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Control of charcoal rot disease of okra plants using certain chemical plant resistance inducers

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Abstract

This study was aimed to determine the efficacy of certain chemical inducers on incidence of charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) in okra okra (*Abelmoschus esculentus* (L.) under greenhouse and field conditions. Pathogenicity of 12 isolates of *Macrophomina phaseolina* was carried out on okra under greenhouse conditions. These isolates capable to infect okra plants caused charcoal rot on the basil stem with various degrees of diseases severity. Isolate No. 3 caused the highest charcoal rot severity (70.15%) followed by isolate No. 6 (58.69%). The positive effect of four inducer chemicals, i.e salicylic and ascorbic acids at concentrations 50, 100, 200 ppm; Benzothiadiazole, Bion at 200, 400, 800 ppm and humic acid at 500, 1000, 2000 ppm on the induction of systemic resistance in okra against charcoal rot disease and its effect on growth parameters and green fruit yield components were studied. *In vitro*, all tested chemical inducers able to suppress growth of *M. phaseolina*. The highest decrease in linear growth of *M. phaseolina* was noticed with Bion at concentration 800 ppm (66.96%). Under greenhouse conditions, all the tested chemical inducers significantly decreased charcoal rot severity at all concentrations compared with control. The reduction of charcoal rot in okra was enhanced by increasing chemical inducers concentration. Ascorbic acid was the most effective chemical inducers as they greatly retarded charcoal rot caused by *M. phaseolina* especially at the higher concentration (200 ppm). While, salicylic acid at 50 ppm recorded the lowest protection of charcoal rot severity. Under field conditions, the percentages of charcoal rot severity were significantly reduced due to soaking the seeds before sowing in any of chemical inducers in both trial seasons (2013 and 2014). The most effective inducer was humic acid at 2000 ppm (84.44 and 85.65% reduction of charcoal rot severity), followed by ascorbic acid at 200 ppm (79.79 and 79.28% reduction of charcoal rot severity) in the first and the second growing seasons, respectively. Also, these treatments significantly increased growth parameters i.e. plant height and number of branches plant^{-1} as well as, green fruit yield parameters i.e. number of green fruit plant^{-1}; weight of green fruit plant^{-1} (gm); and total green fruits (ton/acre). Generally, humic acid gave the best results in all growth and green fruit yield parameters under field conditions especially at higher concentration (2000 ppm).

Key words: okra, charcoal rot, chemical inducers, *Macrophomina phaseolina*, humic acid.

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Introduction

Okra (*Abelmoschus esculentus* L.), is an important fruit vegetable crop of the tropical and subtropical regions of the world. In Egypt, it is one of the most popular vegetables and considered a valuable source of calcium, iron and vitamins. It has been grown for its edible green pods which can be used as fresh, canned, frozen, or dried food. Okra dry seeds are reported to contain 18-20% oil and 20-25% crude protein. It has an average nutritive value of 3.21 that is higher than tomato, eggplant and most cucurbits (El-Sheikh & Mohammed, 2009). In okra, it is possible to raise green and seed yield as well as the quality by improving agricultural practices. Among different plant pathogens attacks okra, *Macrophomina phaseolina* (Tassi), is considered as one of the most destructive soil borne pathogen of okra (Hafiz, 1986 and Khanzada *et al*., 2012). It has very wide host range causing diseases on more than 500 cultivated and wild plant species worldwide (Jones and Canada, 1994). The fungus can also cause hallow stem, root rot, pre-emergence and post-emergence damping-off. *Macrophomina phaseolina* is most often seen during summer weather (Gulya, et al., 2002). About 5- 100% yield losses due to this disease have been reported (Vyas, 1981). *Macrophomina phaseolina* does not survive more than seven days in its mycelial form but its sclerotia can survive over ten months in soil (Ghaffar & Akhtar, 1968). It usually develops when soil temperature is 27-35°C for 2 to 3 weeks (Yang & Navi, 2003). This pathogen is difficult to control because of its persistence in soil and wide host range. Some chemicals are effective in controlling charcoal rot disease, but these chemicals are expensive and not environmentally friendly. Therefore, alternative measures are being tested, including induced resistance using biotic and abiotic treatments. The phenomenon of systemic induced resistance, in which resistance to disease is enhanced in tissues distant from the site of the inducing treatment conducted earlier in time, has been extensively reported for a number of plant/pathogen systems and has been the subject of recent reviews (Hammerschmidt, 1999). The majority of these studies have been conducted under controlled environment conditions. However, it has been demonstrated under field conditions for a limited number of plant/pathogen interactions. Induced resistance in some plants against root diseases has been was reported (Sarwar, et al., 2005). Benzothiadiazole (BTH, Bion), was recently identified by scientists at Novartis as a novel disease control compound, and has been promoted as a safe, reliable, and nonphytotoxic plant protection agent. Exogenous application of BTH to wheat and Arabidopsis leaves activate a number of systemic acquired resistance (SAR)-associated genes, leading to enhanced plant protection against various pathogens (Abdel-Monaim, et al., 2011; Lawton, et al., 1996). Humic acid (HA) suspensions based on potassium humates have been applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases (Abdel-Monaim, et al., 2011), stimulating plant growth through increased cell division, as well as optimizing uptake of nutrients, water and stimulating soil microorganisms (Chen, et al., 2004). Also, salicylic acid and ascorbic acid as antioxidants which safe to human and environment had been used successfully to control some plant diseases such as *Fusarium* wilt in chickpea (Nighat Sarwar et al., 2005), peanut root rot (Mahmoud, et al., 2006), *Fusarium* wilt in tomato (Abdel-Monaim, 2010) and root rot and wilt in pepper (Abdel-Monaim & Ismail 2011). The main objectives of this research were to study the efficacy of certain chemical inducers on incidence of charcoal rot disease in okra under greenhouse and field conditions and its effect on growth parameters and yield components.

Materials and methods

Isolation and identification of the causal pathogen: *Macrophomina phaseolina* was isolated from okra plants infected with charcoal rot disease collected from different locations of New Valley Governorate, Egypt. The infected plant tissues bearing fungal sclerotia were selected. The tissue was cut into 5 mm long and 2-3 mm thick pieces. The pieces were surface sterilized with1% NaOCl solution for about 2 minutes followed by thoroughly washing with sterilized water. The sterilized pieces were transferred to potato dextrose agar (PDA) medium in 9 cm diameter Petri plates and incubated at 27±1 °C for 5 days (Abdel-Monaim, 2011). Purification of the isolated fungus was carried out using hyphal tip technique as described by Dhingra and Sinclair (1985). The isolated fungus was identified according to their morphological characters according Barnett and Hunter (1986). Subcultures of the obtained isolates were kept on PDA slants and stored at 4^{o}C for further studies.

Preparation of fungal inoculum: The inoculum of *M. phaseolina* was prepared from one week old culture grown on 100 ml potato dextrose (PD) broth medium in flask (500 ml) and incubated at 27±1°C according to Muthomi, et al. (2007). The content of the flask was homogenized in a blender for one min. The resulted cultures were used for soil infestation in a greenhouse experiment for studying the pathogenicity test.

Pathogenicity tests: Pathogenicity test of *M. phaseolina* (11 isolates) was carried out at New Valley Agricultural Research Station, New Valley, Egypt using okra El-Balady cultivar in plastic pots (30-cm-diam.) containing sterilized sandy loam soil. Okra seeds were surface sterilized by immersing them in 1% sodium hypochlorite solution for 2 min then washed several times with sterilized water. Five seeds were sown in each pot and maintained in the greenhouse. After fifteen days of sowing, pots were inoculated with 100 ml homogenate culture (10^{3}cfu/ml) prepared before, using inoculation method described by Saikia, et al. (2006). Four pots were used for each isolate as replicates, each pot content 5 seedlings. The control was inoculated with the same amount of autoclaved PD medium without fungal inoculum. Disease severity index was determined after 45 days from inoculation with a diseases scale proposed by Mengistu, et al. (2007) based on 0-5 scale according percentage of foliage yellowing or necrosis (0= 0%, 1 = 1-25%, 2 = 26-50%, 3= 51-75%, 4= up to 76%, 5= completely dead plants), and diseases severity (%) was calculated using the following formula:

$$\text{Charcoal rot severity (\%)} = \sum \frac{(n \times v)}{5\ N \times 100}$$

Where, n= number of plants in each category, v= numerical values of symptoms category, N = total number of numerical values of symptoms categories.

Effect of certain inducing resistance compounds on the linear growth of *M. phaseolina in vitro*: Salicylic acid and ascorbic acid were tested at concentrations of 50, 100 and 200 ppm. In addition, Bion was tested at concentrations of 200, 400, 800 ppm. Moreover humic acid was tested at concentrations of 1000, 2000, 4000 ppm. The amount required for obtaining a known concentration of the desired chemical was calculated and added aseptically to a known amount of sterilized PDA medium immediately before pouring in plates, and then plates were inoculated with the desired isolate of *M. phaseolina* then incubated at 27±1°C. Linear growth of the tested isolates was measured when the control plates (medium free of chemical inducers) reached full growth and the average growth diameter was calculated. Each treatment was represented by 3 plates as replicates. Percentage of growth inhibition of pathogen was calculated using the formula below:

$$\text{Inhibition (\%)} = (A - B)/A \times 100$$

Where, A = Colony diameter of pathogen in control, B = Colony diameter in treated plates.

Effectiveness of certain chemical inducers for controlling charcoal rot disease under greenhouse conditions: Fifteen days old okra seedlings (5 seedlings /pot) were injected with 50 µl of chemical inducers listed in Table 1 by sterile syringe at the base of stem with different concentrations. Three days after treatment, pots were inoculated with homogenate suspension of *M. phaseolina* isolate (10^3 cfu ml^{-1}, 100 ml^{-1} pot). Four pots were used as a replicates. The whole experiment was designed as complete randomized block design (CRBD). Disease severity (%) was determined for the next 45 days by the formula as suggested by Mengistu, et al. (2007).

Field experiments: The experiment was carried out in the Experimental Farm of New Valley Agric. Res. Station during two successive growing seasons; 2013 and 2014, for controlling charcoal rot diseases of okra in naturally infested soil. The seeds were soaked in chemical inducers viz. salicylic acid (50, 100, 200 ppm); ascorbic acid (50,100,200 ppm); Benzothiadiazole, Bion (200, 400, 800 ppm) and humic acid (500, 1000, 2000 ppm) for 6 hrs. In control treatment, seeds were soaked in water only for the same time. The treatments were distributed in a complete randomized block design with four replicates, the experimental plot area was 10.5 m^2 (3×3.5 m) containing five rows, each row was 3.5 m in length and distance between rows was 60 cm. All treatments were sown in hills 25 cm apart on one side of row ridge and two seeds per hill (plant population = 56000 plants $acre^{-1}$ approximately). Growing plants were thinned to leave one plant/hill just before first irrigation. Normal cultural procedures know for commercial okra production other than the applied treatments were followed. Charcoal rot severity was recorded on a random sample of plants of the plots (20 plants) three months after planting according to Mengistu, et al. (2007). Fruits harvesting were done every three days. Ten plants were randomly chosen in each plot to determine the following characters (El-Sheikh & Mohammed, 2009): Plant height cm (at the end harvesting), number of branches plant^{-1} (at the end harvesting), number of green fruit /plant, weight of green fruits plant^{-1} (g) fruits were picked with all pedicels in the morning every three days, green fruit length (cm), green fruit diameter (cm) and total green fruits yield (ton acre^{-1}).

Statistical analysis: In all experiments the least significant difference (LSD) at 0.05 confidences was determined according to Gomez and Gomez (1984).

Results and Discussion

Pathogenicity tests: Isolation trails from rotted plants collected from different localities of New Valley Governorate, Egypt yielded 12 fungal isolates which were identified as *Macrophomina phaseolina*. Results illustrated in Fig. (1) show that all the obtained isolates (12 isolates) caused charcoal rot on the basil stem with various degrees of diseases severity. *Macrophomina phaseolina* isolate No. 3 caused the highest charcoal rot severity (70.15%) followed by isolate No. 6 (58.69%). While, the other isolates except No. 4 and No. 11 caused moderate infection of okra plants (24.25-58.69%). Isolates No. 4 and No. 11 were the weak ones for causing charcoal rot (12.58 and 14.14 %, respectively).

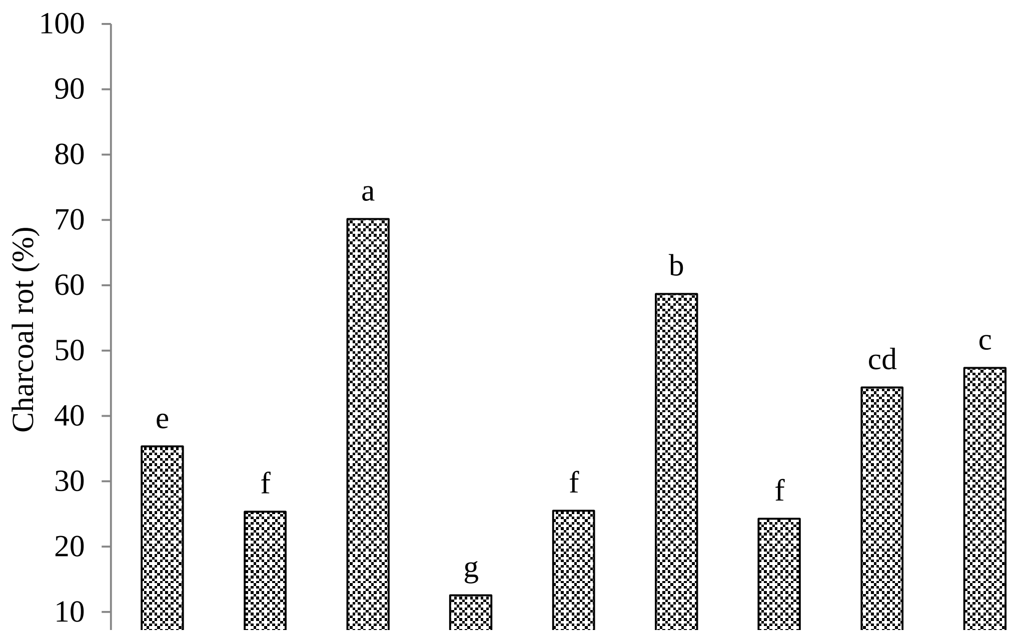

Figure1. Pathogenicity tests of *Macrophomina phaseolina* isolates.

Table 1: Effect of certain chemical inducers on the linear growth of *M. phaseolina in vitro*.

Treatments	Concentrations (ppm)	Inhibition (%)
Salicylic acid	50	15.36
	100	22.56
	200	25.23
	Mean	21.05
Ascorbic acid	50	35.26
	100	45.58
	200	50.14
	Mean	43.66
Bion	200	45.26
	400	56.68
	800	66.96
	Mean	56.30
Humic acid	500	25.16
	1000	36.59
	2000	47.85
	Mean	36.53
LSD at 0.05 for:		
Treatments (T) =	5.23	
Concentrations (C) =	4.29	
Interactions (T×C) =	8.96	

Effect of different concentrations of certain resistance inducing agents on the linear growth of *M. phaseolina*: Data shown in Table (1) demonstrate that the linear growth of *M. phaseolina* was significantly decreased due to the effect of all the tested chemical inducers. The decrease in linear growth was increased by increasing of chemical inducers concentration. The highest decrease in linear growth of *M. phaseolina* was noticed with Bion at concentration 800 ppm (66.96%) followed by Bion at 400 ppm (56.68%), while the lowest decreased of *M. phaseolina* liner growth was recorded in case of SA at 50 ppm (15.36%) followed by SA at 100 ppm (22.56). Generally, Bion recorded the highest suppressed growth of *M. phaseolina* and SA recorded the lowest ones in this respect.

Effectiveness of chemical inducers for controlling charcoal rot disease under greenhouse conditions: A mark reduction in infection by *M. phaseolina* was observed with chemical inducers treated okra plants; while sterilized distilled water treated plants (control) exhibited heavy infection (Table 2). The disease severity in treated plants with any chemical inducers was significantly lower than those plants treated with sterilized distilled water. The reduction of charcoal rot in okra was enhanced by increasing chemical inducers concentration. Ascorbic acid was the most effective chemical inducers as they greatly retarded charcoal rot caused by *M. phaseolina* especially at the higher concentration (200 ppm). Soaking of okra seeds in the chemical solution (200 ppm) of ascorbic acid for 6 hr. before planting reduced charcoal rot infection from 66.36 in control to 8.56% in seed treated. While, salicylic acid at 50 ppm recorded the lowest protection of charcoal rot severity (46.87% protection).

Table 2: Effect of certain chemical inducers on charcoal rot severity of okra under greenhouse conditions.

Treatments	Concentrations (ppm)	Charcoal rot (%)	Protection (%)
Salicylic acid	50	35.26	46.87
	100	24.15	63.61
	200	15.26	77.00
	Mean	24.89	62.49
Ascorbic acid	50	22.45	66.17
	100	17.42	73.75
	200	8.56	87.10
	Mean	16.14	75.67
Bion	200	25.36	61.78
	400	20.14	69.65
	800	15.24	77.03
	Mean	20.25	69.49
Humic acid	500	32.36	51.24
	1000	20.36	69.32
	2000	12.25	81.54
	Mean	21.66	67.36
Control		66.36	-
LSD at 0.05 for:			
Treatments (T) =		3.26	
Concentrations (C) =		3.96	
Interactions (T×C) =		7.08	

Field experiments: All chemical inducers with the tested concentrations exhibit significant protection against charcoal rot disease caused with *M. phaseolina* compared with control in both growing seasons 2013 and 2014), and the protection against charcoal rot diseases increased by increasing concentrations of any chemical inducers (Table 3). However, the most effective inducer was humic acid at 2000 ppm (84.44 and 85.65 reduction of charcoal rot severity) followed by ascorbic acid at 200 ppm (79.79 and 79.28% reduction of charcoal rot severity) in the first and the second growing seasons, respectively. Conversely, okra plants treated with SA at 50 ppm showed the lowest protection against charcoal rot disease, while recorded 45.37 and 44.96 % reduction of charcoal rot severity in the first and the second growing seasons, respectively.

All tested chemical inducers significantly increased the tested growth parameters i.e. plant height and branches number per plant compared with control treatment in both growing seasons (Table 4 and 5). The most effective chemical inducers on plant height (165.47 and 128.36 cm in the first and the second growing season respectively) was humic acid at 2000 ppm, that followed by ascorbic acid at 200 ppm (156.36 and 120.36 during the growing seasons, respectively). Conversely, effect of SA for increasing plant height was lower compared with the others. The same trend was also observed in case of number of branches per plant, while okra seeds were soaked in humic acid at 2000 ppm recorded the highest branch number per plant (6.21 and 5.69) in both growing seasons followed by Bion at 800 ppm (5.96) in the first season and humic acid at 1000 ppm (5.96 and 4.78, respectively in the two seasons), however SA recoded the lowest ones in both seasons.

Table 3: Effect of certain chemical inducers on charcoal rot severity of okra under field conditions during 2013 and 2014 growing seasons in New Valley governorate, Egypt.

Treatments	Concentrations (ppm)	Season 2013		Season 2014	
		Charcoal rot (%)	Protection (%)	Charcoal rot (%)	Protection (%)
Salicylic acid	50	23.14	45.37	20.14	44.96
	100	16.25	61.64	12.14	66.82
	200	12.36	70.82	10.29	71.88
	Mean	17.25	59.28	14.19	61.22
Ascorbic acid	50	20.14	52.46	18.45	49.58
	100	11.5	72.85	9.56	73.87
	200	8.56	79.79	7.58	79.28
	Mean	13.4	68.37	11.86	67.58
Bion	200	20.02	52.74	20.49	44.00
	400	13.56	67.99	14.09	61.49
	800	9.25	78.16	9	75.40
	Mean	14.28	66.30	14.53	60.30
Humic acid	500	18.56	56.19	18.09	50.56
	1000	10.25	75.80	8.25	77.45
	2000	6.59	84.44	5.25	85.65
	Mean	11.8	72.14	10.53	71.22
Control		42.36	-	36.59	-
LSD at 0.05 for:					
Treatments (T) =		2.96		3.61	
Concentrations (C) =		3.96		3.85	
Interactions (T×C) =		6.05		6.59	

There was a significant effect with the chemical inducers on the tested quantitative parameters i. e. number of green fruit plant^{-1}; weight of green fruit plant^{-1} (gm); and total green fruits (ton/acre) and non-significantly effect on green fruit length (cm); green fruits diameters (cm) (Table 4 and 5) compared with control. These parameters increased with increasing concentrations of chemical inducers. The most effective inducers was humic acid at 2000 ppm for all quantitative parameters of green fruit yield, whoever recorded highly number of fruit plant^{-1} (25.46 and 22.14), fruit yield plant^{-1} (296.35 and 216.42 gm), green fruit length (7.24 and 7.25 cm); green fruits diameters (3.05 and 2.93 cm) and total green fruits yield acre $^{-1}$ (9.37 and 7.24 ton acre $^{-1}$ compared with 12.28 and 10.23, 92.89 and 85.36, 5.53 and 5.00, 2.04 and 2.01, 3.15 and 2.96 in control treatment in both growing seasons, respectively. On the other hand, okra seeds treated with SA recorded the lowest increase of all quantitative parameter and green fruit yield in both seasons.

Discussion

Okra (*Abelmoschus esculentus* L.), is one of the most important vegetable crops. Soil borne diseases including root rot, wilt and charcoal rot cause important considerable losses in yield. Charcoal rot is a widespread root and stem disease of okra caused by the soil-inhabiting fungus *Macrophomina phaseolina*. The disease is more damaging in years with extended periods of hot, dry weather. Isolation trails from rotted okra plants yielded 12 isolates of *M. phaseolina* confirming to other reports (Khanzada, et al., 2012; Hafiz, 1986). Pathogenicity test demonstrated that all the obtained isolates able to infect okra plants caused typical charcoal rot symptoms with different percentages of disease severity.

Table 4: Effect of certain chemical inducers on growth parameters and quantitative parameter of green fruit yield of okra under field conditions during 2013 growing season in New Valley Governorate, Egypt.

Treatments	Concen. (ppm)	Plant height (cm)	No. of branches plant^{-1}	No. of green fruit plant^{-1}	Weight of green fruit plant^{-1}(gm)	green fruit Length (cm)	Green fruits diameters (cm)	Total green fruits (Ton/acre)
Salicylic acid	50	102.36	4.25	13.56	155.32	5.96	2.11	5.27
	100	112.53	5.25	16.58	196.36	6.36	2.25	6.97
	200	119.86	5.65	18.69	215.63	6.89	2.31	7.56
	Mean	111.58	5.05	16.28	189.10	6.40	2.22	6.60
Ascorbic acid	50	115.48	4.59	15.25	177.42	6.36	2.28	6.60
	100	125.36	5.65	19.63	229.36	6.86	2.35	7.90
	200	156.36	5.48	22.53	260.45	7.02	2.58	8.97
	Mean	132.40	5.24	19.14	222.41	6.75	2.40	7.82
Bion	200	109.36	4.29	14.25	170.25	5.82	2.19	6.36
	400	115.86	5.25	17.02	199.36	6.48	2.29	7.26
	800	125.36	5.96	18.96	218.36	6.99	2.17	7.97
	Mean	116.86	5.17	16.74	195.99	6.43	2.22	7.19
Humic acid	500	116.36	4.96	17.45	198.29	6.66	2.63	7.03
	1000	130.52	5.89	22.14	265.36	6.96	2.85	7.53
	2000	165.47	6.21	25.46	296.35	7.24	3.05	9.37
	Mean	137.45	5.69	21.68	253.33	6.95	2.84	7.97
Control		88.36	3.42	12.28	92.89	5.53	2.04	3.15
LSD at 0.05 for:								
Treatments (T)	=	6.23	0.53	1.02	7.63	ns	ns	1.02
Concentrations (C)	=	5.25	0.82	1.55	5.69	ns	ns	0.96
Interactions (T×C)	=	11.09	ns	2.36	12.58	ns	ns	1.88

Table 4: Effect of certain chemical inducers on growth parameters and quantitative parameter of green fruit yield of okra under field conditions during 2014 growing season in New Valley Governorate, Egypt.

Treatments	Concen. (ppm)	Plant height (cm)	No. of branches plant^{-1}	No. of green fruit plant^{-1}	Weight of green fruit plant^{-1}(gm)	green fruit Length (cm)	Green fruits diameters (cm)	Total green fruits (Ton/acre)
Salicylic acid	50	99.36	3.63	12.36	115.36	5.42	2.01	4.57
	100	105.36	3.96	13.96	125.86	5.96	2.25	5.02
	200	109.36	4.02	14.25	138.69	6.32	2.41	5.96
	Mean	104.69	3.87	13.52	126.64	5.90	2.22	5.18
Ascorbic acid	50	109.06	4.25	14.52	144.25	6.02	2.53	5.37
	100	115.42	4.96	16.89	158.69	6.85	2.61	5.90
	200	120.36	5.26	19.86	199.36	7.01	2.71	6.86
	Mean	114.95	4.82	17.09	167.43	6.63	2.62	6.04
Bion	200	102.36	3.68	13.25	128.36	5.69	2.12	4.97
	400	109.36	4.05	14.96	140.14	5.86	2.33	5.27
	800	115.36	4.18	16.86	160.52	6.45	2.41	5.97
	Mean	109.03	3.97	15.02	143.01	6.00	2.29	5.40
Humic acid	500	111.96	4.36	14.59	140.02	6.23	2.61	5.86
	1000	119.00	4.78	17.85	170.00	6.99	2.75	6.60
	2000	128.36	5.69	22.14	216.42	7.25	2.93	7.24
	Mean	119.77	4.94	18.19	175.48	6.82	2.76	6.57
Control		85.36	3.02	10.23	85.36	5.00	2.01	2.96
LSD at 0.05 for:								
Treatments (T)	=	5.59	0.51	1.09	6.29	ns	ns	1.09
Concentrations (C)	=	5.63	0.71	1.29	5.29	ns	ns	0.88
Interactions (T×C)	=	10.89	ns	2.42	10.96	ns	ns	1.02

The use of fungicides to control soilborne diseases of economically important crops has been used in agriculture for many years. However, recently the use of chemical has been reduced for several reasons, including pollution of environment, particularly ground water and food supplies. Recently, an increasing desire to reduce the use of fungicides is seen through the attempts to develop integrated pest managements approaches, where natural resources are put to maximum use. Chemically induced resistance (IR) is a suitable strategy to utilize natural defenses of the plant to control pathogens. This phenomenon has been studied at the molecular level and has proved to be mediated by salicylic acid and associated with a number of defense responses and genes (Ton, et al., 2005). Induced resistance was reported to be activated by exogenous application of salicylic acid (Abdel-Monaim, et al., 2011), ascorbic acid (Abdou, et al., 2001), benzothiodiazole, Bion (Lawton *et al*., 1996 and Abdel-Monaim, et al., 2011) and humic acid (Abdel-Monaim, et al., 2011). In the present study, it was planning to investigate the possibility of minimizing the infection with charcoal rot disease of okra using SA, ascorbic acid, Bion, humic acid at three different concentrations as resistance inducer. Application of these compounds to the medium significantly inhibited the growth of *M. phaseolina* at different concentrations compared to the control. Moreover, the reduction percentage of linear growth of *M. phaseolina* increased by increasing the concentration of these compounds and reached their maximum reduction at concentration 800 ppm of Bion. Also, the obtained data revealed that all chemical inducers caused significant reduction charcoal rot disease either in pot or field experiments compared with the control treatment. Generally, ascorbic acid was the most effective chemical inducers as they greatly retarded charcoal rot caused by *M. phaseolina*, especially at the higher concentration (200 ppm). While SA was recorded the lowest reduction ones. On the other hand, all treatments improved plant growth and increased of yield parameters compared with untreated plants (control), Okra seeds soaked with humic acid, especially at high concentration (2000 ppm) recorded the highest increase of all growth parameters *viz.* plant height, number of branches per plant and quantitative parameter of fruit yield *viz.* number of green fruit plant^{-1}; weight of green fruit plant^{-1} (gm); green fruit length (cm); green fruits diameters (cm) and total green fruits (ton/acre), while SA recorded the lowest ones, especially at lower concentration (50 ppm). Several mechanisms that mediate the disease protection induced by different chemicals have been demonstrated, including blocking of disease cycle, the direct inhibition of pathogen growth (Abdel-Monaim, 2011; Thompson, et al., 2000) and the induction of resistance to plant against pathogen infection (Ahn, et al., 2005). Exogenous application of Bion to wheat, soybean and Arabidopsis leaves activate a number of systemic acquired resistances (SAR)-associated genes, leading to enhanced plant protection against various pathogens (Lawton, et al., 1996 and Abdel-Monaim, et al., 2011). Also, application of salicylic and ascorbic acids (antioxidant compounds) resulted in accumulation of pathogenesis related proteins (PRs), which have been defined as plant proteins that are induced in pathological and related situations (Hassan, et al., 2006). Salicylic and ascorbic acids initially proposed to bind to catalase and ascorbate peroxidase. The binding of salicylic and ascorbic acids to suck enzymes might lead to the formation of a phenolic radical involved in lipid peroxidation. Lipid products can active defense gene expiration (Farmer, et al., 1998). Anderson (1988) confirmed that SA was responsible for the accumulation of phytoalexins in viable tissues. Application of HA enhances the activity of antioxidants such as α-tocopherol, α-carotene, superoxide dismutases, and ascorbic acid concentrations in turf grass species. These antioxidants may play a role in the regulation of plant development, flowering, and chilling of disease resistance (Dmitriev, et al., 2003). Humic acid may increase the permeability of plant membranes and enhance the uptake of nutrients. Moreover, HA may also improve soil nitrogen uptake and facilitate the uptake of potassium, calcium, magnesium, and phosphorus, making these nutrients more mobile and available to plant root systems (Piccolo et al., 1992).

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