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Use of α -Lactalbumin, β -Lactoglobulin and Lactoferrin for Controlling Cucumber Powdery Mildew Disease under Plastic House Conditions

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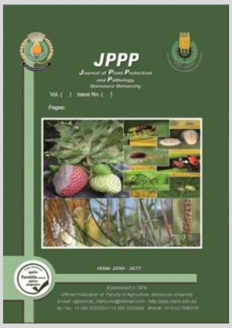

ABSTRACT

The effect of native cow's whey and whey proteins α-lactalbumin, β-lactoglobulin, and lactoferrin was studied under the plastic house conditions for controlling the cucumber powdery mildew disease caused by *Sphaerotheca fuliginea* (Schlecht.) Pollaci, prophylactically and curatively sprays application during 2019 and 2020 growing seasons with improvement the total cucumber product (yield/plant). Disease severity percentages, area under disease progressive curve (AUDPC), final disease severity, efficacy percentage, microscopic observations, growth parameters, yield components and enzyme activities were investigated. Obtained results of prophylactically application indicated that the disease severity was decreased significantly ($P \leq 0.05$) by the application of whey 40% dilution which scored 10 and 6%, followed by a mixture of β-lactoglobulin + α-lactalbumin + lactoferrin (10.4 and 6.4%), and lactoferrin (20.3 and 16.4%), compared to Topas 100 (9.3 and 4.3%) and control treatment (62.3 and 59.1%) during 2019 and 2020 growing seasons respectively. Furthermore, disease severity percentages were higher through curatively application than prophylactically. Consequently, the final disease severity, AUDPC values were decreased, while efficacy percentage, growth parameters, yield components and enzyme activities were increased during the experiment period with the selected treatments either under prophylactically or curatively application during the two successive growing seasons. Photography of light and scanning electron microscopy showed that the selected treatments affected the fungal hyphae, conidiophores and conidia at 24 hr after the application of selected treatments.

Keywords: Powdery mildew, Cucumber, β-lactoglobulin, α-lactalbumin, Lactoferrin.

INTRODUCTION

Cucurbits grown worldwide under open field, plastic house and greenhouse conditions are seriously damaged by the powdery mildew disease (on leaf, stem and fruit) caused by *Sphaerotheca fuliginea* (Schlecht.) Pollaci. Usage of chemical fungicides and repeating the application times are considered the most effective strategies for controlling the causal fungus under commercial production of cucurbits. That can led for three of serious problems as follows, inconvenience habitat for beneficial microorganisms, resistance against chemical active ingredients and environmental pollution (Jänsch *et al*., 2006). For solving the pervious problems, alternative ways to chemical fungicides could be a better choice for controlling the powdery mildew disease of cucurbits. Bettiol *et al*., (1999) obtained a good results in the controlling of *S. fuliginea* which cause a zucchini squash powdery mildew disease when used an environment-friendly product such as row cow milk. Obtained results with milk, surely related with its content such as vitamins, enzymes, oil, fatty acids, lactose, casein, lactoferrins, minerals and salts. Medeiros *et al*. (2012) suggested that, milk and whey could be used as alternatives to chemical fungicides, because they are less expensive and give a protection level closed to those obtained by synthetic fungicides. Promising results were also obtained when whey of row cow milk was used against zucchini squash and cucumber powdery mildews (Bettiol *et al*., 2008). Known whey proteins such as lactoferrin (LA), α-lactalbumin (ALA) and β-lactoglobulin (BLG), lactoperoxidase, glycomacropeptides and immunoglobulins are considered as antiviral, antioxidants and antimicrobial agents. Commercial production of lactoferrin mainly comes from milk of several mammals such as cows (García-Montoya *et al*., 2012). Generally, lactoferrin consists of two-lobe with four domain polypeptide and the peptide linker between two-lobe is the one feature that distinguishes lactoferrin from transferrins (Lambert *et al*., 2005). Lactoferrin is considered as a multifunctional protein involved and takes part in a broad spectrum of important physiological processes, including iron absorption regulation, exhibition of antioxidants and antimicrobial properties (Baker, 1994; Sato *et al*., 1996).

Previous findings of Wang *et al*. (2012) established that the effect of esterified lactoferrin (ELF) and lactoferrin on blue mold caused by *Penicillium expansum* in apple fruit might be associated with the direct fungi-toxic property against the pathogen and the elicitation of defense-related enzymes in fruit. Besides lactoferrin, the most abundant constituents of whey are α-lactalbumin and β-lactoglobulin. Milk or milk components have been recently used for controlling some of plant viruses (Liakot *et al*., 2001) and studies of Abdelbacki *et al*., (2010) demonstrated that esterified whey proteins such as α-

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lactalbumin, β-lactoglobulin, and lactoferrin were effective for inhibition the tomato yellow leaf curl virus on tomato plants through the antiviral activities. The previous studies done by Sudisha *et al*., (2011) established that the application of milk against downy mildew disease caused by *Sclerospora graminicola* in pearl millet induced the systemic responses toward the pathogen. Raymond and Ferguson (2014) observed that fermented milk by-products prevented the buildup of powdery mildew pathogen (*Podosphaera xanthii*) of cucumber and zucchini squash when applied directly to the plants. Recent studies achieved by Kamel and Afifi (2020) indicated that cow's milk and whey dilutions (40 and 50% dilution in water) effectively reduced the powdery mildew signs and symptoms caused by *S. fuliginea* on infected cucumber plants with the four sprays treatment which was the most effective treatment.

Depending on the preliminary results obtained by Kamel *et al*., (2017) that disease severity percentage of cucumber powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht.) Pollaci, significantly exhibited by the effective cow's milk and whey 50 and 40% dilution, the work objective was designed to investigate which whey protein (α-lactalbumin, β-lactoglobulin, and lactoferrin) accounts for the highest management level of the same disease.

MATERIALS AND METHODS

Plant materials

Seeds of cucumber (*Cucumis sativus* L.) Barracuda F1 (Seminis Company, batch number 0161531598) were provided by the Horticultural Research Institute, Agricultural Research Center. Cucumber seeds were sown into plastic trays 84 wells (5 × 5 × 7 cm/well) filled with autoclaved commercial peat-based substrate (Peat moss and vermiculite 1:3 W/W). Barracuda seedlings 30-days old were transplanted into a plastic house located in Kafr El-Sheikh governorate (Sakha), Egypt, at the rate of 10 plants/5 m in a row.

Whey preparation and whey proteins

For obtaining the whey, the fresh cow milk heated in water bath at 72 ºC for 20 sec and immediately transferred to another water bath heated at 42 ºC for 1 hr. After that, rennin enzyme was added in ratio 1ml/kg milk and incubated immediately at 42 ºC for 3 hr to make the gelatinous materials of the curd. Consequently, gelatinous materials of the curd were filtered through cheese cloth to obtain the whey. Lactoferrin (95% protein) was obtained from Armor Proteins Company (Saint-Brice en Cogles, France), while β-lactoglobulin (97.8% protein) and α-lactalbumin (97.46% protein) were obtained from Davisco Food International, USA.

Pots trial

Plastic pots 30 cm diameter were disinfected using sodium hypochlorite solution (5%) for 30 min and later rinsed with sterile distilled water. Plastic pots were filled with autoclaved (121 ºC for 2 hr.) sandy loam soil mixture 1:1 (5 kg/pot). For each pot, two cucumber Barracuda seedlings (30 days old) were transplanted under controlled greenhouse (greenhouses of Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University) conditions (temperature 28 ºC, relative humidity 84±2% and 14 hr day light).

For an inoculation process, the first five true expanded cucumber Barracuda leaves were gently sprayed with water (to make a water film). Then, detached cucumber leaves fully covered with powdery mildew lesions (obtained from plastic house at Sakha, Kafr El-Sheikh, Egypt) were used for dusting the conidia to the new seedling leaves.

Barracuda seedlings were treated prophylactically (24 hr before inoculation process) and curatively (10 days after inoculation process) by the following treatments:

1. Seedlings treated with lactoferrin (100mg/l)
2. Seedlings treated with α-lactalbumin (100mg/l)
3. Seedlings treated with β-lactoglobulin (100mg/l)
4. Seedlings treated with mixture of lactoferrin (100mg/l), α-lactalbumin (100mg/l) and β-lactoglobulin (100mg/l)
5. Seedlings treated with whey at dilution of 40%
6. Seedlings treated with the fungicide Topas 100 EC (Penconazole 10.2% W/W) at the rate of 0.25 ml/l,
7. Seedlings treated with water as control treatment.

The concentration of 100 mg/l of each substance was chosen as the most effective one against the disease compared to 50, 200 and 300 mg/l (data not shown). Tween-20 (V/V) 0.2% was added to each treatment as a surfactant. Complete randomized design with three replicates for each treatment (each replicate consists of 6 Barracuda seedlings) was used in this experiment. Disease severity percentage was recorded at zero time, 7, 14, 21 and 28 days after treatment application process.

Plastic house experiment

Cucumber Barracuda seedlings 30 days old were transplanted (10 plants/5 m in a row) into a plastic house size 9 × 40 m located at Sakha, Kafr El-Sheikh governorate, Egypt. Barracuda seedlings were treated prophylactically (24 hr before inoculation process) and curatively (10 days after inoculation process) as mentioned above in the section of pots trial. The experiment was conducted during 2019 and 2020 using a complete randomized block design. Plastic house temperature was recorded during these two seasons (25±3 and 24±2 °C) and relative humidity (85±2 and 80±2 %), respectively. Fertilization, irrigation and weed management were applied according to the recommendations of Ministry of Agriculture for commercial cucumber production. For evaluation the effect of treatments on disease development, the disease severity, final disease severity, efficacy % and area under disease progress curve (AUDPC) were estimated.

Disease assessment

During the experiment period, disease severity % was recorded at zero time, 7, 14, 21 and 28 days. The percentage of cucumber leaf surface covered with powdery mildew lesions (disease severity) was estimated five times in each replicate. Based on the increasing of disease severity, 10 plants/replicate/treatment were scored. Disease severity percentage was scored based on the scale of 0 to 6 described by Yan *et al*., (2006). A zero to 6 disease scale was designed to describe the disease symptom development, where 0 = no symptoms; 1 = very small lesions with <1% leaf area covered with mycelium; 2 = 1-5% leaf area covered with mycelium; 3 = 6-20% leaf area covered with mycelium; 4 = 21-40% leaf area covered with mycelium; 5 = 41-60% leaf area covered with mycelium and 6 = >60% leaf area covered with mycelium.

Disease severity was calculated according to the following equation described by Descalzo *et al*., (1990):

$$R = [\sum (a \times b)/N \times K] \times 100$$

Where R = disease severity %, a = number of leaves with symptoms rated, b = numerical value of each grade, N = total number of evaluated plants and K= highest degree of symptoms in the used scale.

Final disease severity was scored at the end of the experiment time, while Efficacy % of the used treatments was calculated according to the following formula:

Efficacy % = [DS% of control treatment – DS% of treatment/ DS% of control treatment] × 100,

where, DS = disease severity.

Disease severity was scored five times with one week interval from the first time of treatment application. Disease severity percentage was used to estimate the area under disease progress curve (AUDPC) based on the equation of Campbell and Madden (1990) as follows:

$$AUDPC = \sum ((Y_i + Y_{i+1})/2\ (t_{i+1} - t_i)$$

Where Y = disease severity, t = time interval sequences and i = cumulative number of evaluations.

Microscopic examination

Samples of naturally infected cucumber leaves with powdery mildew pathogen were examined microscopically (light microscope Leica DM100 and scanning electron microscope) to observe the mode of action of lactoferrin, β-lactoglobulin, α-lactalbumin and whey at 40% dilution on conidiophores and conidia of *S. fuliginea* after 24 hr from the weekly treatment application. Scanning electron microscope (SEM) examination was processed according to methods described by Harley and Ferguson (1990). Cucumber leaves with powdery mildew pathogen were cut into small pieces (approx., 5 mm^2) and fixed immediately in glutaraldehyde 3% with 0.2 M phosphate buffer pH 7.2 for 24 hr at 4 ºC. Then followed by exposure to 1% Osmium tetraoxide (O_SO_4) for 1 hr at 25 ºC. Consequently, samples were passed through ascending concentrations of acetone for dehydration process and then sputter-coated with gold and prepared for observation.

Photographing by light microscope was done at the Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Egypt, while scanning electron microscope (Jeol JSM6510LV) photos were taken in the Central Laboratory, Faculty of Agriculture, Mansoura University, Egypt.

Growth parameters and yield components

For growth parameter measurements, plant height, fresh and dry weight and chlorophyll content were estimated during tow growing seasons. Fresh and dry weight of whole plant were measured by gram unit, while the greenness (SPAD unit) or relative content of chlorophyll was measured using a portable chlorophyll meter (Minolta SPAD-502, Japan) according to the method described by Torres-Netto *et al*., (2005). Plant height was measured from the plant base till the plant top by centimeter unit. Yield components such as number of fruits/plant, weight of fruits/plant (kg), mean weight of fruit (g) and mean product/plastic house (ton) were also estimated.

Enzyme activity assessment

For measurement the enzyme activity, 0.5 g of treated and untreated cucumber fresh leaves were homogenized in 3 ml of 50 mM of TRIS buffer (pH 7.8) containing 1mM EDTA-Na_2 and polyvinylpyrrolidone 7.5%. All homogenates were centrifuged 12000 rpm for 20 min at 4 ºC, then the supernatant was replaced for measuring the total soluble enzyme activity using spectrophotometer (UV-160A, Shimadzu, Japan). Peroxidase activity was measured according to the protocol described by Hammerschmidt *et al*. (1982), while the protocol of Coseteng and Lee (1978) was used for polyphenol oxidase measurement.

Statistical analysis

Analysis of variance (ANOVA) test of CoStat software was used for data processing. Experiments were designed as randomized complete and randomized complete block design. The differences between means were compared using Tukey's HSD at $P \leq 0.05$ (Ott, 1984).

RESULTS AND DISCUSSION

Results

Obtained results indicate that whey proteins (lactoferrin, β-lactoglobulin, α-lactalbumin) and whey at 40% dilution can be applied prophylactically or curatively as eco-friendly alternatives to synthetic fungicides against cucumber powdery mildew pathogen.

Effect of whey and whey proteins through pots trial

Preliminary results presented in Table (1) of pots trial indicate that the disease severity percentage of cucumber powdery mildew at the end of the experiment period was significantly reduced when β-lactoglobulin (10.9%), α-lactalbumin (9.8%), Lactoferrin (7.1%), mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (6.4%) and whey at dilution 40% (6.2) were applied. The most effective treatment was whey at dilution 40% (6.2%), followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (6.4%) with no significant differences between them (Table 1) in comparison to Topas 100 (6%) and control treatment (41.4%).

Table 1. Effect of whey and whey proteins in management of cucumber powdery mildew disease in pots trial

Treatment	Disease severity %				
	Zero time	7 days	14 days	21 days	28 days
Lactoferrin (A)	0	2.0	3.8	5.9	7.1 ^{d}
β-lactoglobulin (B)	0	2.9	4.8	7.7	10.9 ^{b}
α-lactalbumin (C)	0	2.6	4.5	7.3	9.8 ^{c}
Mixture (A + B + C)	0	1.5	2.7	4.1	6.4 ^{e}
Whey 40%	0	1.2	2.4	3.9	6.2 ^{e}
Topas 100 EC	0	1.0	2.5	3.7	6.0 ^{e}
Control	0	8.3	15.7	27.3	41.4 ^{a}
L.S.D $_{at\ 0.05}$	--	--	--	--	0.653

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

Effect of treatments on disease development under plastic house condition

Plastic house experiment was conducted during 2019 and 2020 cucumber growing seasons and divided into 2 parts. The first one was treated prophylactically application (Table 2), while the second was curatively (Table 3). Data summarized in Table (2) of prophylactically application show that the disease severity was exhibited significantly by the whey 40% dilution which scored 10 and 6%, followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (10.4 and 6.4%), and Lactoferrin (20.3 and 16.4%), compared to Topas 100 (9.3 and 4.3%) and control treatment (62.3 and 59.1%) during 2019 and 2020 growing seasons respectively.

On the other hand, disease severity percentages were higher through curatively application (Table 3) than prophylactically. Data presented in Table (3) of curatively application show that the disease severity was exhibited significantly by the whey 40% dilution which scored 30 and 26.1%, followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (30.2 and 26.8%), and Lactoferrin (31.7 and 27.8%), compared to Topas 100 (29.3 and 25.3%) and control treatment (73.7 and 68.4%) during 2019 and 2020 growing seasons respectively.

Consequently, AUDPC values were decreased during the experiment period with all treatments either under prophylactically or curatively application during 2019 and 2020 growing seasons (Fig. 1 and 2).

Data in Figure (1) concerning the year 2019 show that the AUDPC under prophylactically and curatively application were exhibited significantly by the whey 40% dilution which scored 107.8 and 698.95, followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (130.2 and 696.5), and Lactoferrin (365.05 and 708.4), compared to Topas 100 (102.55 and 692.3) and control treatment (992.95 and 1380.75), respectively. Similar trend was obtained during the next year 2020 as summarized in Figure (2), where the AUDPC under prophylactically and curatively application was also exhibited significantly by the whey 40% dilution which scored 126 and 618.45, followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (74.2 and 616), and Lactoferrin (276.5 and 634.9), compared to Topas 100 (48.65 and 611.8) and control treatment (926.45 and 1321.3), respectively.

Table 2. Effect of whey and whey proteins in management of cucumber powdery mildew disease treated prophylactically during 2019 and 2020 growing seasons

Treatment	Disease severity % during 2019				
	Zero time	7 days	14 days	21 days	28 days
Lactoferrin (A)	0	10	14.7	17.3	20.3 [c]
β-lactoglobulin (B)	0	17	23.2	27.3	31.4 [b]
α-lactalbumin (C)	0	15	21.3	24.7	29.3 [b]
Mixture (A + B + C)	0	2	5.2	6.2	10.4 [d]
Whey 40%	0	0	4.3	6.1	10.0 [d]
Topas 100 EC	0	1	3.7	5.3	9.3 [d]
Control	0	25	38.7	47.0	62.3 [a]
L.S.D $_{at\ 0.05}$	--	--	--	--	1.883
Disease severity % during 2020					
Lactoferrin (A)	0	7.2	10.7	13.4	16.4 [d]
β-lactoglobulin (B)	0	13.2	20.4	23.7	27.7 [b]
α-lactalbumin (C)	0	11.3	18.1	20.7	25.1 [c]
Mixture (A + B + C)	0	1.3	2.1	4.0	6.4 [e]
Whey 40%	0	0.0	12	3.0	6.0 [e]
Topas 100 EC	0	1.3	1.4	2.1	4.3 [f]
Control	0	22.7	35.9	44.2	59.1 [a]
L.S.D $_{at\ 0.05}$	--	--	--	--	1.040

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

Table 3. Effect of whey and whey proteins in management of cucumber powdery mildew disease treated curatively during 2019 and 2020 growing seasons

Treatment	Disease severity % during 2019				
	Zero time	7 days	14 days	21 days	28 days
Lactoferrin (A)	14.4	25.5	27.7	29.3	31.7 [cd]
β-lactoglobulin (B)	13.1	28.3	33.2	35.2	38.2 [b]
α-lactalbumin (C)	13.7	27.3	30.5	32.3	34.7 [bc]
Mixture (A + B + C)	14.7	26.3	27.3	28.3	30.2 [cd]
Whey 40%	15.0	26.3	27.7	28.7	30.0 [cd]
Topas 100 EC	15.3	26.6	27.7	28.0	29.3 [d]
Control	15.3	37.3	52.7	64.3	73.7 [a]
L.S.D $_{at\ 0.05}$	--	--	--	--	3.831
Disease severity % during 2020					
Lactoferrin (A)	13.0	21.5	23.7	25.1	27.8 [d]
β-lactoglobulin (B)	12.4	24.3	29.4	31.7	36.2 [b]
α-lactalbumin (C)	12.7	23.3	27.1	28.2	30.9 [c]
Mixture (A + B + C)	13.2	21.3	23.1	23.6	26.8 [de]
Whey 40%	14.0	21.7	23.3	23.3	26.1 [ef]
Topas 100 EC	14.3	21.4	23.2	23.0	25.3 [f]
Control	14.1	34.9	51.9	60.7	68.4 [a]
L.S.D $_{at\ 0.05}$	--	--	--	--	0.764

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

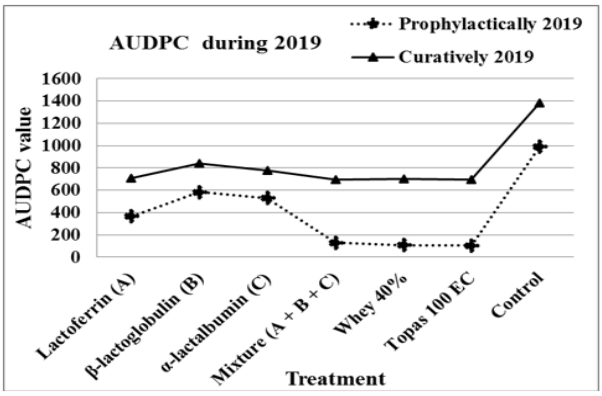

Fig. 1. Effect of used treatments on AUDPC under prophylactically and curatively application during 2019 growing season.

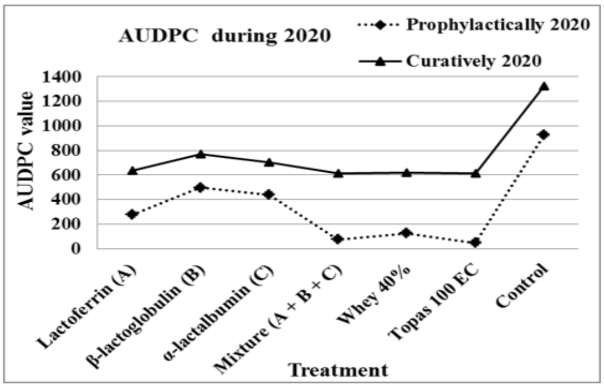

Fig .2. Effect of used treatments on AUDPC under prophylactically and curatively application during 2020 growing season.

Due to the application of selected treatments prophylactically or curatively, the recorded final disease severity and efficacy percentages were affected positively during the 2019 and 2020 growing seasons (Table 4 and 5). During 2019 growing season, the recorded final disease severity percentage was significantly decreased with all treatments when compared with the fungicide and control treatments (Table 4). The most significant effective treatment applied prophylactically and curatively in reducing of disease severity was the diluted whey 40% (10 and 30%), followed by followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (10.4 and 30.2%), and Lactoferrin (20.3 and 31.7%), compared to Topas 100 (9.3 and 29.3%) and control treatment (62.3 and 73.7%), respectively (Table 4). Consequently, the recorded efficacy percentage was significantly high with the diluted whey 40% (83.9 and 59.3%), followed by followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (83.3 and 59%), and Lactoferrin (67.4 and 57%), compared to Topas 100 (85.1 and 60.2%), respectively (Table 4).

Similar trend was obtained during the next season 2020 as shown in Table (5), where the final disease severity was exhibited significantly with increment of efficacy under prophylactically and curatively application by the whey 40% dilution, followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin, and Lactoferrin, compared to Topas 100 and control treatment, respectively (Table 5).

Table 4. Effect of whey and whey proteins in management of cucumber powdery mildew disease treated prophylactically and curatively during 2019 growing season

Treatment	Growing season 2019			
	Final disease severity (%)		Efficacy (%)	
	Prophylactically	Curatively	Prophylactically	Curatively
Lactoferrin (A)	20.3 ^{c}	31.7 ^{cd}	67.4	57.0
β-lactoglobulin (B)	31.4 ^{b}	38.2 ^{b}	49.6	48.1
α-lactalbumin (C)	29.3 ^{b}	34.7 ^{bc}	52.9	52.9
Mixture (A + B + C)	10.4 ^{d}	30.2 ^{cd}	83.3	59.0
Whey 40%	10.0 ^{d}	30.0 ^{cd}	83.9	59.3
Topas 100 EC	9.3 ^{d}	29.3 ^{d}	85.1	60.2
Control	62.3 ^{a}	73.7 ^{a}	--	--
L.S.D $_{at\ 0.05}$	1.883	3.831	--	--

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

Table 5. Effect of whey and whey proteins in management of cucumber powdery mildew disease treated prophylactically and curatively during 2020 growing season

Treatment	Growing season 2020			
	Final disease severity (%)		Efficacy (%)	
	Prophylactically	Curatively	Prophylactically	Curatively
Lactoferrin (A)	16.4 ^{d}	27.8 ^{d}	72.3	59.4
β-lactoglobulin (B)	27.7 ^{b}	36.2 ^{b}	53.1	47.1
α-lactalbumin (C)	25.1 ^{c}	30.9 ^{c}	57.0	54.8
Mixture (A + B + C)	6.4 ^{e}	26.8 ^{de}	89.2	60.8
Whey 40%	6.0 ^{e}	26.1 ^{ef}	89.8	61.8
Topas 100 EC	4.3 ^{f}	25.3 ^{f}	92.7	63.0
Control	59.1 ^{a}	68.4 ^{a}	-	-
L.S.D $_{at\ 0.05}$	1.040	0.764	-	-

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

Microscopic observation

Photography of light and scanning electron microscopy show that the selected treatments affected the fungal hyphae, conidiophores and conidia at 24 hr after treatment application (Fig. 3 and 4). Shrinking, abnormalities, alterations, twisting, plasmolysis and finally collapsing of the fungal hyphae, conidiophores and conidia were observed (Fig. 3 and 4).

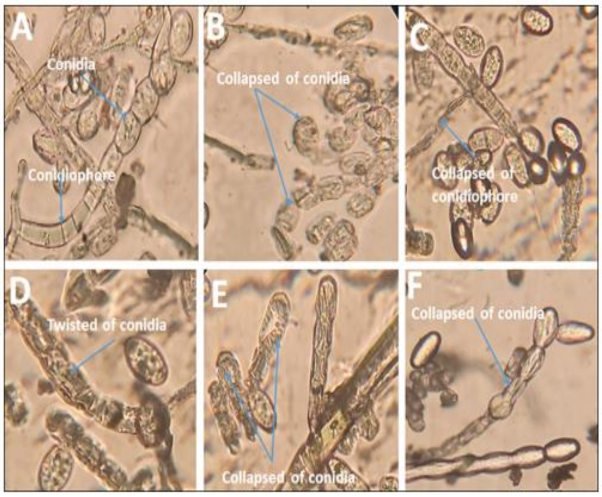

Fig. 3. Light microscopy observations of conidiophores and conidia of *S. fuliginea* collected from cucumber leaf surface sprayed with the selected treatments, where A: Normal conidia and conidiophores (untreated control), B: Lactoferrin treatment, C: α-lactalbumin treatment, D: β-lactoglobulin, E: Mixture (B + C + D) and F: Whey 40%, X 45.

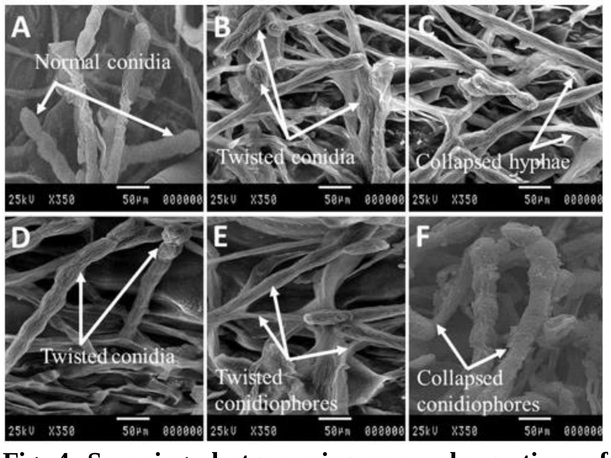

Fig. 4. Scanning electron microscopy observations of hyphae, conidiophores and conidia of *S. fuliginea* collected from cucumber leaf surface sprayed with the selected treatments, where A: Normal conidia (untreated control), B: Lactoferrin treatment, C: α-lactalbumin treatment, D: β-lactoglobulin, E: Mixture (B + C + D) and F: Whey 40%. Scale bar 50µm.

Effect of treatments on growth parameters

Data in Table (6) indicate that, all treatments exhibit a significant effect with all of selected growth parameters compared to the control treatment during the two successive growing seasons 2019 and 2020. The fungicide Topas 100 gave the most effective results on the plant height (255.8 and 251.8 cm), followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (244.6 and 244.8 cm), compared to the control treatment (170.4 and 172.2 cm) during 2019 and 2020, respectively (Table 6). Consequently, the recorded chlorophyll (SPAD unit) was higher with the used mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (37.2 and 38.4), compared to the control treatment (22.4 and 22.9) during 2019 and 2020, respectively. The same trend was observed with the fresh and dry weights, where the used mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (238.9 and 239.8 g), compared to the control treatment (198.9 and 199.8 g) during 2019 and 2020, respectively with the fresh weight. While the used mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin scored (26.8 and 27.3 g), compared to the control treatment (16.3 and 16.6 g) during 2019 and 2020, respectively with the dry weight (Table 6).

Table 6. Effect of whey and whey proteins on growth parameters of cucumber plants treated prophylactically during 2019 and 2020 growing seasons

Treatment	Growth parameter							
	Plant height (cm)		Chlorophyll (SPAD unit)		Fresh weight (g)		Dry weight (g)	
	2019	2020	2019	2020	2019	2020	2019	2020
Lactoferrin (A)	241.0 b	242.2bc	35.9 b	36.4b	235.6 abc	237.4b	24.9 b	25.2b
β-lactoglobulin (B)	239.3 b	242.1bc	33.4 d	34.2d	230.7 bc	232.4cd	22.8 c	23.1c
α-lactalbumin (C)	243.7 b	244.3b	32.8 e	33.7d	229.7 c	231.2d	22.2 c	23.0c
Mixture (A + B + C)	244.6 b	244.8b	37.2 a	38.4a	238.9 a	239.8a	26.8 a	27.3a
Whey 40%	240.3 b	241.2c	32.9 de	33.8d	233.7 abc	234.8c	23.2 c	24.2bc
Topas 100 EC	255.8 a	251.8a	34.2 c	35.3c	237.3 ab	238.7ab	27.7 a	27.9a
Control	170.4 c	172.2d	22.4 f	22.9e	198.9 d	199.8e	16.3 d	16.6d
L.S.D at 0.05	8.298	2.510	0.526	0.911	6.756	2.268	1.242	1.607

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

Effect of treatments on yield components

Yield components such as number of fruits/plant, weight of fruits/plant (kg), mean weight of fruit (g) and mean product/plastic house (ton) were also affected positively in relation to application of selected treatments (Table 7). The whey 40% exhibits the most effective results on the number of fruits/plant (38 and 39), followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (37.9 and 38.3), compared to the control treatment (22.3 and 23.4) during 2019 and 2020, respectively (Table 7). No significant differences were occurred between whey 40% and a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin. The same trend was observed with the weight of fruits/plant (kg), mean weight of fruit (g) and mean product/plastic house (ton) during 2019 and 2020, respectively (Table 7).

Table 7. Effect of whey and whey proteins on yield components of cucumber plants treated prophylactically during 2019 and 2020 growing seasons

Treatment	Yield component							
	No. of fruit/plant		Mean weight fruit (g)		Weight of fruit/ Plant (kg)		*Mean product/ plastic house (ton)	
	2019	2020	2019	2020	2019	2020	2019	2020
Lactoferrin (A)	34.9 c	36.2 cd	81.3 a	81.2 a	2.84 c	2.94 bcd	2.27 c	2.35 b
β-lactoglobulin (B)	32.0 d	33.7 d	80.8 a	80.0 a	2.58 d	2.69 d	2.06 d	2.15 c
α-lactalbumin (C)	32.4 d	34.3 d	80.1 a	80.0 a	2.59 d	2.74 cd	2.07 d	2.19 c
Mixture (A + B + C)	37.9 b	38.3 bc	81.0 a	81.1 a	3.07 b	3.11 bc	2.45 b	2.49 b
Whey 40%	38.0 b	39.0 b	80.4 a	80.0 a	3.05 b	3.12 b	2.44 b	2.49 b
Topas 100 EC	43.2 a	44.3 a	81.0 a	81.2 a	3.50 a	3.59 a	2.80 a	2.87 a
Control	22.3 e	23.4 e	76.7 b	77.2 b	1.71 e	1.81 e	1.37 e	1.45 d
L.S.D at 0.05	0.882	2.393	1.617	1.349	0.154	0.372	0.157	0.152

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*. *= 1 plastic house area 360 m^2 (800 plants)**

Effect of treatments on enzyme activity

Peroxidase and polyphenol oxidase activities were investigated. Data summarized in Table (8) show that whey 40% and whey proteins have a vital role in the management of cucumber powdery mildew through the activation of antioxidant enzymes such as peroxidase and polyphenol oxidase which are involved in the self-defense mechanisms of plants. In case of peroxidase, the highest activity of defense-related enzyme was found with whey 40% (1.141 MmH_2O_2 $g^{-1}FW$ Min^{-1}), followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (1.028), compared to the control treatment (0.321) and fungicide treatment (0.639) during 2020 growing season (Table 8). The same trend was found with the polyphenol oxidase activity, where whey 40% scored (0.213 µmol $g^{-1}FW$ Min^{-1}), followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin (0.201), compared to the control treatment (0.028) and fungicide treatment (0.058) during 2020 growing season (Table 8).

Table 8. Effect of whey and whey proteins on the enzyme activity of cucumber plants treated prophylactically during 2020 growing season

Treatment	Enzyme activity	
	Peroxidase	Polyphenol oxidase
Lactoferrin (A)	1.014 b	0.193 c
β-lactoglobulin (B)	0.734 d	0.078 e
α-lactalbumin (C)	0.839 c	0.089 d
Mixture (A + B + C)	1.028 b	0.201 b
Whey 40%	1.141 a	0.213 a
Topas 100 EC	0.639 e	0.058 f
Control	0.321 f	0.028 g
L.S.D at 0.05	0.027	0.003

***values in the same column followed by the same letter (Tukey's HSD) are not significantly different at *P≤ 0.05*.**

Discussion

The reduction in profit for crop producers, distributors and high prices for consumers is mainly due to economic losses caused by plant pathogens especially fungi. Providing adequate protection strategies based on "green" and eco-friendly alternatives against fungal diseases are urgently needed to reduce the synthetic fungicide load on the environment. In addition, their advantages for consumers, who ready to pay more for pesticide-free plant products. Using whey of cow's milk product as a cheaper and eco-friendly alternative to synthetic fungicide in management of powdery mildew disease of zucchini squash was initiated in 1999 (Bettiol 1999). Whey protein makes up 20% of cow's milk with α-lactalbumin, β-lactoglobulin and lactoferrin, and minor proteins such as bovine serum protein and immunoglobulins (Wal 2004). The implementation of milk-based products requires a better understanding of their modes of action. According to Medeiros *et al*., (2012) and Crisp *et al*., (2006) the use of milk and whey is, in general, less expensive than fungicides, and has the advantage of achieving the same level of control. The economic viability of whey in disease management will depend on cost and benefits for the growers, which in turn will depend on the availability of the product and transportation costs from the dairy industries to the target farm. Whey is very cheap and available, because it is a by-product (waste) of the processing of fresh milk and can be an environmental problem if not adequately disposed of by the dairy industry. Depending on our preliminary results obtained by Kamel *et al*., (2017) that disease severity percentage of cucumber powdery mildew caused by *S. fuliginea* (Schlecht.) Pollaci, significantly exhibited by the effective cow's milk and whey 50 and 40% dilutions. So, the main target was to investigate which whey protein (α-lactalbumin, β-lactoglobulin, and lactoferrin) accounts for the highest level of management the same disease. The obtained results indicate that whey proteins (Lactoferrin, β-lactoglobulin, α-lactalbumin) and whey at 40% dilution can be applied prophylactically or curatively against cucumber powdery mildew pathogen. Obtained results of prophylactically and curatively applications show that the disease severity was exhibited significantly by the whey 40% dilution, followed by a mixture of β-lactoglobulin + α-lactalbumin + Lactoferrin, and Lactoferrin during 2019 and 2020 growing seasons. It was clear that, disease severity percentages were higher through curatively application than prophylactically. Consequently, AUDPC values were decreased during the experiment period with all treatments either under prophylactically or curatively application during the two successive seasons. These results are in agreement with those obtained by Bettiol (1990), in which the powdery mildew disease of squash could be inhibited by using the whey dilutions. Belanger and Labbe (2002) reported that the reduction of disease severity percentages of powdery mildew upon the use of milk or whey, may happened through an induction of systemic resistance, stimulation the multiplication of antagonistic microorganisms existing on leaf surfaces and or germicidal effect. Previous studies of Ravensberg *et al*. (2006) established that, treated tomato, sweet pepper, rose and cucumber with bovine milk sprays exhibited the powdery mildew disease and they found that the lactoperoxidase which known as antimicrobial component, was active in the disease management. Crisp *et al*. (2006) found that the application of fresh milk or whey on grapevine leaves colonized by *Erysiphe necator*, resulted in collapsing and damaging of the hyphae, conidiophore and conidia within 24 hr after treatment. The authors suggested that the observed effect on the pathogen may related to the production of free radicals and the action of lactoferrin (an antimicrobial component of milk). They also suggested that milk, whey, lactoferrin, and mixtures of oil plus bicarbonate are potential alternatives to sulfur and synthetic fungicides for the control of powdery mildew in grapevines. Also, lactoferrin, iron binding glycoprotein, binds to the membranes of various bacteria and fungi, causing damage to membranes and loss of cytoplasmic fluids (Samaranayake *et al*., 2001). Medeiros *et al*. (2012) observed that milk increased the bacterial population on leaf surfaces of cucumber and zucchini, and these bacteria were able to suppress the powdery mildew pathogen. Light and scanning electron microscopy examinations show that the selected treatments (β-lactoglobulin, α-lactalbumin, lactoferrin and whey 40%) affected the fungal hyphae, conidiophores and conidia at 24 hr after treatment application. Abnormality of size and shape as well as shrinking, alterations, twisting, plasmolysis and finally collapsing of the fungal hyphae, conidiophores and conidia were observed. These results are in agreement with those obtained by Kamel and Afifi (2020) who reported that cow's milk and whey dilutions (40 and 50% dilution in water) effectively reduced the powdery mildew signs and symptoms caused by *S. fuliginea* on infected cucumber plants with shrinking, twisting, alterations, plasmolysis and collapsing of the fungal hyphae, conidiophores and conidia. Powdery mildew is one of the biotic stresses affecting plant parameters and plant productivity, where, it causes an increase of free radicals in a plant which cause plant acquisition self-defense through antioxidant enzymes [Catalase (CAT), peroxidases (POX) and polyphenol oxidase (PPO)]. While, the production of ROS early during the infection process could be used by plants as weapons in defense responsive system, while continuous production might lead to susceptibility reaction and initiate of programming cell death (Ketta, 2015 and Ketta *et al*., 2017).

In conclusion, cow's whey and whey proteins such as α-lactalbumin, β-lactoglobulin and lactoferrin can be used effectively as a powerful alternatives to synthetic fungicides against cucumber powdery mildew caused by *Sphaerotheca fuliginea* fungus with improvement the total cucumber product (yield/plant).

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استخدام α-lactalbumin و β-lactoglobulin و lactoferrin في مكافحة مرض البياض الدقيقي للخيار تحت ظروف الصوب البلاستيكية

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تمت دراسة تأثير شرش اللبن البقري الأصلي وبروتينات شرش اللبن α-lactalbumin و β-lactoglobulin و lactoferrin تحت ظروف الصوب البلاستيكية في مكافحة مرض البياض الدقيقي للخيار الناجم عن فطر *Sphaerotheca fuliginea* (Schlecht.) Pollaci، باستخدام الرش الوقائيً والعلاجي خلال موسمي النمو 2019 و 2020 مع امكانية تحسين الانتاجية الإجمالية لمحصول الخيار (المحصول / نبات). تم فحص النسب المئوية للشدة المرضية ، والمساحة الواقعة تحت المنحنى التدريجي للمرض (AUDPC) ، وشدة المرض النهائية ، ونسبة الفعالية ، والتغيرات الميكروسكوبية ، ومعايير النمو ، ومكونات المحصول وأنشطة بعض الإنزيمات. أشارت النتائج المتحصل عليها من التطبيق الوقائي إلى أن الشدة المرضية انخفضت معنويا ($P \leq 0.05$) في حالة تطبيق شرش اللبن المخفف بنسبة 40٪ والذي سجل 10 و 6٪ ، يليه خليط من (β-lactoglobulin + α-lactalbumin + Lactoferrin) والذي سجل 10.4 و 6.4٪ ثم ال Lactoferrin والذي سجل 20.3 و 16.4٪ مقارنة بـالمبيد الفطري Topas 100 والذي سجل 9.3 و 4.3٪ ومعاملة المقارنة (62.3 و 59.1٪) خلال موسمي 2019 و 2020 على التوالي. علاوة على ذلك ، فان نسب الشدة المرضية كانت أعلى في حالة التطبيق العلاجي منها في التطبيق الوقائي. وفقًا لشدة المرض النهائية ، فقد انخفضت قيم AUDPC ، بينما زادت نسبة الفعالية ومعايير النمو ومكونات المحصول وأنشطة بعض الإنزيمات خلال فترة التجربة مع المعاملات المختارة سواء تحت التطبيق الوقائي أو العلاجي خلال موسمي النمو. أُظهر التصوير بالميكروسكوب الضوئي و الإلكتروني أن المعاملات المختارة أثرت على الهيفات الفطرية ، و الحامل الكونيدي و الكونيديا بعد 24 ساعة من التطبيق.