. 2nd Edition Wiley, New York.

HEIL, M., RM. BOSTOCK, 2002. Induced Systemic Resistance (ISR) Against Pathogens in the Context of Induced Plant Defences. **Annuals of Botany**, 89, 503-512.

HWANG, BK., NK. SUNG, 1989. Effect of metalaxyl on capsidiol production in stems of pepper plants infected with *Phytophthora capsici*. **Plant Disease**, 73, 748-751.

HWANG, BK., YJ. KIM 1990. Capsidiol production in pepper plants associated with age-related resistance to *Phytophthora capsici*. **Korean Journal of Plant Pathology**, 6, 193-200.

HWANG, BK., JY. SUNWOO, YJ. KIM, BS. KIM, 1997. Accumulation of β-1,3-glucanase and chitinase isoforms, and salicyclic acid in the DL-β-amino-n-butyric acid-induced resistance response of pepper stems to *Phytophthora capsici*. **Physiological and Molecular Plant Pathology**, 51, 305-322.

JAKAB, G., V. COTTIER, V. TOQUIN, G. RIGOLI, L. ZIMMERLI, JP. METRAUX, B. MAUCH-MANI, 2001. β-Aminobutyric acid-induced resistance in plants. **European Journal of Plant Pathology**, 107, 29-37.

JANJUN, L., I. ZINGEN-SELL, H. BUCHENAUER, 1996. Induction of resistance of cotton plants to Verticillium wilt and of tomato plants to Fusarium wilt by 3-aminobutryric acid and methyl jasmonate. **Journal of Plant Disease and Protection**, 103(3), 288-299.

KIM, YJ., BK. HWANG, 1994. Differential accumulation of β-1,3-glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of *Phytophthora capsici*. **Physiological and Molecular Plant Pathology**, 45, 195-209.

KIM, YC., KA. BLEE, J. ROBINS, AJ. ANDERSON, 2001. Oxycom^{TM} under field and laboratory conditions increases resistance responses in plants. **European Journal of Plant Pathology**, 107, 129-136.

KUC, J. 1995. Sytemic induced resistance. **Asp. Appl. Biol.,** 42, 235-242.

KUC, J. 2000. Development and future direction of induced resistence in plants. **Crop Protection**, 19, 859-861.

KÜÇÜKKÖMÜRCÜ, D., A. ERKILIÇ, Y. CANIHOŞ, 2002. Patlıcanda Fusarium Solgunluğuna Karşı Salisilik asit, Trifluralin ve Ölü Fusarium Spor Süspansiyonu ile Dayanıklılığın Teşviki. **Ç.Ü.Ziraat Fakültesi Dergisi**, 17(1), 91-98.

LOTAN, T., R. FLUHR, 1990. Xylanase, a novel elicitor of pathogenesis-related proteins in tobacco, uses a nonethylene pathway of induction. **Plant Physiology**, 93, 811-817.

LYON, GD., AC. NEWTON, 1997. Do resistance elicitors offer new opportunities in integrated disease control stratagies? **Plant Pathology**, 46, 636-641.

MANANDHAR, HK., HJ. LYNGS-JORGENSEN, SB. MATHUR, V. SMEDEGAARD-PETERSEN, 1998. Resistance to rice blast induced by ferric chloride, di-potassium hydrogen phosphate and salicyclic acid. **Crop Protection**, 17(4), 323-329.

MAUCH, F., B. MAUCH-MANI, T. BOLLER., 1988. Antifungal hydrolases in pea tissue: II..Induction of fungal growth by combination of chitinase and β-1,3-glucanase. **Plant Physiology**, 88, 936-942.

MILLS, PR., WKS. WOOD, 1984. The effect of polyacylic acid, acetylsalicyclic acid and salicyclic acid on resistance of cucumber to *Coletotrichum lagenarium*. **Phytopath. Z.,** 111, 209-216.

NAKASHITA, H., K. YOSHIOKA, M. YASUDA, T. NITTA, Y. ARAIS, S. YOSHIDA, I. YAMAGUCHI, 2002. Probenazole induces systemic acquired resistance in tobacco through salicyclic acid accumulation. **Physiological and Molecular Plant Pathology**, 61, 197-203.

PALVA, TK., M. HURTIG, P. SAINDRENAN, ET. PALVA, 1994. Salicyclic acid induced resistance to *Erwinia caratovora* subsp. *caratovora* in tobacco. **Molecular Plant-Microbe Interaction**, 7, 356-363.

PEREZ, L., ME. RODRIGUEZ, F. RODRIGUEZ, C. ROSON, 2003. Efficacy of acibenzolar-S-methyl, an inducer of systemic resistance against tobacco blue mould caused by *Peronospora hyoscyami* f.sp. *tabacina*. **Crop Protection**, 22, 405-413.

RASKIN, I. 1992. Role of Salicyclic Acid in Plants. **Annu. Rev. Plant Physiol. Plant Mol. Biol**., 43, 439-463.

SIEGRIST, J., D. GLENEWINKEL, C. KOLLE, M. SCHMIDTKE, 1997. Chemically induced resistance in green bean against bacterial and fungal pathogens. **Journal of Plant Disease and Protection**, 104, 599-610.

SUNWOO, JY., YK., YK. LEE, BK. Hwang, 1996. Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-ß-amino-n-butyric acid. **European Journal of Plant Pathology,** 102, 663-670.

TOSI, L., M. GIOVANNETTI, A. ZAZZERINI, G. DELLA TORE, 1988. Influence of mycorrhizal tobacco roots, incorporated into the soil, on the *Thielaviopsis basicola*. **Journal of Phytopathology**, 122, 186-189.

ÜSTÜN, AS., AT. ERCOSKUN, 1994. Bazı Uyarıcıların, Kökboğazı Yanıklığına (*Phytophthora capsici* Leon.) Duyarlılığı Farklı Biberlerin (*Capsicum annuum* L.) Meyvelerinde Kapsidiol Miktarına Etkisi. **Turkish. Journal of Biology**, 18, 173-188.

ÜSTÜN, AS. 1995. Effects of the Some Elicitors on the Capsidiol Amount in the Leaves of Peppers (*Capsicum annuum* L.) Having Different Sensitivities to Root Rot (*Phytophthora capsici* Leon.). **Journal of Turk. Phytopathology**, 24(3), 101-114.

ZIADI, S., S. BARBEDETTE, JF. GODARD, C. MONOT, D. LE CORRE, D. SILUE, 2001. Production of pathogenis-related proteins in the cauliflower (*Brassica oleraceae* var. *botrytis*)-downy mildew (*Peronospora parasitica*) pathosystem treated with acibenzolar-S-methyl. **Plant Pathology**, 50, 579-586.

ZIMMERLI, L., JP METRAUX, B. MAUCH-MANI, 2001. Beta-aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fungus *Botrytis cinerea*. **Plant Physiology**, 126, 517-523.