

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Sugar Beet Leaf Extracts in Response to Exogenous Application of Resistance Inducers to Manage Sugar Beet Powdery Mildew
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The effect of foliar application of some chemical resistance inducers *i.e.*, chitosan, ascorbic acid, salicylic acid and tap water as a control were tested to evaluate their efficacy to induce systemic resistance against *Erysiphe betae*, the causal agent of sugar beet powdery mildew (Herkl cv.) versus the traditional fungicide Opera under greenhouse and field conditions at Gemmeiza Agric. Res. Sta., Agric. Res. Cent. (ARC), Egypt during two successive winter seasons (2014/2015 and 2015/2016). Under greenhouse conditions, results revealed that spraying plants with all the tested IRCs reduced the disease severity of powdery mildew. The fungicide Opera was the most effective treatment in reducing disease severity and increasing the efficacy followed by chitosan and salicylic acid. Also, all treatments significantly increased total phenols and orthodihydric (OD) phenol content of the leaves compared to the control at 15, 30 and 45 days after inoculation. The GC-MS analysis of methanol extracts of 60 days old leaves of sugar beet plants under the greenhouse conditions, which have been treated with chitosan, ascorbic acid, salicylic acid as inducers to the systemic resistance of sugar beet against *Erysiphe betae* recorded the highest concentration (peak area %) for all bioactive phytochemical compounds such as flavonoids, alcohols, aldehydes, aromatic compounds, fatty acid methyl esters, terpenoids, phenolics and steroids that can be postulated for antimicrobial activity compared to control (tap water). Under field conditions, results indicated that the fungicide Opera was the most effective treatment for controlling powdery mildew followed by ascorbic acid. Meanwhile, all treatments showed lower effect than the fungicide Opera in this regard but higher than control. All treatments increased root yield per feddan compared to the control. The fungicide Opera and salicylic acid gave the highest root yield per feddan followed by ascorbic acid then chitosan. Moreover, salicylic acid and the fungicide Opera gave the highest percentages of total soluble solids (T.S.S.) and sucrose percentages, whereas salicylic acid, ascorbic acid and fungicide Opera gave the highest percentages of purity in sugar beet roots.

Keywords: *Erysiphe betae*, Chitosan, Sugar beet, Powdery mildew, Ascorbic acid, Salicylic acid, Phenolic compounds, GCMS studies, Oxidative enzymes.

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops worldwide (Francis *et al.*, 2007). It is a moderate crop; on the other hand, it can be grown in a wide-range of climatic conditions. In Egypt, Sugar beet is ranked as the second crop for sugar production after sugar cane (Eweis *et al.*, 2006). Over and above 20 fungi, oomycetes and 10 bacterial species have been defined as pathogens of sugar beet (Harveson *et al.*, 2009). At least 10 of these species cause symptoms and severe damage on the leaves of sugar beet. The causal agent of powdery mildew, *Erysiphe betae* of sugar beet foliage causes severe damage in the root yield which affects negatively the total crop production (Kontradowitz and Verreet, 2010). Under severe attack, root yield production is reduced down to 22% as well as sucrose contents in the roots decreased to 13% (Karaoglanidis and Karadimos, 2006), consequently declined yield quantity and quality (El-Fahar and Abou El-Magd, 2008).

Powdery mildew has been successfully managed by using fungicides (Karaoglanidis and Karadimos, 2006 and Kontradowitz and Verreet, 2010). Conversely, fungicides can pollute the environment, be phytotoxic to the host and increase the resistance of phytopathogens toward fungicides (Sierotzki *et al.*, 2000 and Ishii *et al.*, 2001). For that reason, the application of alternative management strategies is essential to decrease the negative impacts of fungicides on the plant, human being and the environment. These alternatives should be low cost-effective and less toxic to plants. Furthermore, controlling powdery mildew might take place through inducing systemic resistance (ISR) (Reuveni and Reuveni, 1995). Chito-saccharides have been successfully applied as a plant growth promoter and disease control agent (Trotel-Aziz *et al.*, 2006 and Aziz *et al.*, 2006). Moreover, chitosan stimulates pathogenesis-related response genes (PR genes) in the plants probably by inducing chitinase and β -1, 3-glucanase activities (Aziz *et al.*, 2006). Exogenous application of salicylic acid (SA) could lessen the intensity of late leaf spot disease and increase the phenol contents in the plants (Meena *et al.*, 2001). In addition, ascorbic acid has been reported as successful inducer for plant resistance against pathogens (Khan *et al.*, 2001).

Plants synthesize different types of secondary metabolites in response to both biotic and abiotic stresses (Dixon, 2001). Therefore, productions of these compounds enhance the resistance level of plants in towards plant pathogenic viruses, bacteria and fungi to avoid their attack (Rohloff and Bones, 2005). Distinguished examples of these compounds include alkaloids, flavonoids, phenols, terpenoids, steroids, tannins, etc. (Shahidi *et al.*, 2008). In the last few years, gas chromatography-mass spectrometry (GC-MS) has been firmly established as a key technological platform for metabolite profiling in the plant. Recently such machines are increasingly becoming more conventional (Robertson, 2005 and Kell *et al.*, 2005).

The current research study aimed to evaluate the efficacy of chitosan, ascorbic acid and salicylic as a successful inducers for systemic resistance against sugar beet powdery mildew. Moreover, to investigate the biochemical changes of sugar beet

leaves in response to the exogenous application of these treatments by using gas chromatography-mass spectrometry GCMS analysis.

Materials and Methods

The current research study was carried out at Agricultural Research Station of Gemmeiza, Agricultural Research Centre (ARC) during two successive seasons; 2014/2015 & 2015/2016.

a. Preparation of the tested Chemical resistance inducers substances:

According to the molecular weight, 100 mM, of chitosan, salicylic acid (Abada and Eid, 2014) and ascorbic acid (El-Gamal *et al.*, 2007) were prepared to accomplish the present study.

Opera fungicide:

Opera 18.3% SE (Pyraclostrobin/Epoxiconazole), BASF Company for Chemicals and Pesticides has been applied with the recommended dose to manage powdery mildew as a reference in this study.

b. Preparation of powdery mildew conidial inoculum:

Infected sugar beet leaves were collected from severely mildewed field grown plants during March 2014. They were gently shaken over potted-8-week age beet plants. Infected leaves of the newly mildewed plants were used as a source of conidial inoculum in the present investigation.

Greenhouse experiment:

This experiment has been designed in a randomized complete blocks method with three replicate pots (no.35), the seeds of sugar beet were planted, 10 seeds each. After 3 weeks, seedlings were thinned to 3 per pot and fertilized with 1% solution of NPK (75:150:50). Thirty days after planting, foliar parts of the plants were sprayed with each of the experimented substances by the aid of a manual low-pressure sprayer four times within 2-week intervals. Arabic gum as an adhesive material was added before spraying. At 60 days after planting, plants were artificially inoculated with spores of *E. betae* produced on the powdered leaves according to the method described by Whitney *et al.* (1983) and Lewellen and Schrantd (2001). Percentage of disease severity, leaf biochemical components and enzyme activity were assessed three times at 15, 30 and 45 days after inoculation.

At 15, 30 and 45 days after inoculation, six plant leaves were randomly selected from each pot to analyze total phenols, orthodihydric phenols (OD) and enzymatic activity.

Biochemical activity mediated by the synthesis of allelochemicals in plant leaves of the potted experiment:

Following the colorimetric method of analysis using Folin-phenol reagent at 650 nm, the total phenols were estimated according to the method adopted by Bray and Thorpe (1954). Orthodihydric phenols (OD) were measured spectrophotometrically at a 515nm wavelength as described by Arnou (1937).

*Gas Chromatography-Mass Spectroscopy (GC-MS) analysis:**a. Extraction procedure:*

The fresh leaves were cleaned, shade dried and powdered by using an electric grinder. Then, 5 gm of the powder has been weighed out and dissolved in 100 ml of Methanol, followed by extracts filtration. The filtered extracts have been incubated at 40°C to evaporate the solvent.

b. (GC-MS) analysis:

The GC/MS analysis was performed using a Thermo scientific, trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 mm, 0.25 mm, 0.25 µm film thickness). For GC/MS detection an electron ionization system with ionization energy of 70 eV was applied, Helium gas has been used as the carrier gas at a constant flow rate of 1 mL/min. The injector and MS transfer line temperature was set up at 280°C.

Vol. oil: The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C as a final temperature at an increasing rate of 5°C/min (hold 5 min).

Sap: the oven temperature was programmed at an initial temperature 150°C (hold 4 min) to 280°C as a final temperature at an increasing rate of 5°C/min (hold 4 min).

Unsap: The oven temperature was programmed at an initial temperature 50°C (hold 2 min) to 150°C at an increasing rate of 7°C/min then to 270°C at an increasing rate of 5°C/min (hold 2 min) then to 310°C as a final temperature at an increasing rate of 3.5°C/min (hold 10 min).

The quantification of all the identified components has been investigated using a percent relative peak area. Tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST (National Institute Standard and Technology), Willy library data of the GC/MS system (wileyonlinelibrary.com).

Field experiments:

Field trials were conducted under natural infestation at Agricultural Research Station of Gemmeiza in seasons 2014/2015 and 2015/2016. Plants were sprayed four times at two-week intervals, starting from 30 days after planting as described under pot experiment. The experiments were designed as randomized complete blocks method with three replicate plots. Each plot comprised four rows; 5 m long and 0.8 m between rows. Sugar beet seeds cv. Herkl were planted at 25 cm distance between hills. Cultural practices were made as usual.

Disease severity (D.S.) was recorded on powdered plants three times; after 15, 30 and 45 days of inoculation. Symptoms were scored visually for at least 40 leaves of 10 plants according to the scale of 0 to 9, where 9 = 90-100% of visible, mature leaf area covered with mildew (Whitney *et al.*, 1983 and Lewellen and Schrandt, 2001). The following formula was used to calculate the disease severity:

$$\text{Severity \%} = \frac{\sum (\text{Each category} \times \text{number of leaves in each category})}{\text{The total leaf number} \times \text{the highest category}} \times 100$$

The Efficacy of each treatment in reducing powdery mildew severity was calculated as a percentage using the formula of Derbalah *et al.* (2011).

$$\text{Efficacy \%} = \frac{(\text{DSC}-\text{DST})}{\text{DSC}} \times 100$$

Where: DSC: Disease severity under control.

DST: Disease severity under treatment.

At the end of the experiment (180 days after planting), harvested plants and roots were weighed out and quality traits (percentages of T.S.S., sucrose content and purity) were tested in fresh roots.

Statistical analysis:

The obtained data were subjected to analysis of variance, simple correlations and regressions using SPSS Virgin 18 according to Steel and Torrie (1960). Duncan's multiple range tests were also applied to compare means (Duncan, 1955).

Results and Discussion

Effect on the disease severity:

Data presented in Table 1 show that significant reduction of powdery mildew disease severity in all treated plants compared to control. Generally, the exogenous application of chitosan and salicylic acid reduced significantly the disease severity compared to chemical application with Opera fungicide. Results exhibited that the effect of chitosan and canola oil against powdery mildew began 30 days after inoculation. Previously, chitosan has been reported as an effective compound against many plant diseases. Abada and Eid (2014) found that spraying cantaloupe plants with chemicals inducer of resistance, i.e. chitosan and salicylic acid at 100 mM against cantaloupe downy mildew disease reduced the severity of downy mildew with significant increase to the plant length and foliage fresh weight of cantaloupe plants under greenhouse conditions and caused significant reduction to the disease with significant increment to the harvested fruit yield and their T.S.S. under field experiment in comparison with control treatment. Farouk *et al.* (2008) found that chitosan could suppress downy mildew severity in the infected cucumber plants. Also, chitosan has been reported as plant growth promoter besides being an active in controlling diseases (Aziz *et al.*, 2006 and Trotel-Aziz *et al.*, 2006). Moreover, chitosan has the ability to induce defense responses systems in plants (Aziz *et al.*, 2006). Salicylic acid and ascorbic acid have been extensively studied, applied and reported as inducers for pathogen related response genes (PR genes) to enhance the plant resistance against phytopathogens (Farouk *et al.*, 2008; Tsrar and Bieche, 1999 and Deepthi and Reddy, 2013).

Table 1. Disease severity (%) of sugar beet powdery mildew *Erysiphe betae*, 2014-2015 on the treated plants with Chitosan, Ascorbic acid, Salicylic acid and fungicide Opera and their efficacy (%) compared to control

Treatment	% Disease severity after infection /days				Efficacy %
	15	30	45	Mean	
Chitosan	12.96 c*	7.41 cd	8.52 c	9.63	84.00
Ascorbic acid	21.30 b	14.81 b	16.43 b	17.51	70.90
Salicylic acid	12.04 c	13.58 bc	14.81 b	13.48	77.60
Opera	5.56 c	4.01 d	5.15 c	4.91	91.85
Control	49.67 a	61.73 a	69.14 a	60.18	0.00

* In the same column, means followed by the same letter are not significantly different at 5% level.

Effect of exogenous application with chitosan, ascorbic acid, salicylic acid, Opera on plant chemical components:

Total phenols and orthodihydric phenolic compounds have been estimated (Table 2). Results showed that total phenols and orthodihydric phenolic compounds were increased significantly in 30 days age of plants. The total phenols and orthodihydric phenolic compounds were increased significantly in response to different treatments compared to control. Exogenous application of chitosan, salicylic acid, ascorbic acid and Opera on infected sugar beet plants with powdery mildew increased significantly the total phenols to 5.54, 4.80, 4.10 and 3.99 mg/g fresh weight, respectively compared to 2.11 mg/g fresh weight in the control, 45 days after inoculation with the causal agent of powdery mildew. However, exogenous application of chitosan, salicylic acid, ascorbic acid and Opera on infected sugar beet plants with powdery mildew increased orthodihydric phenolic compounds to 0.70, 0.66, 0.63 and 0.60 mg/g fresh weight, respectively compared to 0.30 mg/g fresh weight in the control, 45 days after inoculation with powdery mildew. The total phenolics and orthodihydric phenols are great inducers to plant resistance against biotic stimuli. Phenols oxidized to highly toxic orthodihydric phenols by polyphenoloxidase (Vidhyasekaran, 1973). Accumulation of phenols at the infection site acts as the first step in mobilized defense system in plants which can be translocated by plants and enzymatically converted into defensive substance at the site of attack (Matern and Kneusal, 1988). Total phenols and orthohydric phenols were increased in parallel with disease development as found by Saraswathi and Reddy (2012). The role of phenolic compounds is essential in the host plant-pathogens interaction and well established. Constitutive phenolic compounds indirectly converse the resistance through activation of post-infection responses in the host plant. Orthohydric phenolic compounds contents induced the defense response against infection of groundnut with *Sclerotium rolfsii*, These phenolic compounds are considered as resistant factors because of their ability to act on oxidation and may be responsible for the formation of substances toxic to the pathogen or causing inactivation of hydrolytic enzymes produced by pathogens as suggested by Patil and Dimond (1967).

Table 2. Effect of Chitosan, Ascorbic acid, Salicylic acid and Opera fungicide on the total phenols and orthodihydric phenols in sugar beet plants infected by powdery mildew compared to control, 2014-2015

Treatment	Total phenols "mg/g fresh weight" after infection/ days				Orthodihydric phenols "mg/g fresh weight" after infection/ days			
	15	30	45	Mean	15	30	45	Mean
Chitosan	2.65ab*	3.13b	5.54a	3.77	0.56a	0.59a	0.70a	0.62
Ascorbic acid	2.93a	3.78a	4.10b	3.60	0.54a	0.61a	0.63a	0.59
Salicylic acid	3.10a	4.16a	4.80ab	4.02	0.50a	0.52a	0.66a	0.56
Opera	3.18a	3.67ab	3.99b	3.61	0.44a	0.47a	0.60a	0.50
Control	2.14b	2.26c	2.11c	2.17	0.28b	0.30b	0.30b	0.27

* In the same column, means followed by the same letter are not significantly different at 5% level.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis:

The GC-MS analysis of methanol extracts of 60 days old leaves of sugar beet plants, which have been treated with chitosan, ascorbic acid, salicylic acid as inducers to the systemic resistance of sugar beet against *Erysiphe betae* compared to control (tap water) under the greenhouse conditions, resulted in detection a number of active compounds. The GC-MS chromatogram with peak area is given in Figs. (1, 2, 3 and 4).

In general, the important phytochemical compounds in the same 15 retentions times in all foliar application of the tested compounds, chitosan, ascorbic acid, salicylic acid and control (tap water) were recognized as shown in Table (3). The phytochemical compounds were reported and arranged according to their peak number, retention time (RT), concentration (peak area %), Probability, compound name, molecular formula (MF) and molecular weight (MW) for all treatments.

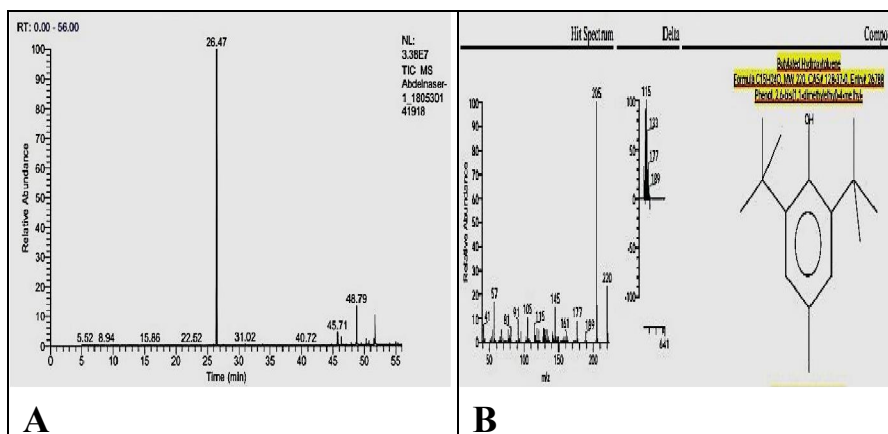


Fig. 1. GC-MS chromatogram of methanol leaves extract of sugar beet plants treated with chitosan. (A) Peak 2 with the retention time 26.47 was identified as phenol, 2, 4-bis (1, 1-dimethylethyl) and as the major phyto-component of the plant while other peaks were of the various phyto-components present.(B) GC-MS mass spectrum and molecular structure of phenol, 2, 4-bis (1, 1-dimethylethyl).

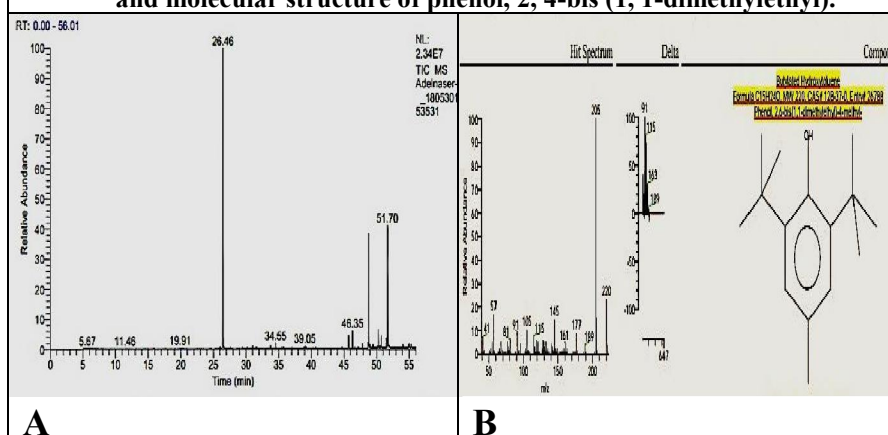


Fig. 2. GC-MS chromatogram of methanol leaves extract of sugar beet plants treated with ascorbic acid. (A) Peak 2 with the retention time 26.47 was identified as phenol, 2, 4-bis (1, 1-dimethylethyl) and as the major phyto-component of the plant while other peaks were of the various phyto-components present.(B) GC-MS mass spectrum and molecular structure of phenol, 2, 4-bis (1, 1-dimethylethyl).

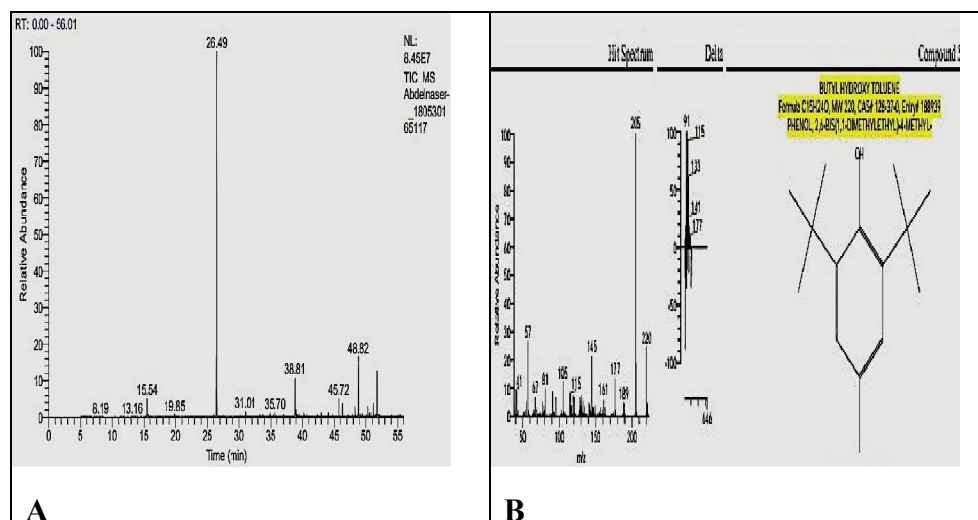


Fig. 3. GC-MS chromatogram of methanol leaves extract of sugar beet plants treated with salicylic acid. (A) Peak 2 with the retention time 26.47 was identified as phenol, 2, 4-bis (1, 1-dimethylethyl) and as the major phyto-component of the plant while other peaks were of the various phyto-components present. (B) GC-MS mass spectrum and molecular structure of phenol, 2, 4-bis (1, 1-dimethylethyl).

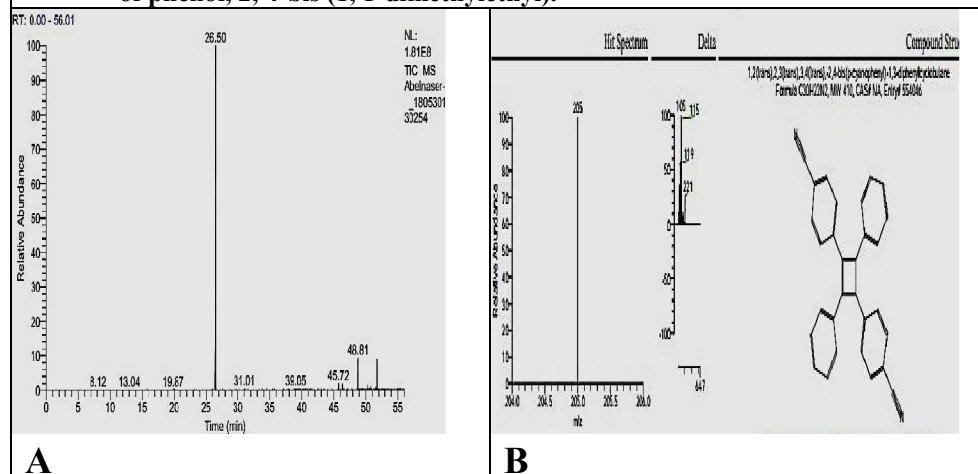


Fig. 4. GC-MS chromatogram of methanol leaves extract of sugar beet plants treated with tap water as a control. (A) Peak 2 with the retention time 26.50 was identified 1,2(trans),2,3(trans),3,4(trans),-2,4-bis(p-cyanophenyl)-1,3-diphenylcyclobutane and as the major phyto-component of the plant while other peaks were of the various phyto-components present. (B) GC-MS mass spectrum and molecular structure of 1,2 (trans),2,3(trans),3,4(trans),-2,4-bis(p-cyanophenyl)-1,3-diphenylcyclobutane.

Table 3. GC-MS analytical report of extracted sugar beet leaves sprayed with the tested compounds and their biological properties

P. No.	Treatment	*			Compound Name	*	
		R.T.	Area %	Prob.		M.W.	M.F.
1	Chitosan	26.07	0.42	14.47	â-Ionon-5,6-epoxide	208	C13H20O2
	Ascorbic acid	26.07	0.35	24.00			
	Salicylic acid	26.07	0.36	26.97			
	Control	26.08	0.21	18.56			
2	Chitosan	26.47	63.04	45.23	Phenol, 2,6-bis (1,1-dimethylethyl)-4-methyl-(CAS) 1,2(trans),2,3(trans),3,4(trans),-2,4-bis(p-cyanophenyl)-1,3-diphenylcyclobutane	220	C15H24O
	Ascorbic acid	26.46	40.56	70.11			
	Salicylic acid	26.48	50.33	67.67			
	Control	26.50	72.01	11.84			
3	Chitosan	27.56	0.32	8.61	Ingol 12-acetate	408	C22H32O7
	Ascorbic acid	27.56	0.39	3.66			
	Salicylic acid	27.53	0.29	58.73			
	Control	27.55	0.16	9.53			
4	Chitosan	31.02	0.62	13.38	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-Spiro[4.4]non-3-en-2-one, 4-methyl-3-(1H-tetrazol-5-yl)1-oxa-	224	C13H20O3
	Ascorbic acid	31.05	0.73	4.54			
	Salicylic acid	31.00	1.09	20.84			
	Control	31.01	0.42	6.10			
5	Chitosan	34.55	0.23	17.74	Phthalic acid, butyl octyl ester	334	C20H30O4
	Ascorbic acid	34.55	1.05	9.77			
	Salicylic acid	34.88	0.73	11.58			
	Control	34.55	0.14	8.76			
					(9E)-9-Hexadecen-1-ol	240	C16H32O
					Phthalic acid,2,7-dimethyloct-7-en-5-yn-4-yl isobutyl ester	365	C22H28O4

Continued

6	Chitosan	39.83	0.15	8.71	1H-Indole-3-acetic acid, 4,5,6,7-tetrahydro-2,6,6- trimethyl-4-oxo-1-phenyl-	311	C19H21NO3
	Ascorbic acid	39.26	0.31	8.48	cis-11-Eicosenoic acid	310	C20H38O2
	Salicylic acid	39.25	0.28	13.34	((Z)-11-Eicosenic acid)		
	Control	39.27	0.09	10.60	(n-Octadecylsuccinic anhydride)	352	C22H40O3
7	Chitosan	44.73	0.46	7.68	Oleic Acid dihydroxypropyl ester (CAS)	356	C21H40O4
	Ascorbic acid	44.74	0.51	5.03	Oleic Acid 9-Octadecenoic acid (Z)-HO	282	C18H34O2
	Salicylic acid	44.72	0.35	8.65	Oleic Acid dihydroxypropyl ester (CAS)	356	C21H40O4
	Control	44.73	0.17	38.87			
8	Chitosan	45.71	3.46	8.09	1-Eicosanol n-Eicosanol	298	C20H42O
	Ascorbic acid	45.72	2.43	5.15	n-Tetracosanol-1 Lignoceric alcohol	354	C24H50O
	Salicylic acid	45.72	2.92	10.19	1-Eicosanol n-Eicosanol	298	C20H42O
	Control	45.72	1.51	6.57	Stenol 1-Octadecanol (CAS)	270	C18H38O
9	Chitosan	46.35	2.25	25.10	Diisooctyl phthalate	390	C24H38O4
	Ascorbic acid	46.35	2.92	32.01			
	Salicylic acid	46.36	2.13	29.43	Bis(6-methylheptyl) phthalate		
	Control	46.35	1.39	42.50	Dioctyl phthalate	390	C24H38O4
10	Chitosan	47.21	0.15	17.87	Quercetin 7,3',4'-trimethoxy	344	C18H16O7
	Ascorbic acid	47.19	0.34	5.33	Docosane (CAS)	310	C22H46
	Salicylic acid	47.20	0.26	8.70			
	Control	47.20	0.11	11.60			

Continued

11	Chitosan	47.86	0.70	6.14	2,2-di deuteron octa decanal	268	C18H34D2 O
	Ascorbic acid	47.87	0.76	6.91			
	Salicylic acid	47.87	0.31	6.73			
	Control	47.86	0.30	5.92			
12	Chitosan	48.79	8.87	6.14	1-Docosene	308	C22H44
	Ascorbic acid	48.80	14.82	5.84			
	Salicylic acid	48.81	8.92	6.03	1-Docosanol (CAS)	326	C22H46O
	Control	48.81	6.21	8.89			
13	Chitosan	49.47	0.62	24.33	(Bis (2-ethylhexyl) isophthalate)	390	C24H38O4
	Ascorbic acid	49.47	0.59	33.60			
	Salicylic acid	49.47	0.49	45.10			
	Control	49.47	0.42	21.16			
14	Chitosan	50.30	1.72	29.27	Squalene	410	C30H50
	Ascorbic acid	50.30	2.91	28.91			
	Salicylic acid	50.30	1.73	41.74			
	Control	50.30	1.16	17.72			
15	Chitosan	51.68	7.47	8.61	1-Eicosanol	298	C20H42O
	Ascorbic acid	51.70	18.38	5.73			
	Salicylic acid	51.71	7.30	4.59			
	Control	51.71	7.20	4.61	n-Tetracosanol-1	354	C24H50O

(*) (P. No.) Peak No. (R.T.) Retention time. (Prob.) Probability, (M.W.) Molecular weight. (M.F.) Molecular formula

The obtained results revealed that exogenous applications of chitosan, ascorbic acid and salicylic acid on sugar beet plants recorded the highest concentration (peak area %) for all phytochemical compounds compared to control, except the major component (Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-) which was found in retention time (26.4) with concentration as peak area % (63.04, 40.56, 50.33%), respectively. Butylated Hydroxytoluene (Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-) phyto-component was identified as Phenolic compound with variable activities such as antioxidant, antifungal and antibacterial activities (Vinod *et al.*, 2016, Teresa *et al.*, 2014 and Chuah *et al.*, 2015). Whereas, 1,2(trans), 2,3(trans),3,4(trans),-2,4-bis(p-cyanophenyl)-1,3-diphenyl cyclobutane, was the major component in control treatment (72.01%), which has been identified as catechol-o-methyl-transferase-inhibitor decrease glutamate pyruvate transaminase.

Biological activities of phenolic compound (1,2(trans),2,3 (trans),3,4(trans),-2,4-bis(p-cyanophenyl)-1,3-diphenyl cyclobutane) are based on Dr. Duke's: Phytochemical and Ethnobotanical Databases (1992). In the retention time (26.0) the compound á-Ionon-5,6-epoxide (3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-) has been detected with concentrations or peaks areas % (0.42, 0.35, 0.36 and 0.21%) in all foliar application treatments with tested compounds chitosan, ascorbic acid, salicylic acid and control (tap water), respectively, which was identified as alcohols Phytochemical with antimicrobial activity (Sita, 2009). In retention time (27.5) the phyto-component Ingot 12-acetate were detected in the foliar application of the tested compounds chitosan and ascorbic acid with a concentration 0.32 and 0.39% whereas 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- phyto-component were detected in the same retention in the foliar application of the tested compound salicylic acid and control with a concentration 0.29 and 0.16%. Both of them identified as terpenes compounds and have an antibacterial activity (Mujeeb, 2014).

The phyto-component (3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl) has been detected in the retention time (31.0) with concentration 0.62 and 1.09% in response to chitosan and salicylic acid treatments. Also, this phyto-component has been identified as a ketone with antibacterial, antimicrobial and antioxidant activities (Ahamath and Sirajudeen 2014 and Madkour *et al.*, 2017). Whereas the phyto-component Spiro[4.4]non-3-en-2-one, 4-methyl-3-(1H-tetrazol-5-yl)-1-oxa has been detected in the concentration 0.73% in response to ascorbic acid treatment, also Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl- was detected in the same retention time in the untreated plants (control) with concentration 0.42%. The phyto-component Phthalic acid, butyl octyl ester has been identified in the retention time (34.5) with concentration 0.23 and 1.05% in response to chitosan and ascorbic acid treatments, respectively. Phthalic acid, 2,7-dimethyl oct-7-en-5-yn-4-yl isobutyl ester as an antibacterial active compound has been detected with concentration 0.14% in the untreated plants (Ajoke *et al.*, 2014). Whereas, in retention time (34.8) the phyto-components (9E)-9-Hexadecen-1-ol as fatty alcohol with antimicrobial activities with concentration 0.73% was found in response to salicylic acid treatment (Kale *et al.*, 2015). In retention time (39.83), the phyto-component 1H-Indole-3-acetic acid, 4,5,6,7-tetrahydro-2,6,6-trimethyl-4-oxo-1-phenyl- was detected with concentration 0.15% in chitosan treatment which was identified as auxin-like substance plant growth promoting. In retention time (39.2) the phyto-component cis-11-Eicosenoic acid with concentrations 0.31 and 0.28% was detected in ascorbic acid and salicylic acid treatments and was identified as fatty acid and has an antibacterial, antioxidant and antimicrobial activity (Gulfraz *et al.*, 2011) whereas the phyto-component 2,5-Furandione, dihydro-3-octadecyl- was detected with concentration 0.09% in control treatment. In retention time (44.7), two phyto-components 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (1-Monoolein) with concentrations 0.46, 0.35 and 0.17% were detected in chitosan, salicylic acid and control (tap water) treatments, respectively and phyto-component Oleic acid with concentration 0.51% was detected in ascorbic acid treatment. Both of them were identified as fatty acid esters and have antioxidant and antimicrobial activities (Guiheneuf *et al.*, 2016).

In retention time (45.7) phyto-component 1-Eicosanol (n-Eicosanol) with concentrations 3.46 and 2.92% was detected in chitosan and salicylic acid treatments and identified as aliphatic alcohol and has antifungal and antioxidant (Farina *et al.*, 2014) whereas in the same retention time the phyto-component n-Tetracosanol-1 with concentration 2.43% was detected in ascorbic acid treatment and was identified as lignoceryl alcohol and has an antibacterial effect (Kotan *et al.*, 2014). In control treatment phyto-component 1-Octadecanol (Stenol) with concentration, 1.51% was detected and identified as aliphatic alcohol. The phyto-component 1,2-Benzenedicarboxylic acid, dioctyl ester (Dioctyl phthalate) was detected in retention time (46.3) in all foliar application treatments with tested compounds chitosan, ascorbic acid and salicylic acid with higher concentrations or peak area % (2.25, 2.92 and 2.13%), respectively than the concentration of control (tap water) treatment (1.39%). This phyto-component was identified as phthalate ester and has an antibacterial and antifungal effect (Sastry and Rao, 1995). In retention time (47.2) phyto-component Quercetin 7,3',4'-tri methoxy with concentration 0.15% was detected in chitosan treatment and was identified as a flavonoid and has anti-oxidant, antimicrobial and antiviral activities (Justyna *et al.*, 2014), whereas in the same retention time (47.2) phyto-component Docosane (CAS) with concentrations 0.34 and 0.26 % was detected in ascorbic acid and salicylic acid treatments, respectively more than concentration of control (tap water) treatment (0.11%). This component was identified as alkane and has anti-bacterial and anti-fungal activities and anti-viral activities (Yamunadevi *et al.*, 2013).

The phyto-component 2,2-di deuteron octa decanal was detected in retention time (47.8) in all foliar application treatments using the tested compounds, chitosan, ascorbic acid and salicylic acid with higher concentrations or peaks areas % (0.70, 0.76 and 0.31%), respectively than the concentration of control (tap water) treatment (0.30%). This phyto-component was identified as Aldehyde and has an anti-microbial activity (Guiheneuf *et al.*, 2016). In retention time (48.8) phyto-component 1-Docosene with concentrations (8.87, 14.82 and 8.92%) was detected in chitosan, ascorbic acid and salicylic acid treatments, respectively. This phyto-component was identified as hydrocarbon compound and has antibacterial activity (Samejo *et al.*, 2010), whereas, in control treatment the phyto-component 1-Docosanol (CAS) was detected with concentration (6.21%). This component was identified as saturated fatty alcohol and has an antiviral activity (Katz *et al.*, 1991).

In retention time (49.47), the compound (Bis(2-ethylhexyl) isophthalate) was detected with concentration or peak area % (0.62, 0.59, 0.49 and 0.42%) in all foliar application treatments with the tested compounds chitosan, ascorbic acid, salicylic acid and control (tap water), respectively and identified as phthalate ester and has an antibacterial and antifungal activity (Moorthy and Boominathan, 2011). The phyto-component Squalene was detected in retention time (50.3) in all foliar application treatments with tested compounds, chitosan, ascorbic acid and salicylic acid with higher concentrations or peaks areas % (1.72, 2.91 and 1.73%), respectively than the concentration of control (tap water) treatment (1.16%). This phyto-component was identified as a triterpene and acts as antibacterial, (Sermakkani and Thangapandian, 2012). In retention time (51.7) phyto-component 1-Eicosanol with concentrations

(7.47, 18.38 and 7.30%) was detected in chitosan, ascorbic acid and salicylic acid treatments, respectively. This phyto-component was identified as aliphatic alcohol compound and has antifungal and antioxidant activity (Farina *et al.*, 2014), whereas, in control treatment the phyto-component n-Tetracosanol-1 was detected with concentration (7.20%). This component was identified as lignoceryl alcohol and has an antibacterial effect (Kotan *et al.*, 2014).

Field experiments:

Effect of the experimented substances on the severity of powdery mildew of sugar beet and root yield conducted in the field of Gemmeiza Experimental Station under natural infection in two successive seasons *i.e.*, 2014-2015 and 2015-2016.

Data in Table 4 indicate that all treatments under study efficiently decreased the disease severity in both seasons of experimentation comparable to the non-treated control, generally. While spraying with Opera fungicide was highly effective in controlling the disease. However, the other substances were equally affecting the disease severity (Table 4).

Table 4. Effect of the tested compounds on the management of powdery mildew and the produced root yield, experimental field of Gemmeiza, during the two successive seasons

Treatment	% Disease severity during			% Efficacy	Yield "ton/fed." During			% Yield increase
	2014/2015	2015/2016	Mean		2014/2015	2015/2016	Mean	
Chitosan	14.44b*	18.52b	16.48	58.49	19.77bc	18.48c	19.12	14.91
Ascorbic acid	12.96b	15.74b	14.35	63.85	21.80ab	20.18b	20.99	26.15
Salicylic acid	14.81b	18.37b	16.59	58.22	20.63b	21.96a	21.29	27.96
Opera	4.81c	6.11c	5.46	86.24	24.43a	22.89a	23.66	42.21
Control	37.78a	41.63a	39.71	0.00	18.33c	16.61d	16.64	0.00

*In the same column, means followed by the same letter are not significantly different at 5% level.

Results of the effectiveness of treatments under study on the produced beet roots are presented in Table (4). It was found that all treatments caused a significant increase in yield production, in general. Opera fungicide was superior in increasing the production and all treatments equally affected positively the root production. Logically, as a result of managing infection of sugar beet with powdery mildew due to spraying with substances under study, root productivity was significantly increased as shown in Table (5).

Table 5. Effect of the tested compounds on quality traits (total soluble solids, sucrose and purity percentages) of sugar beet plants naturally infected by powdery mildew

Treatment	% Total soluble solids during			% Sucrose during			%Purity during		
	2014/ 2015	2015/ 2016	Mean	2014/ 2015	2015/ 2016	Mean	2014/ 2015	2015/ 2016	Mean
Chitosan	23.87a*	22.87a	23.37	20.72a	18.51ab	19.61	86.98ab	80.99b	83.99
Ascorbic acid	24.00a	21.40ab	22.70	21.28a	17.87ab	19.58	88.70a	83.49ab	86.09
Salicylic acid	24.80a	23.13a	23.97	21.67a	19.78a	20.73	87.05ab	85.92a	86.49
Opera	24.50a	22.90a	23.70	20.64a	19.54a	20.09	84.26b	85.40a	84.83
Control	21.40b	18.92b	21.16	17.37b	16.47b	17.09	78.72c	77.93c	78.32

*In the same column, means followed by the same letter are not significantly different at 5% level.

Total soluble solids (T.S.S.), sucrose and the purity percentage of the obtained sucrose were determined in beet roots and results are shown in Table (5). It was found that all treatments improved the percentages of T.S.S., sucrose content and sucrose purity compared to the control. Except for the obtained sucrose after spraying with ascorbic acid in seasons (2014/2015 and 2015/2016), insignificant differences were found for treating with any of substances.

These results are in consistency with the findings obtained by Francis *et al.* (2007); EL-Fahar and Abou El-Magd (2008) and Kontradowitz and Verreet (2010), where they reported that powdery mildew of sugar beet decreased root yield and yield quality and controlling the disease causing an increase substantially the root yield and yield quality.

Conclusion

The results of our investigation indicate that fungicide Opera could decrease the fungal activity without increasing the plant resistance, while chitosan, salicylic acid and ascorbic acid induced many phenolic compounds, which affect positively in the plant tolerance in response to the fungal infection without affecting the quality and percentage of sugar production. Finally, exogenous application of chemical inducers should start before infection to assist the plant resistance against different pathogenic attack.

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كيماوية لمكافحة مرض البياض الدقيقي

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أمراض النبات- مركز البحوث الزراعية.

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يهدف البحث إلى تقييم فاعلية رش المجموع الخضري لبنجر السكر لاستحضات المقاومة الجهازية باستخدام بعض المواد الكيميائية مثل الشيتوزان وحمض الأسكوربيك وحمض الساليسيليك كبدائل للمبيدات الفطرية لمقاومة مرض البياض الدقيقي في بنجر السكر صنف "هركل" المتسبب عن الفطر *Erysiphe betae* مقارنة بالرش بماء الصنبور أو المبيد الفطري أوبرا (كمعاملة مقارنة) تحت ظروف الصوبة والحقل بمحطة بحوث الجميزة محافظة الغربية مركز البحوث الزراعية خلال موسمي الزراعة 2014/ 2015 و 2015/ 2016. تحت ظروف الصوبة أظهرت النتائج أن رش النباتات بالمعاملات السابقة خفضت شدة الإصابة بمرض البياض الدقيقي بالمقارنة برش الأوراق بالماء والمادة اللاصقة فقط كمعاملة مقارنة. كان مبيد الفطريات أوبرا هو العلاج الأكثر فعالية في الحد من شدة المرض يليه معاملة الشيتوزان ومعاملة حمض الساليسيليك. وقد أظهرت كل المعاملات زيادة معنوية في محتوى الأوراق من الفينولات الكلية والارثوفينول عند تقديرها على فترات 15، 30، 45 يوم من العدوى الصناعية بالمسبب المرضي بالمقارنة بالنباتات المعاملة بالماء والمادة اللاصقة فقط كمعاملة مقارنة. أظهر التحليل الكروماتوجرافي الغازي المرتبط بمطياف الكتلة (GC-MS) لمستخلصات الميثانول لأوراق بنجر السكر عند تقديرها في عمر 60 يوماً تحت ظروف الصوبة، والتي تمت معاملتها بالشيتوزان وحمض الأسكوربيك وحمض الساليسيليك لاستحضات المقاومة الجهازية في بنجر السكر ضد فطر *Erysiphe betae* زيادة في المركبات الكيميائية الحيوية النشطة مثل الفلافينويدات، الكحول، الألدهيدات، المركبات العطرية، استرات ميثيل الأحماض الدهنية، التربينويد والمركبات الفينولات والتي يفترض أن لها نشاط مضاد للميكروبات بالمقارنة بالنباتات المعاملة بالماء والمادة اللاصقة فقط كمعاملة مقارنة. تحت ظروف الحقل أوضحت النتائج أن المعاملات بالمبيد الفطري أوبرا كان أفضل المعاملات رشاً على الأوراق في مقاومة مرض البياض الدقيقي في بنجر السكر يليه المعاملة بحمض الأسكوربيك. وفي الوقت نفسه، أظهرت جميع المعاملات تأثير أقل للمبيد الفطري أوبرا في هذا الصدد، ولكن أعلى من النباتات المعاملة بالماء والمادة اللاصقة فقط كمعاملة مقارنة. وأظهرت جميع المعاملات زيادة في محصول جذور بنجر السكر للقدان مقارنة بالمعاملة بالماء والمادة اللاصقة فقط كمعاملة مقارنة. أعطى مبيد الأوبرا وحمض الساليسيليك أعلى محصول جذور بنجر السكر للقدان يليه حمض الأسكوربيك ثم الشيتوزان. علاوة على ذلك أعطى حمض الساليسيليك والمبيد الفطري أوبرا أعلى النسب المئوية من إجمالي المواد الصلبة الذائبة (T.S.S) ونسبة السكر، في حين أعطى حمض الساليسيليك وحمض الأسكوربيك والمبيد الفطري أوبرا أعلى النسب المئوية للنقاء في جذور بنجر السكر.