

ORIGINAL PAPER

## Controlling Cucumber Powdery Mildew using Cow Milk and Whey under Greenhouse Conditions

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### ABSTRACT

The role of raw cow's milk and whey (40 and 50% dilution in water) and number of sprays against cucumber powdery mildew caused by *Sphaerotheca fuliginea* was studied in greenhouse experiments during 2017/18 and 2018/19. As a result of number of sprays, the disease severity percentage and area under the disease progress curve (AUDPC) were affected by the number of sprays with raw milk and whey. The number of sprays played an important role in controlling powdery mildew, and significant differences have occurred among all applied sprays. Four sprays with intervals of one week was the effective treatment in reducing disease severity, AUDPC values, compared to other treatments, *i.e.*, two and three sprays in comparison to the fungicide as well as a control treatment. Additionally, treated plants showed a significant increase in plant growth and yield parameters in case of spraying four times. Also, the activity of defense-related enzymes (catalase, peroxidase, and polyphenoloxidase) and amino acid proline was high in response to increasing the number of sprays at four times compared with untreated plants. Light and scanning electron microscopic observations showed a clear change in the morphology of *S. fuliginea* spores, in addition to hyphae degradation and loss the vitality of conidia as well as rupturing the content of conidia. The results obtained also indicated that cow's milk and whey dilution effectively reduced the powdery mildew signs and symptoms on infected cucumber plants at 50% with the four sprays treatment being the most effective treatment.

**Key words:** Cucumber, *Cucumis sativus*, enzyme, greenhouse, Cow milk, *Sphaerotheca fuliginea*, Whey

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### INTRODUCTION

Worldwide, the cultivated cucurbits under greenhouse conditions are mainly infected and damaged by powdery mildew pathogen *Sphaerotheca fuliginea*, which attacks leaves, stems, and even fruits. The chemical fungicides are used as the main strategy to control the fungal diseases affecting cucurbits production under commercial conditions (Kimati *et al.*, 1997). Usage of chemical fungicides especially repeating the application times contributes to environmental pollutions and resulted in resistant populations of *S. fuliginea* to the used fungicides (McGrath, 1996). So, searching for alternatives to chemical fungicides or reducing

their application times is the urgent target. In this respect, Bettiol *et al.* (1999) used milk, an eco-friendly product to control squash powdery mildew caused by *S. fuliginea*. So, they suggested that foliar sprays of aqueous dilutions of fresh, non-pasteurized cow's milk can be quite effective in controlling the powdery mildew on zucchini where the weekly foliar sprays using an aqueous solution of 40% milk reduced the powdery mildew disease severity by 85-90%. Also, using milk with different concentrations gave a good reduction in powdery mildew disease severity and this may be attributed to its contents of fatty acids, proteins, sugars, salts, enzymes, minerals, and vitamins. In this regard, Crisp *et al.* (2006) suggested that management of powdery mildew on grapevine could be done through potential alternatives to synthetic fungicides such as whey, milk individually or mixed with oils or bicarbonate. Whey of raw cow milk was also used to control the powdery mildew of squash and cucumber (Bettiol *et al.*, 2008). On the other hand, Smith *et al.* (2003) mentioned that the milk-based foliar sprays of pumpkin infected naturally with *Sphaerotheca fuliginea*, muskmelon infected with *S. fuliginea* and tomato infected with *Leveillula taurica* reduced the powdery mildew signs and symptoms under open field conditions.

Also, powdery mildew disease severity on pumpkin plants was reduced by 85-90% when 40% of milk was applied as foliar sprays (Ferrandino and Smith 2007). Recently, Kamel *et al.* (2017) reported that whey dilutions (50 and 40%) followed by milk dilutions (50 and 40%) were significantly effective in reducing the foliar symptoms of cucumber powdery mildew disease severity by 43-74%.

An efficient anti-oxidative system comprising of the enzymatic, as well as non-enzymatic antioxidants, is involved in scavenging or detoxification of excess ROS (Noctor *et al.*, 2007). As for the role of antioxidant enzymatic components, *i.e.* catalase (CAT), peroxidase (POX), and polyphenoloxidase (PPO) play a vital role during high ROS levels, thereby and protect plants from pathogen attack (Hafez *et al.*, 2016). Whereas non-enzymatic antioxidants include phenolics, ascorbate, glutathione, carotenoids, and tocopherols (Gill and Tuteja, 2010)

The present work was conducted to study the role of raw cow milk and whey 40 and 50% dilutions and the number of sprays against cucumber powdery mildew caused by *S. fuliginea* under greenhouse conditions.

## MATERIALS AND METHODS

### Plant source:

The efficacy of cow milk and whey with two dilutions (40 and 50%) was evaluated under greenhouse conditions to determine the best number of sprays against *S. fuliginea* the causal agent of cucumber powdery mildew compared to fungicide Topas 100 EC (Penconazole 10.2% W/W, Syngenta Company). In this respect, seeds of cucumber (*Cucumis sativus* L., Barracuda F1) batch No. 0161531598 (Seminis Company) were obtained kindly from the Horticultural Research Institute, Agricultural Research Center (ARC). Rectangular plastic trays (5×5×7 cm) were filled with autoclaved commercial potting medium (peat-based container medium, containing peat moss and vermiculite). Seedlings 30-days old of cucumber cv. Barracuda were transplanted at the rate of 10 plants per 5 m in a row in the greenhouse located in Sakha, Kafr El-Sheikh governorate, Egypt. Recommendations of the Ministry of Agriculture for irrigation, fertilization and weed control were applied precisely.

### Milk and whey preparation:

Cow's raw fresh milk, unpasteurized and unhomogenized was obtained from Animal Research Station located in Sakha, Kafr El-Sheikh governorate, Egypt. The whey was prepared from cow's fresh milk by heating the milk at 72°C in a water bath for 20 sec then transferred immediately to water bath heated at 42°C for 1 hr. For obtaining the gelatinous materials of the curd, rennin enzyme was added 1ml/L milk and then incubated at 42°C for 3 hr. For obtaining the whey, cheesecloth was used for the filtration process of the gelatinous materials of the curd.

### Experimental design:

This experiment was conducted during 2017/2018 and 2018/2019 as complete randomized blocks design. The treatments were spraying fresh milk and whey, each with two dilutions (40 and 50%) compared to the fungicide Topas-100 EC at the ratio 0.25ml/L and untreated control, in a greenhouse (9 x 40 m). To determine the best number of sprays on controlling cucumber powdery mildew, three replicates were sprayed with each dilution of each tested substance, viz. two sprays, three sprays and four sprays treatments with one week interval. Foliar spray was carried out when the natural infection by powdery mildew was appeared on the first five true expanded leaves. Control treatment and fungicide Topas 100 EC at ratio 0.25 ml/L. were used for comparison. For studying the effect of number of sprays on disease development, the final disease severity percentage, efficacy %, area under disease progress curve (AUDPC) and r-value were estimated.

### Disease assessment:

Seven days after the appearance of powdery mildew symptoms, disease severity was assessed five times during the experiment, zero time, 7, 14, 21 and 28 days. The disease severity (percentage of the leaf surface covered with powdery mildew symptoms) was evaluated based on the scale of 0 to 6 according to Yan, *et al.*, 2006, and calculated according to the equation described by Descalzo *et al.* (1990) as follow:

$$R = \left[ \frac{\sum (a \times b)}{N \times K} \right] \times 100.$$

**Where:** R =disease severity %, a =number of infected leaves rated, b = numerical value of each grade, N =total number of examined plants, K = the highest degree of infection in the scale.

The efficacy of the treatments was calculated according to the following formula:

$$\text{Efficacy \%} = \frac{\text{DS\% control} - \text{DS\% treatment}}{\text{DS\% control}} \times 100$$

The method described by Pandey *et al.* (1989) was used for calculation area under disease progress curve (AUDPC) as follow:

**AUDPC** =

$$D [1/2 (Y_1 + Y_k) + Y_2 + Y_3 + \dots + Y_{(k-1)}]$$

**Where:** D = days between each successive two readings,  $Y_1$  = first disease record.  $Y_k$  = last disease record.

$$\text{r-value} = \frac{1}{t_2 - t_1} \left( \log_e \frac{X_2}{1 - X_2} - \log_e \frac{X_1}{1 - X_1} \right)$$

**Where:**

$X_1$  = Disease severity (%) at  $t_1$  date.

$X_2$  = Disease severity (%) at  $t_2$  date.

$t_2 - t_1$  = Time interval in days between two observations.

### Growth and yield parameters:

Plant height, chlorophyll content, fresh and dry weights were also estimated (Data are the average of 2017/2018 and 2018/2019). Plant height was taken from the base of cucumber plants to the top by centimeter unit, while chlorophyll content was measured by portable leaf chlorophyll meter (Minolta SPAD-502, Japan) to determine the greenness or relative content of leaves to analyze the photosynthetic pigments according to the method of Torres-Netto *et al.* (2005) in treated and untreated fully expanded cucumber leaves. Yield parameters such as number of fruits/plant, mean weight fruit (g), weight of fruits/plant (kg) and mean product/ greenhouse (ton) were investigated, (Data are the average of 2017/2018 and 2018/2019).

### Enzymes activity and proline:

For the three enzyme assays and amino acid proline, 0.5 g of fresh treated cucumber leaf was homogenized at 0–4°C in 3 mL of 50mM TRIS buffer (pH 7.8), containing 1mM EDTA- $\text{Na}_2$  and 7.5% polyvinylpyrrolidone. The homogenates were centrifuged (12,000 rpm, 20 min, 4°C), the enzymes activity was determined spectrophotometrically. The measurements were carried out at 25°C, using the model UV-160A spectrophotometer. The activity of catalase (CAT) was measured according to Aebi (1984). Peroxidase (POX) activity was

measured as described by Hammerschmidt *et al.* (1982). Polyphenoloxidase (PPO) activity was measured according to Malik and Singh (1980). Proline was measured as described by Bates *et al.* (1973).

### Microscopic observations:

Light microscope (Leica DM1000) examination was used to study the mode of milk and whey action on conidiophores and conidia of *S. fuliginea* on naturally infected cucumber leaves with powdery mildew sampled at 24 h after the tested treating with substances every week (5 times). Photographing by microscope was done at the Agricultural Botany Dept., Faculty of Agriculture, Kafrelsheikh University, Egypt.

For scanning electron microscope (SEM), cucumber leaves bearing lesions of powdery mildew (with and without treatment) were processed according to Harley and Ferguson (1990). Tissue pieces of ~4 mm<sup>2</sup> were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 24 h at 4°C, followed by exposure to osmium tetroxide (1% OsO<sub>4</sub>) for 1 h at 25°C. Samples were dehydrated by passing through ascending concentrations of acetone and dried till the critical point. Samples were sputter-coated with gold. The examination and photographing were done using a Jeol Scanning Electron Microscope (JSM-T.330 A) in the Central Laboratory, Faculty of Agriculture, Mansoura University, Egypt.

### Statistical analysis:

All experiments were designed with complete randomized block design. WASP software (Web Agriculture Stat Package) was used for analysis of variance (ANOVA). The comparisons of means were determined by Duncan's multiple range tests, at  $P \leq 0.05$  (Gomez and Gomez, 1984).

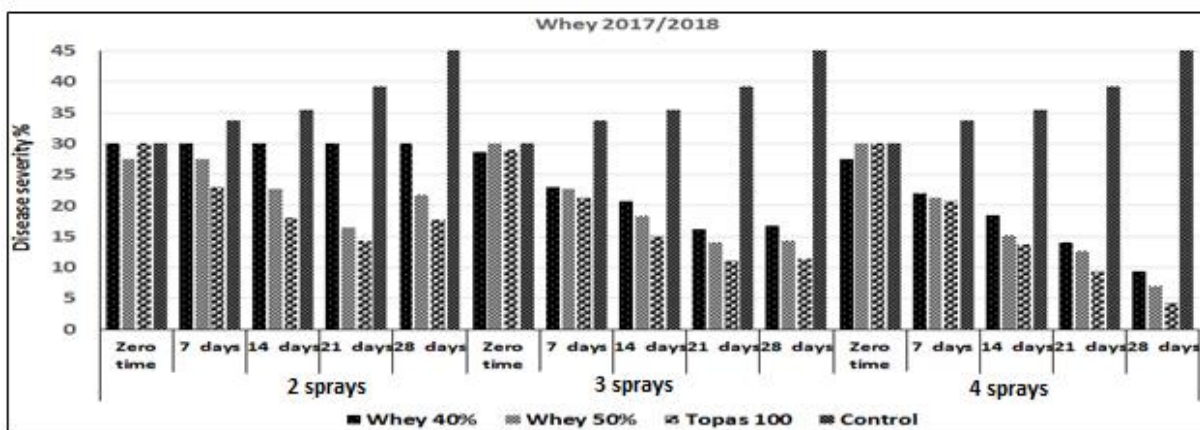
## RESULTS

Nowadays, the major ways of modern, safe and sustainable in agriculture depend on eco-friendly disease management strategies. Naturally infected cucumber plants by powdery mildew were foliar sprayed two, three and four times with natural compounds such as raw milk and whey in dilution of 40 and 50 % compared to fungicide and control treatment. The obtained results indicated that whey and milk could be used as a curative treatment, as a result of their antifungal activities observed in these products.

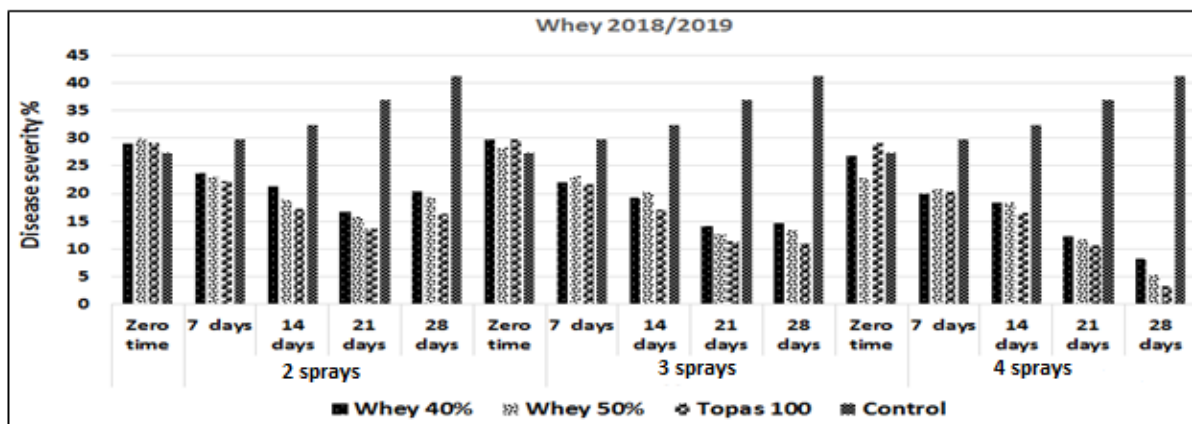
**1- Effect of whey on controlling cucumber powdery mildew:**

The present results show that 50% dilution of whey was very effective than 40% in reducing the disease severity percentages of cucumber powdery mildew during 2017/2018 (Fig. 1). The number of sprays was a very effective factor, as shown in Fig. (1) which indicates that spraying cucumber plants for four times with 50% whey was more effective than 2 and 3 sprays, where the recorded disease severity values were 21.7, 14.3 and 7.0 % for the tested treatments, *i.e.*, 2, 3 and 4 sprays, respectively compared to fungicide treatment which recorded 17.7, 11.3 and 4.3 %, respectively.

The experiment was repeated in 2018/2019 and the obtained results confirmed that diluted whey at 50% was more effective than diluted whey at 40% in reducing disease severity percentages (Fig. 2). In this respect, the number of sprays was a very effective factor in reducing infection with powdery mildew as shown in Fig. (2) where, four sprays treatment in case of diluted whey at 50% was effective in reducing disease severity% than treatments of two and three sprays, being 19.3, 13.3 and 7.3 %, respectively, compared to fungicide treatment which recorded 16.3, 11.0 and 5.3 %, respectively.



**Figure (1): Effect of diluted whey at 40 and 50 % and number of sprays (2, 3 and 4) on disease severity percentages during 2017/2018.**



**Figure (2): Effect of diluted whey at 40 and 50 % and number of sprays (2, 3 and 4) on disease severity percentages during 2018/2019.**

The effect of diluted whey at 40 and 50 % with 2, 3 and 4 sprays treatments against powdery mildew pathogen was evaluated. In this respect, the number of sprays affected positively the final disease severity percentage, efficacy %, area under disease progress curve (AUDPC) and r-value

(Table, 1). The recorded final disease severity percentages due to using the effectively diluted whey at 50 % with two, three and four sprays were 21.7, 14.3 and 7.0 %, respectively, compared to the fungicide treatment which recorded 17.7, 11.3 and 4.3 %, respectively, at the same conditions during

2017/2018. Similar results were obtained during 2018/2019 (Table 1). Consequently, the recorded efficacy percentages were significantly higher with using diluted whey (50 %) for 4 sprays treatment.

Also, AUDPC values and r-value were significantly reduced by spraying cucumber plants four times with whey 50% (Table 1).

**Table (1): Effect of whey on some disease parameters of cucumber powdery mildew caused by *S. fuliginea* during 2017/18 and 2018/19.**

Treatment	No. of sprays	Final disease severity (%)		Efficacy (%)		AUDPC		r-value	
		2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
Control	-	45.3 a	41.3 a	-	-	1023.a	933.1a	0.022a	0.021a
Whey 40%	2	21.3 b	20.3 b	52.9	50.8	612.2b	604.5b	-0.015b	-0.015b
	3	16.7 c	14.7 cd	63.1	64.4	578.2bc	543.9c	-0.023c	-0.029de
	4	9.4 f	8.3 f	79.2	79.9	510.7de	479.9de	-0.043e	-0.046g
Whey 50%	2	21.7 b	19.3 b	52.1	53.3	616.7b	604.5b	-0.010b	-0.019bc
	3	14.3 d	13.3 d	68.4	67.8	540.1cd	546.0c	-0.031d	-0.031e
	4	7.0 g	7.3 f	84.5	82.3	474.3e	490.7d	-0.057f	-0.052h
Topas 100 fungicide	2	17.7 c	16.3 c	60.9	60.5	554.1cd	530.6c	-0.022c	-0.024cd
	3	11.3 e	11.0 e	75.1	73.4	472.2e	484.1d	-0.038e	-0.037f
	4	4.3 h	5.3 g	90.5	87.2	425.9f	448.7e	-0.075g	-0.064i

Values in the same column followed by the same letter are not significantly differed at  $P < 0.05$  level.

## 2- Effect of cow milk on controlling cucumber powdery mildew:

Data in Fig. (3) indicate that diluted cow milk at 40, 50% was more effective in inhibiting and reducing the severity of cucumber powdery mildew when the plants were sprayed weekly as mentioned before. In this respect, four sprays treatment with cow milk 50% achieved the lowest disease severity (5.7%) when it was applied every week followed by treatments sprayed 3 and 2 times, which recorded 11.7 and 17.3% disease severity, respectively. Topas fungicide treatment recorded the lowest percentage of disease severity, where it recorded 4.3%. On the other hand, the recorded disease severity % in control treatment was 43.7% at 28 days of application.

Also, results indicate that cow milk 50% was very effective than 40% in reducing the disease severity percentages of cucumber powdery mildew during 2017/2018 (Fig. 3).

It is clear from the obtained results (Fig. 3) that the number of sprays was a very effective factor in controlling powdery mildew on cucumber plants where spraying plants 4 times with cow milk 50% and fungicide treatment were more effective than two and three sprays treatments in reducing disease severity percentage compared to control treatment.

The trial was repeated during 2018/2019 and the obtained results showed the same trend obtained during 2017/2018 to confirm that milk in dilution 50% was very effective than 40% in

reducing the disease severity percentages (Fig. 4). Also, the number of sprays was a highly effective factor and four sprays with cow milk 50% and fungicide treatment were more effective than 2 and 3 sprays in reducing the cucumber powdery mildew disease severity compared to control treatment.

Data presented in Table (2) show the effect of number of sprays, two, three and four using cow milk 40 and 50 % against cucumber powdery mildew. In this respect, all values of final disease severity percentage, efficacy %, area under disease progress curve (AUDPC) and r-value were affected by the number of sprays. However, all the three tested sprays treatments reduced final disease severity, area under the disease progress curve (AUDPC) and r-value of cucumber powdery mildew compared to control treatment. In contrast, four sprays treatment achieved the highest efficacy % of controlling the disease on sprayed cucumber plants with cow milk 40 and 50%. Also, spraying cucumber plants with cow milk 40 and 50% showed the highest reduction of AUDPC (499.3) and (460.9) during 2017/2018 and 2018/2019 and (432.7) and (427.0) AUDPC, during 2017/ 2018 and 2018/2019, respectively, followed by using three and two sprays treatment, compared to control treatment which recorded 1011.8 and 932.1 for AUDPC, respectively, during 2017/2018 and 2018/2019. However, Topas fungicide recorded the highest reduction of

AUDPC (409.2 and 420 AUDPC, respectively, during 2017/2018 and 2018/2019) when the plants were sprayed four times. Also, the highest

reduction of r-value was recorded when cucumber plants were sprayed 4 times.

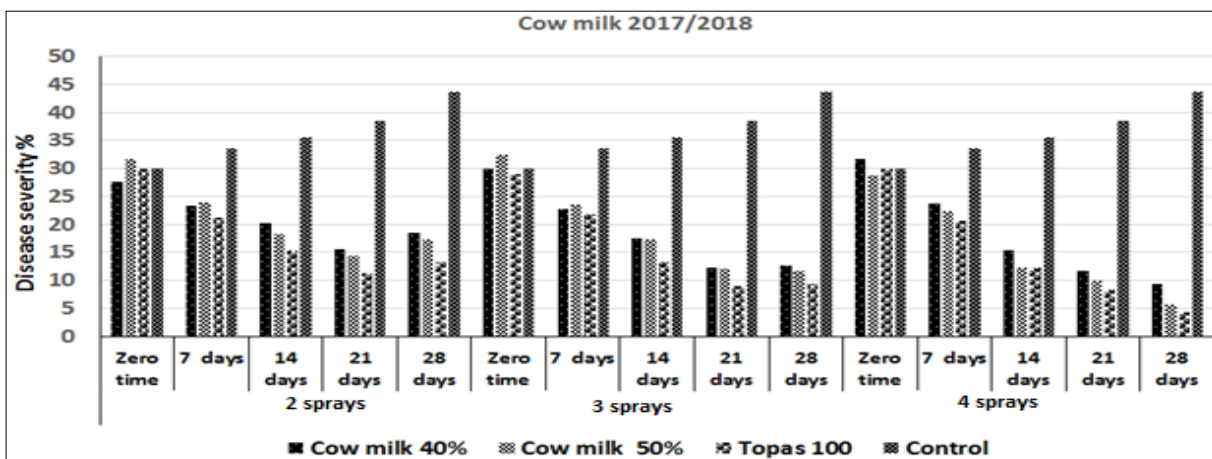


Figure (3): Effect of diluted cow milk at 40 and 50 % and number of sprays (2, 3 and 4) on disease severity percentages at zero time, 7, 14, 21 and 28 days during 2017/2018.

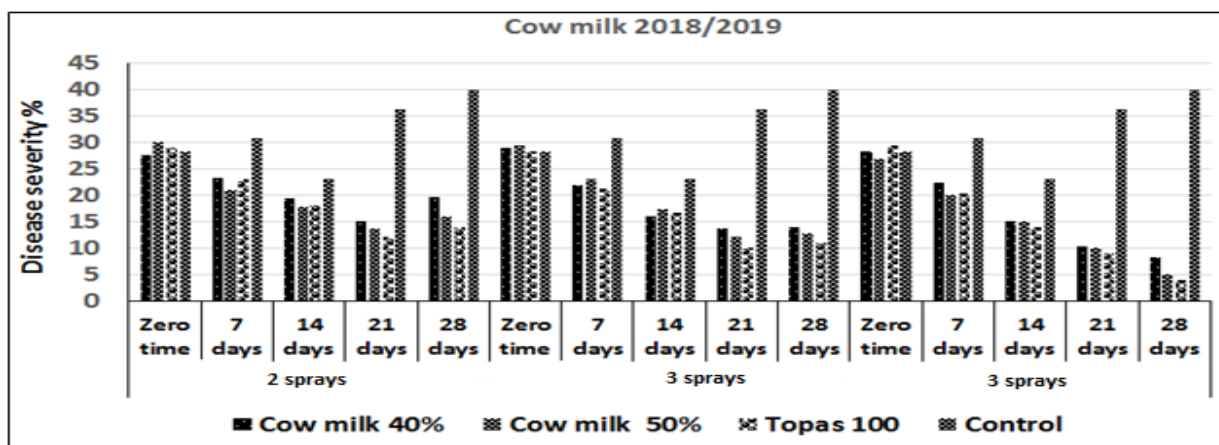


Figure (4). Effect of diluted cow milk at 40 and 50 % and number of sprays (2, 3 and 4) on disease severity percentages at zero time, 7, 14, 21 and 28 days during 2018/2019.

Table (2): Effect of cow milk on some disease parameters of cucumber powdery mildew caused by *S. fuliginea* during 2017/18 and 2018/19.

Treatment	No. of sprays	Final disease severity (%)		Efficacy (%)		AUDPC		r-value	
		2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
Control	-	43.7 a	40.0 a	-	-	1011.8a	932.1a	0.019a	0.017a
Cow milk 40%	2	18.5 b	19.7 b	57.7	50.8	574.3b	568.4b	-0.017b	-0.014b
	3	12.7 c	14.0 d	71.2	65.0	516.9cd	512.4d	-0.036de	-0.030cd
	4	9.3 d	8.2 f	78.7	79.5	499.3de	460.9e	-0.050f	-0.049f
Cow milk 50%	2	17.3 b	16.0 c	60.4	60.0	567.8b	527.8c	-0.026c	-0.027c
	3	11.7 c	12.7 d	73.2	68.3	525.0 c	513.1d	-0.043ef	-0.034de
	4	5.7 e	5.0 g	86.9	87.5	432.7f	427.0e	-0.063g	-0.064g
Topas 100 fungicide	2	13.3 c	14.0 d	69.6	65.0	485.4e	521.5cd	-0.034d	-0.030cd
	3	9.4 d	11.0 e	78.5	72.5	442.4f	473.5 e	-0.045f	-0.038e
	4	4.3 e	4.0 h	90.2	90.0	409.2g	420.4f	-0.075h	-0.076h

- Values in the same column followed by the same letter are not significantly differed at  $P < 0.5$  level.

### 3- Effect of cow milk and whey on some growth parameters of treated cucumber plants:

All of plant height, chlorophyll content, fresh and dry weights were significantly affected as a result of treating cucumber plants with cow milk and whey 40 and 50 % three times compared to the chemical fungicide and control treatment (Table 3). Plant height of cucumber was significantly increased when cow milk and whey 40 and 50%

were sprayed four times comparing with control treatment followed by those sprayed two and three sprays (Table 3). Interestingly, spraying cow milk and whey at 50% four times was the best treatment in increasing chlorophyll content, fresh and dry weights to be similar in their effects with those treated with chemical fungicide followed by those treated two and three sprays at the same dilution.

**Table (3): Effect of cow milk and whey applications on some growth parameters of treated cucumber plants, data are the average of 2017/18 and 2018/19.**

Treatment	No. of sprays	Plant height (cm)		Chlorophyll (SPAD)		Fresh weight (g)		Dry weight (g)	
		Cow milk	Whey	Cow milk	Whey	Cow milk	Whey	Cow milk	Whey
Control	-	174.7f	170.3f	21.3e	19.8f	202.1g	199.3i	16.6g	15.9g
Dilution 40%	2	234.3e	227.4e	33.5d	31.9e	224.0f	221.9h	19.2f	18.7f
	3	240.2de	235.5d	34.3cd	31.7e	229.8d	228.1f	21.8de	20.2e
	4	250.1bc	242.3bc	36.2ab	33.5bcd	233.5c	230.7e	24.8c	22.9d
Dilution 50%	2	236.8e	226.5e	34.2cd	32.7cde	226.4e	223.8g	21.1e	19.4ef
	3	244.9cd	236.3d	35.4bc	33.1bcd	229.9d	227.2f	22.7d	20.0e
	4	256.0ab	245.5b	37.4a	34.3ab	238.4a	234.4c	27.1b	26.7b
Topas 100 fungicide	2	236.0e	237.1d	34.3cd	32.0de	231.7cd	231.7d	23.9c	24.3c
	3	245.0cd	241.4c	35.4bc	33.7bc	235.3b	236.2b	26.7b	26.8b
	4	259.0a	257.8a	35.5bc	35.7a	239.2a	240.1a	30.6a	31.2a

- Values in the same column followed by the same letter are not significantly differed at  $P < 0.05$  level.

### 4- Effect of cow milk and whey on some yield parameters of treated cucumber plants:

Data presented in Table (4) show that all yield parameters such as number of fruits/plant, mean weight fruit (g), the weight of fruits/plant (kg) and mean product/ greenhouse (ton) were investigated. Cow milk, whey at 40 or 50% dilutions and fungicide using any number of sprays significantly

affected the cucumber yield components compared to control treatment. No significant differences were found between the chemical fungicide and cow milk at 50% when sprayed four times on mean product/ greenhouse (ton), being 2.89 and 2.78 ton, respectively, followed by whey at the same dilution.

**Table (4): Effect of cow milk and whey on some yield parameters of treated cucumber plants, data are the average of 2017/18 and 2018/19.**

Treatment	No. of sprays	No. of fruit/plant		Mean weight fruit (g)		Weight of fruit/plant (kg)		Mean product/ greenhouse (ton)	
		Cow milk	Whey	Cow milk	Whey	Cow milk	Whey	Cow milk	Whey
Control	-	21.3f	20.8f	77.3c	77.4c	1.65h	1.61f	1.32f	1.29e
Dilution 40%	2	32.7e	30.3e	80.2b	80.2b	2.62g	2.43e	2.10de	1.98d
	3	36.3d	34.4d	80.3ab	81.4ab	2.91e	2.80d	2.33de	2.24c
	4	42.3b	39.7bc	81.2ab	81.2ab	3.43b	3.22bc	2.74ab	2.58b
Dilution 50%	2	33.7e	31.2e	81.7ab	80.9ab	2.75f	2.52e	2.20e	2.02d
	3	37.3d	34.3d	80.7ab	82.0a	3.01d	2.81d	2.41cd	2.24c
	4	42.7ab	40.0b	81.2ab	81.5ab	3.47b	3.26b	2.78a	2.61b
Topas 100 fungicide	2	34.3e	34.2d	80.2b	80.3b	2.75f	2.75d	2.20e	2.20c
	3	39.2c	38.2c	81.5ab	81.4ab	3.20c	3.11c	2.56bc	2.49b
	4	44.3a	43.8a	81.8a	82.0a	3.62a	3.59a	2.89a	2.87a

- Values in the same column followed by the same letter are not significantly differed at  $P < 0.05$  level.

### 5- Effect of cow milk and whey on activities of defense related enzymes in treated cucumber plants:

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-defence through antioxidant enzymes (CAT, POX. and PPO). Data presented in Fig. (5) show that cow milk and whey have a vital role in controlling powdery mildew where they activated main plant self-defenses through antioxidant enzymes.

Dilutions of both cow milk and whey 50% and using them four times recorded the highest activities of defence-related enzymes, *i.e.*, catalase, peroxidase and polyphenoloxidase in addition to the amino acid proline. In this respect, spraying cow milk at 50% four times caused the highest activities of catalase enzyme ( $32.9 \text{ mM H}_2\text{O}_2 \text{ g}^{-1} \text{ FW Min}^{-1}$ ) comparing with control and other treatments. Also, application whey 50% four times was more effective in increasing the activities of catalase to be  $28.8 \text{ mM H}_2\text{O}_2 \text{ g}^{-1} \text{ FW Min}^{-1}$  than other treatments and control. Also, cow milk and whey at 50% increased the activities of peroxidase more than other treatments and control, being 1.202 and  $1.121 \text{ mM H}_2\text{O}_2 \text{ g}^{-1} \text{ FW Min}^{-1}$ , respectively, when cucumber plants were sprayed four times. Also, spraying 50% dilution of cow milk or whey was more effective dilution in increasing the activities of polyphenoloxidase enzyme than the other dilution (40%) and other treatments, being  $0.109$  and  $0.100 \mu \text{ mol/min}^{-1} \text{ g}^{-1}$  (FW) with milk and whey, respectively.

On the other hand, there was a clear increment in determining activities of proline as a related defense amino acid as a result of spraying cucumber plants with cow milk or whey 50%. The recorded activity values were  $0.173$  and  $0.167 \mu \text{ mol/min}^{-1} \text{ g}^{-1}$  (FW), respectively.

The lowest activity of the CAT, POX and PPO enzymes as well as proline amino acid was obtained when the fungicide Topas was applied in compare with the other tested treatments.

### 6- Microscopic observations on *S. fuliginea*, the causal agent of cucumber powdery mildew:

Light microscope examinations show that cow milk and whey at 50% affected the size and shape as well as caused shrinking of treated hyphae, conidiophores and conidia of *S. fuliginea* on naturally infected cucumber leaves with powdery mildew at 24 h post-treatment with four sprays (Fig. 6). Also,

scanning electron microscope examination of the fungal structures taken from cucumber leaf surfaces treated with milk as well as whey at 50 % dilution showed abnormalities and alterations in the conidia, conidiophores and hyphae of *S. fuliginea* compared to control treatment (Fig. 7). Moreover, twisting and plasmolysis of hyphae and conidiophores, conidial shrinking and collapsing were also observed (Fig. 7. B and C).

## DISCUSSION

Through the dairy industry, elimination of whey which is a by-product of fresh milk processing is very necessary because it could be an environmental problem. Control of powdery mildew diseases infecting various crops through cheaper and safe alternatives to fungicides such as whey of fresh milk was initiated in 1999 (Bettiol, 2001). So, the main target of this research is to test both cow milk and whey by two dilutions (40 and 50%) with two, three and four sprays treatments for controlling powdery mildew in cucumber plants. The obtained results indicated that spraying cucumber plants infected with powdery mildew, with cow milk and whey dilutions 40 and 50% four times were able to reduce disease severity percentage. Also, different dilutions of cow milk, as well as whey, gave a good reduction in powdery mildew area under disease progress curve, efficacy percentage and r-value through 2017/2018 and 2018/2019. These results are in agreement with Bettiol (2001) who reported that powdery mildew of squash could be controlled through the suppression of disease by using whey dilutions. Also, Crisp *et al.* (2005 and 2006) found that disease severity of powdery mildew of grapevine could be reduced through whey application. This may be due to its contents which consist of oil, fatty acids, proteins, sugar, salts, enzymes, vitamins and minerals (Stadnik and Bettiol, 2001). Also, Medeiros *et al.* (2006) reported that application of milk as foliar spray was reported to be participated with increasing of beneficial bacterial populations which may help in suppression process of the pathogen causing powdery mildew on cucumber and zucchini. In addition, Modler *et al.* (1998) and Ravensberg *et al.* (2006) observed that bovine milk was very effective in the control process of powdery mildew in tomato, rose, sweet pepper and cucumber because of lactoperoxidase, which known as an important factor in the anti-microbial system.



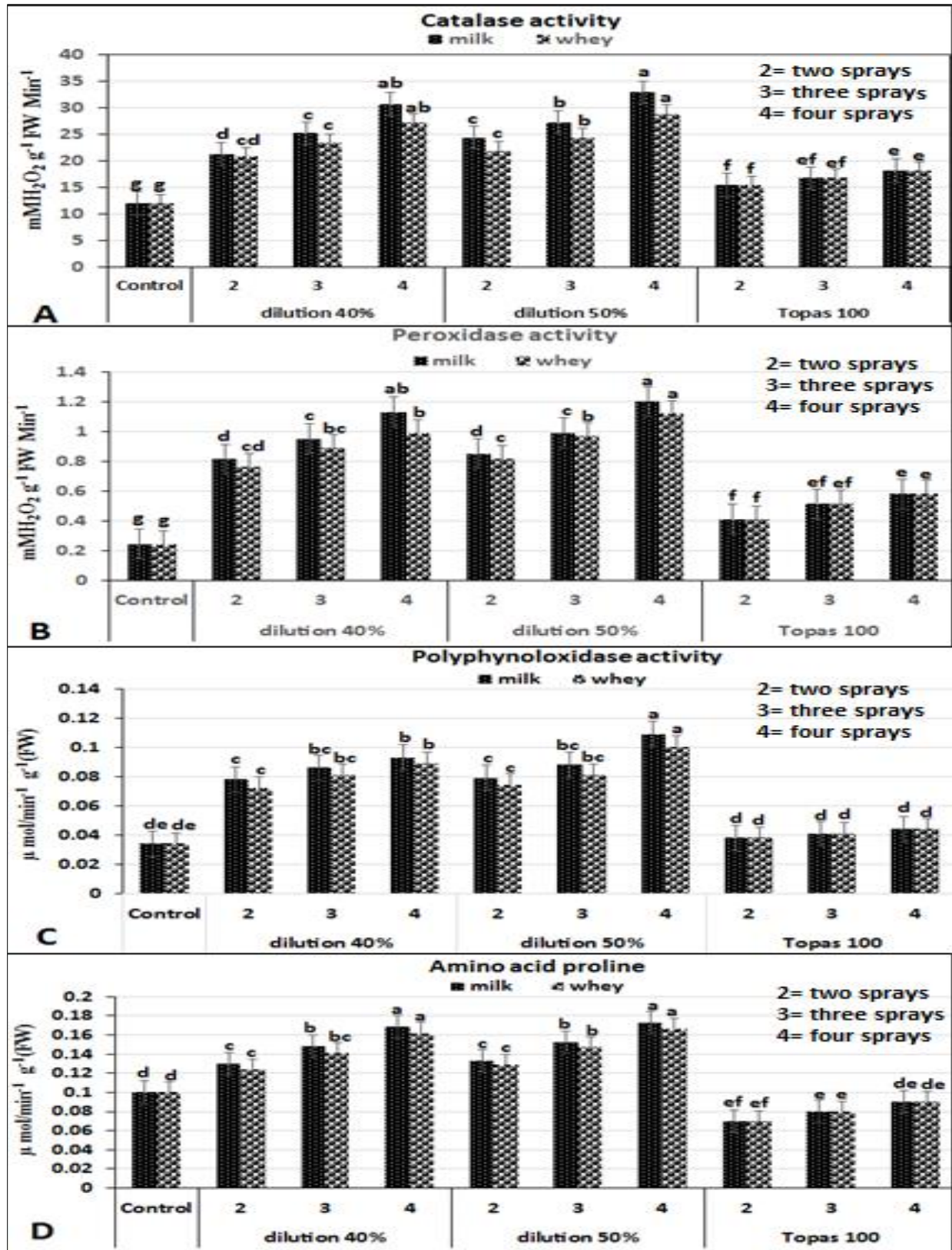
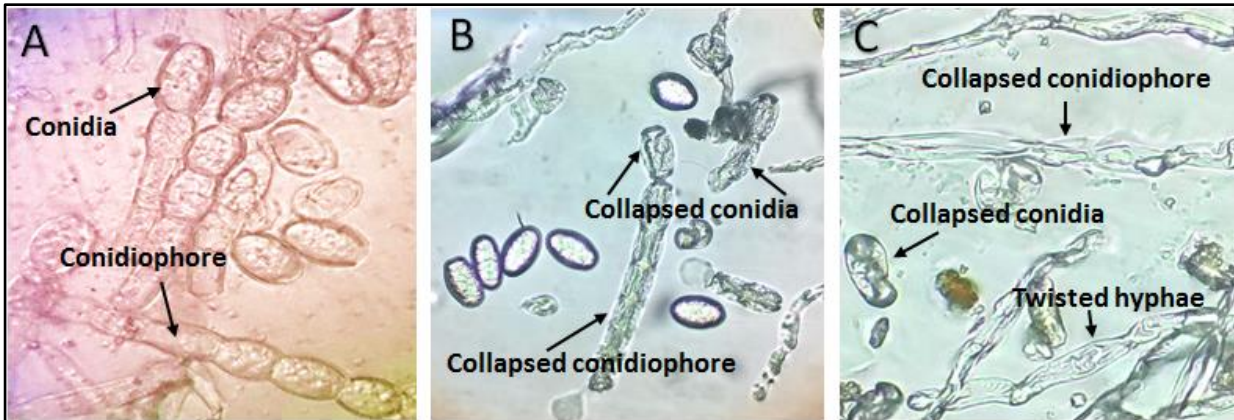
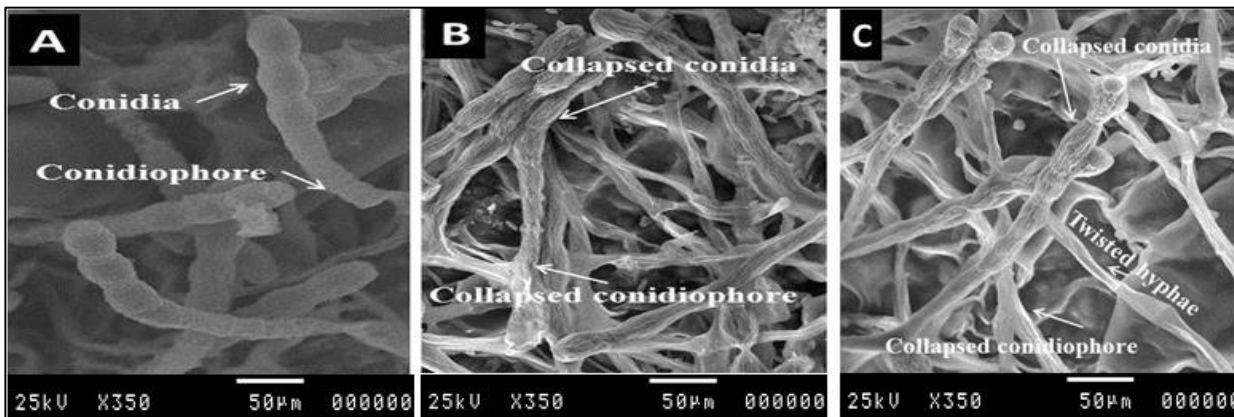


Fig. (5): Effect of cow milk and whey on activities of defense related enzymes in treated and untreated cucumber plants, A= catalase, B= Peroxidase and C= Polyphenoloxidase activity and D= Amino acid proline.



**Figure (6):** Light microscope observations of hyphae, conidiophores and conidia of *S. fuliginea* collected from cucumber leaf surface sprayed with cow milk or whey 50 % showing, **A:** Untreated control with normal conidia and conidiophores (black arrows). **B:** cow milk 50 % and **C:** whey 50 %, showing twisting of hyphae and conidiophores, conidial shrinking and collapsing (black arrows), (400X).



**Figure (7):** Scanning electron microscope observations of hyphae, conidiophores and conidia of *S. fuliginea* taken from cucumber leaf surface sprayed with cow milk or whey 50 % showing, **A:** Untreated control with normal conidia and conidiophores (white arrows). **B:** cow milk 50 % and **C:** whey 50 %, showing twisting of hyphae and conidiophores, conidial shrinking and collapsing (white arrows).

Another suggested active component of milk and whey is lactoferrin which is responsible for damage and collapse of *U. necator* conidia at 48 h than at 24 h after exposure the majority of the damage caused to hyphae after treatment with milk, whey occurred within 24 h of application. 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). Possibly, components of milk other than lactoferrin also caused damage to the hyphae, or the interaction with other components of milk that increased the activity of lactoferrin against hyphae

(Crisp *et al.*, 2003). Reduction of disease severity of powdery mildew in grapevines was reported by Tzeng and DeVay (1989) to be associated with the production of free radicals when sulfur-rich amino acids, riboflavin and methionine exposure to light. The same action could be happened with milk or whey solids when influenced by solar radiations including UV and result in the production of free radicals. So the timing of application (on bright, warm days) is important factor to maximize the production of free radicals than an application on cooler and cloudy days. Crisp *et al.* (2006) explained that milk and its components could produce oxygen free radicals (ROS), which attacks

the pathogen directly in the infection sites during natural sunlight. 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). the different metabolic and developmental processes that involve the interaction between ROS and hormones when high proportions ROS in plants include closing the stomata (Neill *et al.*, 2008), programmed cell death (Bethke and Jones, 2001), control of apical root organization (Jiang and Feldman, 2003) and gaining tolerance to both biotic and abiotic stresses (Miller *et al.*, 2008). ROS is constantly reduced/erased by plant-based antioxidant defence systems that maintain it at certain stable levels under stressful conditions (Tuteja *et al.*, 2012).

The results indicated that the 50% dilution of both cow milk and whey, when used four times, recorded the highest activities of defence-related antioxidant enzymes, *i.e.*, catalase, peroxidase and polyphenoloxidase which play an important role of increasing defence of plant to infection by powdery mildew. Various enzymatic antioxidants comprise of superoxide dismutase, catalase, peroxidase and polyphenoloxidase (Foyer and Noctor, 2003 and Sarhan *et al.*, 2020). Increased activities of many antioxidant enzymes have been observed in plants to combat oxidative stress induced by various stresses and also to maintain cellular homeostasis (Almeselmani *et al.*, 2006). Maintenance of a high antioxidant capacity to scavenge the toxic ROS has been linked to an increase in tolerance of plants to these diseases stress (Hasanuzzaman *et al.*, 2013).

Also, our results showed that amino acid proline showed noticeable increasing with four sprays of cow milk and whey at a dilution 50%. This may be attributed to milk and its derivatives content of high proline which has a role in low deleterious on plant. Proline is considered one of the self-defense tools for plant when plant is under stress. Scano *et al.* (2014) reported that the chemical composition analysis done by GC-MS of cow milk revealed that the aqueous fraction obtained by the extraction procedure was rich in short-chain hydroxylated carboxylic acids, such as lactic acid, succinic acid, fumaric acid, malic acid, 2-hydroxyglutaric acid, and gluconic acid; long-chain stearic and palmitic acids were also found. Among free amino acids, proline, serine, valine, glycine, alanine and glutamine were detected.

Light and scanning electron microscope examinations showed that cow milk and whey at 50% affected the size and shape as well as caused shrinking of treated hyphae, conidiophores and conidia of *S. fuliginea* collected from naturally infected cucumber leaves with powdery mildew at 24 h post-treatment. These results are in harmony with Kamel *et al.* (2017) who mentioned that microscopic observations showed abnormal shape, shrinking and collapsing of conidia with twisting and plasmolysis of conidiophores and hyphae of *Sphaerotheca fuliginea* when they were treated with many dilutions of both raw milk and whey. Also, Crisp *et al.* (2006) found that application of fresh milk or whey on *Uncinula necator*, which causes the powdery mildew of grapevine, caused collapse of hyphae and conidia within 24 h post-treatment. They hypothesized that the observed collapse of hyphae and conidia was related to increased production of free radicals. This may be due to several milk properties such as chemical and/or physical barrier could be responsible for reducing the attachment of the fungus to the cucumber leaf surfaces in addition to direct suppression of the pathogen. Slightly basic pH of milk could be related to the development disturbance of the fungus.

## CONCLUSION

The authors recommend using cow milk and whey dilutions 40 and 50% under four sprays could be promising tools as potential alternatives to chemical fungicides against cucumber powdery mildew because of their efficacy, safety and environment eco-friendly in addition to, whey is less expensive because it considered as a waste of industrial milk process. In addition, they have a vital role and significantly effective against cucumber powdery mildew. So, we need more studies to better understand the mechanisms of cow milk and whey in controlling plant diseases.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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