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Role of some Non-traditional Compounds in Managing Sugar Beet Powdery Mildew

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Certain non-traditional compounds such as compost tea, humic acid and sulphated canola oil "Sulplex." were tested as foliar application to evaluate their efficacy to induce systemic resistance against *Erysiphe betae*, the causal agent of powdery mildew in sugar beet (Herkl cv.) versus the traditional fungicide Opera under greenhouse and field conditions at Gemmeiza Agricultural Research Station, Agricultural Research Center (A.R.C.), Egypt during two successive winter seasons (2014/2015 and 2015/2016). Under greenhouse conditions, results revealed that spraying plants with all the tested compounds reduced disease severity of powdery mildew. The fungicide Opera was the most effective treatment in reducing disease severity and increasing the efficacy followed by "Sulplex", compost tea and humic acid. Also, all treatments significantly increased total phenols and orthodihydric (OD) phenol content of the leaves compared to the control 15, 30 and 45 days after inoculation. Also, spraying plants with the tested compounds increased the activity of peroxidase (PO), polyphenoloxidase (PPO) and chitinase enzymes compared to the control, 15, 30 and 45 days after artificial inoculation. Inthe detection of chitinase activity by using SDS-glycol chitin polyacrylamide gel electrophoresis the molecular weight for all samples ranged from 28 to 30 kDa. high intensity band expressing the highest chitinase activity was found in compost-tea treatment followed by "Sulplex". Moreover, control, fungicide Opera and humic acid treatments showed low chitinase activity. Under field experiments, results indicated that the fungicide Opera was the most effective treatment for controlling powdery mildew followed by Sulplex and compost tea. Meanwhile, humic acid was the lowest effective one in this regard. All treatments increased root yield per feddan compared to the control. The fungicide Opera and Sulplex gave the highest root yield per feddan followed by compost tea then humic acid. Moreover, compost tea and Sulplex gave the highest percentages of total soluble solids (T.S.S.), sucrose percentages in beet roots and purity.

Keywords: Chitinase, sucrose%, compost tea, *Erysiphe betae*, humic acid, oxidative enzymes, phenolic compounds, powdery mildew, sugar beet and sulphated canola oil "Sulplex.".

Sugar beet (*Beta vulgaris* L.) is considering one of the most important sugar crops in many countries all over the world (Francis *et al*., 2007). In Egypt, it is ranked as the second crop for sugar production after sugar cane (Eweis *et al*., 2006). One of the important plant pathogens that attack sugar beet foliage growth causing a great reduction in the root yield is powdery mildew caused by *Erysiphe betae* (Kontradowitz and Verreet, 2010). Under severe attack, up to 22% reduction in root yield was recorded as well as, 13% reduction in sucrose content in roots took place (Karaoglanidis and Karadimos, 2006), consequently lessened yield and yield quality (El-Fahar and Abou El-Magd, 2008).

Powdery mildew has been successfully controlling with fungicides (Karaoglanidis and Karadimos, 2006 and Kontradowitz and Verreet, 2010). However, these fungicides can pollute the environment, be phytotoxic to the host and a probably resistance in plant pathogens toward fungicides can be built up (Sierotzki *et al*., 2000 and Ishii *et al*., 2001). Accordingly, the application of alternative control methods is required to reduce the harmful effect of fungicides. These alternatives should be economic and of low toxicity for plants. Furthermore, controlling powdery mildew might take place through inducing systemic resistance (ISR) (Reuveni and Reuveni 1995).

Foliar application of compost tea has been using successfully for many years to control foliar plant diseases (Scheuerell and Mahaffee, 2002 and Litterick *et al*., 2004). In addition, essential plant oils have also inhibitory activity on the growth of pathogens. (Kaur and Arora, 1999). Sulphated canola has suggested being one of the substitutes, where the elemental sulphur was used as an efficient fungicide (Williams and Cooper, 2004). Thus, sulphur containing oil can successfully be used in controlling the pathogen (Pohoreski, 2004). Chitinase catalyzes the hydrolysis of chitin, a β-1, 4-linked polymer of N-acetyl-D-glucosamine (GlcNAc), which is the main structural component of fungal cell walls and arthropod integuments.

Plants do not have chitin, but they do have chitin-degrading enzymes. It was hypothesized that plant chitinases can degrade chitin in the fungal cell walls to directly affect the viability of the invading fungal pathogen and to release short chitin fragments (chitooligosaccharides) that can act as a general elicitor of plant innate immunity (Shibuya and Minami, 2001 and Passarinho and de Vries, 2002). The cell walls of the ascomycetes always contain chitin and β-glucans, and divisions within the hyphae, called "septa", are the internal boundaries of individual cells (or compartments) (Alexopoulos *et al*., 1996)

The current study was planned to evaluate the efficacy of humic acid, sulphated canola oil "Sulplex." and compost tea as a foliar application for inducing systemic resistance against sugar beet powdery mildew. In addition, biochemical changes in sugar beet leaves associated with the application of these treatments were also investigated.

Materials and Methods

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

Preparation of substances used in the study:
Host plant:

Sugar beet (*Beta vulgaris* L.) seeds cultivar (Herkl cv.) provided by Maize and Sugar Crops Dis. Res. Dept., Plant Pathol. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt were used in the present study

Preparation of Compost tea:

Compost mixed as animal manure and plant product was supplied by Agriculture Service Center Compost (production unit), Fac. Agric. Moshtohor, Benha Univ. Egypt. Analysis of the used compost presented in Table (1). To prepare compost tea, 1 Kg of compost was soaked in a large barrel containing 5 litres of tap water for one day. Thereafter, the compost extract was sieve through cheesecloth and the extract was diluted to 200 ml/L water (Litterick *et al*., 2004 and Zinati, 2005).

Table 1. Analysis of the used compost type

Characters	Analysis
Bulk density, kg/m^3	610
pH	7.2
EC ds/m	2.1
Organic carbon %	20.7
Organic mater %	35.5
Total nitrogen %	1.13
C/N ratio	18.3
Total phosphorus %	1.03
Total potassium %	2.11
Weed germination	Nil
Phytopathogens	Nil
Root feeding nematodes	Nil

Preparation of sulphated canola oil "Sulplex":

Canola oil (100% pure oil) was obtained from Al-Ghurair Foods (LLC), Dubai under the trade name "Jenan". Sulphated canola oil "Sulplex" was prepared as described by Pohoreski (2004). A volume of 477 ml of conc. H_2SO_4 was added to 3 kg of canola oil, mixed and allowed to stand for 18h. Thereafter, 3.88 L of NaOH (3N) was added slowly to the mixture and left for 24h. The sulphated oil was then removed from the top of the mixture, diluted with deionized water at a volume ratio of 1:4 and the pH of the resulting solution was adjusted to a pH of about 4 to 5 with 3 N NaOH.

Preparation of humic acid:

Humic acid (analytical grade, assay 99%) was obtained from Fluka Chemika. An aqueous solution of 400 mg/L was prepared from humic acid using deionized water.

The fungicide:

Opera 18.3% SE (pyraclostrobin / Epoxiconazole), BASF Company for Chemicals and Pesticides was used at the recommended dose in the controlling of powdery mildew as a reference in this study.

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-weeks age beet plants. Infected leaves of the newly mildewed plants were used as a source of conidial inoculum in the present investigation.

Greenhouse experiment:

In an experiment designed in a randomized complete blocks method with three replicate pots (no.35), seeds of sugar beet were planted, 10 seeds per pot. After 3 weeks, seedlings were thinned to 3 per pot and fertilized with 1% solution of NPK (75:150:50). After 30 days of planting, plants were sprayed with each of the experimented substances by the aid of a manual low pressure sprayer four times at 2 week intervals. Arabic gum as an adhesive material was added before spraying. At 60 days after planting, plants were artificially sprayed with spores of *E. betae* produced on the powdered leaves according to the method described by Whitney *et al*. (1983) and Lewellen and Schrandt (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 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). Ortho dihydirc phenols (OD) were measured spectrophotometrically at 515 nm wave length as described by Arnow (1937).

Measurement of enzymatic activity:

Peroxidase, polyphenoloxidase and chitinase activities were determined in leaf extracts prepared according to the method of Tuzun *et al.* (1989). Peroxidase activity was estimated spectrophotometrically by measuring the oxidation of pyrogallol in the presence of H_2O_2 at wave length 425nm, according to Allam and Hollis (1972). Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

Polyphenoloxidase activity was determined using the spectrophotometer procedure at 495 nm, as described by Matta and Dimond (1963). Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/30 min.

Chitinase activity was measured spectrophotometrically according to Boller and Mauch (1988). Colloidal chitin was used as a substrate and dinitrosalicylic acid reagent to measure reducing sugars. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

Determination of chitinase activity by using SDS-glycol chitin polyacrylamide gel electrophoresis:

The enzyme extracted from treated and non-treated sugar beet leaves which harvested at the third time point 45 days from artificial inoculation of sugar beet plants with *E. betae*, by cutting them at the leaf base level and the supernatant was prepared according to method of Tuzun *et al*. (1989) was used to detect chitinase activity by SDS-glycol chitin polyacrylamide gel using a modified coomassie brilliant blue method according to Liau and Lin (2008). The image data of SDS-glycol chitin polyacrylamide gel analyzed with totallap1D Gel software.

Field experiments:

Field trials were conducted under natural infestation at Agricultural Research Station of Gemmeiza in seasons of 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 the 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 disease severity:

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

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

$$\text{Efficacy \%} = [(DSC-DST)/DSC] \times 100$$

Where: DSC: Disease severity under control.
DST: Disease severity under treatment.

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

Statistical analysis:

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

Results and Discussion

Effect of the tested compounds on the severity of sugar beet powdery mildew and the content of the total phenols as well as orthodihydric phenols:

Generally, all foliar applications with the investigated compounds on inducing the systemic resistance of plants against *Erysiphe betae* significantly reduced the disease severity of powdery mildew (Table 2). Opera fungicide was found to be the most effective treatment in reducing disease severity (4.32 %) recording an increase in the efficacy of reducing powdery mildew severity by 92.80%, followed by "Sulplex". The least significant reductions in decease severity were recorded for the humic acid treatment. Similar results were recorded by Pasini *et al*. (1997) who found that the usage of "Sulplex" reduced significantly the infection of rose leaves by powdery mildew compared with the control treatment. Moreover, "Sulplex" effect seemed to be of equal effect to that of conventional chemicals fungicide.

Table 2. Effect of the tested substances on the severity of sugar beet powdery mildew under greenhouse conditions

Treatment	% Disease severity after days				Efficacy %
	15	30	45	Mean	
Compost tea	12.03 c	7.41c	12.00 c	10.48	82.48
Sulplex	9.26 cd	8.64 c	8.15 cd	8.68	85.49
Humic acid	21.66 b	20.09 b	17.94 b	19.90	66.93
Opera	5.18 d	3.73.01c	4.01 d	4.32	92.80
Control	50.22 a	60.93 a	68.34 a	59.83	0.00

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

In case of compost tea treatment, a significant reduction in the incidence of tomato early blight was associated with a pronounced increase in the tomato yield of plants treated with compost prepared at a ratio of 1:5 compost to water (Tsror and Bieche, 1999). Compost tea shows multiple modes of activity in suppressing plant disease such as inducing plant resistance against pathogens, antibiosis as well as competing on soil nutrients with the pathogens (Deepthi and Reddy, 2013). They added that a preventive application of compost tea before pathogen infection is necessary to optimize the control of this plant pathogen.

Total phenols and orthodihydric phenols were estimated and results are tabulated in Table (3). Data indicated that spraying plants with "Sulplex" exhibited the highest amount of total phenols (4.76 mg g-1 fresh weight) followed by compost tea (3.79 mg g-1 fresh weight), and humic acid (3.69 mg g-1 fresh weight) compared with Opera fungicide (2.81 mg g-1 fresh weight) at 45 days after inoculation. Meanwhile, the control exhibited the least amount (1.25 mg g-1 fresh weight). In the case of orthodihydric phenols, the highest increases were detected in plants treated with compost tea, "Sulplex" (0.82 and 0.79 mg g-1 fresh weight, respectively at 45 days after inoculation).

Table 3. Effect of the tested substances on the total phenols and orthodihydric phenols in the leaves of sugar beet infected with powdery mildew under greenhouse conditions

Treatment	Total phenols "mg/g fresh weight" after days				Orthodihydric phenols "mg/g fresh weight" after days			
	15	30	45	Mean	15	30	45	Mean
Compost tea	3.65 a	4.02 a	3.79 ab	3.82	0.53 a	0.78 a	0.82 a	0.71
Sulplex	3.78 a	4.38 a	4.76 a	4.31	0.45 ab	0.73 a	0.79 ab	0.66
Humic acid	2.88 b	3.29 b	3.69 ab	3.28	0.41 b	0.47 b	0.57 ab	0.48
Opera	2.08 c	2.72 c	2.81 b	2.53	0.37 b	0.39 b	0.57 b	0.44
Control	1.23 d	1.22 d	1.25 c	1.23	0.22 c	0.18 c	0.21 c	0.20

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

On the other hand, humic acid and opera fungicide exhibited the least amounts of orthodihydric phenols in plants. It is well known that total phenolics and orthodihydric phenols are two important components that induce resistance of plants against pathogens, *e.g.* phenols are oxidized to highly toxic orthodihydric phenols by enzymatic action (polyphenoloxidase) (Vidhyasekaran, 1973). According to MaternandKneusal (1988), the first step involves the rapid accumulation of phenols at the infection site, which acts as mobilized defence system which can be translocated by plants and enzymatically converted into defensive substance at the site of the attack.

Effect of the tested compounds on the activity of peroxidase, polyphenoloxidase and chitinase enzymes:

The increase in the activity of peroxidase, polyphenoloxidase and chitinase enzymes within sugar beet leaves as a result of treatments application was found to be the highest in comparison with the control (Table, 4). "Sulplex" and compost tea recorded the highest peroxidase activity, being 0.65 and 0.63, respectively at 30 days after inoculation. The highest polyphenoloxidase activity was recorded in plants treated with "Sulplex" and compost tea (0.27 and 0.25, respectively). In the case of chitinase activity, plants treated with "Sulplex" and compost tea recorded the highest values (0.59 and 0.56, respectively). Humic acid and opera were the least effective treatments in stimulating the activities of these three enzymes. Many researchers have reported the oxidative roles of enzymes during plant infection with fungal pathogens.

Table 4. Effect of the tested substances on enzymatic activity in the leaves of sugar beet infected with powdery mildew under greenhouse conditions

Treatment	Days	*Peroxidase activity in leaves aged (days)	*Polyphenoloxidase activity in leaves aged (days)	**Chitinase activity in leaves aged (days)
Compost tea	15	0.55	0.19	0.54
	30	0.65	0.25	0.56
	45	0.57	0.22	0.55
	Mean	0.59	0.22	0.55
Sulplex	15	0.58	0.18	0.38
	30	0.63	0.23	0.58
	45	0.61	0.27	0.59
	Mean	0.61	0.22	0.52
Humic acid	15	0.55	0.12	0.38
	30	0.57	0.19	0.40
	45	0.55	0.17	0.41
	Mean	0.56	0.16	0.40
Opera	15	0.50	0.12	0.31
	30	0.57	0.18	0.44
	45	0.55	0.17	0.37
	Mean	0.54	0.16	0.37
Control	15	0.50	0.11	0.23
	30	0.53	0.16	0.27
	45	0.55	0.11	0.26
	Mean	0.53	0.13	0.26

* Activities expressed as change in absorbance/ 5 min./g fresh weight

** Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released / gram fresh weight tissue / 60 minutes.

Peroxidases are oxidoreductive enzymes that have a critical role in several metabolic processes such as oxidation of phenols (Reuveni *et al*. 1991), suberization (Bernards *et al*., 1999) and reinforcement of cell walls in plants (Dean and Kuc, 1987). Ride (1983) reported that an increase in peroxidase activity enhances lignification in response to infection, which may restrict the penetration of the pathogen. Polyphenoloxidase is involved in the formation of melanin compounds in the necrotic tissues (Mayer, 1987). Polyphenoloxidase catalyzes the oxygen-dependent oxidation of o-dihydroxyphenols to o-quinones, which are more toxic to pathogens than the former one. In addition, many studies have shown that PPO is induced in response to mechanical wounding, fungal and bacterial infection and by treatment with signaling molecules such as jasmonic acid /methyl jasmonate (MeJA), systemin and salicylic acid (Constabel *et al*., 2000 and Stewart *et al.*, 2001). These enzymes play important roles in oxidizing phenols to produce quinons (the more fungi toxic compounds) and subsequently control diseases (Bi and Zhang, 1993). According to Hammerschmidt and Kuc (1982), the resistance of plants towards pathogens invasion takes place through lignin production, lignification and enhancing the activity of peroxidase in plants. Moreover, chitinase enzyme plays important role in hydrolyzing the cell wall of the pathogen (Tian *et al*., 2006 & Barilli *et al*., 2010).

Detection of chitinase activity:

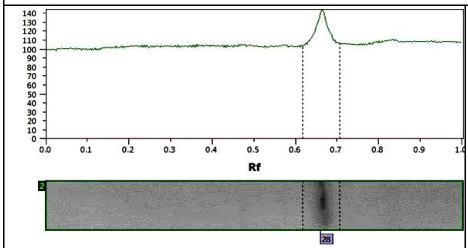
Proteins isolated from sugar beet leaves were separated in a glycol chitin embedded SDS-PAGE and then stained according to Liau and Lin (2008) as shown in Fig (1) to determine chitinase activity. Table 5 shows that the dark zones that can be visualized on the gel at the position of the chitinase activity where glycol chitin was degraded. The samples are loaded in lanes 2.3.4.5.6 while, lane1 is loaded with the protein standard ladder (Thermo Scientific Broad Range Protein Ladder 10-260 kDa). The molecular weight for all samples ranged from 28 to 30kDa.

Table 5.Analysis tools software to Lab Image of SDS-glycol chitin polyacrylamide gel of protein of sugar beet leaves sprayed with the tested substances by using totallab1D Gel & Essential Lab Image (©2015 Totallab all content)

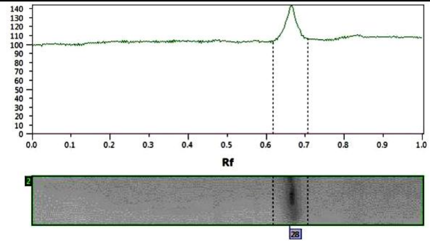
Treatment	Lane	Bands	MW (kDa)	Rf	Peak Height	Bands volume
*Control	2	1	28.000	0.660	140.60	188207.00
Opera	3	1	28.363	0.650	138.48	178806.00
Humic acid	4	1	30.390	0.615	146.98	190604.00
Sulplex.	5	1	30.230	0.617	240.48	274805.00
Compost-tea	6	1	29.856	0.624	248.31	292196.00

* Protein of sugar beet leaves sprayed with tap water as a control treatment.

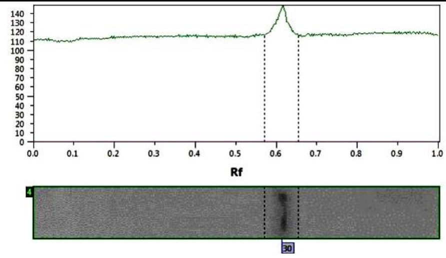
A dark zone at MW of about 30 kDa was observed in lane 6 as a high intensity band expressing the highest chitinase activity in compost-tea treatment (the highest peak and bands volume) followed by "Sulplex." treatment which was observed in lane 5. Control treatment, Opera fungicide and Humic acid ones were observed in lanes 2, 3 and 4 showed the least activity of chitinase enzyme (weak bands with lower peak heights and bands volume). Many of studies have described the use of compost extracts in controlling leaf diseases. In these studies, compost extracts have been used successfully to control leaf diseases caused by *Botrytis cinerea* and *Venturia inaequalis*, powdery and downy mildews, sometimes with effectiveness similar to conventional fungicides (Hoitink *et al*., 1997 and Litterick *et al*., 2004). Other also reported increases in activities of peroxidase, polyphenoloxidase and phenylalanine ammonia- lyase in okra plants (Siddiqui *et al*., 2009) as well as increases in peroxidase, β-1,3-glucanase and chitinase in tomato and onion plants (Haggag and Saber, 2007). On the other hand, Sulphated canola oil increased the activities of chitinase, peroxidase and polyphenoloxidase enzymes as a safe chemical for improving the nutritional status and inducing phytoresistance of common beans grown in soil infected with *Sclerotium rolfsii* (Eid and Abbas, 2013).



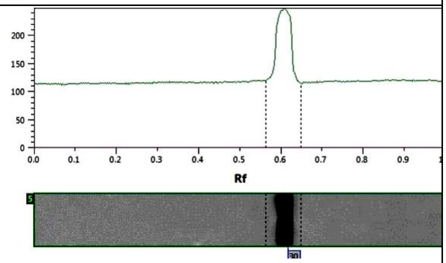
(Lane 2) protein of sugar beet leaves sprayed with tap water



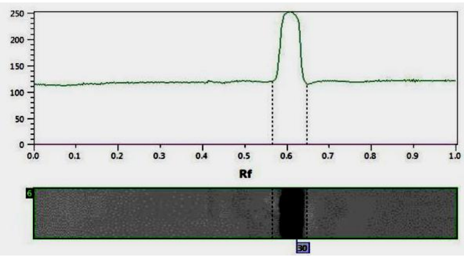
(Lane 3) protein of sugar beet leaves sprayed with opera fungicide



(Lane 4) protein of sugar beet leaves sprayed with humic acid

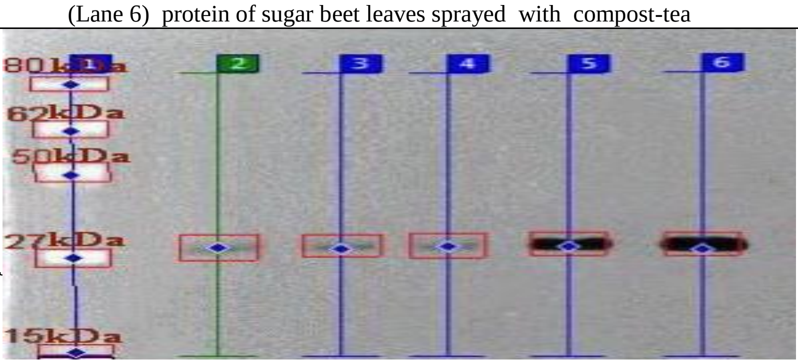


(Lane 5) protein of sugar beet leaves sprayed with sulphated canola oil "Sulplex."



(Lane 6) protein of sugar beet leaves sprayed with compost-tea

Fig. 1.



Lab Image of SDS-glycol chitin polyacrylamide gel of protein of sugar beet leaves sprayed with the tested compounds by using totallab1D Gel & Essential Lab Image (©2015 Totallab all content) to detect chitinase activity by using SDS-glycol chitin polyacrylamide gel electrophoresis.

Field experiments:

Significant variations in disease severity and yield per feddan were recorded for the used treatments (Table 6). Combined data over two seasons showed that the opera fungicide was the most effective treatment in controlling powdery mildew, (4.62 % disease severity and 87.96 % efficacy), followed by "Sulplex" and compost tea with disease severity 9.83% and 13.78% & efficacy 74.37% and 64.05%, respectively.

Table 6. Effect of the tested compounds on the management of sugar beet powdery mildew and the produced root yield, experimental field at Gemmeiza, in two successive growing seasons

Treatment	% Disease severity during			% Efficacy	Yield ton / feddan During:			% Yield increase
	2014/ 2015	2015/ 2016	Mean		2014/ 2015	2015/ 2016	Mean	
Compost tea	10.74 c	16.82 c	13.78	64.05	21.32 ab	21.38 ab	21.35	28.07
Sulplex	8.15 d	11.50 d	9.83	74.37	23.60 a	23.10 a	23.35	40.07
Humic acid	20.74 b	25.46b	23.10	39.73	18.62 bc	18.93 bc	18.78	12.64
Opera	3.94 e	5.29 e	4.62	87.96	23.82 a	22.35 ab	23.08	38.48
Control	7.89 a	38.77 a	38.33	0.00	17.24 c	16.09 c	16.67	0.00

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

Humic acid was the least effective treatment in this regard (23.10 % disease severity and 39.73 % efficacy). All treatments showed enhancements in yield per feddan over the respective untreated control. "Sulplex" and Opera fungicide recorded the highest root yield per feddan (23.35 and 23.08, respectively) and yield increase (40.07% and 38.48%, respectively), followed by compost tea. These results are in agreement with those obtained by Pasini *et al*. (1997) who recorded that treating rose with canola oil reduced significantly plant infection with powdery mildew and such an effect seemed to be equal to that of conventional chemical fungicide. Also, Azam *et al*. (1998) found that using canola oil at a rate of 0.5% controlled successfully grapevine powdery mildew under shade house conditions without causing plant damage. In case of compost tea, its application before pathogen infection is necessary to optimal control of plant pathogens through multiple modes of activities, *e.g.* induced resistance, antibiosis and competition (Deepthi and Reddy, 2013). The rate of application of 1:5 compost to water was found effective in reducing the incidence of tomato early blight caused by *Alternaria solani* and increased the yield of tomato (Tsror and Bieche, 1999). A mixture of azoxystrobin and pyraclostrobin with either osterol demethylatio –inhibiting fungicides (DMIs), difenoconazole or cyproconazole provided a better control efficiency of sugar beet powdery mildew compared to the single application each mixture partner (Karaoglanidis and Karadimos, 2006). Results concerning the total soluble solids (T.S.S.) and sucrose in beet roots, in addition to purity percentage, are presented in Table 7. These contents were significantly affected by these investigated chemical treatments.

Table 7. 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
Compost tea	25.73a	24.00a	24.87	22.61a	21.69a	22.15	87.89a	90.37a	89.13
Sulplex	24.60ab	23.53ab	24.07	21.24ab	20.82a	21.03	86.37a	88.57a	87.47
Humic acid	22.40bc	22.07bc	22.23	19.15b	18.72b	18.94	85.92a	84.90a	85.41
Opera	23.40ab	21.43c	22.42	19.64b	17.92b	18.78	83.60ab	84.67a	84.14
Control	20.76c	19.44d	20.10	16.20c	15.95c	16.07	75.19b	4.76b	74.98

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

In this concern, compost tea and "Sulplex" recorded significantly the highest increases in sugar beet contents of both T.S.S and sucrose percentage followed by opera fungicide. On the other hand, humic acid recorded the least significant effects on both T.S.S and sucrose percentages in sugar beet. Compost tea and "Sulplex" treatments recorded the highest increase in the percentage of purity in sugar beet among the investigated treatments while humic acid treatment recorded the least increases in purity percentage and, such increase seemed to be insignificantly higher than the control. These results are in agreement with those reported by Francis *et al*. (2007); El-Fahar and Abou El-Magd (2008) and Kontradowitz and Verreet (2010), who reported that powdery mildew of sugar beet decreased root yield and yield quality.

References

Alexopoulos, C.J.; Mims, C.W. and Blackwell, M. 1996. Introductory Mycology. Wiley. pp. 218–223.

Allam, A.I. and Hollis, J.P. 1972. Sulfide inhibition of oxidase in rice roots. *Phytopathology*, **62**: 634-636.

Arnow, L.E. 1937. Colorimetric determination of the component of 2, 4-dihydroxyphenyl alanine- tyrosine mixtures. *J. Boil. Chem.,* **118**: 531-537.

Azam, M.G.N.; Gurr, G.M. and Magarey, P.A. 1998. Efficacy of compound based on canola oil as a fungicide for control of grapevine powdery mildew caused by *Uncinula necator*. *Aust. Plant Pathol.,* **27**: 116 -118.

Barilli, E.; Prats, E. and Rubiales, D. 2010. Benzothiadiazole and baba improve resistance to *Uromyces pisi* (Pers) Wint. In *Pisum sativum* L. with an enhancement of enzymatic activities and total phenolic content. *Eur. J. Plant Pathol.,* **128**: 483-493.

Bernards, M.A.; Fleming, W.D.; Liewellyn, D.B.; Priefer, R.; Yang, X.; Sabatino, A. and Plourde, G.L. 1999. Biochemical characterization of the suberization associated anionic peroxidase of potato. *Plant Physiol.,* **12**: 135-145.

Bi, Y. and Zhang, W.Y. 1993. On changes respiratory rate, ethylene evolution and peroxidase activity of the infected melon. *Acta Phytopathol. Sinica,* **23**(1): 69-73.

Boller, T. and Mauch, F. 1988. Colorimetroric assay for chitinase. *Methods Enzymol.,* **161**: 430-435.

Bray, H.G. and Thorpe, W.V. 1954. Analysis of phenolics compounds of interest in metabolism. *Meth. Biochem. Anal.,* **1:** 27-52.

Constabel, C.P.; Yip, L.; Patton, J.J. and Christopher, M.E., 2000. Polyphenoloxidase from hybrid poplar, cloning and expression in response to wounding and herbivory. *Plant Physiol.,* **124**: 285-295.

Dean, R.A. and Kuc, J. 1987. Rapid lignification in response to wounding and infection as a mechanism for induced systemic protection in cucumber. *Physiol. Plant Pathol.,* **24**: 33-42.

Deepthi, K.P. and Reddy, P. N. 2013. Compost teas- an organic source for crop disease management. *Int. J. Biol. Res.,* **2**: 51-60.

Derbalah, A.S.; El-Mahrouk, M. S. and El-Sayed, A.B. 2011. Efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani*. *Plant Pathol. J.,* **10** (3): 115-121.

Duncan, D.B. 1955. Multiple ranges and multiple F. test. *Biometrics,* II: 1-42.

Eid, Kh. E. and Abbas, M.H.H. 2013. Safety chemicals as tools for improving the nutritional status and inducing phytoresistance of common beans grown in soil infested with *Sclerotium rolfsii*. *J. Appl. Sci. Res.,* **9**(8): 5283-5293.

El-Fahar, Samia, A. and Abou El- Magd, B.M. 2008. Effect of infected sugar plants with powdery mildew disease on invertase activity and certain quality characteristic. *J. Agri. Res. Kafr El- Sheikh Univ.,* **34**(4): 940-949.

Eweis, M.; El-Kholy, S.S. and El- Sabee, M.Z. 2006. Antifungal efficacy of chitosan and its thiourea derivatives upon the growth of some sugar beet pathogens. *Inter. J. Boil. Macromal*., **38**: 1-8.

Francis, S.A., Brett, R.C.; Michael, A.J.; John, W. and Michael A.C. 2007. Comparison of ITS sequences from UK and North American sugar-beet powdery mildews and the designation of Erysiphe betae. *Mycol. Res.*, III: 204-212.

Haggag, Wafaa, M. and Saber, M.S.M. 2007. Suppression of early blight on tomato and purple blight on onion by foliar sprays of aerated and non-aerated compost teas. *J. Food Agric. Environ.*, **5**: 302-309.

Hammerschmidt, R. and Kuc, J. 1982. Lignifications as mechanisms for induced systemic resistance in cucumber. *Physiol. Plant Pathol.,* **20**: 61.

Hoitink H.A.J.; Stone A.G. and Han D.Y. 1997. Suppression of plant diseases by composts. *Hort. Sci.,* **32**: 184-187.

Ishii, H.; Fraaije, B.A. and Sugiyama, T. 2001. Occurrence and molecular characterization of strobinlurin resistance in cucumber powdery mildew and downy mildew. *Phytopathology*, **91**: 1166–1171.

Karaoglanidis, G.S. and Karadimos, D.A. 2006. Efficacy of strobilurins and mixtures with DMI fungicides in controlling powdery mildew in field grown sugar beet. *Crop protec.*, **25**: 977-983.

Kaur J. and Arora D. 1999. Antimicrobial activities of species. *Int. J. Antimicrob. Agents,* **12**: 257–262

Kontradowitz, L. and Verreet, J.A. 2010. Assessment of resistance and virulence in the pathosystem sugar beet (*Beta vulgaris*) / powdery mildew (*Erysiphe betae*)-development of basics for an effective powdery mildew resistance breeding. *J. Plant Dis. Protec.*, **117**(2): 49-54.

Lewellen, R.T. and Schrandt J.K., 2001. Inheritance of powdery mildew resistance in sugar beet derived from *Beta vulgaris* sub sp. maritima. *Plant Dis.,* **85**: 627-631.

Liau, C.Y. and Lin, C.S. 2008. A modified Coomassie Brilliant Blue G 250 staining method for the detection of chitinase activity and molecular weight after polyacrylamide gel electrophoresis. *J. Biosci. Bioeng.,* **106**: 111–113.

Litterick, A.M.; Harrier, L.; Wallace, P.; Watson, C.A. and Wood, M. 2004. The role of un-composted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production–a review. *Critical Rev. Plant Sci.*, **23**(6): 453-479.

Matern, U., and Kneusal, R.E. 1988. Phenolic compounds in disease resistance. *Phytopathology,* **78**:153-170.

Matta, A. and Dimond, A.E. 1963. Symptoms of fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. *Phytopathology*, **53**: 574-578.

Mayer, M.A. 1987. Polyphenoloxidases in plants- recent progress, *Phytochemistry*, **26**(1): 11-20.

Pasini, C.; D'Aquila, F.; Curir, P. and Gullino, M. L. 1997. Alternative strategies to control rose powdery mildew. Bulletin. OIL./SROP, **20**:84-87.

Passarinho, P., and de-Vries, S.C. 2002. Arabidopsis chitinase: A genomic survey. In The Arabidopsis Book, C.R. Somerville and E.M. Meyerowitz, (Eds) (Rockville, MD: American Society of Plant Biologists), doi/10.1199/tab.002, http://www.aspb.org/publications/arabidopsis.

Pohoreski, A. 2004. Sulphur-containing oils for controlling plant pathogens and stimulating nutrient uptake. Patent WO 2005112648 A8.

Reuveni, M. and Reuveni, R. 1995. Efficacy of foliar sprays of phosphates in controlling powdery mildews in field grown nectarine, mango trees and grapevines. *Crop Protec.*, **14**: 311–314.

Reuveni, R.; Shimoni, M. and Crute, I.R. 1991. An association between high peroxidase activity in lettuce (*Lactuca sativa*) and field resistance to downy mildew (*Bremia lactucae*). *J. Phytopathology*, **132**(4): 312-318.

Ride, J.P. 1983. Cell walls and other structural barriers in defense In: J.A. Callow, (Ed) Biochemical Plant Pathology. Wiley- Inter Science, New York, pp. 215-236.

Scheuerell S.J. and Mahaffee W.F. 2002. Compost tea: principles and prospects for plant disease control. *Compost Sci. Utilization*, **10**: 313-338.

Shibuya, N., and Minami, E. 2001. Oligosaccharide signalling for defence responses in plant. *Physiol. Mol. Plant Pathol.*, **59**:223–233.

Siddiqui Y., Meon S., Ismail R. and Rahmani M. 2009. Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. *Biol. Cont.*, **49**: 38-44.

Sierotzki, H.; Wullschleger, J. and Gisi, U. 2000. Point mutation in cytochrome gene conferring resistance to strolilurin fungicides in *Erysiphe graminis f.sp. tritici* field isolates. *Pestic. Biochemis. Phys.*, **68**, 107–112.

Steel, P.G.D. and Torrie, J.H. 1960. Principle and Procedures of Statistics McMraw Hill Book CO. Inc., New York, 481p.

Stewart, R.J.; Sawyer, B.J.B.; Bucheli, C.S. and Robinson, S.P., 2001. Polyphenoloxidase is induced by chilling and wounding in pineapple. *Aust. J. Plant Physiol.*, **28**: 181-191.

Tian, S.; Wan, Y.; Qin, G. and Xu, Y. 2006. Induction of defense responses against Alternaria rot by different elicitors in harvested pear fruit. *Appl. Microbiol. Biotechnol.*, **70**: 729-734.

Tsror, L. and Bieche, P.J. 1999. Biological control of early blight tomatoes. *Acta Hort.*, **487**: 271-273.

Tuzun, S.; Rao, M.N.; Vogeli, U.; Schardl, C.L. and Ku, J.A. 1989. Induced systemic resistance to blue mold: early induction and accumulation of *ß*-1,3-gluconases, Chitinase and otherpathogenisis-related proteins (b-proteins) in immunized tobacco. *Phytopathology,* **79**: 979-983.

Vidhyasekaran, P. 1973. Possible role of ortho-dihydroxy phenolics in grapevine anthracnose disease resistance. *Indian J. Exp. Boil.,* **13**: 473-475.

Whitney, E.D.; Lewellen, R.T. and Skoyen, I.O. 1983. Reaction of sugar beet to powdery mildew: genetic variation association among testing procedures and resistance breeding. *Phytopathology*, **73**: 183-185.

Williams, J.S. and Cooper, R.M. 2004. The oldest fungicide and newest phytoalexin - a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathol.,* **53**: 263-279.

Zinati, M. Gladis, A. 2005. Compost in the 20^{th} Century: A tool to control plant diseases in nursery and vegetable crops. *Hort. Technol.,* **15**: 61-66.

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دور بعض المركبات غير التقليدية في مقاومة مرض البياض الدقيقي في بنجر السكر

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تم تقييم فاعلية رش محاليل بعض المركبات غير التقليدية مثل كمبوست الشاي وزيت الكانولا الكبريتي وحمض الهيوميك كبدائل للمبيدات الفطرية لمقاومة مرض البياض الدقيقي في بنجر السكر صنف "هركل" المتسبب عن الفطر *Erysiphe betae,* بالمقارنة بالمبيد الفطري أوبرا تحت ظروف الصوبة والحقل بمحطة بحوث الجميزة محافظة الغربية مركز البحوث الزراعية خلال موسمي الزراعة ٢٠١٤ /٢٠١٥ و ٢٠١٥/٢٠١٦. تحت ظروف الصوبة أظهرت النتائج أن رش النباتات بالمعاملات السابقة خفضت شدة الاصابة بمرض البياض الدقيقي بالمقارنة برش الأوراق بالماء والمادة اللاصقة فقط كمعاملة مقارنة. الرش بالمبيد الفطري أوبرا كان أكثر المعاملات خفضاً لشدة الاصابة ثم زيت الكانولا الكبريتي، وكمبوست الشاي. وقد أظهرت كل المعاملات زيادة معنوية في محتوى الاوراق من الفينولات الكلية والارثوفينول عند تقديرها علي فترات ١٥، ٣٠، ٤٥ يوم من العدوى الصناعية بالمسبب المرضي بالمقارنة بالنباتات المعاملة بالماء والمادة اللاصقة فقط كمعاملة مقارنة. كذلك أظهرت كل المعاملات زيادة في النشاط الانزيمي لانزيمات البيروكسيديز والبولي فينول أكسيديز والشيتينيز عند تقديرها علي فترات ١٥، ٣٠، ٤٥ يوم من العدوي الصناعية بالمسبب المرضي بالمقارنة بالنباتات المعاملة بالماء والمادة اللاصقة فقط كمعاملة مقارنة. وباستخدام تقنية الفصل الكهربي في بيئة البولي أكريلاميد جل لدراسة نشاط المشابه الانزيمي لانزيم الشيتينيز أظهرت المعاملات حزم مختلفة الكثافة ذات وزن جزئي يترواح بين ٢٨ و ٣٠ كيلو دالتون. أظهرت معاملات رش الاوراق بكمبوست الشاي حزمة ذات كثافة عالية وبالتالي نشاط عالي للمشابه الانزيمي لانزيم الشيتينيز ثم معاملتي زيت الكانولا الكبريتي أعطت حزمة ذات كثافة متوسطة وبالتالي نشاط متوسطة للمشابه الانزيمي لانزيم الشيتينيز بينما أعطت معاملات رش الاوراق بحمض الهيوميك أو بالماء والمادة اللاصقة فقط كمعاملة مقارنة أو بالمبيد حزمة ذات كثافة منخفضة وبالتالي نشاط منخفض للمشابه الانزيمي لانزيم الشيتينيز. تحت ظروف الحقل أوضحت النتائج أن المعاملة بالمبيد الفطري أوبرا كان أفضل المعاملات رشاً علي الاوراق في مقاومة مرض البياض الدقيقي في بنجر السكر يليه معاملات زيت الكانولا الكبريتي وكمبوست الشاي بينما المعاملة بحمض الهيوميك كانت الأقل في هذا الصدد. وزادت جميع المعاملات من محصول الجذور للفدان بالمقارنة بمعاملة رش الاوراق بالماء والمادة اللاصقة فقط كمعاملة مقارنة .أعطى المبيد الفطري أوبرا وزيت الكانولا الكبريتي أعلى محصول جذور للفدان يليه كمبوست الشاي. وعلاوة على ذلك أعطى كمبوست الشاي وزيت الكانولا الكبريتي أعلى النسب المئوية من المواد الصلبة القابلة للذوبان (T.S.S)، والنسب المئوية للسكروز في جذور البنجر والنقاء.