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Impact of some Treatments on Sugarbeet Powdery Mildew

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Abstract

Effect of two types of vegetable oils (neem and soybean), two types of plant extracts (bougainvillea blossoms and Garlic), KH_2PO_4 and salicylic acid (SA) as inducers and Eminent fungicide on sugarbeet powdery mildew disease, were tested to study their effect *in vitro* and *in vivo* against *Erysiphe betae* the causal pathogen of Sugarbeet powdery mildew. The reduction of spore germination was increased with concentration increasing in vitro. Most effective concentration was studied on powdery mildew disease of three varieties of sugar beet in greenhouse, all treatments had a significant effect in reducing disease severity compared to control Untreated plants, bougainvillea blossoms extract and Eminent fungicide were the most effective treatments. Enzymes, phenols, sugars, proteins and chlorophyll contents of leaves were evaluated. Bougainvillea blossoms extract exhibited high contents of sugars, phenols, protein, pigments and antioxidant enzymes.

Keywords: *Fungicide, Soy bean Oil, neem oil, plant extracts, KH_2PO_4, Salycilic acid (SA), Sugar beet, sugar beet powdery mildew, chemical composition, antioxidant enzymes.*

Introduction

Sugar beet (*Beta vulgaris* L.) is an important crop for its high content of sucrose after sugarcane. Under field conditions, several pathogenic fungi attack growing sugar beet plants causing serious diseases (El-Kholi *et al*., 1994 and El-Kholi, 2000). Powdery mildew (*Erysiphe betae*) is the most serious and destructive foliar disease in many sugar beet growing regions of the world, causing sugar yield losses of up to 30% (Weltzien and Ahrens, 1977 and Ruppel, 1995). Application of fungicides is still the effective method for controlling these diseases. The wide spread use of the chemical fungicides has become a subject of research concern due to their harmful effect on non-target organisms as well as their possible carcinogenicity. Some reports, which have done, on field isolates of *E. betae* indicated that some of these isolates were fungicide resistant's (Fernández-Aparicio *et al*., 2009).

Recently, plant extract, and vegetable oils, such as neem (*Azadirachta indica*), garlic (*Allium sativum*), have been used to control powdery mildew fungi (Singh *et al*., 1991; Daayf *et al*., 1995; Abd-El-Sayed, 2000 and Tohamy *et al*., 2002). Also, it has been reported that some phosphate salts induce systemic resistance against various pathogens including powdery mildew of cucumber (Reuveni *et al*., 1993 and Reuveni *et al*., 1995). Salicylic acid was used by many researchers to study their effect as chemical agent for induce plants resistance against many pathogens (Zeier, *et al* 2004).

The objective of this work was to evaluate the efficacy of some oils, chemical inducers and plant extracts to control powdery mildew disease on sugar beet, compare with commercial eminent fungicide.

Material and Methods

The tested fungicide used in this study was Eminent 12.5 % EW (Tetraconazole). This fungicide was applied at rate of 1ml/l, 0.5ml/l and 0.25 ml/l water. Soyabean and Neem oils were obtained from El Captain Company for Extracting Natural Oils, Plants & Cosmetics, Cairo, Egypt and applied at concentration of 4%, 2% and 1%. Salicylic acid (SA) ($C_7H_6O_3$) is a monohydroxybenzoic acid, a type of phenolic acid and a beta hydroxy acid) was applied at concentration of 100ppm, 50ppm and 25ppm and potassium phosphate mono basic salt (KH_2PO_4) manufactured by El Nasr Pharmaceutical chemicals co. Abu Zaabal, Egypt, and was applied at concentration of 100mM, 75m and M50mM. Bougainvillea blossoms extract, garlic extract.

Garlic extraction:

Garlic (*Allium sativum*) bulb, 100 g of plant material were cut, placed in a blender in sterilized distilled water at the ratio of 1:1 w/v and blended for 10 minutes. The plant material residues were filtered through cheesecloth. The filtrate was centrifuged at 3000 rpm for 10 minutes and separated to obtain the extract (Abd-El-Sayed, 2000). Garlic extract was used at concentration 20, 10 and 5%.

Bougainvillea blossoms aqueous Extraction

Crushed blossoms 50 g were dipped in 500 ml of the distilled water for 48 hours at room temperature in a conical flask and shaken periodically. The extract filtered and filtrates evaporated under reduced pressure on rotary evaborator to obtain the crude extract (Dhankhar *et. al.*, 2013). Bougainvillea extract was used at concentration 5, 2.5 and 1.25%.

Effect of different treatments on germination of *Erysiphe betae* conidia in laboratory.

According to the methods described by Nair et *al*. (1962). Conidia of *E. betae* were harvested from only young leaves of Sugar beet to avoid the presence of old conidia, lesions were gently shaken first by a glass rod to discard any old conidia presented on such young leaves. The new conidia, which formed on conidiophores after four to six hours, were spread on dry clean glass slides previously received 0.1 ml. From each one of the previously prepared individual treatments at concentrations which included in Table (1). However glass slides prepared with sterilized distilled water were served as a control treatment. These conidia were examined microscopically to determine the number of spores that had germinated. Each slide was placed on a U-shaped glass rod in a moist chamber made up of sterile Petri dish lined with filter paper saturated with sterile distilled water. Petri dishes were incubated at 25 ± 1.5^0C (Awad *et al*., 1990) for 24 hours before examination. Three slides were used as replicates for each particular treatment. The percentage of germination was based on counts of 300 conidia.

% Spore germination=

$$\frac{\textbf{No. of germinated spores}}{\textbf{Total number of spores}} \times 100$$

The percentage of treatment efficiency in the reduction of conidia germination was calculated using the following equation:

% Treatment efficiency =

$$\frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Greenhouse experiments:

Seeds of three varieties of Sugar beet (Baraca, Euklid and Clavius) were obtained from the Agriculture Research Center, Giza, Egypt, and then cultivated in pots (30cm). After 12 weeks from cultivated, plants were sprayed with salicylic acid at the concentration of 100ppm, potassium phosphate salt (KH_2PO_4) at the concentration of 100mM, oils (Neem and Soybean) at the concentration of 4%, Bougainvillea blossoms extract, garlic extract and Eminent fungicide at the concentration of 1ml/l. All treatments were applied three times in 15 day intervals between each spray. Percentage of disease severity was assessed after 12 days from each spraying.

Assessment of disease

Powdery mildew scale from 0 to 5 according to Paulus *et al*., (1982) was used to assessment the disease, where R0 = no powdery mildew colony, R1 = 10%, R2 = 35%, R3 = 65%, R4 = 90 and R5 = 100% colonies/leaf.

Disease severity (%) =

$$\frac{[\sum \text{(rating no.)} \times \text{(no. leaves in rating category)} \times (100)]}{\text{(Total no. leaves)} \times \text{(highest rating value)}}$$

The percentage of treatment efficiency in the reduction of powdery mildew severity was calculated using last equation.

Challenge inoculation

Inoculation was accomplished by shaking powdery mildew heavily diseased Sugar beet plants over the treated plants at a height of about 30cm. Inoculated plants were kept in greenhouse until disease assessment was undertaken. Inoculation was done 2 days after foliar application with treatments (Strobel and Kuc, 1995).

Biochemical changes:

Healthy and infected leaves of different varieties were taken and different biochemical changes were assayed as follows:-

Reducing and total soluble sugars were determined calorimetrically by using potassium ferricyanide solution according to Schales and Schales (1945). The reducing sugers and total soluble sugars were calculated as mg glucose /100g f.wt. the non reducing sugars calculated from difference between total soluble sugars and reducing sugars. Phenolic compounds were colorimetrically assayed by Folin-Ciocalteu solution as described by Shahidi and Naczk, (1995).The phenolic compounds concentration was expressed as mg gallic acid / 100 g f.wt. Chlorophyll a, b and carotenoids were extracted and estimated according to Lichtenthaler and Wellburn (1985) as follows:- 0.5g/25ml fresh leaves were homogenized with acetone 100% then filtered and wash the residue with acetone until filtrate become colorless the absorbance was determined using spectrophotometer at 662 ,645 nm for chl a and chl b respectively and 470 nm for carotenoids. The results were calculated as mg/g fresh weight.

Enzymes assay

Tissue were homogenized with potassium phosphate buffer (100 mM, pH=7.0) containing 0.1 mM EDTA and 1 % polyvinyl pyrrolidone

(PVP) (W/V) at 4ºC. Homogenate was centrifuged at 15000 ×g for 15 min at 4º C. Supernatant was considered as enzyme crude extract and used to measure the activities of guaiacol peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL).POD activity (E.C 1.11.1.7) measured from absorbance changes at 470 nm per min using spectrophotometer according to Hammer Schmidt *et al*. (1982). Unit of enzyme (IU) equal 0.01 ΔOD. min-1.at 470 nm. PPO activity (EC 1.14.18.1) was measured according to Benjamin and Montegomery (1973). One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per min at 420 nm. PAL activity (EC 4.3.1.5) was quantified by the method of Beaudoin-Eagan and Thorpe (1985). One unit of enzyme activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 per hour at 290 nm using spectrophotometer. The enzymes activities were expressed as unit/mg protein. Soluble protein concentration was estimated to calculate specific activity of enzymes. Proteins concentration was quantified in the crude extract by the method of Bradford (1976) using bovine serum albumin as a standard. All chemical determinations were carried out on UV-Vis spectrophotometer UV 9100 B, Lab-Tech.

Statistical analysis

All data were subjected to one way analysis of variance (ANOVA) followed by means separation through least significant difference (L.S.D.) test at $P < 0.05$ level (Snedecor and Cochran, 1980).

Results and Discussion

Laboratory test:

Effect of the tested treatments on germination of *Erysiphe betae* conidia "*in vitro*":

The data in Table (1) reveal that all tested plant extracts significantly reduced the percentage of conidia germination compared with the control. The high reduction was achieved by Bougainvillea extract at concentration 5g/l (97.6 % efficiency) and Emenint fungicide at concentration 1ml/ l (97.6 % efficiency) followed by Neem oil 4% (95% efficiency) and garlic extract at concentration 20% (93.9 % efficiency) compared to control treatment (0.0). Generally the reduction in spores germination was significantly increased by increasing concentration of all tested treatments. This results are in agreement with several researches on effect of plant extracts and plant oils on spore germination which were found to be effective in controlling obligate diseases including powdery mildew all over the world (Achimu & Schlosser, 1992; Rovesti *et al*., 1992; Singh *et al*., 1992; Ahmed, 1995; El-Naggar, 1997; Amadioha, 1998; Abdel-Megid *et al*., 2001; Sallam Minaas, 2001; Haroun, 2002 and Nada, 2002). The effect of plant extracts might be mainly due to the inhibitory effects of the antifungal compounds in the extracts on germination of the fungal spores, since some of these extracts in preventive treatment completely prevented infection (Daayf *et al*. 1995, El-Naggar, 1997; Singh *et al*. 1999 and Haroun, 2002). Singh and Singh (1983) tested the efficacy of various plant extracts and oils against *E. polygoniand* reported that the conidial germination was considerably reduced in all the extracts and oils at 100 ppm.

Greenhouse experiments:

Data presented in Table (2) indicated that all tested treatments significantly reduced the percentage of powdery mildew incidence and severity compared with the control treatment. These results in agreement with Nada (2002) reported that spraying squash plants in greenhouse and field with eight essential oils as preventative and curative treatments gave sufficient control to disease in most cases. Thyme essential oil completely prevented the disease incidence in the field. Ko *et al.* (2003) found that soya bean oil was greatly reduced the severity of tomato powdery mildew caused by *Oidium neolycopersici*. Treatments with Baraca variety, i.e., in Eminent was higher significant followed by Bougainvillea extract, Soybean oil, Neem oil, garlic extract (0.00, 2.47, 10.53, 15.86 and 16.92 %) respectively compared to control (73.17%) so the most efficiency treatments were Eminent followed by Bougainvillea extract, Soybean oil, Neem oil, garlic extract (100.00, 96.62, 85.61,78.32 and 76.88%) respectively. Disease severity in the Euklid variety was decreased significantly and the best treatment was Eminent followed by Bougainvillea extract, KH_2PO_4, Soybean oil and Salicylic acid (SA) (0.00,0.60, 6.53, 9.41 and 14.32%) respectively compared to control (69.76%) and the efficiency was (100.00, 99.14, 90.64, 86.51 and 79.47%) respectively.

Table 1. Effect of different treatments on germination of *Erysiphe betae* conidia "*in vitro*"

Treatment	Conc.	% Germination	% Efficiency	Conc.	% Germination	% Efficiency
Neem oil	4%	4.3^{HI}	95.0	KH_2PO_4	100mM	57.3^{BC}
	2%	7.3^{GHI}	91.7		75mM	39.0^{E}
	1%	$15F^{GH}$	83.0		50mM	41.7^{DE}
Soybean oil	4%	6.3^{GHI}	92.8	garlic extract	20%	5.3^{GHI}
	2%	9.3^{GHI}	89.4		10%	7.7^{GHI}
	1%	16.3^{FG}	81.5		5%	9.3^{GHI}
Salicylic acid (SA)	100ppm	40.3^{E}	54.5	Bougainvillea extract	5%	2.0^{I}
	50ppm	52.3^{CD}	41.0		2.5%	8.3^{GHI}
	25ppm	66.3^{B}	25.2		1.25%	21.0^{F}
Control (water)	-	88.67^{A}	0.0	Emenint	1m/L.	2.0^{I}
L.S.D.		11.4			0.5m/L.	6.3^{GHI}
					0.25m/L.	13.3^{FGHI}

Different letters refer to significant difference ($P \leq 0.05$).

On the other hand, disease severity in Clavius variety was higher in treatments with Eminent and Bougainvillea extract followed by KH_2PO_4, Garlic extract and Salicylic acid (SA) (0.00,0.00,12.91, 17.71 and 18.01%) respectively compared to control (98.07%) by the same way efficiency was higher in the same treatment which was (100.00, 100.00, 86.84, 81.94 and 81.64%) respectively. This results agreement with Tohamy, *et al.* (2002) who using neem extracts on cucumber plant reduced the powdery and downy mildews disease severity compared with control. Rettinassababady, *et al* (2000) found that neem seed kernel extract (5%) was most efficient in suppressing the disease with increased yields.

Table 2. Effect of two oils, two inducers, two plant extracts and Eminent fungicide on disease severity of powdery mildew on three varieties of Sugarbeet plants and efficiency of the treatments; after three times of spraying.

Treatment \ Varieties	Baraca		Euklid		Clavius	
	%Disease Severity	% Efficiency	%Disease Severity	% Efficiency	%Disease Severity	% Efficiency
Neem oil	15.86^{C} ±0.85	78.32	23.71^{B} ±0.82	66.01	28.73^{B} ±0.94	70.70
Soybean oil	10.53^{D} ±0.80	85.61	9.41^{D} ±0.81	86.51	24.60^{C} ±0.81	74.92
Salicylic acid (SA)	23.62^{B} ±0.82	67.72	14.32^{C} ±0.82	79.47	18.01^{D} ±0.82	81.64
KH_2PO_4	25.34^{B} ±0.82	65.37	6.53^{E} ±0.82	90.64	12.91^{E} ±0.81	86.84
garlic extract	16.92^{C} ±1.25	76.88	21.72^{B} ±1.25	68.86	17.71^{D} ±0.82	81.94
Bougainvillea extract	2.47^{E} ±0.04	96.62	0.60^{F} ±0.08	99.14	0.00^{F} ±0.00	100.00
Eminent	0.00^{E} ±0.00	100.00	0.00^{F} ±0.00	100.00	0.00^{F} ±0.00	100.00
Control	73.17^{A} ±0.82	0.00	69.76^{A} ±0.82	0.00	98.07^{A} ±0.81	0.00
L.S.D.	**0.961914**		**0.958940**		**0.889263**	

Data presented as the means of three replicates ± SD. Different letters refer to significant difference (P≤ 0.05).

The inhibitory effect may be attributed to the formation of a physical barrier, which prevent fungal penetration and reduced disease incidence, also, it formed a continuous membrane that permits diffusion of oxygen and carbon dioxide but inhibits the passage of water and promotes a healthy physiological state of the plant (Han, 1990). Rettinassababady *et al*. (2000) found that significant reduction in blackgram powdery mildew incidence due to foliar spray of neem oil (3%) might be due to the presence of sulphur containing compounds viz., nimbidin and azadirachtin.

Jedperales, and Leysa, (2012) found that the phytochemical screening revealed the presence of alkaloids, saponins, tannins, reducing sugar and cardiac glycosides in the leaves of *Bougainvillea glabra*. Different parts of Bougainvillea glabra have antibacterial potential when used on two gram-positive bacteria, except for flowers against two gram-positive bacteria. Bougainvillea glabra also had an interesting source of antimicrobial agents.

Biochemical changes:

Oxidative enzymes activity:

Data presented in Table (3) revealed that the effect of treatments on Polyphenol oxidase (PPO), Peroxidase (POD) and phenylalanine ammonia lyase (PAL) activities in three varieties of Sugarbeet plants after three times of spraying, all treatments resulted in significant increase in enzymes activity in all varieties compared to control. This increase in enzymes activity are in agreement with those reported by Reuveni *et al.* (1997); Abd-El-Kareem (1998) and El-Habbak, (2003).

Many plant enzymes are involved in defense reaction against plant pathogen. These included oxidative enzymes such as peroxidase and polyphenoloxidase which catalyses the formation of lignin and other oxidative phenols that contribute to formation of defense barriers for reinforcing the cell structure (Avdiushko *et al*., 1993). Enzyme activity played an important role in plant disease resistance through increasing plant defense mechanisms that are considered the main tool of varietal resistance (Takuo *et al*., 1993).

PPO activity in Baraca variety exhibited the highest value with Bougainvillea extract (16632.7) followed by Neem oil (13892.9) and then garlic extract (12584.8). Also in Euklid variety garlic extract gave the highest activity (19582.82) followed by Bougainvillea extract (17830.39). While Clavius had the lowest PPO activity where neem extract gave the highest value (7403.97 followed by Bougainvillea extract (6902.81) in comparing with control (3078.33, 1864.46 and 3121.23) respectively.

POD enzyme had the highest value in treatment with Bougainvillea extract (514.72) followed by garlic extract (177.83) in Baraca variety, while KH_2PO_4 (239.12) followed by Bougainvillea extract (203.36) in Euklid variety, but Salicylic acid (SA) (685.96) followed by soy bean oil (366.28) in Clavius variety compared to control (76.95, 21.76 and 71.59) respectively. Gao and Zhang (2013) note that the resistance of pear to *P. piricola* could be greatly increased by the treatment of Salicylic acid (SA). Alkahtani *et al.* (2011) reported that defense system increase in PAL activity is application of SA probably enhanced the induced closely associated with biosynthesis of active resistance against powdery mildew in cucumber metabolites, such as phytoalexins, phenols, lignins.

Table 3. Effect of two oils, two inducers, two plant extracts and Eminent fungicide on Polyphenoloxidase, Peroxidase and phenylalanine ammonia lyase activities in three varieties of Sugarbeet plants after three times of spraying.

Varieties / Treatment	Baraca			Euklid			Clavius		
	PPO	**POD**	**PAL**	**PPO**	**POD**	**PAL**	**PPO**	**POD**	**PAL**
Neem oil	13892.9^{B} ±1.63	151.85^{D} ±0.82	74.41^{F} ±0.82	6092.27^{D} ±1.63	129.47^{F} ±0.82	66.14^{B} ±1.63	7403.97^{A} ±1.64	293.53^{C} ±1.63	102.42^{C} ±0.82
Soybean oil	10892.3^{D} ±0.82	110.56^{E} ±0.82	53.20^{G} ±0.82	6485.26^{C} ±1.63	182.92^{D} ±1.63	75.95^{A} ±0.82	6005.23^{F} ±2.45	366.28^{B} ±1.63	105.46^{B} ±0.82
Salicylic acid (SA)	10728.8^{E} ±1.64	110.34^{E} ±0.82	79.10^{E} ±0.82	5331.33^{E} ±0.81	163.75^{E} ±1.63	65.16^{B} ±0.82	6432.16^{E} ±1.63	685.96^{A} ±1.63	49.05^{F} ±0.82
KH_2PO_4	10352F ±1.64	161.28^{C} ±0.81	131.23^{A} ±0.82	5153.76^{F} ±1.64	239.12^{A} ±0.82	44.79^{E} ±0.82	6513.45^{C} ±1.62	182.62^{F} ±1.63	110.36^{A} ±0.82
garlic extract	12584.8^{C} ±1.62	177.83^{B} ±0.81	83.00^{D} ±0.47	19582.82^{A} ±1.64	195.02^{C} ±0.82	60.24^{C} ±0.82	6472.73^{D} ±0.82	221.72^{E} ±1.25	74.11^{D} ±0.82
Bougainvillea extract	16632.7^{A} ±1.63	514.72^{A} ±0.82	104.36^{B} ±0.82	17830.39^{B} ±0.82	203.36^{B} ±1.63	53.27^{D} ±1.63	6902.81^{B} ±1.67	229.961^{D} ±0.82	102.55^{C} ±0.82
Eminent	2564.08^{H} ±1.63	86.57^{F} ±0.81	89.42^{C} ±1.63	3059.16^{G} ±0.82	80.56^{G} ±0.81	40.88^{E} ±0.81	5703.84^{G} ±1.59	71.27^{G} ±0.81	58.73^{E} ±0.82
Control	3078.33^{G} ±1.64	76.95G ±1.25	52.29^{G} ±1.63	1864.46^{H} ±3.26	21.76^{H} ±0.82	20.19F ±1.64	3121.23^{H} ±0.81	71.59^{G} ±0.82	18.54^{G} ±0.82
L.S.D.	**5.3776**	**3.0501**	**3.651**	**5.9094**	**4.1168**	**4.1215**	**5.5643**	**4.6157**	**2.8268**

Data presented as the means of three replicates ± SD. Different letters refer to significant difference ($P \leq 0.05$).

Peroxidase activity was related to resistance and tolerance against powdery and downy mildew. PODs have been supposed to be mainly present in cell wall and reported to catalyze the oxidation of various organic compounds like phenolics, lignin or suberin resulting in reinforcement of host plant cell walls against pathogenic agents (Wang, *et al* 2004).

Induced resistance in cucumber plants with K_2HPO_4 increased the activity of peroxidase and chitinase enzymes (Irving and Kuc 1990). Also, increased accumulation of peroxidase in phosphate treated leaves is strongly indicate the possible role or peroxidase in defense mechanism (Gamil 1995; Reuveni *et al.,* 1995 and Abd-El-Sayed, 2000). Orober *et al.* (1998) recorded that the foliar application of phosphate induced systemic acquired resistance in cucumber against powdery mildew (*Sphaerotheca fuliginea*). As a further consequence of phosphate application, activities of typical defense-related enzymes like peroxidase and polyphenoloxidase increased in all parts of the induced plants.

Induced resistance in cucumber plants with acetylsalicylic acid (aspirin) led to an increase activity of peroxidase polyphenol-oxidase and phenylalanine ammonia lyase (Schneider and Ullrich, 1994).

On the other hand, PAL enzyme was elevated in Baraca variety treated with KH_2PO_4 (131.23) followed by Bougainvillea extract (104.36), while soy bean oil (75.95) followed by Neem (66.14) in Euklid variety, but KH_2PO_4 (110.36) followed by soy bean oil (105.46) Clavius variety compared to control (52.29, 20.19 and 18.54) respectively.

Plants treated with Salicylic acid (SA) evolved enzymatic and non-enzymatic protection mechanisms that could efficiently scavenge excess ROS. SOD and POD are important enzymes in such action. SOD is able to protect cells from oxidant stress, which constitutes the first line of defenses against ROS (Alscher, 2002), also Li, and Steffens, 2002 and Chen, *et al* 2010 demonstrated that the activity of PAL and PPO activity in the SA-treated leaves was relatively higher than that in control leaves.

Gao and Zhang (2013) demonstrate that SA could induce the systemic acquired resistance and strengthen antiviral capability of pear against *P. piricola*.

Chemical constituents:

Reducing, non reducing and total soluble sugars.

The present data in Table (4) show that all tested treatments resulted in higher contents of non reducing, reducing and total soluble sugars than control.

In Baraca variety treated by Bougainvillea extract gave the highest contents of Reducing, non reducing and total soluble sugars followed by neem oil. While Euklid variety had the highest non reducing and total sugars in plant treated with Bougainvillea extract and soybean oil followed by neem oil, but Clavius variety had the highest non reducing and total sugars contents by soy bean oil followed by KH_2PO_4. On the other hand, reducing sugars in Euklid variety scored the highest value in soybean oil treatment followed by Bougainvillea extract. Clavius variety gave the highest reducing sugars content in neem oil treatment followed by garlic extract.

Resistance to powdery mildew was positively correlated with a low sugar content in the leaves of resistant cultivars (Helal *et al.*, 1978; El-Shanawani *et al*. 1990 and Mohamed, 1994), while high sugar content in the susceptible cultivars was reported by (Omar (1977), Helal *et al.* (1978) and Farahat (1980). Growth of powdery mildews is favored by a high carbohydrate level of their hosts (Yarwood, 1978). Similar results were obtained by (Awad, 2000) who reported that sugars tended to increase the susceptibility of detached leaves to fungal parasites by providing an extra source of energy for the invader.

Protein and Phenolic compounds:

Data in Table (5) show that all treatments resulted in high significant variation in contents of protein and phenols in the three varieties compared to control, Bougainvillea extract was the best treatments which gave the highest protein content (0.0151, 0.0152 and 0.0152) compared to control (0.0115, 0.0125 and 0.0111) in Baraca, Euklid and Clavius varieties respectively, and highest phenolic compounds content (1.161, 0.572 and 0.7127) compared to control (0.292, 0.4323 and 0.0813) in the same varieties.

This increase in the total phenol levels gave surely an increase in the capability of plants to defense against disease infection process and disease development, The increase in total phenol contents was in agreement with those reported by Daayf *et al.*, (1995 and 1997) and Nada (2002).

Mostafa (1986) showed that powdery mildew host resistance was due to phenolic compounds with markedly fungistatic properties presented before infection.

Since the role of secondary metabolic substances, such as phenolic compounds, on disease resistance mechanisms are well known (Kalaichelvan and Nagarajan, 1992).

Table 4. Effect of two oils, two inducers, two plant extracts and Eminent fungicide on Non reducing (N. R. S.), reducing (R. S.) and total soluble (T.S.S.) sugars in three varieties of Sugarbeet plants after three times of spraying.

Varieties / Treatment	Baraca			Euklid			Clavius		
	N. R. S.	R. S.	T.S.S.	N. R. S.	R. S.	T.S.S.	N. R. S.	R. S.	T.S.S.
Neem oil	14.36^{B} ±0.02	14.36^{B} ±0.01	28.72^{B} ±0.01	11.32^{B} ±0.07	14.78^{D} ±0.01	26.10^{B} ±0.08	4.79^{E} ±0.07	18.66^{A} ±0.04	23.45^{C} ±0.06
Soybean oil	4.36^{F} ±0.02	9.56^{C} ±0.02	13.92^{F} ±0.01	9.58^{C} ±0.01	19.14^{A} ±0.02	28.72^{A} ±0.02	37.86^{A} ±0.05	12.60^{C} ±0.03	50.47^{A} ±0.02
Salicylic acid (SA)	3.91^{G} ±0.01	15.22^{A} ±0.01	19.13^{D} ±0.02	2.19^{G} ±0.01	14.35^{E} ±0.01	16.54^{F} ±0.01	8.42^{D} ±0.45	8.67^{E} ±0.12	17.09^{E} ±0.42
KH_2PO_4	10.41^{D} ±0.12	8.69^{E} ±0.04	19.10^{D} ±0.01	3.49^{F} ±0.01	15.65^{C} ±0.01	19.14^{D} ±0.016	15.26^{B} ±0.06	9.13^{D} ±0.02	24.40^{B} ±0.06
garlic extract	13.46^{C} ±0.01	9.14^{D} ±0.01	22.60^{C} ±0.01	4.78^{E} ±0.01	14.35^{E} ±0.02	19.13^{D} ±0.01	4.80^{E} ±0.02	14.32^{B} ±0.05	19.12^{D} ±0.03
Bougainvillea extract	16.95^{A} ±0.01	15.23^{A} ±0.01	32.18^{A} ±0.03	12.19^{A} ±0.02	16.53^{B} ±0.03	28.72^{A} ±0.01	10.72^{C} ±0.09	12.65^{C} ±0.04	23.37^{C} ±0.08
Eminent	6.06^{E} ±0.01	8.71^{E} ±0.01	14.78^{E} ±0.01	7.84^{D} ±0.01	13.91^{F} ±0.01	21.75^{C} ±0.01	10.44^{C} ±0.01	8.67^{E} ±0.05	19.11^{D} ±0.04
Control	3.52^{H} ±0.01	9.55^{C} ±0.01	13.07^{G} ±0.02	4.78^{E} ±0.08	12.62^{G} ±0.01	17.40^{E} ±0.08	2.10^{F} ±0.04	7.46^{F} ±0.02	9.56^{F} ±0.02
L.S.D.	0.1541	2.07163	3.80789	0.1413	1.08032	5.11126	0.5766	8.4951	0.000019

Data presented as the means of three replicates ± SD. Different letters refer to significant difference ($P \leq 0.05$).

Moreover, the toxic phenolic compounds in plant cells acting through the structure of bond form with cell wall components of plant tissues (Mahadevan and Sridhar, 1986), enhance host resistant by stimulating host defense mechanisms (Subba Rao *et al.*, 1988), prevent the extent of fungal growth in plant tissues and penetrate the microorganisms and cause considerable damage to the cell metabolisms (Kalaichelvan and Elangovan, 1995).

Table 5. Effect of two oils, two inducers, two plant extracts and Eminent fungicide on protein and phenolic content in three varieties of Sugarbeet plants after three times of spraying.

Varieties / Treatment	Baraca		Euklid		Clavius	
	Protein	Phenolic	Protein	Phenolic	Protein	Phenolic
Neem oil	0.0129^{C} ±0.0002	0.673^{B} ±0.0008	0.012^{D} ±0.0008	0.41^{F} ±0.0081	0.0115^{F} ±0.00008	0.1917^{F} ±0.0004
Soybean oil	0.0138^{B} ±0.0001	0.472^{F} ±0.0008	0.0135^{BC} ±0.00005	0.491^{C} ±0.0008	0.0143^{C} ±0.00008	0.341^{E} ±0.0008
Salicylic acid (SA)	0.014^{B} ±0.00008	0.662^{C} ±0.0008	0.0132^{BC} ±0.00008	0.472^{D} ±0.0008	0.0121^{E} ±0.00008	0.351^{D} ±0.0008
KH_2PO_4	0.0128^{C} ±0.0001	0.4823^{E} ±0.001	0.0142^{BA} ±0.00006	0.462^{D} ±0.0008	0.0147^{B} ±0.0001	0.441^{C} ±0.0008
garlic extract	0.0122^{D} ±0.0001	0.532^{D} ±0.0008	0.0132^{BC} ±0.00008	0.541^{B} ±0.0008	0.0113^{GF} ±0.00008	0.4613^{B} ±0.0012
Bougainvillea extract	0.0152^{A} ±0.00008	1.161^{A} ±0.002	0.0151^{A} ±0.00004	0.572^{A} ±0.0008	0.0152^{A} ±0.00008	0.7127^{A} ±0.0012
Eminent	0.0116^{E} ±0.00008	0.293G ±0.0006	0.0142^{BA} ±0.00003	0.432^{E} ±0.0008	0.0132^{D} ±0.0001	0.351^{D} ±0.0008
Control	0.0115^{E} ±0.00008	0.292^{G} ±0.0008	0.0125^{DC} ±0.00008	0.4323^{E} ±0.0066	0.0111^{G} ±0.00008	0.0813^{G} ±0.0012
L.S.D.	0.0004	0.0035	0.001	0.0131	0.0004	0.0034

Data presented as the means of three replicates ± SD. Different letters refer to significant difference ($P \leq 0.05$).

Photosynthetic pigments:

Data in Table (6) demonstrated that all treatments gave significant increased in photosynthetic pigments in the three varieties but the most significant treatments were Bougainvillea extract and Eminent fungicide which were the best.

Ahmed (1995) reported that garlic was the most effective in inhibiting disease infection of *S. fuliginea*. The reduction of disease intensity may be due to the increment in chlorophyll content in cucumber plants, which was reduced as a result to powdery mildew infection.

Conclusion

The uses of vegetable oils (neem and soybean), two types of plant extracts (bougainvillea blossoms and Garlic), KH_2PO_4 and salicylic acid (SA) as inducers and Eminent fungicide induced resistance in sugarbeet plant against powdery mildew disease, bougainvillea blossoms extract was the best treatment in disease controlling, preventation the disease incidence, high content of sugars, phenols, protein, pigments and antioxidant enzymes.

Table 6. Effect of two oils, two inducers, two plant extracts and Eminent fungicide on photosynthetic pigments (chlorophyll (A), chlorophyll (B) and carotenoids) in three varieties of Sugarbeet plants after three times of spraying.

Varieties / Treatment	Baraca			Euklid			Clavius		
	Chl(A)	Chl (B)	Caroti	Chl(A)	Chl (B)	Caroti	Chl(A)	Chl (B)	Caroti
Neem oil	8.32^{C} ±0.09	3.33^{D} ±0.09	6.68^{C} ±0.12	8.76^{C} ±0.09	1.46^{F} ±0.04	5.67^{E} ±0.12	6.54^{E} ±0.06	2.14^{D} ±0.08	5.71^{E} ±0.12
Soybean oil	3.72^{G} ±0.09	3.39^{C} ±0.06	4.40^{G} ±0.09	4.80^{F} ±0.12	2.61^{E} ±0.08	6.46^{C} ±0.06	5.15^{G} ±0.12	1.47^{F} ±0.05	5.73^{F} ±0.08
Salicylic acid (SA)	7.10^{D} ±0.05	1.28^{G} ±0.13	6.03^{E} ±0.01	6.37^{E} ±0.14	2.45^{D} ±0.12	5.58^{F} ±0.17	8.17^{D} ±0.06	0.44^{G} ±0.13	6.86^{C} ±0.09
KH_2PO_4	6.27^{E} ±0.09	3.20^{E} ±0.04	5.60^{F} ±0.07	6.76^{D} ±0.09	2.89^{C} ±0.07	6.34^{D} ±0.17	8.26^{C} ±0.08	3.51^{C} ±0.08	5.46^{G} ±0.07
Garlic extract	5.73^{F} ±0.14	1.82^{F} ±0.08	6.21^{D} ±0.04	4.32^{G} ±0.08	1.26^{G} ±0.05	4.41^{G} ±0.12	5.18^{F} ±0.07	1.69^{E} ±0.12	6.17^{D} ±0.05
Bougainvillea extract	10.85^{A} ±0.09	5.15^{A} ±0.05	9.71^{A} ±0.14	9.06^{A} ±0.05	5.31^{A} ±0.09	7.22^{A} ±0.05	10.52^{B} ±0.12	4.82^{B} ±0.06	7.11^{B} ±0.06
Eminent	9.18^{B} ±0.04	3.52^{B} ±0.09	8.29^{B} ±0.09	8.71^{B} ±0.20	4.64^{B} ±0.16	6.90^{B} ±0.04	11.13^{A} ±0.08	5.24^{A} ±0.07	7.28^{A} ±0.08
Control	3.39^{H} ±0.12	1.33^{H} ±0.09	2.94^{H} ±0.07	3.15^{H} ±0.05	0.73^{H} ±0.01	3.59^{H} ±0.12	4.75^{H} ±0.08	0.19^{H} ±0.01	3.28^{H} ±0.08
L.S.D.	0.3346	0.2957	0.3047	0.4026	0.3168	0.4104	0.3139	0.3194	0.2868

Data presented as the means of three replicates ± SD. Different letters refer to significant difference (P≤ 0.05).

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تأثير بعض المعاملات على البياض الدقيقى فى بنجر السكر

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الملخص

تم إستخدام نوعين من الزيوت النباتية (النيم و فول الصويا) ونوعين من المستخلصات النباتية (أزهار الجهنمية والثوم) واستخدم فوسفات احادى البوتاسيوم وسليسليك آسيد كمستحثات للمقاومة بالاضافة إلى استخدام مبيد فطرى إيمينتت كمقارنة و ذلك لمكافحة مرض البياض الدقيقى على بنجر السكر، استخدمت المعاملات فى ثلاث تركيزات لدراسة تأثيرها على انبات جراثيم الفطر فى المعمل هناك علاقة عكسية بين انبات الجراثيم وتركيز المعاملة. فكلما زاد التركيز انخفض انبات الجراثيم.

تم دراسة تأثير التركيز الأكثر فاعلية من جميع المعاملات على مكافحة مرض البياض الدقيقى فى الصوبة على ثلاثة اصناف من البنجر فأظهرت المعاملات تأثير معنوى فى خفض شدة الإصابة بالمقارنة بالنباتات الغير معاملة و اظهرت المعاملة بمستخلص الجهنمية و المبيد إيمننت أعلى كفاءة فى خفض شدة الاصابة.

كذلك تم دراسة تأثير المعاملات على نشاط انزيم البيروكسيديز والبولى فينول اوكسيديز والفينيل الانين امونياليز، وعلى محتوى الفينولات، السكريات، البروتينات ومحتوى الاوراق من الكلوروفيل فلوحظ زيادة فى نشاط الانزيمات وكذلك زيادة بمحتوى الفينولات والسكريات والبروتينات فى المعاملات موضع الدراسة و خاصة مستخلص الجهنمية.