Mycobiology

Research Article

Induction of Defense-Related Physiological and Antioxidant Enzyme Response against Powdery Mildew Disease in Okra (*Abelmoschus esculentus* L.) Plant by Using Chitosan and Potassium Salts

Mona H. Soliman[1,2,*] and Riad S. R. El-Mohamedy[3]

[1]*Biology Department, Faculty of Science, Taibah University, Yanbu 464 29, Kingdom of Saudi Arabia*
[2]*Botany and Microbiology Department, Faculty of Science, Cairo University, 12613 Giza, Egypt*
[3]*Plant Pathology Department, National Research Centre, Cairo 12622, Egypt*

Abstract Foliar sprays of three plant resistance inducers, including chitosan (CH), potassium sorbate (PS) ($C_6H_7kO_2$), and potassium bicarbonates (PB) ($KHCO_3$), were used for resistance inducing against *Erysiphe cichoracearum* DC (powdery mildew) infecting okra plants. Experiments under green house and field conditions showed that, the powdery mildew disease severity was significantly reduced with all tested treatments of CH, PS, and PB in comparison with untreated control. CH at 0.5% and 0.75% (w/v) plus PS at 1.0% and 2.0% and/or PB at 2.0% or 3.0% recorded as the most effective treatments. Moreover, the highest values of vegetative studies and yield were observed with such treatments. CH and potassium salts treatments reflected many compounds of defense singles which leading to the activation power defense system in okra plant. The highest records of reduction in powdery mildew were accompanied with increasing in total phenolic, protein content and increased the activity of polyphenol oxidase, peroxidase, chitinase, and β-1,3-glucanase in okra plants. Meanwhile, single treatments of CH, PS, and PB at high concentration (0.75%, 2.0%, and/or 3.0%) caused considerable effects. Therefore, application of CH and potassium salts as natural and chemical inducers by foliar methods can be used to control of powdery mildew disease at early stages of growth and led to a maximum fruit yield in okra plants.

Keywords Antioxidant enzymes, Induce resistance, Okra, Powdery mildew

Okra (*Abelmoschus esculentus* L.), is one of the most important vegetable crops belongs to the family *Malvaceae*, which grown in tropical, subtropical and warm temperate regions around the world. It is also popular vegetable for home garden and a good source to fulfill the energy needed to the body. Moreover, it provides vitamin A, B, C, protein, minerals, and iodine. Okra vegetable crop recently recorded a considerable yield losses range between 17% and 86.6%, when attacked by powdery mildew disease to all plant parts infected i.e., leaves, stem and fruits [1-3]. Reuveni *et al.* [4] tested the efficacy of various chemical inducers of systemic resistance against powdery mildew disease. In the same manner, an observed decrement in powdery mildew severity in pepper plants as exposed to foliage sprays of sodium or potassium bicarbonates (PB) was recorded [5]. Deliopoulos and colleagues [6, 7] reported that these salts were used as pesticide ingredients and horticultural fungicide, in the United States and United Kingdom, respectively. Several physiological mechanisms must be considered for the chemical compounds to be registered as fungicides, such as pH elevation on the leaf surface and collapse of fungal cell walls due to K^+ imbalance or dehydration of fungal spores [8, 9].

Potassium bicarbonates ($KHCO_3$) and PS ($C_6H_7kO_2$) have been demonstrated to inhibit several fungal pathogens infecting vegetables and ornamental crops at the post-harvest stage [10-12]. Antimicrobial and antifungal activity of potassium sorbate (PS) as a weak acid are also studied [13, 14]. PS was the highly effective compound used to

Mycobiology 2017 December, **45**(4): 409-420
https://doi.org/10.5941/MYCO.2017.45.4.409
pISSN 1229-8093 • eISSN 2092-9323

*Corresponding author
E-mail: riadelmohamedy@yahoo.com

Received August 12, 2017
Revised November 13, 2017
Accepted November 22, 2017

control orange molds caused by *Penicillium digitatum* and management of several food-borne fungal pathogens for plants infected with *P. italicum* [15, 16]. Furthermore, PB as chemical inducer is characterized by lower risk of fungal resistance development due to its multiple modes of action, lower cost and shorter application to harvest interval, as well as its potential role to reduce the environmental impact of synthetic fungicides [9]. Chitosan (CH) is a biopolymer, a chitin derivative, a compound that is eco-friendly and characterized by unique properties such as bioactivity and biocompatibility [17]. Several studies reported that CH could increase the yield, reduce transpiration, and induce more resistance to viral, bacterial, and fungal infections [18-20]. Regards to biochemical and physiological processes, CH can stimulate vital mechanism of plants at the single cells, tissues and molecular changes based on expression of genes [21-23].

Some of the unique properties of CH may specifically stimulate plant reactions and inhibit the microorganisms. Moreover, one of the most important factors affecting the biological activity of this biopolymer is the molecular weight [24-28]. In recent years, the importance of CH as plant growth promoter and pathogen control agent has been evaluated [29, 30]. CH as a biopolymer can stimulate the immune system in plant to pathogen infection resistance [31]. Faoro *et al.* [32] showed that the sprayed plants with CH recorded a decrement of systemically infection caused by powdery mildew pathogen *Blumeria graminis* f. sp. *hordei*. Enhancement levels of defense related antioxidant enzymes [such as protease inhibitors, β-1,3-glucanases, peroxidases (PODs), polyphenol oxidases (PPOs)] was demonstrated in leaves and also in some rhizomes grown under field condition and treated with CH as natural inducer [33].

Research works of certain plant resistance inducers (PRI) on growth, yield attributes, and fruit yield of okra is almost rare. Considering the above facts, our work was conducted to study the effect of CH and potassium salts, PS and PB as PRIs for inducing systemic protection in okra plants infected with powdery mildew and for improving the vegetative growth as well as final yield. Some biochemical mechanisms and antioxidant responses were also investigated.

MATERIALS AND METHODS

Procurement of culture and seed. The fungal pathogen inoculum was isolated from naturally infected okra plants, Okra seeds (cv. Baladi), obtained from Department of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

Pathological studies.

Greenhouse experiments: Okra seeds were sown in plastic pots (25-cm-diam.) containing loamy soil, four seeds/pot. Ten pots were used for each treatment. Irrigation were added as needed. The efficiency of PRI such as CH, PS, and PB in controlling okra powdery mildew were evaluated under artificial inoculation with conidia of *Erysiphe cichoracearum* for plants implanted in greenhouse conditions.

Preparation of *E. cichoracearum* inoculum and pathogenicity: The powdery mildew fungal inoculum was obtained from freshly infected leaves of naturally infected okra plants. Conidia were gently brushed into 100-mL distilled water with 5 mL of Tween-20 then counted by haemocytometer to give a mixture of 5×10^5 conidia/mL. For plant inoculation, the upper surfaces of all the leaves were sprayed with a conidial suspension delivered by a hand sprayer [34].

The following treatments of PRI by CH at concentrations of 0.25%, 0.5%, and 0.75% (w/v), PS at 0.5%, 1.0%, and 2% (w/v), and PB at 1%, 2%, and 3% (w/v) were applied alone and in combined treatments for evaluate their efficacy against powdery mildew. Plant inoculation was carried out by dusting of okra leaves with conidia of *E. cichoracearum*. All tested PRIs were applied as twice sprays on the okra plants four days after pathogenicity. Inoculated plants were covered with polyethylene bags overnight. Control plants were sprayed with distilled water under similar conditions. Observations were recorded on the development symptoms daily for a period of 15 days after inoculation.

Disease assessment. Powdery mildew disease severity was estimated into 5 categories according to Descalzo *et al.* [35] based on the following scale: 0 = no visual infection, 1 = 1–5% infections, 2 = 6–25%, 3 = 26–50%, and 4 = more than 50% of leaf area covered by fungal colonies. Final disease assessment was conducted at 11 days after each spray in each treatment.

Conidia production of *E. cichoracearum* on treated okra leaves were also evaluated. Leaves were detached gently at the early morning and immersed in screw cap jars containing 100 mL of distilled water. Conidia were released from lesions using a brush, and then counted using a haemocytometer. Fungal conidia/cm^2 of leaf area were counted in each treatment.

Field experiments. Based on data obtained from greenhouse experiments, the most effective treatments of the tested PRI were chosen and applied under field conditions to evaluate their efficiency in control of powdery mildew disease, and evaluate their effects on vegetative growth and yield parameters of okra. Fungicide, Tri-Miltox forte (2.5 g/L) was used as comparison treatment. Field experiment was carried out under condition of El-Qanater, El-Khairiya, Qualubiya Governorate, Egypt. Field experiments consisted of 36 plots designed in a randomly complete block design with four replicated plots for each particular treatment. Okra seeds (cv. Baladi) were planted in all plots.

The following treatments (T1, CH 0.75%; T2, PS 2.0%; T3, PB 3.0%; T4, CH 0.5% + PS 1.0%; T5, CH 0.75% + PS 2.0%; T6, CH 0.5% + PB 2.0%; T7, CH 0.75% + PB 3.0%; T8, Tri-Miltox forte 2.5 g/L; and T9, control) were applied as twice foliar spray at 7–8 leaf growth stage during 15 days intervals.

In addition, Tri-Miltox forte (2.5 g/L) was sprayed twice every 15 days started after 20 days of sowing date. Application with tap water was used for control. Assessment of powdery mildew disease severity was carried as mentioned before. Vegetative growth and yield parameters plant height (cm), number of branches per plant, pod length (cm), average weight of single pod (g), and total fresh yield of pods (ton/fed) were estimated in 20 plants and 25 pod samples taken randomly from each treatments as well as control.

Biochemical studies. Determination of the activity of different enzymes responsible for diseases resistance in treated okra plant with resistance inducers grown under greenhouses was carried out. Protein content, catalase activity, total phenolic, the activity of POD, PPO, chitinase, and β-1,3-glucanase enzymes was estimated in sprayed and control plants as follows.

Leaves plant samples for biochemical analysis were taken after the second spray of each treatment. Extraction from okra leaves were prepared as follows: A representative samples, 10 g of each, were cut into small portions and immediately plunged into 95% boiling ethanol for ten minutes to kill the tissues. The extraction was resumed in a soxhlet apparatus by using 75% ethanol as an extractant until the percolate was colorless (8–10 hr). The combined ethanolic extracts were filtered and evaporated to near dryness on a mild water bath, 60°C. The dried residue was re-dissolved in 10 mL of 50% iso-propanol and used for chemical analysis as follows:

Estimation of total phenolic content. Total phenolic content in okra leaves of each treatment was determined according the methods described by Descalzo *et al.* [35] as follows: Okra leaves was immersed in liquid nitrogen, homogenized in 80% methanol (1 g plant material in 10 mL) and stored at −20°C. Later, the homogenate was centrifuged at 15,000 ×g for 30 min at 4°C. The pellet was discarded. After addition of ascorbic acid (0.1 g/5 mL), the homogenate was evaporated in rotary evaporator at 65°C 3 times for 5 min. The residues were dissolved in 5 mL of 80% methanol. For the determination, 0.02 mL methanol extract was incubated for 1 hr with 0.5 mL Folin-Ciocalteu reagent, 0.75 mL of Na_2CO_3 solution (20%) and 8-mL water. Total phenolic content was assayed spectrophotometrically at 767 nm. Gallic acid (0–5 mg) and methanol were used as a standard and blank, respectively. Total phenolic were estimated as gallic acid/g (mg) plant material. Activity of all enzymes was determined spectrophotometric as unit/g fresh weight (fw) by measuring the change in absorbance at different weave lengths refers to enzymes using Shimadzu UV-2401 PC UV-Vis recording spectrophotometer (Molecular Biology Lab., National Research Center NRC, Egypt).

Determination of POD activity. POD is routinely assayed by measuring the change in absorbance at 470 nm due to guaiacol oxidation in the presence of hydrogen peroxide and the enzyme was assayed every 30-sec intervals using Shimadzu UV-2401 PC UV-V is recording spectrophotometer in a 1-mL light path cuvettes. The reaction mixture (unless otherwise stated) contained in a volume of 3 mL, 8 μmoles hydrogen peroxide, 60 μmoles guaiacol, 50 μmoles sodium acetate buffer, pH 5.6 and POD at concentrations which gave a linear response over a period of 3 min. The reaction was initiated by introducing the enzyme and mixing, all assays were carried out at 25°C. A unite of POD activity was defined by monitoring the changes in the optical density (OD) values per minute due to amount of enzyme [36].

Determination of PPO activity. PPO activity was determined by measuring the initial rate of quinine formation, as indicated by an increase in absorbance at 420 nm [37]. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min. The sample cuvette contained 2.95 mL of 20 mM catechol solution in 0.1 M phosphate buffer, pH 6.0 and 0.05 mL of the enzyme solution. The blank sample contained only 3 mL of substrate solution. Phenylalanine ammonialyase activity was determined according to Coseteng and Lee [38]. Two hundred microliters of sample crude extract was transferred to a 3-mL light path cuvettes containing 2.5 mL of 0.03 M L-phenylalanine dissolved in 0.05 M sodium borate buffer, pH 8.8. The reaction mixture was then incubated in water bath at 37°C for 1 hr, after cooling the unit of phenylalanine, ammonialyase activity was calculated as one OD change per minute at 290 nm.

Determination of chitinase activity. The chitinase activity was determined by colorimetric method [39] by using colloidal chitin as a substrate and dinitrosalicylic acid as a reagent to measure reducing sugars. The colloidal chitin substrate was prepared from chitin powder as the method described by Boller and Mauch [40], by milting 25 g of chitin and then suspended in 250 mL of 85% phosphoric acid and kept at 4°C for 24 hr. The mixture was blended in 2 L of distilled water and then centrifuged at 2,500 ×g for 20 min. This washing step repeated twice and the colloidal chitin suspension in the final wash was adjusted to pH 7.0 and collected by centrifugation and stored at 4°C. Chitinase activity was assayed in test tube containing the reaction mixture consists of 1 mL of 1% colloidal chitin in 50 mM sodium acetate buffer pH 6.6 and 1 mL sample. The mixture was incubated for 1 hr in controlled water bath at 37°C and centrifuged 2,500 ×g for 20 min. The concentration as mM of N-acetyl-glucose amine (NAGA) in the supernatant was determined using absorbance at 540 nm and the unit of chitinase activity was calculated by the expressed NAGA equivalent released/g fresh weight tissue/60 min.

Determination of β-1,3-glucanase activity. The enzyme solution (100 μL) was mixed with 200 μL of 0.2% (w/v)

laminarin (Sigma, St. Louis, MO, USA) dissolved in 0.1 M sodium phosphate buffer, pH 6.0 and incubated at 30°C for 30 min. The reaction was determined by adding di-nitro-salicylic acid solution and boiling the reaction mixture for 5 min. The absorbance at 540 nm was measured and the unit was defined as the amount of the enzyme that released reducing sugar equivalent to 1 µg glucose per minute under the above conditions [41].

Determination of catalase and total soluble protein. Okra leaves were collected 10 days after inducers application and were excised, weighed (100 mg) and ground with a pestle in an ice cold mortar with 2 mL of 0.05 M Na_2HPO_4/NaH_2PO_4 (pH 7.0) buffer. The homogenate was centrifuged at 4°C for 20 min at 15,000 ×g. The supernatant was used for the assay of catalase activity and total soluble proteins. Catalase activity was measured according to Sun *et al.* [42]. Enzyme extract [20] was added to 3 mL of HP_2-PO_4 buffer (0.16 mL of HP_2 to 100 mL phosphate buffer, pH 7.0) and the breakdown of HP_2 was measured at 240 nm in a spectrophotometer. An equivalent amount of buffer containing H_2O_2 was used as reference. The enzyme activity was expressed in enzyme units/mg protein where one enzyme unit was defined as a change of 0.01 absorbance min caused by the enzyme aliquot. Soluble proteins in the supernatant were estimated according to Sun *et al.* [42] and expressed as mg/g fresh weight.

Statistical analysis. Data of all measurable characters were subjected to statistical analysis according to Chance and Maehly [43]. Duncan's multiple range test was used for means separation [44]. The statistical analysis procedures were kindly carried out by Statistical Consulting Office, National Research Centre, Egypt.

RESULTS

Pathological studies.

Control of powdery mildew on okra plants in green house: Different concentrations of PRIs i.e., CH, PS, PB were applied as single or in combined treatments (CH + PS and CH + PB) for controlling powdery mildew on okra plants under artificially infection with *E. cichoracearum* under greenhouse conditions. Data in Table 1 clearly expressed that all treatments of inducers were highly effective to reduce the powdery mildew disease severity in inoculated plants compared with untreated control. Reduction in disease severity was increased by increasing PRIs concentration. The most significant treatments were CH at 0.5% or 0.75% (w/v) plus PS at 1.0% or 2.0%, PB at 2.0% or 3.0%, which reduced powdery mildew disease severity by 64.7–75.0%. Meanwhile, single treatments of CH, PS, or PB at high concentration (0.75%, 2.0%, and 3.0%) caused considerable disease reductions (57.3%, 55.8%, and 58.8%) respectively. The lowest effects against powdery mildew disease severity observed with low concentrations of PS and CH respectively.

Data represented in (Table 2) also showed that the same trend of results in (Table 1) as all treatments have significantly reduced conidial production (conidia/mm^2 of lesion caused by *E. cichoracearum*). CH + PS or PB are reported as the inhibitory treatments; these treatments reduce conidial production from 82.8% to 94.2%, meanwhile, single treatments of CH, PS, or PB caused reduction range from 50.2% to 88.2% after the second spray.

Control of powdery mildew on okra plants under field conditions: The promised effective treatments of PRIs in controlling powdery mildew under greenhouse experiments were conducted and applied for controlling powdery mildew

Table 1. Disease severity in okra plants in response to spraying with different plant resistance inducers under artificially inoculation with *Erysiphe cichoracearum* in green house

Plant resistance inducer	Concentration (%)	Powdery mildew disease severity					
		1st spray	R (%)	2nd spray	R (%)	Mean	R (%)
CH	0.25	2.3b	32.2	1.9b	41.4	2.10	38.2
	0.5	1.9b	42.2	1.6b	52.8	1.75	48.5
	0.75	1.7b	47.8	1.2b	64.4	1.45	57.3
PS	0.5	3.2a	30.2	1.8b	46.4	2.50	26.4
	1.0	2.1b	38.4	1.6b	52.8	1.85	45.5
	2.0	1.7b	48.2	1.3b	60.6	1.50	55.8
PB	1.0	1.9b	42.4	1.6b	52.8	1.75	48.5
	2.0	1.6b	51.8	1.5b	55.8	1.55	54.4
	3.0	1.6b	52.8	1.2b	64.2	1.40	58.8
CH + PS	0.5 + 1.0	1.5b	55.2	0.9bc	72.2	1.20	64.7
	0.75 + 2.0	1.3bc	60.2	0.8bc	75.0	1.05	69.1
CH + PB	0.5 + 2.0	1.4bc	58.6	0.8bc	73.8	1.10	67.6
	0.75 + 3.0	1.2bc	62.2	0.5bc	84.4	0.85	75.0
Control		3.4a	0.0	3.4a	0.0	3.40	0.0

Values with the same letters in each column are not significantly differed ($p \leq 0.05$).
R, Reduction percent; CH, chitosan; PS, potassium sorbate; PB, potassium bicarbonates.

Table 2. Reduction of produced conidia of *Erysiphe cichoracearum* in okra leaves in response to different plant resistance inducers under artificially inoculation in green house

Plant resistance inducer	Concentration (%)	Reduction (%) of conidia/mm^2 of lesion		
		One spray	Two sprays	Mean
CH	0.25	40.0	50.2	45.1
	0.5	55.4	70.4	62.9
	0.75	60.2	77.8	69.0
PS	0.5	44.2	64.4	54.3
	1.0	50.0	71.2	60.6
	2.0	57.2	77.2	67.2
PB	1.0	55.4	70.8	63.1
	2.0	60.2	76.4	68.3
	3.0	74.2	88.2	81.2
CH + PS	0.5 + 1.0	70.8	82.8	76.8
	0.75 + 2.0	75.0	91.0	85.5
CH + PB	0.5 + 2.0	72.2	90.0	81.1
	0.75 + 3.0	80.4	94.2	87.3
Control		0.0	0.0	0.0

CH, chitosan; PS, potassium sorbate; PB, potassium bicarbonates.

Table 3. Disease severity of powdery mildew in okra leaves as affected by different treatments of plant resistance inducers under field conditions during 2016 season

Plant resistance inducer	Concentration (%)	Powdery mildew disease severity					
		1st spray	R (%)	2nd spray	R (%)	Mean	R (%)
CH	0.75	1.20b	42.8	1.37b	57.2	1.28	51.6
PS	2.0	1.29b	38.1	1.25b	60.9	1.27	52.1
PB	3.0	0.90c	57.1	0.89c	72.1	0.89	66.4
CH + PS	0.5 + 1.0	0.9bc	42.8	1.00c	68.7	0.95	64.2
	0.75 + 2.0	1.00c	52.3	0.88c	78.5	0.94	64.5
CH + PB	0.5 + 2.0	0.80c	61.9	0.83c	74.0	0.81	69.4
	0.75 + 3.0	0.66cd	68.7	0.55d	82.8	0.62	76.6
Tri-Miltox forte (2.5 g/L)		0.76cd	63.8	0.60d	73.2	0.63	76.3
Control		2.1a	0.0	3.2a	0.0	2.65	0.0

Values with the same letters in each column are not significantly differed ($p \leq 0.05$).
R, reduction percent; CH, chitosan; PS, potassium sorbate; PB, potassium bicarbonates.

disease on okra plants under field conditions during 2016. The effects of such treatments on vegetative growth and yield of okra plants were also investigated and comparative treatment was by fungicide, Tri-Miltox forte (2.5 g/L).

Table 3 indicated that all PRIs treatments have significantly reduced powdery mildew on treated plants compared with control. Integrative treatments of CH and PS or PB were the most effective treatments which reduce powdery mildew by 42.8–68.7% and 68.7–82.8% after the first and second spray respectively and the mean reduction after the two sprays was 64.2–76.6%. Meanwhile, single treatments of CH, PS, and PB showed considerable control of powdery mildew after the second spray, as the disease severity reduced by 57.2%, 60.9%, and 72.1%, respectively. PS 2.0% and CH 0.75% showed the lowest effect against powdery mildew (38.1% and 42.8%) after the first spray.

The efficacy of applied PRIs treatments on some vegetative growth and yield parameters of okra plants were also recorded. Table 4 clearly showed that all PRIs treatments improved both vegetative growth (plant height, number of branches) and yield parameters (number of pods/plant, pod length, pod weight, yield/ton fed) of treated okra plants compared with untreated control. The highest records of vegetative growth and yield parameter were observed with combined treatments of CH and PB. The highest effective treatment in okra yield/fed was CH + PB at 0.75% + 3.0% followed by concentration CH + PB at 0.5% + 2.0% and Tri-Miltox forte (2.5 g/L). They increased okra plants by 64.3%, 60.7%, and 60.7%, respectively. CH + PS at 0.5% + 1.0% or 0.75% + 2.0% and PB at 3.0% treatments show concealable yield increasing (53.6%, 57.1%, and 57.1%, respectively). Meanwhile, the least records of increase in okra yield were with the PS and CH treatments.

Biochemical studies. Effects of different concentrations of PS, PB, and CH separately and in combination as PRIs

Table 4. Vegetative growth and yield parameters of okra plants as affected by different treatments of plant resistance inducers under field conditions during 2016 season

Plant resistance inducer	Concentration (%)	Vegetative growth		Yield attributes				
		Plant height (cm)	No. of branches per plant	No. of pods per plant	Pod length (cm)	Pod weight (gm)	Yield per ton fed	Increasing (%)
CH	0.75	104b	3.2b	26b	8.0c	13.2b	4.1c	46.4
PS	2.0	92a	2.2a	22a	7.2b	13.4b	3.1b	39.3
PB	3.0	112b	4.1c	23a	6.4a	14.0c	4.4c	57.1
CH + PS	0.5 + 1.0	100b	3.4b	25b	7.2b	13.2b	4.3c	53.6
	0.75 + 2.0	104b	3.2b	22a	9.0c	14.2c	4.4c	57.1
CH + PB	0.5 + 2.0	108b	3.8b	24b	9.4c	14.8c	4.5cd	60.7
	0.75 + 3.0	110b	4.0c	27b	8.8c	14.4	4.6cd	64.3
Tri-Miltox forte (2.5 g/L)		105b	3.2b	24	9.2c	14.0c	4.5d	60.7
Control		85a	2.1a	21a	6.4a	12.2a	2.8a	0.0

Values with the same letters in each column are not significantly differed ($p \leq 0.05$).
CH, chitosan; PS, potassium sorbate; PB, potassium bicarbonates.

Table 5. Accumulation of phenolic, activity of PPO and POD enzymes in okra leaves in response to spraying with different plant resistance inducers under artificially inoculation with *Erysiphe cichoracearum* in green house condition

Plant resistance inducer	Concentration (%)	Phenolic		PPO		POD	
		mg/100 g leaves	Change (%)	PPO activity	Change (%)	POD activity	Change (%)
CH	0.25	0.511bc	24.0	1.79b	10.4	1.3b	18.1
	0.5	0.614c	49.0	1.88b	16.0	1.4b	27.2
	0.75	0.632c	53.3	2.10c	29.6	1.8c	63.6
PS	0.5	0.492b	19.4	1.74b	7.4	1.2b	9.0
	1.0	0.612c	48.5	1.98c	22.2	1.4b	27.2
	2.0	0.623c	51.2	2.32d	43.2	1.6b	45.4
PB	1.0	0.592c	43.6	1.95c	20.3	1.5b	36.3
	2.0	0.638c	54.8	2.28d	40.7	1.8c	63.6
	3.0	0.688cd	66.9	2.44d	50.6	2.0c	81.8
CH + PS	0.5 + 1.0	0.740d	79.6	2.60d	60.4	1.9c	72.7
	0.75 + 2.0	0.788d	91.2	2.90e	79.0	2.1c	90.9
CH + PB	0.75 + 2.0	0.772d	87.3	2.78d	71.6	2.0c	81.8
	1.0 + 3.0	0.810d	96.6	3.05e	88.2	2.1c	90.9
Control (inoculated and untreated)		0.412b	0.0	1.62b	0.0	1.1b	0.0
Control (noninoculated and untreated)		0.323a	0.0	1.40a	0.0	0.8a	0.0

Values with the same letters in each column are not significantly differed ($p \leq 0.05$).

on activity of antioxidant compounds (such as phenolic) as well as antioxidant enzymes in okra leaves during growth of okra plants under artificially inoculation with powdery mildew caused by *E. cichoracearum* were investigated.

Total phenolic content: Results in Table 5 reported that the total phenolic content (non-enzymatic antioxidant defense systems) was increased with 96.6% over control when treated with mixed chemical inducers CH + PB at 1.0% + 3.0% concentrations. Measuring phenolic content as mg per 100 g leaves showed gradual increase in amounts estimated in a similar trend by increasing concentration of treatments in individual manner for CH, PS, and PB, as data clear that phenolic content was (0.632, 0.623, and 0.688 mg/100 g leaves), respectively. On the other hand, the maximum values were 0.788 mg/100 g leaves and 0.810 mg/100 g leaves, obtained in okra leaves when sprayed with combined treatments, CH + PS at 0.75% + 2.0% and CH + PB at 1.0% + 3.0% mixed concentrations, in respective order.

PPO activity: Regards to the effect of the plant induce resistance agents on PPO activity, all different inducers produced an increasing in PPO in all inducers with different levels of treatments (Table 5). Data indicated the effects of CH + PB and CH + PS treatments on PPO were PPO was increased up to (3.05 and 2.90 unit/mL) in comparison with control (1.62 unit/mL). On the other hand, the lowest increase in PPO activities were induced by applied treatment (CH, PS, and PB) when data recorded (2.10, 2.32, and 2.44 unit/mL). The highest level of PPO activity was determined in treatment (CH + PB) at the concentrations (1.0% + 3.0%), at higher concentrations for combined inducers showed enhanced activities of PPO in comparison with other tested treatments. In contrast, the lowest activity of PPO as percentage change was represented at CH treatment (29.6%).

POD activity. POD activity was affected also by treatments; data in Table 5 showed maximum increasing in POD activity as percentage change (90.9%) at both treatments of CH + PS and CH + PB, when 0.75% + 2.0% and 1.0% + 3.0% combined concentration are applied. Moreover, it was obvious that, POD activity recorded the same results when okra leaves sprayed with CH + PS and CH + PB, as results showed (2.1 for both treatments). Data referred to effect of CH, PS, and PB treatments on POD activity indicated that POD activity was increased up to (1.8, 1.6, and 2.0 unit/mL). Results obtained in Table 5, it could be concluded that, individual treatments showed more pronounced enhancement for POD activity. In contrast, combined treatments have showed increasing in POD activity but with non-significant difference between them.

Illustrated data by Table 5 represented that PRI in respective order with applied treatments CH, PS, and PB as individual treatments and in gradual increase in concentration, showed an increment in both non-enzymatic and enzymatic antioxidant defense systems in okra leaves. The obtained results showed high defense when mixed chemical inducers applied as foliar spray against powdery mildews of okra plants.

Effect on chitinase and β-1,3-glucanase activity. Chitinase activity was affected by treatments as the data presented in Table 6 revealed that the different chemical inducers induced different levels of chitinase activities in okra leaves. The highest level was 106.6% and 89.1% as percentage change over control of chitinase activity by applied treatment (CH + PB and CH + PS), respectively. Moreover, the lowest chitinase activities were 43.3% over control for PS as individual treatment at 2% concentration. Meanwhile, CH and PS showed similar results that were (1.76 and 1.72 unit/mL), respectively. Furthermore, PB showed a moderate effect with 1.94 unit/mL and indicated that the maximum value of chitinase activity was observed in okra leaves treated with combined chemical inducers at 1.0% + 3.0% showed (106.6%) in relative to its control. β-1,3-glucanase activity also analyzed from sprayed okra leaves plants by individual and mixed chemical inducers that induced different levels of activities. Results clearly indicated that, activity of β-1,3-glucanase increased with increasing the concentrations of chemical inducers (CH, PS, and PB) as single treatments. More increment was recorded at CH + PB (1.0% + 3.0%) and CH + PS (0.75% + 2.0%) treatments were 90.0% and 80.0%, respectively. On the other hand, lower value of β-1,3-glucanase activity (37.5% change over control) was observed in PS as single treatment. Whereas, the mixed lower concentrations of CH + PS (0.5% + 1.0%) and CH + PB (0.75% + 2.0%), indicated a similar or nearly results (63.7% and 68.7%), respectively.

Data in Table 6 revealed that there was noticeably difference in antioxidant enzymes activities among the employed chemical inducer treatments in okra leaves. Meanwhile, when the treatments used individually showed a higher significant increment in chitinase activity than β-1,3-glucanase activity for (CH and PS) treatments as results recorded (46.6% and 43.3% over control) and β-1,3-glucanase activity which recorded (41.8% and 37.5%). Opposite results obtained in okra leaves treated with PB showed an increment in β-1,3-glucanase activity (65.6% change over β-1,3-glucanase activity 61.6%). The best significant increase was obtained at (1.0% + 3.0%) for CH + PB treatment and 106.6% for chitinase activity.

Effect on catalase activity and protein content.

Catalase activity: Catalase activity has been affected due to different concentrations of tested chemical inducer in

Table 6. Chitinase and β-1,3-glucanase enzymes activity in okra leaves in response to spraying with different plant resistance inducers under artificially inoculation with *Erysiphe cichoracearum* in green house condition

Plant resistance inducer	Concentration (%)	Chitinase		β-1,3-Glucanase	
		Activity (mL)	Change (%)	Activity (mL)	Change (%)
CH	0.25	1.42d	18.3	1.80b	12.5
	0.5	1.57dc	30.8	2.10c	31.2
	0.75	1.76c	46.6	2.27c	41.8
PS	0.5	1.32d	10.0	1.72b	7.5
	1.0	1.56d	30.0	2.02c	26.2
	2.0	1.72c	43.3	2.20c	37.5
PB	1.0	1.62c	35.0	2.08c	30.0
	2.0	1.86c	55.0	2.35c	46.8
	3.0	1.94c	61.6	2.56c	65.6
CH + PS	0.5 + 1.0	2.01b	67.5	2.62d	63.7
	0.75 + 2.0	2.27b	89.1	2.88d	80.0
CH + PB	0.75 + 2.0	2.20b	83.3	2.70d	68.7
	1.0 + 3.0	2.48b	106.6	3.04e	90.0
Control (inoculated and untreated)		1.20	0.0	1.60b	0.0
Control (noninoculated and untreated)		2.30a	0.0	2.10a	0.0

Values with the same letters in each column are not significantly differed ($p \leq 0.05$).
CH, chitosan; PS, potassium sorbate; PB, potassium bicarbonates.

okra leaves, maximum catalase activity was (457.3, 425.9, and 405.1 U/mg protein) at (0.5% PS, 0.25% CH, and 1.0% PB) concentrations as individual inducer, respectively (Table 7). Meanwhile, minimum value in catalase (175.3 U/mg protein) was mentioned at 1.0% + 3.0% of CH + PB treatment and then decreased over control, which recorded (220.4 U/mg protein). Moreover, data showed that catalase activity was (522.0 U/mg protein) for control, plants (inoculated and untreated). Recorded results revealed that, lower concentrations of different individual chemical inducers (CH, PS, and PB), caused significant increase in catalase activity. In contrast, treatments in combination at higher concentrations produced a significant decrease in comparison with individual treatments. The highest percentage change for catalase activity (66.4%) was recorded in CH + PB treatment, while PS treatment showed the lowest catalase activity as percentage change (46.4%).

Protein content: Our results clearly revealed that, foliar spraying of bio-stimulants "chitosan" and other chemical inducers significantly had influence on protein content accumulation in okra plant under artificial infection with powdery mildew. Results in Table 7 mentioned that by gradual increase in different concentrations of chemical inducer, there are increments in protein content either for treatments used individually or in mixtures. The highest value of protein content was (341.4 mg/g fw) at CH + PB (1.0% + 3.0%) mixed concentration compared with control plants (inoculated or non-inoculated) which recorded (225 and 276 mg/g fw), respectively.

Furthermore, the increase in PS concentration led to decreasing of protein content and rise in catalase activities in okra leaves (Table 7). PS treatment indicated the minimum increasing in protein content where recorded (302.2 mg/g fw) and percentage change (34.0%) in comparison with other treatments. The extent of increases under combined effects of CH + PB and CH + PS were 51.2% and 46.3%, respectively. In different way, the highest catalase activity (457.3 U/mg protein) was observed under PS treatments condition when used in a single treatment, whereas, the lowest catalase activity (175.3 U/mg protein) was recorded under combined treatments condition (CH + PB). The application of tested chemical inducer treatments induced significant stimulation effect on protein content in okra plant under artificial infection with powdery mildew. Results in Table 7 showed that by gradual increase in different concentrations of chemical inducer, there was increment in protein content either for treatments used as individually or as mixtures. The highest value for protein content was (341.4 mg/g fw) with CH + PB (1.0% + 3.0%) mixed concentration compared with control plants (inoculated or non-inoculated) which recorded (225 and 276 mg/g fw), respectively.

DISCUSSION

There is a worldwide trend to explore new alternatives approaches for controlling plant diseases, giving priority to methods that are efficient reliable and safe for environment. Induce of host resistance by using PRI such as CH and other safe chemicals such as potassium salts seems to be one of these new approaches to substitute or at least to decrease the usages of fungicides in plant disease control. In the present study, our data indicate that using CH as individually or mixed with potassium salts successfully controlled powdery mildew disease in okra plants either with *E. cichoracearum* under greenhouse condition or naturally infection under field condition. We use different levels of chemical inducer treatments as potassium salt (sorbate and bicarbonate) tested and as well as CH. The

Table 7. Catalase activity and protein content in okra leaves in response to spraying with different plant resistance inducers under artificially inoculation with *Erysiphe cichoracearum* in greenhouse condition

Plant resistance inducer	Concentration (%)	Catalase activity		Protein content	
		Unit/mg protein	Change (%)	mg/g fresh weight	Change (%)
CH	0.25	425.9b	18.4	253.3b	12.2
	0.5	345.5c	33.8	277.2b	22.8
	0.75	248.5d	52.4	318.8c	41.2
PS	0.5	457.3b	12.4	246.5b	9.2
	1.0	374.7c	28.2	270.0b	19.6
	2.0	279.7d	46.4	302.2c	34.0
PB	1.0	405.1b	22.4	265.0b	17.4
	2.0	254.7d	51.2	319.7c	41.6
	3.0	233.8d	55.2	323.3c	43.2
CH + PS	0.5 + 1.0	228.6d	56.4	316.7c	40.3
	0.75 + 2.0	202.5d	61.2	330.3c	46.3
CH + PB	0.75 + 2.0	206.7d	60.4	317.9c	40.8
	1.0 + 3.0	175.3c	66.4	341.4d	51.2
Control (inoculated and untreated)		522.0a	0.0	225.8a	0.0
Control (noninoculated and untreated)		220.4d	0.0	276.0b	0.0

The specific activity of catalase activity was expressed in terms of unit of H_2O_2 decomposed per min per mg of protein.
CH, chitosan; PS, potassium sorbate; PB, potassium bicarbonates.

required concentration to control pathogen was differed from each other, either when used individually or in combination. Data cleared that, the application of CH combined with potassium bicarbonate and PS offers a commercially acceptable, economically viable, and effective alternative to control of powdery mildew in okra leaves.

Spraying okra plants after inoculation with *E. cichoracearum* by CH at 0.75% (w/v) reduce powdery mildew severity by 64.4%, and spore production by 77.8%. Moreover, mixed treatments such as CH + PB lead to increasing in phenolic content (53.3% to 96.6%), PPO (29.6% to 88.2%), POD (63.6% to 90.9%), chitinase (46.6% to 106.6%), β-1,3-glucanases (41.8% to 90.0%), catalase (52.4% to 66.4%), and protein content (41.2% to 51.2%) in leaves of treated plants. These results are in consistent with other studies demonstrating that suppression of microbial strains varies in response to different organic and inorganic salts. In addition, results indicated that the degree of fungi inhibition was concentration dependent and the maximum value for decrement in mycelia growth was positively correlated with the concentration of salt tested [10, 45]. Our results indicated that application of CH as foliar spray method significantly reduced the severity of powdery mildew disease in okra leaves where increased by 20% in yield and an enhanced improvement in controlling powdery mildew disease of tomatoes when applied with CH and significant reduction in local and systemic infection caused by *Blumeria graminis* f. sp. *hordei*, respectively [32, 46].

Physiologically CH is an effective elicitor of systemic acquired resistance, improved controlling pathogens, as well as stimulate the immune system for plant resistance to broad infection [31, 47]. Barley plants infected with *Blumeria graminis* f. sp. *horde* showed a reduced locally and systemically infection by powdery mildew pathogen when sprayed with CH [32]. Meanwhile, activation of protective mechanisms in plant tissues and inhibition of the growth of taxonomically different pathogens can be recorded in previous studies [48]. Moreover, physical barriers are formed around the penetration sites of pathogens, preventing them from infecting other healthy tissues. These bioactive derivatives can activate H^+-ATPases, depolarizing biological membranes and inducing other series of events. In addition, on the molecular changes, some defensive genes can be activated by CH through octadecanoid signaling pathways and oligouronides [49]. In the same trend, in bacterial cells permeability of the outer and inner membrane was increased and ultimately causing release of cellular contents [50].

Treatments with different concentrations of potassium salts and CH as antimicrobial compound showed an elevation in PPO and POD activity as compared to untreated plants during the present work. Same trend of results obtained with [49] who mentioned that CH can induce elicitation of the POD and PPO activity in palm roots. Protease inhibitors, β-1,3-glucanases, POD, PPO as an antioxidant defense enzymes were elevated in leaves and also in rhizomes in plants grown in field condition and treated with CH [33]. Chitinases and β-1,3-glucanase are members of a group of plant pathogenesis-related (PR) proteins showed a close relationship with resistance against pathogens that can be induced by CH [51-53]. These PR proteins may play a role in host plant defense systems by degrading the cell walls of some phytopathogens [54-56]. High-level expression of defense-related proteins such as POD and PPO in CH treated plants might involve in the formation of lignin to control the entry and movement of fungal pathogens in the plant system. Antioxidant enzyme activity as (POD and PPO) can be increased and associated with decreases in disease severity involved in plant pathogenic fungi, bacteria and viruses in a number of resistant interactions [57-60]. Seed germination, yield enhancement was stimulated in many crop species such as faba bean under effect of CH [61], cucumber [62], corn [63], green bean [64], barley [32], and curcumin [33].

The application of CH as elicitors with chemical inducers may be considered as a promising tool in production of biologically active phytoalexins and other secondary metabolites such as phenolic that have a structural function in lignification and an antibiotic activity [65, 66]. In this study, the CH mixed with PB and PS treatments increased the activity of phenolic in infected okra leaves. These results indicate that the response mechanisms induced by combined CH treatments are positive in controlling disease attack. In lines with the present results, caffeoylshimick acids (sinapic, p-coumaric, and feluric derivatives), were reported as the major phenolic constitutive compounds in date palm roots as influence of CH biopolymer [51]. Phenolic concentration increment considered as a suitable substrate for the oxidative reactions catalyzed by PPO that produce fungitoxic polymerisation of quinones, consuming oxygen and so creating an incompatible environment for fungal growth [67, 68]. Both PPO and POD could generate reactive oxygen species molecules such as hydrogen peroxide (H_2O_2); that inhibit pathogens directly or generate other free radicals with antimicrobial effects; which can scavenged with POD [69-71]. Catalase activity (CAT), which catalyses conversion of hydrogen peroxide into water and oxygen, is the major H_2O_2-scavenging enzyme in all aerobic organisms. Results reported in the present work, suggested that CAT activities was stimulated in production over control.

Conversely, our results indicated that, sprayed inoculated okra plants with PS (2.0%) and/or PB (3.0%) twice applications lead to decreasing of powdery mildew severity by 60.6% and 64.2%, spore production by the pathogen by 77.2% and 88.2%. Moreover, such treatments enhancing defense resistance proteins and increasing in phenol continent by (51.2% and 66.9%) and enhancement of PPO (43.2% and 50.6%), POD (45.4% and 81.8%), chitinase (43.3% and 61.6%), β-1,3-glucanases (37.5% and 65.6%), catalase (46.4% and 55.2%), and protein content (34.0% and 43.2%) in leaves of treated plants.

In addition to inhibiting effects of CH, other potassium salts showed effects on growth inhibition of powdery

mildew in okra leaves. Many investigators [72] have tested the efficacy of various chemical inducers of systemic resistance against powdery mildew disease. Pepper plants showed inhibition of powdery mildew severity as influenced by foliage sprays of sodium bicarbonate or PB. These salts were registered as pesticide ingredients and horticultural fungicide in the United States and United Kingdom [6, 7]. Physiological mechanism of these inhibiting effects may be due to changes in pH medium, which became more alkaline as bicarbonate concentration increased. Besides changes to pH that affect fungal growth, bicarbonates may also affect membrane permeability and alter physiological pathways such as oxidative phosphorylation [10]. In our study, the best controlling effects for pathogen was obtained at elevated concentrations of CH + PB at (1.0% + 3.0%) or CH + PS at (0.75% + 2.0%). Moreover, some chemical inducers such as K_2HPO_4, dichlro-isooicotinic acid, and ethephone ($C_2H_6ClO_3P$) have significantly reduced powdery mildew and leaf spot diseases of squash plants [73].

Protein is the major constitute of the cell structure, is required for the most enzymatic reaction. In okra plants, as demonstrated in Table 6, a faster response on protein synthesis recorded a significant increase (51.2% and 46.3%) over control was in combined treatments CH + PB or CH + PS, respectively. These results could be attributed to the stimulator effects of CH application when mixed with potassium salts. Higher protein content in okra plants treated with CH, may reflect the degree of assimilation of inorganic nitrogen and promoting protein synthesis in leaves, improve photosynthetic rate, and increase the synthesis as well as accumulation of plant photosynthetic products. CH also can increase soluble sugar content and enhance the activity of protease conversion to protein and increasing free amino acid content, which has obvious inhibiting effect for many plant pathogenic fungi. All pervious explanation are in agreement with [74, 75]. The present findings demonstrate that PRI in future can be used on a commercial scale for controlling such diseases especially under system used for protective cultivation. With results such as those reported here, foliar applications of natural and inorganic chemical inducers when used in combination, could become effective components of disease management system in okra plants.

The foliar applications of CH and potassium salts at vegetative stage could inhibit the powdery mildew caused by *Erysiphe cichoracearum* DC and improve the biochemical pathways during growth of okra plants, which resulted from increased of defense enzymatic systems. Hence, the high-level accumulation of PR-protein, POD, PPO, chitinase and β-1,3-glucanase, catalase and protein content in plant leaves might have collectively contributed to the induced resistance in treated plants. Among the concentrations, 0.75% CH, 3.0% PB and 2.0% PS had superiority for controlling plant pathogen and accelerate metabolic profile in tested okra plant over control treatments. Therefore, application of CH at concentration of 0.75% or 1.0% in combination with inorganic inducers may be recommended for okra plant. However, more experiments should be conducted in different locations and seasons to valid conclusion regarding the CH foliar application for yield improvement of okra.

REFERENCES

1. Sridhar TS, Sinha P. Assessment of loss caused by powdery mildew (*Erysiphe cichoracearum*) of okra (*Hibiscus esculentus*) and its control. Indian J Agric Sci 1989;59:606-7.
2. Kiss L. A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manag Sci 2003; 59:475-83.
3. Younes NA, Abo-Elyousr KA. Screening of some Okra (*Abelmoschus esculentus* L.) genotypes to powdery mildew resistance and yield under agro-climatic conditions of Assiut, Egypt. Int J Agric Econ Dev 2014;2:59-76.
4. Reuveni M, Agapov V, Reuveni R. Suppression of cucumber powdery mildew (*Sphaerotheca fuliginea*) by foliar sprays of phosphate and potassium salts. Plant Pathol (Lond) 1995;44: 31-9.
5. Fallik E, Ziv O, Grinberg S, Alkalai S, Klein JD. Bicarbonate solutions control powdery mildew (*Leveillula taurica*) on sweet red pepper and reduce the development of postharvest fruit rotting. Phytoparasitica 1997;25:41-3.
6. Deliopoulos T, Kettlewell PS, Hare MC. Fungal disease suppression by inorganic salts: a review. Crop Prot 2010;29: 1059-75.
7. Greenway DL. Potassium bicarbonate (073508) and sodium bicarbonate (073505) fact sheet. Washington, DC: U.S. Environmental Protection Agency; 1999.
8. Ziv O, Zitter TA. Effects of bicarbonates and film-forming polymers on cucurbit foliar diseases. Plant Dis 1992;76:513-7.
9. Horticultural Development Company. Use of potassium hydrogen carbonate for powdery mildew control. Final report on HDC Project CP48. East Malling: Horticultural Development Company; 2005.
10. Olivier C, Halseth DE, Mizubuti ES, Loria R. Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Dis 1998;82:213-7.
11. Palou L, Smilanick JL, Usall J, Viñas I. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis 2001;85:371-6.
12. Arslan U, Ilhan K, Karabulut OA. Evaluation of food additives and low toxicity compounds for the control of bean rust and wheat leaf rust. J Phytopathol 2006;154:534-41.
13. Reuveni M, Agapov V, Reuveni R. Controlling powdery mildew caused by *Sphaerotheca fuliginea* in cucumber by foliar sprays of phosphate and potassium salts. Crop Prot 1996;15:49-53.
14. Suhr KI, Nielsen PV. Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. Int J Food Microbiol 2004;95:67-78.
15. Palou L, Usall J, Smilanick JL, Aguilar MJ, Viñas I. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manag Sci 2002;58:459-66.
16. Arslan U, Ilhan K, Vardar C, Karabulut OA. Evaluation of

antifungal activity of food additives against soil-borne phytopathogenic fungi. World J Microbiol Biotechnol 2009; 25:537-43.
17. Dias AM, Cortez AR, Barsan MM, Santos JB, Brett CM, De Sousa HC. Development of greener multi-responsive chitosan biomaterials doped with biocompatible ammonium ionic liquids. ACS Sustain Chem Eng 2013;1:1480-92.
18. Dzung NA, Khanh VT, Dzung TT. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. Carbohydr Polym 2011;84:751-5.
19. Al-Hetar MY, Zainal Abidin MA, Sariah M, Wong MY. Antifungal activity of chitosan against *Fusarium oxysporum* f. sp. *cubense*. J Appl Polym Sci 2011;120:2434-9.
20. Mondal MM, Malek MA, Puteh AB, Ismail MR, Ashrafuzzaman M, Naher L. Effect of foliar application of chitosan on growth and yield in okra. Aust J Crop Sci 2012;6:918-21.
21. Limpanavech P, Chaiyasuta S, Vongpromek R, Pichyangkura R, Khunwasi C, Chadchawan S, Lotrakul P, Bunjongrat R, Chaidee A, Bangyeekhun T. Chitosan effects on floral production, gene expression, and anatomical changes in the *Dendrobium* orchid. Sci Hortic 2008;116:65-72.
22. Hadwiger LA. Multiple effects of chitosan on plant systems: solid science or hype. Plant Sci 2013;208:42-9.
23. Nguyen Van S, Dinh Minh H, Nguyen Anh D. Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatal Agric Biotechnol 2013;2:289-94.
24. Patkowska E, Pięta D, Pastucha A. The effect of biochikol 020 pc on microorganisms communities in the rhizosphere of Fabaceae plants. Polish Chitin Soc Monogr 2006;11:171-8.
25. Abdou Sereih A, Abd-El-Aal S, Sahab AF. The mutagenic activity and its effect on the growth of *Trichoderma harzianum* and *Fuzarium oxysporum*. J Appl Sci Res 2007;3:450-5.
26. No HK, Meyers SP, Prinyawiwatkul W, Xu Z. Applications of chitosan for improvement of quality and shelf life of foods: a review. J Food Sci 2007;72:R87-100.
27. Górnik K, Grzesik M, Romanowska-Duda B. The effect of chitosan on rooting of gravevine cuttings and on subsequent plant growth under drought and temperature stress. J Fruit Ornam Plant Res 2008;16:333-43.
28. Li M, Chen X, Liu J, Zhang W, Tang X. Molecular weight-dependent antifungal activity and action mode of chitosan against *Fulvia fulva* (cooke) ciffrri. J Appl Polym Sci 2011; 119:3127-35.
29. Trotel-Aziz P, Couderchet M, Vernet G, Aziz A. Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. Eur J Plant Pathol 2006; 114:405-13.
30. Aziz A, Trotel-Aziz P, Dhuicq L, Jeandet P, Couderchet M, Vernet G. Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. Phytopathology 2006;96:1189-94.
31. Pichyangkura R, Chadchawan S. Biostimulant activity of chitosan in horticulture. Sci Hortic 2015;195:49-65.
32. Faoro F, Maffi D, Cantu D, Iriti M. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. BioControl 2008;53:387-401.
33. Anusuya S, Sathiyabama M. Effect of chitosan on growth, yield and curcumin content in turmeric under field condition. Biocatal Agric Biotechnol 2016;6:102-6.
34. Reuveni R, Dor G, Raviv M, Reuveni M, Tuzun S. Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system and its control by foliar spray of mono-potassium phosphate. Crop Prot 2000;19:355-61.
35. Descalzo RC, Rahe JE, Mauza B. Comparative efficacy of induced resistance for selected diseases of greenhouse cucumber. Can J Plant Pathol 1990;12:16-24.
36. Rapp A, Ziegler A. Analysis of the phenolcarboxylic acids (hydroxybenzoic acids and hydroxycinnamic acids) in grape leaves, berries, and wine by micro-polyamide thin-layer chromatography. Vitis 1973;12:226-36.
37. Johri S, Jamwal U, Rasool S, Kumar A, Verma V, Qazi GN. Purification and characterization of peroxidases from *Withania somnifera* (AGB 002) and their ability to oxidize IAA. Plant Sci 2005;169:1014-21.
38. Coseteng MY, Lee CY. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J Food Sci 1987;52:985-9.
39. Guleria S, Kumar A. Qualitative profiling of phenols and extracellular proteins induced in mustard (*Brassica juncea*) in response to benzothiadiazole treatment. J Cell Mol Biol 2006; 5:51-6.
40. Boller T, Mauch F. Colorimetric assay for chitinase. Methods Enzymol 1988;161:430-5.
41. Monreal J, Reese ET. The chitinase of *Serratia marcescens*. Can J Microbiol 1969;15:689-96.
42. Sun H, Yang J, Lin C, Huang X, Xing R, Zhang KQ. Purification and properties of a β-1,3-glucanase from *Chaetomium* sp. that is involved in mycoparasitism. Biotechnol Lett 2006;28:131-5.
43. Chance B, Maehly AC. Assay of catalases and peroxidases. Methods Enzymol 1955;2:764-75.
44. Snedecor GW, Cochran WG. Statistical methods. Ames (IW): Iowa State University Press; 1983.
45. Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.
46. Fagundes C, Pérez-Gago MB, Monteiro AR, Palou L. Antifungal activity of food additives *in vitro* and as ingredients of hydroxypropyl methylcellulose-lipid edible coatings against *Botrytis cinerea* and *Alternaria alternata* on cherry tomato fruit. Int J Food Microbiol 2013;166:391-8.
47. Walker R, Morris S, Brown P, Gracie A. Evaluation of potential for chitosan to enhance plant defense: a report for the Rural Industries Research and Development Corporation. Barton: Rural Industries Research and Development Corporation; 2004.
48. Abro MA, Lecompte F, Bardin M, Nicot PC. Nitrogen fertilization impacts biocontrol of tomato gray mold. Agron Sustain Dev 2014;34:641-8.
49. Doares SH, Syrovets T, Weiler EW, Ryan CA. Oligogalacturonides and chitosan activate plant defensive gene through the octadecanoid pathway. Proc Natl Acad Sci U S A 1995;92:4095-8.
50. Vasiukova NI, Zinov'eva SV, Il'inskaia LI, Perekhod EA, Chalenko GI, Gerasimova NG, Il'ina AV, Varlamov VP,

Ozeretskovskaia OL. Modulation of plant resistance to diseases by water-soluble chitosan. Appl Biochem Microbiol 2001;37: 103-9.
51. El Hassni M, El Hadrami A, Daayf F, Ait Barka E, El Hadrami I. Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. Phytopathol Mediterr 2004;43:195-204.
52. Liu H, Du Y, Wang X, Sun L. Chitosan kills bacteria through cell membrane damage. Int J Food Microbiol 2004;95:147-55.
53. Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K. Plant chitinase. Plant J 1993;3:31-40.
54. Van Loon LC, Pierpoint WS, Boller T, Conejero V. Recommendations for naming plant pathogenesis-related protein. Plant Mol Biol Rep 1994;12:245-64.
55. O'Herlihy EA, Duffy EM, Cassells AC. The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. Folia Geobot 2003;38:201-7.
56. Dixon RA, Harrison MJ, Lamb CJ. Early events in the activation of plant defense responses. Annu Rev Phytopathol 1994;32:479-501.
57. Graham LS, Sticklen MB. Plant chitinases. Can J Bot 1994; 72:1057-83.
58. Abbasi NA, Iqbal Z, Maqbool M, Hafiz IA. Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating. Pak J Bot 2009;41:343-57.
59. Sathiyabama M, Charles RE. Fungal cell wall polymer based nanoparticles in protection of tomato plants from wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici*. Carbohydr Polym 2015;133:400-7.
60. Ma Z, Yang L, Yan H, Kennedy JF, Meng X. Chitosan and oligochitosan enhance the resistance of peach fruit to brown rot. Carbohydr Polym 2013;94:272-7.
61. Madadkhah E, Lotfi M, Nabipour A, Rahmanpour S, Banihashemi Z, Shoorooei M. Enzymatic activities in roots of melon genotypes infected with *Fusarium oxysporum* f. sp. *melonis* race. Sci Hortic 2012;135:171-6.
62. Nandakumar R, Babu S, Viswanathan R, Raguchander T, Samiyappan R. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol Biochem 2001;33:603-12.
63. El-Sawy NM, El-Rehim HA, Elbarbary AM, Hegazy ES. Radiation-induced degradation of chitosan for possible use as a growth promoter in agricultural purposes. Carbohydr Polym 2010;79:555-62.
64. Shehata SA, Fawzy ZF, El-Ramady HR. Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. Aust J Basic Appl Sci 2012;6:63-71.
65. Boonlertnirun S, Suvannasara R, Promsomboon P, Boonlertnirun K. Application of chitosan for reducing chemical fertilizer uses in waxy corn growing. Thai J Agric Sci 2011;44:22-8.
66. Jabnoun-Khiareddine H, El-Mohamedy RS, Abdel-Kareem F, Abdallah RA, Gueddes-Chahed M, Daami-Remadi M. Variation in chitosan and salicylic acid efficacy towards soil-borne and air-borne fungi and their suppressive effect of tomato wilt severity. J Plant Pathol Microbiol 2016;6:325.
67. Walker-Simmons M, Hadwiger L, Ryan CA. Chitosans and pectic polysaccharides both induce the accumulation of the antifungal phytoalexin pisatin in pea pods and antinutrient proteinase inhibitors in tomato leaves. Biochem Biophys Res Commun 1983;110:194-9.
68. Lattanzio V, Lattanzio VM, Cardinali A. Role of phenolics in the resistance mechanisms of plant against fungal pathogens and insects. In: Imperato F, editor. Phytochemistry: advances in research. Kerala: Research Signpost; 2006. p. 23-67.
69. Mohammadi M, Kazemi H. Changes in peroxidase and polyphenol oxidase activities insusceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Sci 2002;162:491-8.
70. Benhamou N. Elicitor-induced plant defense pathways. Trends Plant Sci 1996;1:233-40.
71. Mayer AM. Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 2006;67:2318-31.
72. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 2010;48:909-30.
73. Abd-El-Kareem F, El-Mougy NS, El-Gamal NG, Fotouh YO. Induction of resistance in squash plants against powdery mildew and *Alternaia* leaf spot diseases using chemical inducers as protective or therapeutic treatments. Egypt J Phytopathol 2004;32:65-76.
74. Amini J. Induced resistance in potato plants against verticillium wilt invoked by chitosan and acibenzolar-S-methyl. Aust J Crop Sci 2015;9:570-6.
75. Zeng D, Luo X, Tu R. Application of bioactive coatings based on chitosan for soybean seed protection. Int J Carbohydr Chem 2012;2012:104565.