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Impact of various concentrations of mozzarella cheese whey on vegetative growth, chemical composition, and anatomical structure of *Schinus molle* L.

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Using industrial waste in agriculture is a crucial management technique. Cheese whey (CW) is recognized as a significant functional ingredient due to its nutritional components. This study aims to assess the effects of irrigation with various concentrations of mozzarella cheese non-salty whey (MCNSW) and potable water (PW) on the saplings of *Schinus molle* L. The evaluation focuses on vegetative characteristics, chemical composition, and anatomical structures. The research was conducted in the experimental nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the seasons 2020/2021 and 2021/2022. Six treatments were used (MCNSW: PW, v: v) at concentrations of 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, and 0:1 (control). Vegetative characteristics were measured, and chemical composition and anatomical structure were analyzed. The results indicated that plants treated with MCNSW at 1:1 showed the greatest significant improvement, followed by 1:0.75, 1:0.50, 1:0.25, and 1:0, compared to the control plants in both seasons.

**Key words:** Industrial waste; Manufacturing effluent; Dairy waste.

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## **INTRODUCTION**

Water is a critical limiting factor in agriculture, particularly in arid and semi-arid regions (Ahmali et al., 2020). Egypt is recognized as a nation with constrained water resources. With the growing population and increasing scarcity of drinking water, exploring other sources to supply water for agricultural use has become essential. Industrial wastewater, particularly from untreated dairy processing facilities, presents an acceptable alternative, as it contains essential nutrients required by plants and satisfies their water requirements (Seaf Elnasr et al., 2017). Cheese whey is a byproduct of industrial dairy production and is recognized as a valuable functional ingredient due to its rich nutritional composition derived from cheese constituents. Mozzarella cheese whey contains many functional macro elements that contribute to favorable growth, especially nitrogen for vegetative growth, phosphorus for energy, magnesium for chlorophyll, and calcium for cell walls. This byproduct finds application in various industries. However, many dairy processing facilities often waste significant volumes of whey, either by discharging it into the ground or disposing of it alongside the sewage, primarily due to the high costs associated with its utilization. Generally, whey can be categorized into two primary types: sweet and acidic. Sweet whey is a byproduct of cheese manufacturing. Conversely, acid whey is derived from Greek-style yogurt, cottage cheese, and cream cheese (Abd Al-Razeq, 2019). Specifically, mozzarella cheese whey contains various compounds, including noncasein nitrogen, nonprotein nitrogen, total nitrogen, citrate, and lactate (Gernigon et al., 2009).

An estimated 139 billion kilograms of whey are produced globally yearly (Akay and Sert, 2020). In Egypt, approximately 107,891 tons of white cheese were produced by cheese factories in 2021 (Agriculture Directorates of Governorates, 2021). Schinus molle L. belongs to the Anacardiaceae family, commonly called the Brazilian pepper tree. This evergreen tree is characterized by its weeping foliage. Schinus molle L. was chosen as the experimental material for this study as it has various economic and environmental advantages, such as low-combustion wood. Its fruit, leaf extracts, and essential oils have been used as traditional medicines across the tropics. It thrives in various soil and climatic conditions and is commonly used in landscaping. Furthermore, S. molle has demonstrated analgesic, anti-inflammatory, and anti-tumour properties. Additionally, it exhibits significant antibacterial, antiviral, antifungal, and insecticidal properties. Even its limitation-being cultivated only sexually and from seed—is a reason for further study to achieve growth acceleration and promote earlier entry into the reproductive phase (Abou-Dahab et al., 2019).

This study aimed to evaluate the effects of using various concentrations of mozzarella cheese non-salty whey (MCNSW) on the growth, chemical composition, and anatomical structure of *Schinus molle* L. saplings compared to potable water (PW) as a control. This technique can save drinking water and protect the environment from contamination caused by the large volumes of wastewater discharged into sewage pipelines.

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# MATERIALS AND METHODS Set up Experiment

In continuity with a previous study on cheese whey types in Schinus molle (Aboudahab et al., 2017; Abou-Dahab et al., 2019), various concentrations were selected and applied at regular intervals between the concentrations used in the previous studies to determine the most preferable one. The Schinus molle L. plants were obtained with an average height of 26 cm, a diameter of 5.63 mm (measured 5 cm above the soil surface), and an average of 11 leaves. Saplings were transplanted at the beginning of March 2020 and 2021 for both seasons into plastic pots filled with sandy soil weighing 20 kg. The average maximum and minimum temperatures and humidity data recorded in Giza Governorate for the first season were 29 °C, 18 °C, and 55%, respectively, and for the second season, they were 28 °C, 17 °C, and 58%, respectively. The physical and chemical analyses of the soil for both seasons before transplantation are presented in Table

**Table 1.** Average parameters of the soil's physical and chemical analyses of the first (2020/2021) and second (2021/2022) seasons.

Parameter	Average
Physical characteristics	
Texture class	Sand
Sand (%)	97
Silt (%)	1.6
Clay (%)	1.4
рН	7.4
EC (dS/m)	4.16
CaCO₃	0.8
Organic matter (%)	0.5
Chemical characteristics	
Soluble Cations (meq/l)	
Ca <sup>++</sup>	6.2
Mg**	2.8
Na <sup>+</sup>	9.9
K <sup>+</sup>	0.3
Soluble Anions (meq/l)	
CO <sub>3</sub> -	-
HCO₃ <sup>-</sup>	4.8
Cl-	9.00
SO <sub>4</sub>	6.2
Available N (ppm)	0.07
Available P (ppm)	0.009

# Chemical Characterization of Mozzarella Cheese Non-Salty Whey and Treatment Concentration

S. molle L. saplings were subjected to irrigation using six concentrations of mozzarella cheese non-salty whey (MCNSW). MCNSW was used without prior filtration and was directly diluted with potable water (PW). The dilution ratios of the MCNSW to PW (volume: volume) were as follows: 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, and 0:1 (control [PW]). The MCNSW was obtained from the Dairy Technology Unit at the Faculty of Agriculture, Cairo University. All plants received regular irrigation with potable water twice weekly during the summer and once weekly during the winter for one month after transplantation. Manual irrigation (the plants were irrigated with a mix of MCNSW and tap water, which were manually prepared in the field) was conducted at the beginning of April during both seasons. The plants were treated for eleven months. Each concentration of MCNSW was applied using a beaker at a constant rate of 750 ml per pot across all treatments, including those irrigated with PW. The average chemical characteristics of MCNSW concentrations and PW during both seasons are shown in Table 2.

# Plant sampling, measurements, method of analytical and anatomical characters

After eleven months of treatment, plant samples were collected in early March for both seasons. Fifteen saplings from each treatment were randomly selected and harvested. The recorded vegetative characteristics include plant height (cm), number of leaves per plant, main stem diameter (mm), leaf area (cm<sup>2</sup>), fresh and dry weights of leaves (g/plant), and fresh and dry weights of the main stem (g/plant). Additionally, root characteristics were assessed, comprising main root length (cm/plant), number of primary roots per plant, and fresh and dry weights of roots (g/plant). Samples of leaves were dried in an electric oven (Memmert, Tv80 U1, Germany) at 70°C until a constant weight was achieved. The dried leaves were then ground into powder. Subsequent digestion of the leaf samples was performed following the procedure outlined by Piper (1947). The total elemental content was determined as follows: organic carbon (O.C%) as stated by Dean (1974), nitrogen (N%) using the Micro-Kjeldahl method, potassium (K%), magnesium (Mg%), and sulfur (S%) all determined according to AOAC (2000), phosphorus (P%) by the micro-vanadate-molybdate yellow method according to Chapman and Pratt (1961), and calcium (Ca%) as detailed by Isaac and Keber (1971).

**Table 2.** Average parameters of the chemical analyses of MCNSW and PW used for irrigating *Schinus molle* L. saplings during first (2020/2021) and second (2021/2022.) seasons.

	MCNSW concentrations						
Parameters	MCNSW (1:0)	MCNSW (1:0.25)	MCNSW (1:0.5)	MCNSW (1:0.75)	MCNSW (1:1)	PW (control) (0:1)	
рН	4.1	4.26	4.59	4.68	4.83	7.03	
EC (dS/m)	8.20	7.25	7.09	5.98	4.25	0.34	
SAR	5.92	5.20	4.98	4.78	4.47	1.81	
Soluble anion	s mg/l						
HCO <sub>3</sub> <sup>-</sup> + CO <sub>3</sub> <sup>-</sup>	15.94	12.65	11.52	9.54	8.15	1.45	
CL-	18.63	16.25	14.98	12.87	11.23	2.23	
SO <sub>4</sub> -	7.98	6.34	5.68	4.85	4.09	0.91	
Soluble cation	ns mg/l						
Ca++	22.00	18.32	14.54	13.08	10.56	1.03	
Mg <sup>++</sup>	4.02	3.97	3.32	3.11	2.98	0.97	
Na⁺	22.00	19.23	15.75	14.18	11.35	1.73	
K <sup>+</sup>	32.00	27.15	23.65	18.24	15.39	0.14	
Total N (%)	2.86	2.64	2.23	1.98	1.65	0.12	
Total P (%)	0.62	0.58	0.52	0.47	0.40	0.03	
Turbidity	68.00	56.00	43.00	37.00	29.00	0.20	
TSS (ppm)	1498	1334	1287	1124	1065	46	
TDS (ppm)	5248	4632	4038	3763	2637	199.32	
BOD (ppm)	49312	42987	37155	29674	22368	13	
COD (ppm)	65750	53987	46378	40178	37534	49	

PW= Potable water; MCNSW= Mozzarella cheese non-salty whey; SAR= sodium absorption ratio; TSS= Total suspended solids; TDS= Total dissolved solids; BOD= Biochemical oxygen demand; COD= Chemical oxygen demand

The levels of chlorophyll a and b in leaf samples (mg/g) by fresh weight were determined as follows:

Using 85% v/v acetone, fresh leaf samples (0.2 g) with trace quantities of silica quartz and Na<sub>2</sub>CO<sub>3</sub> were homogenized. After that, a glass funnel (G4) was used to filter the acetone extracted. The residue was repeatedly washed with acetone until the filtrate became colorless. A preset quantity of 25 milliliters of combined extract was prepared. spectrophotometer was used to extract a small amount (5 ml) of this extract at wavelengths of 660 and 640 nm (for chlorophylls a and b, respectively) to determine the pigment colorimetrically. As a standard blank, 85% of Acetone was used. Chlorophyll a and b were calculated using a spectrophotometer according to Lichtenthaler (1987) using the following equations: Chlorophyll  $a \text{ (mg/l)} = 9.784E_{660} - 0.99E_{640}$ Chlorophyll  $b \text{ (mg/I)} = 21.426E_{640} - 4.65E_{660}$ 

According to Dubois *et al.* (1956), the total carbohydrate content of dry leaf samples (%) was determined using a spectrophotometer. Anatomical characteristics: transverse sections were prepared in the second season on leaflet specimens (taken from the fifth compound leaf from the top of saplings), according to Nassar and El-Sahhar (1998). Before being embedded in paraffin wax (melting point 52–54°C), all specimens were killed and preserved for at least 48 hours in F.A.A. (10 ml formalin, 5 ml glacial acetic acid,

50 ml 95% alcohol, and 35 ml distilled water). They were then rinsed in 50% ethyl alcohol and dehydrated in a standard butyl alcohol series. Using a rotary microtome, transverse sections ( $20\,\mu\text{m}$  thick) were cut, and after double staining with Safranin and Fast Green sequentially, they were mounted in Canada balsam.

The anatomical characters recorded were: midrib thickness (M.Th.), main vascular bundle width (M.V.B.W.) and length (M.V.B.L.), xylem thickness (X.Th.), mean xylem vessel diameter (M.X.V.D.), phloem thickness (Ph.Th.), lamina thickness (L.Th.), upper epidermis thickness (Up.Epi.Th.), mesophyll tissue thickness (Meso.T.Th.), palisade tissue thickness (Pal.T.Th.), spongy tissue thickness (Spo.T.Th.), and lower epidermis thickness (La.Epi.Th.). The slides were examined using a micrometer eyepiece and a 10×10 stage with a 200 µm field of view. Photographs of transverse sections were taken using a Microscope Optika B-150 equipped with a Toupcam™ digital camera.

#### Statistical Analysis and Experimental Design

Each season was subjected to separate statistical analyses. A combined analysis could not be conducted. Since the soil mixture was the same in both seasons, the observed heterogeneity may be attributed to climatic factors such as temperature, humidity, etc. The study followed a randomized

complete block design (RCBD) with six treatments. Each treatment was performed in three replications, with six plants used in each replicate (108 plants per season). The recorded data were analyzed according to Snedecor and Cochran (1982) using the MSTAT-C (1989) program, with the least significant difference (L.S.D.) assessed at P < 0.05.

# RESULTS AND DISCUSSION Vegetative Characteristics

Tables 3 and 4 illustrate the vegetative and root characteristics of *S. molle* L. saplings as affected by various MCNSW doses during the 2020/2021 and 2021/2022 seasons. The results showed that MCNSW treatments had a greater influence on vegetative development than the control treatment (PW).

## **Plant Height**

The data from both seasons revealed that *Schinus molle* L. saplings irrigated with MCNSW at ratios of 1:1, 1:0.75, or 1:0.50 were significantly taller than those irrigated with MCNSW at 1:0.25 or 1:0, recording heights of 83.33, 75.78, 68.78, 57.39, and 50.52cm, respectively, in the first season, and 82.00, 72.78, 63.61, 46.22, and 42.33cm, respectively, in the second season, as compared to the control (60.78 and 54.05cm in the first and second seasons, respectively).

# **Number of Leaves/plants**

In the first season, a significant increase in the number of leaves per plant was recorded in plants treated with MCNSW at 1:1, followed by 1:0.75 and 1:0.50 (65.28, 53.11, and 42.55, respectively), while plants treated with MCNSW at 1:0.25 and 1:0 recorded the lowest number of leaves per plant (38.95 and 33.17, respectively) in comparison with PW-treated plants (39.06 leaves per plant). The same trend was observed in the second season, with values of 54.78, 47.28, 42.33, 32.67, and 28.06, respectively, compared to control (36.39 leaves per plant).

#### **Stem Diameter**

Irrigating *S. molle* saplings with MCNSW at a rate of 1:1 in both seasons resulted in a significant increase in stem diameter, followed by MCNSW at 1:0.75 and 1:0.50, with averages of 13.06, 11.59, and 10.57mm, respectively, in the first season and 12.18, 11.16, and 10.08mm, during the second season. The thinnest stem diameters were recorded in plants irrigated with MCNSW at 1:0.25 and 1:0, with averages of 8.90 and 7.98mm in the first season and 7.80 and 6.95mm in the second season, as compared to saplings irrigated

with PW (9.45 and 9.00mm in the first and second seasons, respectively).

#### **Leaf Area**

MCNSW at 1:1, 1:0.75, and 1:0.50 significantly enhanced the leaf area of plants, recording 56.17, 50.64, and 44.89 cm², respectively, in the first season and 54.09, 48.32, and 43.82 cm² in the second season. On the other hand, the leaf area significantly decreased when saplings were irrigated with MCNSW at 1:0.25 or 1:0, recording 35.50 and 33.85 cm² in the first season and 34.34 and 31.65 cm² in the second season, respectively, compared with the control saplings (38.61 cm² and 37.77 cm² in the first and second seasons, respectively).

# Leaves Fresh and Dry Weights (FW and DW)

In both growing seasons, irrigation of S. molle with MCNSW at a 1:1 ratio produced the highest fresh weight (FW) and dry weight (DW) of leaves, followed by the 1:0.75 and 1:0.50 treatments. In the first season, FW values were 80.73, 72.17, and 63.04 g/plant, while DW values reached 34.91, 32.05, and 29.01 g/plant, respectively. Similarly, in the second season, FW measurements were 76.44, 66.40, and 57.69 g/plant, with corresponding DW values of 34.48, 31.13, and 28.23 g/plant. Conversely, the lowest FW and DW values were observed in plants irrigated with MCNSW at 1:0.25 and 1:0 ratios. In the first season, the FW and DW values were 44.40 and 35.49 g/plant and 22.80 and 19.83 g/plant, respectively. In the second season, these values declined to 43.69 and 37.75 g/plant (FW) and 23.56 and 21.58 g/plant (DW). These results were significantly lower than those of the control plants, which recorded FW and DW values of 54.12 and 26.04 g/plant in the first season and 48.24 and 25.08 g/plant in the second season.

# Stem Fresh and Dry Weights (FW and DW)

During the first season, the heaviest stem fresh weights (FW) and dry weights (DW) were obtained from plants irrigated with MCNSW at 1:1, followed by 1:0.75 and 1:0.50 ratios, with FWs of 91.75, 81.35, and 73.32 g/plant and DWs of 40.58, 37.12, and 34.44 g/plant, respectively. The same trend was observed in the second season, with stem FWs of 105.76, 99.94, and 93.07 g/plant and DWs of 44.25, 42.31, and 40.02 g/plant in the same treatment. Conversely, the lowest stem FW and DW values in both seasons were recorded from plants treated with MCNSW at 1:0.25 and 1:0 ratios. In the first season, these treatments produced stem FWs of 53.54 and 44.98 g/plant and DWs of 27.84 and 24.99 g/plant.

**Table 3.** Impact of various concentrations of mozzarella cheese whey on vegetative characteristics of *Schinus molle* L. saplings during the first (2020/2021) and second (2021/2022) season.

	Vegetative characteristics									
Treatments	Plant	Number of	Stem	Leaf area	Leaves FW	Leaves DW	Stem FW	Stem DW		
	height (cm)	leaves/ plants	diameter (mm)	(cm <sup>2</sup> )	(g/plant)	(g/plant)	(g/plant)	(g/plant)		
	2020/2021									
MCNSW (1:0)	50.52	33.17	7.98	33.85	35.49	19.83	44.98	24.99		
MCNSW (1:0.25)	57.39	38.95	8.90	35.50	44.40	22.80	53.54	27.84		
MCNSW (1:0.50)	68.78	42.55	10.57	44.89	63.04	29.01	73.32	34.44		
MCNSW (1:0.75)	75.78	53.11	11.59	50.64	72.17	32.05	81.35	37.12		
MCNSW (1:1)	83.33	65.28	13.06	56.17	80.73	34.91	91.75	40.58		
PW (control) (0:1)	60.78	39.06	9.45	38.61	54.13	26.04	63.10	31.03		
LSD <sub>0.05</sub>	2.02	4.53	0.45	1.24	1.55	0.52	1.51	0.50		
			2021/20	)22						
MCNSW (1:0)	42.33	28.06	6.95	31.65	37.75	21.58	65.46	30.82		
MCNSW (1:0.25)	46.22	32.67	7.80	34.34	43.69	23.56	75.73	34.24		
MCNSW (1:0.50)	63.61	42.33	10.08	43.82	57.69	28.23	93.07	40.02		
MCNSW (1:0.75)	72.78	47.28	11.16	48.32	66.40	31.13	99.94	42.31		
MCNSW (1:1)	82.00	54.78	12.18	54.09	76.44	34.48	105.76	44.25		
PW (control) (0:1)	54.05	36.39	9.00	37.77	48.24	25.08	83.71	36.90		
LSD <sub>0.05</sub>	2.03	1.08	0.29	0.91	1.48	0.49	1.83	0.61		

PW= Potable water; MCNSW=Mozzarella cheese non-salty whey

**Table 4.** Impact of various concentrations of mozzarella cheese whey on root characteristics of *Schinus molle* L. saplings during the first (2020/2021) and second (2021/2022) season.

	Root characteristics								
Treatments	Main root length (cm/plant)	Number of main roots/plants	Roots fresh weight (g/plant)	Roots dry weight (g/plant)					
2020/2021									
MCNSW (1:0)	15.50	9.61	31.92	19.64					
MCNSW (1:0.25)	17.78	11.33	41.95	22.98					
MCNSW (1:0.50)	22.11	13.33	61.54	29.51					
MCNSW (1:0.75)	24.50	14.06	71.15	32.71					
MCNSW (1:1)	30.00	16.72	81.00	36.00					
PW (control) (0:1)	19.17	12.50	47.71	24.90					
LSD <sub>0.05</sub>	0.82	0.82	2.27	0.76					
		2021/2022							
MCNSW (1:0)	13.12	7.06	23.35	15.78					
MCNSW (1:0.25)	15.42	9.22	28.25	17.41					
MCNSW (1:0.50)	18.00	12.94	43.02	22.33					
MCNSW (1:0.75)	21.11	15.11	52.09	25.36					
MCNSW (1:1)	24.39	17.11	62.34	28.78					
PW (control) (0:1)	16.42	10.78	33.06	19.02					
LSD <sub>0.05</sub>	0.64	0.43	1.55	0.52					

PW= Potable water; MCNSW=Mozzarella cheese non-salty whey

In the second season, stem FWs decreased to 75.73 and 65.46 g/plant, with corresponding DWs of 34.24 and 30.82 g/plant. All treatment groups showed significant differences compared to control plants, which yielded stem FWs and DWs of 63.10 and 31.03 g/plant in the first season and 83.71 and 36.90 g/plant in the second season.

# Root Characteristics Main Root Length (cm/plant)

MCNSW at the rate of 1:1 followed by MCNSW MCNSW, at a 1:1 ratio, produced *S. molle* saplings with the longest roots (30.00 cm/plant), followed by the

1:0.75 (24.50 cm/plant) and 1:0.50 (22.11 cm/plant) treatments in the first season. This trend continued in the second season, with 24.39, 21.11, and 18.00 cm/plant root lengths for the same treatments The shortest root lengths were observed in plants irrigated with MCNSW at 1:0.25 (17.78 cm/plant in the first season and 15.42 cm/plant in the second season) and 1:0 (15.50 cm/plant in the first season and 13.12 cm/plant in the second season). These measurements were significantly shorter than control saplings, measuring 19.17 cm/plant in the first season and 16.42 cm/plant in the second.

# **Number of Main Roots/plants**

MCNSW concentrations significantly affected the number of main roots in saplings. The highest root numbers were observed in plants treated with MCNSW at 1:1 (16.72 roots/plant), 1:0.75 (14.06 roots/plant), and 1:0.50 (13.33 roots/plant) during the first season. This trend continued in the second season, with root numbers of 17.11, 15.11, and 12.94 roots/plant for the respective treatments. All treatment groups showed significantly higher root numbers than control plants, which produced 12.50 and 10.78 roots/plant in the first and second seasons, respectively. Conversely, the lowest root numbers were recorded in saplings treated with MCNSW at 1:0.25 (11.33 and 9.22 roots/plant) and 1:0 (9.61 and 7.06 roots/plant) during the first and second seasons, respectively.

## Fresh and Dry Weights of Roots (FW and DW)

Root fresh weights (FW) and dry weights (DW) of *S. molle* were significantly enhanced by MCNSW at 1:1, 1:0.75, and 1:0.50 ratios in both growing seasons. In the first season, root FWs reached 81.00, 71.15, and 61.54 g/plant, corresponding DWs of 36.00, 32.71, and 29.51 g/plant for these treatments. The second season showed a similar trend, with FWs of 62.34, 52.09, and 43.02 g/plant and DWs of 28.78, 25.36, and 22.33 g/plant for the respective treatments.

Conversely, MCNSW at 1:0.25 and 1:0 ratios resulted in significantly lower root biomass. First-season measurements showed FWs of 41.95 and 31.92 g/plant and DWs of 22.98 and 19.64 g/plant for these treatments. In the second season, these values declined to FWs of 28.25 and 23.35 g/plant and DWs of 17.41 and 15.78 g/plant. All treatment effects were significant compared to control plants, which produced root FWs and DWs of 47.71 and 24.90 g/plant in the first season and 33.06 and 19.02 g/plant in the second season.

The observed variations in vegetative and root characteristics of S. molle saplings were primarily influenced by MCNSW dilution levels. Optimal dilutions (1:1-1:0.50) improved plant chemical properties, enhancing aerial and root growth. In contrast, undiluted (1:0) and low-dilution (1:0.25) treatments reduced vegetative growth due to elemental toxicity from excessive nutrient concentrations, which impaired absorption and plant development (Aboudahab et al., 2017). The benefits of diluted MCNSW align with findings from organic dairy fertilizers. Expired dairy product powder (EDPP) enhances plant growth by improving soil water retention and nutrient (N, P, K) availability (Eissa *et al.*, 2018). Suspended particles in dairy wastewater can improve soil properties (Abou-Dahab *et al.*, 2019), while dairy waste fertilizers increase soil organic matter by 38% compared to inorganic alternatives, significantly boosting wheat growth and yield (Alharbi *et al.*, 2021). However, undiluted applications may cause soil compaction under acidic conditions and inhibit nutrient-degrading microbes (Sirmacekic *et al.*, 2022). The reduced growth in 1:0 and 1:0.25 treatments likely reflect pH sensitivity, as lower pH conditions impair nutrient uptake (La Torre *et al.*, 2023). Supporting evidence includes:

- Superior wheat growth with EDPP versus inorganic fertilization (Eissa *et al.*, 2018).
- Enhanced soil fertility and crop productivity through enzymatically hydrolyzed dairy waste (Gil et al., 2024)
- Successful dairy wastewater reuse for lettuce and cucumber germination (Lucio *et al.*, 2024).

However, our results contrast with Prazeres *et al.* (2014), who found no effect of cheese whey wastewater salinity (1.75-10.02 dSm<sup>-1</sup>) on tomato cultivars. While initial soil pH (7.4) favored nutrient uptake, prolonged MCNSW application may lead to nutrient accumulation, increasing osmotic pressure and ultimately restricting water uptake and growth. This highlights the importance of optimal dilution and application duration using dairy-based fertilizers.

# Chemical Composition of *S. molle* leaves

Data in Table 5 shows the effect of using MCNSW concentrations as an irrigation source on chemical composition of *S. molle* as compared to PW (control) during both seasons.

#### Total Elements Content of S. molle

MCNSW irrigation treatments at 1:0, 1:0.25, 1:0.50, 1:0.75, and 1:1 significantly increased O.C.% contents of *S. molle*, recording 4.18, 3.91, 3.67, 3.46, and 3.19%, respectively, in the first season. In the second season, O.C. contents were 4.30, 3.93, 3.73, 3.37, and 3.21%, respectively, compared to control plants (1.42 and 1.37% in the first and second seasons, respectively). MCNSW treatments showed higher N% values at 1:0, 1:0.25, 1:0.50, 1:0.75, and 1:1 with percentages of 2.35, 2.24, 2.14, 2.04, and 1.84%, respectively, in the first season compared to PW (1.22%). The second season showed similar results, recording 2.36, 2.27, 2.14, 2.04, and 1.83%, respectively, compared to the control (1.31%).

MCNSW at 1:0 in both seasons gave the highest P% content (0.32% first season; 0.33% second season), followed by 1:0.25 (0.27% both seasons), 1:0.50 (0.23% first season; 0.22% second season), 1:0.75 (0.18% first season; 0.17% second season), and 1:1 (0.12% first season; 0.13% second season), compared to PW (0.05%) and tap water (0.05%) controls. Higher K% values were obtained with MCNSW treatments at 1:0 (3.39% first season; 3.42% second season), 1:0.25 (3.23%; 3.18%), 1:0.50 (3.02%; 2.94%), 1:0.75 (2.79%; 2.80%), and 1:1 (2.59%; 2.55%), compared to control plants (1.16 and 1.15% in first and second seasons, respectively).

Total Ca% content was higher in MCNSW-treated plants at 1:0 (11.37% first season; 11.30% second season), 1:0.25 (11.17%; 11.14%), 1:0.50 (10.83%; 10.81%), 1:0.75 (10.58%; 10.64%), and 1:1 (10.46%; 10.41%), compared to controls (1.12% first season; 1.14% second season).

MCNSW treatments at 1:0 (1.47% first season; 1.49% second season), 1:0.25 (1.34% both seasons), 1:0.50 (1.24%; 1.26%), 1:0.75 (1.14%; 1.18%), and 1:1 (0.92%; 0.99%) showed higher Mg% contents than controls (0.42% first season; 0.40% second season). Higher S% contents were recorded with MCNSW at 1:0 (0.64% first season; 0.71% second season), 1:0.25 (0.53%; 0.60%), 1:0.50 (0.44%; 0.42%), 1:0.75 (0.34%; 0.35%), and 1:1 (0.23%; 0.27%), compared to controls (0.18% first season; 0.22% second season).

# **Chlorophyll Content**

In the first season, the highest content of chlorophyll a was found in plants irrigated with MCNSW at the rate of 1:1 (0.71mg/g) followed by 1:0.75 (0.62mg/g) and 1:0.50 (0.57mg/g). On the other hand, the lowest content of chlorophyll a was obtained from saplings treated with MCNSW at 1:0.25 (0.47mg/g) and 1:0 (0.38 mg/g) as compared to the control plants (0.51mg/g). In the second season, the highest content of chlorophyll a was also shown in saplings irrigated with MCNSW at the rate of 1:1 and 1:0.75 with values of 0.75 and 0.64mg/g, respectively and was insignificant with MCNSW at 1:0.50 (0.0.57mg/g). In contrast, the lowest insignificant content of chlorophyll a was shown in plants irrigated with MCNSW at 1:0.25 (0.52mg/g) and significant in plants treated with MCNSW at 1:0 (0.46mg/g) as compared to the control plants (0.54 mg/g). Chlorophyll b content was significantly increased by treating plants with MCNSW at 1:1, 1:0.75, or 1:50, recording 0.99, 0.88, and 0.79mg/g, respectively, in the first season. The same trend was observed in the second season with values of 1.05, 0.91, and 0.82 mg/g, respectively. On the contrary, irrigating *S. molle* with MCNSW at 1:0.25 gave the lowest significant content of chlorophyll b, followed by MCNSW at 1:0 with values of 0.62 and 0.52 mg/g in the first season and 0.61mg/g and 0.54mg/g, respectively in the second one as compared to untreated plants (0.70 and 0.0.69mg/g, in the first and second seasons, respectively).

# **Total carbohydrates content**

In the first season, irrigating S. molle with MCNSW at 1:1 gave the highest significant content of total carbohydrates, followed by MCNSW at 1:0.75 and 1:0.50 with values of 37.10%, 32.92%, and 28.57%, respectively. On the other hand, plants irrigated with MCNSW at 1:0.25 and 1:0 recorded the lowest insignificant concentrations of total carbohydrates with values of 25.69% and 23.96%, respectively, compared to untreated plants (27.06%). The same trend was observed in the second season, with the highest total carbohydrate contents of 37.95%, 33.10%, and 28.05% for MCNSW at 1:1, 1:0.75, and 1:0.50, respectively, and the lowest concentrations of 25.69% and 23.96% for MCNSW at 1:0.25 and 1:0, respectively, as compared to PW (26.45%). The high nutrients (N, P, K, and Ca) and total carbohydrate content in the CW, in addition to the presence of milk and lactose, which remain in the CW and constitute the main fraction of organic load accumulation under non-saline conditions, have a significant role in plant growth, development, and differentiation throughout the plant life cycle (Abou-Dahab et al., 2019). Whey waste products, which are rich in nutrients, can be utilized in fertilization procedures, particularly as a nitrogen source, according to Akay and Sert (2020). Due to the low pH in MCNSW at 1:0 and 1:0.25 treatments, the plant had difficulty absorbing the elements and nutrients present in the soil irrigated with MCNSW, as the plant may be sensitive to low pH levels, according to La Torre et al. (2023). The results agreed with Eissa et al. (2018), who examined the effects of expired dairy powder (EDDP) on the growth and chemical composition of wheat (Triticum aestivum) plants. The findings demonstrated that, compared to inorganic fertilization (IF), treating plants with EDDP addition increased total chlorophyll and boosted N, P, and K uptake.

**Table 5.** Impact of various concentrations of mozzarella cheese whey on chemical composition, pigments content and total carbohydrates of *Schinus molle* L. saplings during the first (2020/2021) and second (2021/2022) seasons.

Treatments		Total elements content (%)							ontent (mg/g)	Total carbohydrates (%)	
rreauments	O.C	N	Р	K	Ca	Mg	S	Chl a	Chl b	rotal carbonydrates (%)	
2020/2021											
MCNSW (1:0)	4.18	2.35	0.32	3.39	11.37	1.47	0.64	0.38	0.52	23.96	
MCNSW (1:0.25)	3.91	2.24	0.27	3.23	11.17	1.34	0.53	0.47	0.62	25.69	
MCNSW (1:0.50)	3.67	2.14	0.23	3.02	10.83	1.24	0.44	0.57	0.79	28.57	
MCNSW (1:0.75)	3.46	2.04	0.18	2.79	10.58	1.14	0.34	0.62	0.88	32.92	
MCNSW (1:1)	3.19	1.84	0.12	2.59	10.46	0.92	0.23	0.71	0.99	37.10	
PW (control) (0:1)	1.42	1.22	0.05	1.16	1.12	0.42	0.18	0.51	0.70	27.06	
LSD <sub>0.05</sub>	0.08	0.06	0.06	0.10	0.12	0.06	0.06	0.12	0.08	3.76	
					2	2021/20	22				
MCNSW (1:0)	4.30	2.36	0.33	3.42	11.30	1.49	0.71	0.46	0.54	24.61	
MCNSW (1:0.25)	3.93	2.27	0.27	3.18	11.14	1.34	0.60	0.52	0.61	25.34	
MCNSW (1:0.50)	3.73	2.14	0.22	2.94	10.81	1.26	0.42	0.57	0.82	28.05	
MCNSW (1:0.75)	3.37	2.04	0.17	2.80	10.64	1.18	0.35	0.64	0.91	33.10	
MCNSW (1:1)	3.21	1.83	0.13	2.55	10.41	0.99	0.27	0.75	1.05	37.95	
PW (control) (0:1)	1.37	1.31	0.05	1.15	1.14	0.40	0.22	0.54	0.69	26.45	
LSD <sub>0.05</sub>	0.10	0.08	0.06	0.38	0.10	0.08	0.06	0.08	0.06	2.72	

PW= Potable water; MCNSW=Mozzarella cheese non-salty whey

Hajihashemi et al. (2020) studied the effect of different wastewater dilution levels (0%, 25%, 50%, and 100%), and the results indicated that irrigation of two wheat cultivars, Chamran and Behrang, with wastewater significantly reduced photosynthetic parameters like chlorophyll fluorescence, water usage efficiency, and photosynthetic pigments. The loss of photosynthesis was followed by a considerable fall in carbohydrate content. Gorfie et al. (2022) investigated the effect of brewery wastewater on the chemical constituents of the lettuce (Lactuca sativa) crop. Irrigation with 100% wastewater enhanced the nutrient composition of lettuce leaves. La Torre et al. (2023) studied the effect of using sweet whey at 4%, 8%, 12%, 16%, and 20% and drinking water as a control on alfalfa (Medicago sativa L.) and corn (Zea mays L.). The results indicate that diluted whey can safely be used for sustainable agriculture

#### **Anatomical Characters**

Data of the effect of Mozzarella cheese non-salty Increasing the proportion of water in the MCNSW treatments markedly enhanced most anatomical features of *Schinus molle* leaflets. Plants irrigated with MCNSW at 1:1 or 1:0.75 demonstrated significant improvements in leaflet structure and internal tissue anatomy. The highest midvein thickness values were recorded with MCNSW at 1:0.75 (576.00  $\mu m$ ) and 1:1 (568.00  $\mu m$ ). These enhancements were associated with notable increases in the dimensions of the main vascular bundle (length and width) and vascular tissue thickness (xylem and phloem). Specifically, treatment with MCNSW at 1:1 increased midvein thickness, vascular bundle length, xylem thickness, and phloem thickness by 45.32%, 32.87%, 90.22%, and 97.19%,

respectively, compared to the control. The 1:0.75 treatment also showed similar increases of 56.63%, 9.93%, 89.85%, and 87.57%, respectively. The widest xylem vessel diameters were observed under 1:1 and 1:0.75 treatments, recording 14.31 µm and 12.02 µm, respectively. Moreover, plants irrigated with MCNSW at 1:1 and 1:0.75 exhibited notable increases in leaflet lamina thickness (58.82% and 14.33%), upper epidermis thickness (9.69% and 7.51%), lower epidermis thickness (14.04% and 12.20%), mesophyll tissue thickness (69.22% and 18.49%), palisade tissue thickness (35.47% and 6.79%), and spongy tissue thickness (89.42% and 37.16% respectively. In contrast, irrigation with MCNSW at 1:0.50 also promoted specific anatomical improvements compared to the control, although enhancements were less pronounced than those at higher water dilutions.

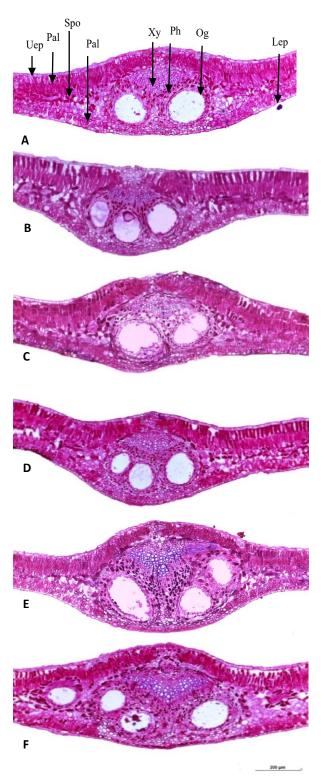
The highest anatomical increments compared with the control were observed in leaflets treated with MCNSW at a 1:1 ratio, recording increases of 35.33%, 12.04%, 3.64%, 7.06%, 18.72%, 5.06%, 11.07%, and 55.77% for xylem and phloem tissue thickness, lamina thickness, upper and lower epidermis thickness, mesophyll tissue thickness, palisade tissue thickness, and xylem vessel diameter, respectively. In contrast, treatment with undiluted MCNSW (1:0) resulted in the lowest anatomical measurements among all S. molle leaflet parameters. These included reductions in midvein thickness (7.55%), main vascular bundle length (32.33%), main vascular bundle width (15.72%), xylem tissue thickness (7.44%), phloem tissue thickness (37.86%), and average xylem vessel diameter (23.48%) when compared with control.

Furthermore, undiluted MCNSW caused notable reductions in lamina characteristics, including leaflet lamina thickness (19.61%), upper and lower (35.00% epidermis thickness and 34.31%, respectively), mesophyll tissue thickness (30.76%), palisade tissue thickness (8.61%), and spongy tissue thickness (26.64%). A similar pattern of decline was also recorded with the 1:0.25 MCNSW treatment, though to a lesser extent. In contrast, MCNSW treatments with higher water content (1:1 and 1:0.75) exhibited marked improvements in all measured anatomical traits of S. molle leaflets compared to the control and other treatments.

The stimulatory effects of each treatment on the anatomical features of S. molle leaflets may enhance plant growth by improving soil's physiological properties and micronutrient availability for plant growth (Dawood et al., 2014). Also, it could be a result of cheese whey's role in determining nutrients, carbohydrates, protein patterns, and water status (Haroun et al., 2003). In this study, a gradual reduction in leaflet structure was observed with decreasing potable water percentage added to MCNSW compared with untreated controls. The highest reduction was recorded in MCNSW at a 1:0 ratio. The deterioration in leaflet measurements may be related to nutrient imbalance, disturbed absorption, or nutrient toxicity from the chloride, calcium, sodium, phosphorus and potassium elements. These results are compatible with the findings of Abou-Dahab et al. (2019), who suggested that cheese whey (CW) may have an adverse effect on soil and ornamental plants due to high salt accumulation, mainly sodium and chloride. Also, this reduction may be attributed to the reduction in vascular tissue thickness (xylem and phloem), palisades, and spongy tissues. Thus, it affects the transfer of water and salts from the soil and photosynthates from the leaf to the rest of the plants and disrupts photosynthesis processes, causing a decrease in plant growth and anatomical parameters.

### **CONCLUSION**

The data demonstrate that using mozzarella cheese non-salty whey (MCNSW) (an industrial effluent) has a noticeable and substantial impact on plants irrigated with its various concentrations of 1:1, 1:0.75, 1:0.50, 1:0.25, and 1:0. Vegetative and root characteristics, total elements, pigment content, total carbohydrates, and anatomical structure were positively affected compared to plants irrigated with tap water in both seasons.



**Figure 1.** Transverse sections through the middle portion of *Schinus molle* L. leaflets as treated cheese whey types during 2021/2022. A: plants irrigated with PW (control) (0:1) B: plants irrigated with MCNSW (1:0) C: MCNSW (1:0.25) D: MCNSW (1:0.50) E: MCNSW (1:0.75) F: MCNSW (1:1) Details: Uep, Upper epidermis; Pal, palisade tissue; Spo, Spongy tissue; Xy, xylem tissue; Ph, Phloem; Og, oil glands; Lep, Lower epidermis.

A		PW				
Anatomical characters	MCNSW	MCNSW	MCNSW	MCNSW	MCNSW	(control)
Characters	(1:0)	(1:0.25)	(1:0.50)	(1:0.75)	(1:1)	(0:1)
M.Th.	412.12	418.16	451.03	576.00	568.00	445.78
M.V.B.L.	132.52	156.40	172.17	270.72	251.17	172.84
M.V.B.W.	251.91	219.58	250.10	328.55	397.11	298.88
X.Th.	50.61	53.78	74.00	103.81	104.01	54.68
M.X.V.D	5.90	9.62	12.01	12.02	14.31	7.71
Ph.Th.	22.35	36.68	40.30	67.47	70.93	35.97
L.Th.	180.29	219.89	232.44	256.40	356.18	224.27
Up.Epi.Th.	10.12	16.05	16.67	16.74	17.08	15.57
Meso.T.Th.	133.67	199.60	202.83	228.76	326.67	193.05
Pal.T.Th.	108.43	139.37	131.79	126.71	160.74	118.65
Spo.T.Th.	54.58	60.21	71.04	102.05	140.93	74.40
La Fni Th	7 16	10.26	12 94	12 23	12 43	10 90

**Table 6.** Impact of various concentrations of mozzarella cheese non-salty whey on anatomical characters of *Schinus molle* L. leaflets during second season (2021/2022).

PW= Potable water; MCNSW= Mozzarella cheese non-salty whey; M.Th.= Midrib thickness; M.V.B.L. Main vascular bundle length; M.V.B.W. Main vascular bundle width; X.Th.= Xylem thickness; M.X.V.D.=Mean xylem vessels diameters; Ph.Th.= Phloem thickness; L.Th.= Lamina thickness; Up.Epi.Th.= Upper epidermis thickness; Meso.T.Th.= Mesophyll tissue thickness; Pal.Th.Th.= Palaside tissue thickness; Spo.T.Th.= Spongy tissue thickness; La.Epi.Th.= Lower epidermis thickness.

This technique can be effective for growing plants using nutrient-rich manufacturing waste while preserving the environment from contamination caused by massive amounts of dairy waste. Since this study was conducted in pots, in an enclosed space with minimal drainage, the system was more prone to nutrient accumulation approaching toxic concentrations. This condition differs from open fields, where the natural rise of groundwater may dilute nutrients and then be washed away by irrigation and drainage.

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