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Eco-friendly management of pepper powdery mildew in Libya by farm animals whey milk, the inducer resistance chemical salicylic acid and Trichoderma harzianum

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المكافحة الصديقة للبيئة لمرض البياض الدقيقي في الفلفل في ليبيا باستخدام مصل حليب حيوانات المزرعة، وحمض الساليسيليك كمادة كيميائية محفزة للمقاومة، وفطر *Trichoderma harzianum*.

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Abstract:

Isolation procedures from phylloplane of pepper leaves showing low infection by *Leveillula taurica,*the causal of powdery mildew yielded many fungal isolates. The isolates of *Trichoderma* strains were selected, purified and identified as: *T. album, T.hamatum, T.koningii,T.harzianum* and *T.viride.* Significant inhibition to the germinated conidia of *L. taurica,* the causal fungus of pepper powdery mildew, *in vitro* was occurred by farm animals' whey milk *i.e.,* camel, cow, goat and sheep compared with the control. Camel′s whey milk was the most efficient one in reducing the germinated conidia of the causal fungus followed by sheep then goat. The tested non-volatile substances of *Trichoderma* strains *i.e., T. album, T.hamatum, T.koningii, T.harzianum* and *T.viride*, also, resulted in significant reduction to the germinated conidia of the causal pathogen in comparison with the control. This reduction was gradually increased significantly by increasing the tested concentration of both whey milk and non-volatile substances of *Trichoderma* strains. Moreover, spraying pepper plants with camel's whey milk, the IRC salicylic acid and the non-volatile substances of the bioagent *T.harzianum*, each alone or in different combinations, caused significant reduction to the severity of the disease with significant increase to the number and weight of the produced pod yield plant^{-1} in comparison with the infected control by the causal fungus. Furthermore, spraying any of these compounds alone was of less effect in this regard compared with spraying their combinations. However, the fungicide Byleton was the superior one in this concern, followed by the combination of the three items of disease management. The estimated photosynthesis pigments *i.e.,* chlorophyll–a, chlorophyll–b and carotenoids, in addition to phenolic compounds, where they increased significantly in pepper leaves due to spraying camel´s whey milk, the IRC salicylic acid and non-volatile substances of the *T. harzianum* compared with the leaves of un-treated and infected plants.

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Keywords: Farm animals, disease management, pepper, photosynthesis pigments, phenolic compounds, powdery mildew, salicylic acid, whey milk.

الملخص

أسفرت إجراءات العزل من سطح أوراق (phylloplane) نبات الفلفل التي أظهرت إصابة خفيفة بفطر *Leveillula taurica*، المسبب لمرض البياض الدقيقي، عن الحصول على العديد من العزلات الفطرية. تم اختيار عزلات من سلالات فطر *Trichoderma*، وتنقيتها، وتصنيفها إلى*T. album* :، و*T. hamatum*، و *T. koningii*، و*T. harzianum*، و *T. viride*.وأظهرت النتائج حدوث تثبيط معنوي لإنبات جراثيم (conidia) فطر *L. taurica*(المسبب لمرض البياض الدقيقي في الفلفل) مخبرياً (*in vitro*) باستخدام مصل (شرش) حليب حيوانات المزرعة، وهي الإبل والأبقار والماعز والأغنام، مقارنة بمعاملة المقارنة (الكنترول). وكان مصل حليب الإبل الأكثر كفاءة في تقليل إنبات جراثيم الفطر المسبب، يليه مصل حليب الأغنام ثم الماعز. كما أدت المواد غير المتطايرة المستخلصة من سلالات *Trichoderma*المذكورة أعلاه إلى خفض معنوي في إنبات جراثيم الفطر الممرض مقارنة بمعاملة المقارنة، حيث ازداد هذا الانخفاض تدريجياً وبشكل معنوي مع زيادة تركيز كل من مصل الحليب والمواد غير المتطايرة لسلالات *Trichoderma.*علاوة على ذلك، أدى رش نباتات الفلفل بمصل حليب الإبل، وحمض الساليسيليك(IRC) ، والمواد غير المتطايرة للعامل الحيوي *T. harzianum*، سواء كل منها على حدة أو بتراكيب مختلفة، إلى انخفاض معنوي في شدة المرض، مع زيادة معنوية في عدد ووزن القرون الناتجة للنبات الواحد مقارنة بالنباتات المصابة وغير المعالجة. كما تبين أن رش أي من هذه المركبات منفرداً كان أقل تأثيراً مقارنة برشها مجتمعة، في حين تفوق مبيد الفطريات "بايلتون (Byleton) "في هذا الصدد، يليه المزيج المكون من العناصر الثلاثة لمكافحة المرض. وأظهرت التقديرات لأصباغ التمثيل الضوئي (الكلوروفيل أ، الكلوروفيل ب، والكاروتينات)، بالإضافة إلى المركبات الفينولية، زيادة معنوية في أوراق الفلفل نتيجة الرش بمصل حليب الإبل، وحمض الساليسيليك، والمواد غير المتطايرة لفطر *T. harzianum*، مقارنة بأوراق النباتات المصابة وغير المعالجة.

الكلمات المفتاحية: حيوانات المزرعة، مكافحة الأمراض، الفلفل، أصباغ التمثيل الضوئي، المركبات الفينولية، البياض الدقيقي، حمض الساليسيليك، مصل الحليب.

1. Introduction

Pepper (Capsicum annum L.) powdery mildew is a highly destructive foliar disease caused by the obligate fungal pathogen Leveillula taurica. It is characterized by patchy, white or brownish powdery growth on the undersides of leaves, often accompanied by yellowing and premature leaf drop. Powdery mildew of pepper is a common fungal disease affecting pepper plants in Libya (Pernezny et al., 2003; Mohamed et al., 2018; Jiménez-Pérez et al., 2024). Recent studies have highlighted that L. taurica is a global challenge for pepper production, affecting a vast range of hosts (Massire et al., 2025).

The use of green and environmentally friendly technologies to control plant diseases has become a research hotspot, especially for vegetables such as pepper (Begum et al., 2019). Furthermore, the characterization of chemical composition and biological activity of various plant extracts has shown significant potential in combating pathogens (Salem & Lakwani, 2024; Kadak & Salem, 2020).

Regarding biological control, the genus Trichoderma is recognized for its ability to act as a biocontrol agent against plant pathogens (Harman, 2007). The isolated Trichoderma strains have been frequently used for the biological management of many plant foliage diseases (Barakat et al., 2014; Xue et al., 2021; Wang et al., 2021; Kredics et al., 2024; Guzmán-Guzmán et al., 2025). The mechanisms of action include mycoparasitism, antibiosis, and competition for resources (Barakat et al., 2014; Tian, 2025).

In addition to biological agents, the phytochemical profiling of plant extracts has provided new insights into their antimicrobial potential (Alshawish et al., 2025). Biochemical studies on alcoholic and aqueous extracts have also demonstrated efficacy in inhibiting antibiotic-resistant bacteria

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(Khalil et al., 2025). Specifically, the antimicrobial activity of plant extracts, such as Dandelion (Taraxacum officinale) and Lichen (Usnea barbata), against Escherichia coli and Staphylococcus aureus has been documented (Salem et al., 2025; Salem, 2024). Moreover, the quantification of bioactive compounds in essential oils, such as those in Sinapis alba, confirms their bactericidal kinetics against clinically relevant pathogens (Soof et al., 2025).

Regarding the physiological impact, infected plants exhibit reduced rates of photosynthesis (Moharam, 2013; Prokopová et al., 2010; Tian et al., 2024). Furthermore, phenolic compounds play a vital role in the natural disease resistance of plants against powdery mildew (Kanday et al., 2024). The isolation and identification of pathogenic bacteria in clinical settings in Libya further underscore the necessity for continued research into antimicrobial polymers and their mechanisms in combating antibiotic resistance (Ben Hsin et al., 2025; Salem & Salem, 2025).

Pepper is liable to infection by many bacterial, fungal and viral diseases as well as physiological disorder. However, fungal diseases, especially powdery mildew disease caused by *L. taurica* is one of the most important diseases that infect pepper, which causes harmful effect to the plant growth and the produced pod yield (Pernezny *et al*., 2003 ; Mohamed *et al.,* 2018 and Jiménez-Pérez , 2018 *et al.,* 2024). Powdery mildew of peppers can develop over a wide range of temperatures in low or high humidity conditions. Due to its wide host range, it can be difficult to control with cultural practices (Pernezny *et al*., 2003).

There is a direct correlation between the percentage of powdery mildew infection of the leaves and yield loss, where one percent mildew infection on the leaves would result in a one percent yield loss or more. The heavy infection by powdery mildew had about 50% loss of production compared to a later, lighter infection (Sudha and Lakshmanan, 2009 and Arici and Özkaya,2022).

Powdery mildew disease, attributed to *Leveillula taurica* (Lév.) Arnaud (Anamorph: *Oidiopsis taurica* (Lév.) Salmon), holds substantial significance for chili cultivation. This cosmopolitan phytopathogenic fungus affects more than 1000 plant species, including members of Compositae and Leguminosae. However, the most crucial host crops belong to Solanaceae (Guigón-López and González-González, 2001 and Cerkauskas *et al.,*2011).

Pepper powdery mildew does not infect the fruit. Under favorable conditions, the fungus reproduces rapidly and spores can germinate and infect a plant in less than 24 hours. Wind-disseminated spores cause secondary infections, which help spread the disease. The disease is most severe on older leaves, but it can occur at any time throughout the season if environmental conditions are favorable. Severe infections early in the season can result in heavy yield losses (Arici and Özkaya, 2022).

Symptoms on pepper plants are manifested through the presence of mycelium and conidiophores emerging through stomata on the underside of leaves, forming whitish powdery spots. On the upper side, these spots exhibit a yellowish to brown coloration, progressing from lower to upper parts of the plants. As the disease advances, symptoms intensify, leading to premature defoliation and adversely affecting crop production (Zheng *et al.,*2013).

With the continuous improvement in people's living standards, food safety problems caused by pesticides and fertilizers have received widespread attention. Therefore, the search for green and environmentally friendly technologies to control plant diseases has become one of the research hotspots of environmental scientists and plant pathologists, especially for vegetables such as pepper (Begum *et al.,* 2019).

Whey is the yellow or clear liquid that separates from milk after it curdles. It consists mostly of water (93% to 94%), while the remaining percentage contains a variety of nutrients. The basic components common to the whey of all animals are: Water: representing the largest percentage (approximately 93-94%). Lactose: milk sugar, constituting about 4-5%. Whey proteins: constituting about 0.6-1% and including alpha-lactoalbumin, immunoglobulins, lactoferrin, and serum albumin. Vitamins and minerals: such as calcium, potassium, phosphorus, sodium, and B vitamins (Liu *et al.,* 2021 and Mirzakulova *et al.,* 2025).

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This work was planned to evaluate the inhibitory effect of farm animals and *Tricoderma* strains *in vitro* on the germination of *L.taurica* the causal of pepper powdery mildew. Also, studying the role of camel′s whey milk, the inducer resistance chemical salicylic acid and non-volatile substances of *T.harzianum* strains, each alone or in different combinations on managing the disease under greenhouse conditions. Finally, biochemical studies *i.e.,* Photosynthsis pigments and phenolic compounds content in pepper leaf tissues of plant treated and un-treated by the tested materials and inoculated with the causal of the disease were also estimated.

2. Materials and Methods

2.1 Isolation, purification and preparation of non-volatile substances of *Trichoderma* strains:

Depending on the procedure of Mohamed (2001), fungal microflora naturally grown on pepper phylloplane (leaf surface) were isolated from healthy leaves, using dilution plate technique. Isolates belonging to *Trichoderma* spp. were chosen and serial dilution plate technique was used to isolate native antagonistic *Trichoderma* spp. isolated on solid soil extract medium. All the isolated *Trichoderma* spp. were picked-up, purified by hyphal tip technique and then identified on the basis of their cultural and morphological features using the description of Rifai (1969) and Bissett (1991).
Trichoderma strains *i.e., T. album, T.hamatum, T.koningii, T.harzianum* and *T.viride* were grown in sterilized gliotoxin medium (Weindling,1934) for 10 days and filtrate through Whatman filter paper (No.4), then sterilized using 0.25 µm syringe filter. The concentrations of 20, 40 and 50 % were prepared using distilled sterile water, each in sterile (100 ml)sterilized glass conical.

2.2. Effect of the tested farm animals' whey milk and non-volatile substances of *Trichoderma* strains on the conidial germination*:*

Freshly collected conidia by sterilized brush from the freshly naturally infected pepper leaves (Hybrid Lilac cv.) by powdery mildew were put in each concentration *i.e.,* 10, 25 and 50 % (using distilled sterile water) of the tested farm animals whey milk *i.e.,* camel, cown, goat and sheep. The non-volatile substances of *Trichoderma* strains *i.e., T. album, T.hamatum, T.koningii, T.harzianum* and *T.viride* were prepared at 10 , 25 and 50% % (using distilled sterile water). Have m1. of the conidial suspension of any of the tested *Trichoderma* strains was placed on two sterilized slides, borne on two glass rods in a sterilized Petri -dish containing a piece of cotton wetted by sterile distilled water to provide high relative humidity. The same was carried out for a spore suspension put in distilled sterile water only as control treatment. The preparations were incubated in an incubator at 25±1 ˚C for 24 hour. Five replications were prepared for each treatment. At the time of slide examination, one drop from lacto-phenolic cotton blue stain was added to fix, kill and stain the germinated conidia. The germinated conidia were calculated in five random microscopic fields and the mean percentages of conidial germination of each treatment was calculated and recorded. Also, the efficacy percentages of these materials on reducing the germinated conidia of the causal pathogen were, also, estimated and recorded.

2.3. Effect of the tested camel´s whey milk, the IRC salicylic acid and non-volatile substances of *T. harzianum* on managing the disease:

Greenhouse experiment was carried out at Azzaytuna University, Libya. Two pepper transplants (Hybrid Lilac cv.) of 25 days old grown in foam trays contained disinfested soil (peat moss + clay+ sand + vermiculite) were transplanted in each plastic pot (30 cm. in diameter) contained disinfested silt soil by 5 % formalin.

The grown plants were divided into two groups and firstly sprayed by sterile water, just before the inoculation by the pathogen. The first group was sprayed by the tested treatment one week before the

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artificial inoculation with the pathogen tested (protective treatment) and the second group was sprayed one week after the artificial inoculation with the pathogen tested (curative treatment). The artificially inoculation was done by shaking of the freshly naturally infected pepper leaves on the grown plants of one months after transplanting.
The prepared camil´s whey milk at 50 %, the IRC salicylic acid at 10 mM and non-volatile substances of *T.harzianum* at 50% , were sprayed , each alone or in different combination ,onto the upper and the lower leaf surfaces of pepper plants (Hybrid Lilac cv,) one month after transplanting until run off. Plants inoculated with the causal fungus only were served as check treatment (control). Plants sprayed with the Byleton fungicide (Tubaconazole) at the concentration of 20 ml 100 L^{-1} were used for comparison. The plants were re-sprayed by the previous treatments 2,4, 6 and 8 weeks after the first spray. Five pots were used for each treatment. The harvested fruits were calculated and weighed each harvest and the averages of No. of pods and their weight for each plant were recorded.

2.4. Disease assessment:

Plants were examined periodically and disease measures were determined using the devised scale (0-5) adopted by Horsfall and Barratt (1945) , where 0 = no symptoms appeared ; 1 = 0.1 to 5% of leaf area covered by the infection ; 2 = more than 5 to 20 % of leaf area covered by the infection ; 2 = more than 20 to 50% of leaf area covered by the infection; 4 = more than 50 to 75% of leaf area covered by the infection; 5 = more than 75% of the leaves of the plant growth covered and burned. Disease symptoms were recorded to estimate the severity of the disease and the final averages were recorded using the following formula:

$$\text{Disease severity \%} = \frac{\Sigma\,(n x v)}{5\,N} \text{ X } 100$$

Where: n = number of infected leaves in each category,
v = numerical values of each category,
N = total number of the infected leaves and
5 = Highest numerical value achievable.

2.5. Values of photosynthetic pigments of pepper plants (Hybrid Lilac cv.) inoculated with *L.taurica* and sprayed with camel´s whey milk, the IRC salicylic acid and non-volatile substances of *T. harzianum*:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids (mg g^{-1} fresh leaves weight) were spectro-photometrically estimated using the method escribed by Metzner *et al.* (1965). Twenty gram fresh weight of pepper leaves (Hybrid Lilac cv.), were taken randomly from five plants represent the categories of the disease. The samples were homogenized in 85% aqueous acetone for 10 min, the centrifuged (10.000 rpm for 10 minutes), and the supernatant was adjusted to 200 ml with 85% acetone and measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. Dilutions were used to determine the concentrations of the pigment fractions. The formula of Arnon (1949) was used to estimate the content of chlorophyll a, chlorophyll b and carotenoids .

2.6. Estimation of total phenolic compounds:

According to the procedure of Zieslin and Ben-Zaken (1993), five g of pepper leaf samples were taken randomly from five plants represent the categories of the disease of the plants treated with the tested treatments of disease management. Each sample was extracted with 10 ml of 80% methanol at 70 °C

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for 15 min. Reaction mixture was containing 1 ml of methanol extracts, 5 ml of distilled sterile water, and 250 µl of Folin–Ciocalteau reagent (1N). This solution was kept at 25±1°C. The absorbance of the developed blue color was measured using a spectrophotometer at 725 nm. Gallic acid was used as the standard. The amount of phenolic compounds was expressed as mg gallic acid g^{-1} leaf sample. The assessment was done one, two and three months after inoculation by *L.taurica.*

2.6. Statistical analysis:
Using the procedures of Snedecor and Cochran (1989), the obtained results were statistically analyzed using the standard procedures for complete randomized block and split designs . Least significant differences (L.S.D) were used to compare data averages at 0.05 level according to Fisher (1924).

3. Results

3.1. Isolation, purification and identification of *Trichoderma* strains:

Isolation procedures from phylloplane of pepper leaves showing low infection by *L.taurica,* the causal of powdery mildew, yielded many fungal isolates. The isolates of *Trichoderma* strains were selected, purified and identified as : . *T. album, T.hamatum, T.koningii, T.harzianum* and *T.viride*

3.2. Effect of different concentrations of farm animals whey milk on conidial germination of *L.taurica*:

Table (1) reveals that significant reduction was occurred to the germinated conidial spores of *L.taurica* due to using any of the farm animals whey milk *i.e.,* camel, cow, goat and sheep, being 27.0, 31.5, 30.3 and 29.6% conidial germination, on the average, respectively. Camel′s whey milk was the most efficient one (70.9 % efficacy) , and the conidial spores failed to germinate at 50% followed by sheep′s whey milk then goat′s whey milk, being 70.9, 68.4 and 67.6% efficacy, on the average. respectively. Control treatment recorded 93.6% conidial germination. on the average. So, camel′s whey milk was used in managing the disease under the greenhouse experiment.

Table 1. Effect of different concentrations of farm animals whey milk on conidial germination of *L.taurica*, 24 hours after incubation at 25±1ºC.

Farm animals whey milk	Average percentages of sporangial germination at conc. (			Mean	% Efficacy
	10	25	50		
Camel	60.6	20.4	0.0	27.0	70.9
Cow	63.0	25.2	6.2	31.5	66.4
Goat	62.0	23.6	5.4	30.3	67.6
Sheep	61.4	22.8	4.6	29.6	68.4
Control **	93.6	93.6	93.6	93.6	-----
Mean	61.8	23.0	4.0	----	-----

* Initial germination percentage was 2.2%.
** Control not calculated in the mean and the initial germination percentage was 1.6%.
L.S.D. at 5 % for : Farm animals (F)= 2.4,Concentrations (C)=2.7 and Fx C = 3.3.

3.3. Effect of the tested non-volatile substances of *Trichoderma* strains on conidial germination of *L.taurica* :

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Data presented in Table (2) indicate that all non-volatile substances of the tested *Trichoderma* strains, *i.e. T. album, T.hamatum, T.koningii, T.harzianum* and *T.viride* caused significant reduction to the germinated conidia of *L.taurica* in comparison with control treatment. Significant reduction to the percentages of the germinated conidia of the causal pathogen was gradually increased by increasing the tested concentration . No germination was occurred at 50% by *T.harzianum* and *T.viride*. In addition, *T.harzianum* was the most efficient one in this regard, being 33.5 % conidial germination, on the average and 64.4% efficacy followed by *T.viride*, being 34.1% conidial germination, on the average and 63.8 % efficacy. Meanwhile, *T.album*, *T.hamatum* and *T.koningii* were the lowest effective ones, being 48.1, 46.9 and 44.9% conidial germination, on the average with 48.9, 50.2 and 52.3% efficacy, respectively. Control treatment recorded 94.2% conidial germination, on the average. Therefore, non-volatile culture filtrate of the fungus *T.harzianum* was used in managing the disease under the greenhouse experiment.

Table 2. Effect of the non-volatile substances (culture filtrate) of the tested *Trichoderma* strains on conidial germination of *L.taurica*, 24 hours after incubation at 25±1ºC.

Trichoderma strains	Average percentage of sporangial germination at conc. (%)			Mean	% Efficacy
	10	25	50		
T.album	70.4	58.0	15.8	48.1	48.9
T.hamatam	68.6	56.4	15.6	46.9	50.2
T.koningii	67.0	55.6	12.2	44.9	52.3
T.harzianum	63.8	36.6	0.0	33.5	64.4
T.viride	64.2	38.2	0.0	34.1	63.8
Control *	94.2	94.2	94.2	94.2	-----
Mean	66.8	49.0	8.7	-----	----

* Initial germination percentage was 1.8%.

** Control not calculated in the mean and the initial germination percentage was 1.6%.

L.S.D. at 5 % for : Trichoderma strains (T)= 2.3,Concentrations (C)=2.9 and Tx C = 3.7.

3.4. Effect of the tested camel′s whey milk , the IRC salicylic acid and non-volatile substances of *T.harzianum* on managing the disease under greenhouse conditions :

Spraying pepper plants with the tested camel′s whey milk (50%), the IRC salicylic acid (10 mM) and non-volatile substances of *T.harzianum* (50%), each alone or in different combinations, exerted significant reduction in the severity of the disease compared with control treatment (Table 3). Moreover, spraying any of the tested materials alone was of low efficiency in this respect than spraying their different combinations. Spraying these materials as curative treatment against the disease was significant in reducing the severity of the disease than curative treatment, being 7.7 and 9.3 % disease severity, on the average, respectively. The highest reduction in the disease severity was obtained by the combination among the three materials (camel′s whey milk, salicylic acid and non-volatile substances of the bioagent *T.harzianum*), being 5.2 % disease severity, on the average (92.6 %efficacy) nearby to the effect of the systemic fungicide Byleton, being 3.9 % disease severity , on the average (94.7 %efficacy). Control plants recorded 63.8% disease severity.

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Table 3. Effect of spraying pepper plants (Hybrid Lilac cv.) with camel′s whey milk, the IRC salicylic acid and non-volatile substances of *T.harzianum* , each alone or in different combinations, on the severity of powdery mildew , greenhouse experiment.

Treatments	% Severity of the disease after spraying the tested materials as		Mean	% Efficacy
	Protective treatment	Curative treatment		
Camel′s whey milk (CWM)	12.0	15.6	13.8	79.2
Salicylic acid (SA)	9.8	12.4	11.1	83.9
T.harzianum(TH)	12.5	15.8	14.2	78.5
CWM+SA	6.8	9.1	8.0	88.2
CWM+TH	6.2	9.0	7.6	88.9
SA+TH	7.1	10.2	8.7	87.2
CWM+SA+TH	4.1	6.3	5.2	92.6
Byleton	3.0	4.8	2.5	94.7
Control (Infected) *	63.8	63.8	63.8	-----
Mean	7.7	9.3	-----	-----

* The values of control treatment not calculated in the assessed mean.

L.S.D. at 0.05 for:

Treatments(T)= 3.3 , Severity of the disease (S)= 1.3 ,

T x S = 3.1 .

3.4. Effect of the tested camel′s whey milk, the IRC salicylic acid and non-volatile substances of *T.harzianum* on the produced green pods and their weight under greenhouse conditions:

Data (Table 4) show that pepper plants (Hybrid Lilac cv.) sprayed with the tested camel´s whey milk, the IRC salicylic acid and non-volatile substances of the bioagent *T.harzianum* , each alone or in different combinations, caused significant increase to the produced green pods and their weight compared with the infected control treatment. In addition, spraying any of these materials alone was of low efficiency in this regard than spraying their different combinations. In addition, protective treatments by the tested materials against pepper powdery mildew showed significant decrease in the number and weight of pods yield plant $^{-1}$ compared with curative treatments, being 27.9 and 24.1 pod plant^{-1} and 416.3 and 385.2 g. plant^{-1}, on the average, respectively. The highest number and weight of the produced pods yield was obtained by the combination among the three treatments (camel´s whey milk, the IRC salicylic acid and *T.harzianum*), being 36.0 pod plant^{-1} and 477.3 g. plant^{-1}, respectively nearby to the produced pod yield from spraying the plants by the systemic fungicide Byleton , being 36.9 pod plant^{-1} and 516.1 g. plant^{-1}, on the average .The infected plants by powdery mildew and un-received any treatments recorded the lowest values of the produced green pods and their weight, being 10.6 pod and 220.0 g plant^{-1}, on the average, respectively.

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Table 4. Effect of spraying pepper plants (Hybrid Lilac cv.) with camel′s whey milk, the IRC salicylic acid and non-volatile substances of *T.harzianum,* each alone or in different combinations, as well as infected plants,, on the average No. of the produced green pods and their weight (g) plant^{-1} under the infection by powdery mildew, greenhouse experiments.

Treatments	Average No. of the produced green pods plant^{-1} under the treatment as		Mean	Average weight of the produced green pods (g) plant^{-1} under the treatment as		Mean
	Protective	Curative		Protective	Curative	
Camel′s whey milk (CWM)	19.8	17.0	18.4	344.6	315.2	329.9
Salicylic acid (SA)	17.0	13.8	15.4	342.7	300.5	321.6
T.harzianum(TH)	20.2	17.8	19.0	366.3	340.8	353.6
CWM+SA	28.0	22.0	30.0	390.0	364.5	377.3
CWM+TH	29.0	23.0	26.0	4001.0	380.0	390.5
SA+TH	34.0	31.0	32.5	460.7	416.8	438.8
CWM+SA+TH	36.2	33.2	36.0	496.3	458.3	477.3
Byleton	38.8	35.0	36.9	528.4	505.7	516.1
Mean	27.9	24.1	26.0	416.3	385.2	81.6
Control (Infected)*	10.6	10.6	10.6	220.0	220.0	230.0
L.S.D. at 0.05	Treatments(T)= 3.1 , No. of pods (N)= 1.5 , T x N = 3.9		------	Treatments(T) = 4.7 , Weight of pods (W)= 4.2, T x W = 5.5		-----

* The values of both controls not calculated in the mean.

3.5. Values of photosynthetic pigments in pepper plants (Hybrid Lilac cv.) inoculated with *L.taurica* and sprayed with camel´s whey milk, the IRC salicylic acid and non-volatile substances of *T. harzianum*:

The estimated photosynthesis pigments *i.e.,* chlorophyll –a, chlorophyll –b and carotenoids were increased significantly in pepper leaves (Hybrid Lilac cv.) due to spraying any of camel´s whey milk, the IRC salicylic acid and non-volatile substances of *T. harzianum* compared with the leaves of un-treated infected plants (Table 5). No significant differences among photosynthesis pigments values of the three items of disease management were detected. The highest values of photosynthesis pigments were found in case of plants sprayed with camel´s whey milk, being 1.52, 1.30 and 0.86 mg g^{-1} fresh leaves weight, on the average, respectively. On the contrary, the lowest values of photosynthesis pigments were found in case of untreated infected plants, being 1.26, 1.08 and 0.75 mg g^{-1} fresh leaves weight, on the average, respectively.

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Table 5. Effect of spraying artificially inoculated pepper plants (Hybrid Lilac cv.) by the causal pathogen of powdery mildew with camel′s whey milk, salicylic acid and non-volatile substances of *T.harzianum*, each alone or in different combinations, as well as infected plants on photosynthesis pigments (chlorophyll-a, chlorophyll-b and carotenoids).

Treatments	Photosynthesis pigments (mg g^{-1} fresh leaves weight)		
	Chlorophyll -a	Chlorophyll –b	Carotenoids
Camel′s whey milk (CWM)	1.52	1.30	0.86
Salicylic acid (SA)	1.44	1.23	0.82
T.harzianum(TH)	1.44	1.23	0.82
Byleton	1.46	1.25	0.83
Control (Infected)	1.26	1.08	0.75
L.S.D. at 0.05	0.11	0.13	0.06

3.6. Effect of camel′s whey milk, salicylic acid, culture filtrate of *T.harzianum*, pathogen and non-pathogen treatment on the content of pepper leaves (Hybrid Lilac cv.) from phenolic compounds under artificial inoculation by the causal pathogen of powdery mildew:

Table (6) reveals that the tested camel′s whey milk, salicylic acid and non-volatile substances of *T.harzianum* resulted in significant increase in the content of phenolic compounds in the leaves of pepper plants (Hybrid Lilac cv.) with low variation in their values compared with the two controls. In addition, the infected plants (without any treatments) recorded the highest figure of total phenolic compounds, being 11.49 mg g plant-1 fresh leaves followed by salicylic acid, being 11.25 mg g plant^{-1} fresh leaves, on the average then by Byleton fungicide (9.30 mg g plant-1 fresh leaves). Both camel′s whey milk and non-volatile substances of *T.harzianum* recorded , to somewhat, similar values, being 8.88 and 8.91 mg g plant^{-1} fresh leaves, on the average , respectively.

Table 6. Effect of camel′s whey milk, salicylic acid, culture filtrate of *T.harzianum* and pathogen treatment on the content of pepper leaves from phenolic compounds under artificial inoculation of pepper by the causal pathogen of powdery mildew

Treatments	Gallic acid in mg g^{-1} fresh leaves weight after (months)				Mean
	Zero time	One	Two	Three	
Camel′s whey milk (CWM)	5.45	8.06	10.78	11.18	8.88
Salicylic acid (SA)	5.45	9.72	13.86	15.95	11.25
T.harzianum(TH)	5.45	8.10	10.82	11.25	8.91
Byleton	5.45	8.86	11.12	12.91	9.30
Control (Infected)	5.45	9.95	14.20	16.34	11.49
L.S.D. at 0.05	n.s.	2.10	2.16	2.15	----

4. DISCUSSION

Pepper powdery mildew, caused by the obligate fungal pathogen *Leveillula taurica* (asexual stage: *Oidiopsis taurica* (Lév.) Salmon 1906, synonym: *Oidiopsis sicula* Scalia 1902), poses a significant threat to pepper (*Capsicum* spp.) cultivation worldwide. The fungus and its asexual

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stage *Oidiopsis taurica* (Lév.) Salmon 1906 affects a variety of crops, including peppers, tomatoes, eggplants, cucumbers, onions, fennels, legumes, sunflowers, cottons, and ornamental plants like nasturtiums and geraniums, as well as weeds and trees, making it a critical threat to global agriculture (Massire *et al*., 2025).
Isolation procedures from phylloplane of pepper leaves showing low infection by *L.taurica,* the causal of powdery mildew yielded many fungal isolates. The isolates of *Trichoderma* strains were selected, purified and identified as : *T. album, T.hamatum, T.koningii, T.harzianum* and *T.viride* .The isolated *Trichoderma* strains were frequently used for biological management of many plant foliage diseases (Barakat *et al*., 2014; Xue *et al*., 2021; Wang *et al.,*2021; Kredics *et al.,*2024 and Guzmán-Guzmán *et al*., 2025) .
The tested camel´s skim milk. IRC salicylic acid and non-volatile substances of *T.harzianum* caused significant reduction to conidial germination of *L.taurica*, the causal of pepper powdery mildew, compared with control treatment. Significant increase in this reduction was gradually increased the increasing the tested concentration. In addition, spraying of pepper plants with camel´s whey milk, the IRC salicylic acid and non-volatile substances of the bioagent *T.harzianum*, each alone or in different combinations, resulted in significant reduction to the severity of the disease with significant increase to the produced green pod and their weight in comparison with the infected control. Furthermore, spraying any of these compounds was of less efficacy in this regard compared with spraying of their combinations. However, the fungicide Byleton was the superior in this regard followed by the mixture of the camel´s whey milk, IRC salicylic acid, and non-volatile substances of *T.harzianum*. Bademiyya and Ashtaputre (2019) mentioned that the infection by pepper powdery mildew causes great reduction in the growth and the produced pods yield.
Chemical control is highly recommended because powdery mildew is an aggressive and destructive disease and satisfactory control without the use of fungicides is unlikely. The role of fungicides in reducing the disease is well known (Mc Grath, 2001 and Malav *et al*., 2024). So, due to the great hazard on the human health due to the residue of agrochemicals in the consume food ,fungicides become unlikely to use . Therefore, great efforts by agro-scientists are spend to search about alternative safely methods to management plant pests. In this respect, this work aimed to evaluate spraying pepper plants with camel´s whey milk, the IRC salicylic acid and the non-volatile substances of the bioagent *T.harzianum*, each alone or in different combinations, on management of pepper powdery mildew.
Production of healthy and safe food free from toxic substances is the desire of the consumers especially that consume freshly like pepper. Therefore, to avoid the use of hazard agrochemicals against diseases, certain protective or curative procedures could be conducted using different non-chemical methods to management such diseases. In this regard, camel´s whey milk, the inducer resistance chemical salicylic acid and non-volatile substances of *T.harzianum* were evaluated for their efficacy in management of pepper powdery mildew and caused desirable management to pepper downy mildew. However in most cases, using such non-traditional management methods did not give adequate results when used alone. Therefore, these methods are preverbal to be used as a mixture .
Harman (2007) reported that genus *Trichoderma* has been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens. Until recently, the principal mechanisms for control have been assumed to be those primarily acting upon the pathogens and included mycoparasitism, antibiosis, and competition for resources and space.

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The studies of mycoparasitism, also have demonstrated that genus *Trichoderma* produces a rich mixture of antifungal enzymes, including chitinases and β-1,3 glucanases. These enzymes are synergistic with each other, with other antifungal enzymes, and with other materials (Barakat *et al.,*2014 and Tian, 2025).

Durrant and Dong (2004) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection. The systemic acquired resistance response is dependent on the plant hormone, salicylic acid..

Bettiol (1999) mentioned that numerous small studies from around the world have validated the use of whey / or milk as spray on powdery mildew on a wide range of plants. Most recently, a spray made of 40% milk and 60% water was effective as chemical fungicides in managing powdery mildew of pumpkins and cucumbers grown in mildew areas. Like other fungicides, milk sprays work best when used preventatively, before the disease can gain a foothold. He added that it does not matter if the milk you use is skim or whole because it is the protein rather than the milk fat that is working on your behalf.

Dairy products of farm milk and/or whey are highly effective natural fungicides against powdery mildew. The proteins, amino acids, and salts in whey milk act as antiseptics, while the lactic acid can alter the pH on the leaf surface to inhibit spore germination. Milk and whey are powerful natural fungicides. The proteins, amino acids, and salts in whey act as antiseptics, and lactic acid alters the leaf surface pH to inhibit spore germination. Research shows these mixtures are often 50–94% as effective as commercial fungicides. Several mechanisms for milk and/or whey are emphasized i.e., eradicate and prevent powdery mildew as: 1. Antiseptic Properties: Proteins like lactoferrin and amino acids in milk/whey release free radicals when exposed to sunlight, causing fungal hyphae to collapse.2. pH Alteration: Lactic acid changes the leaf, creating an environment that inhibits fungal spore germination and damages conidia and Nutrient competition: The sugars and proteins in whey also encourage the growth of beneficial, harmless microbes on the leaf surface that outcompete the powdery mildew fungus (Crisp *et al.,*2006 and Kamel and Afifi, 2020, Liu *et al.,* 2021 and Mirzakulova *et al.,* 2025).

The estimated photosynthesis pigments *i.e.,* Chlorophyll –a, Chlorophyll –b and carotenoids were increased significantly in pepper leaves due to spraying camel´s whey milk, the IRC salicylic acid and non-volatile substances of *T. harzianum* compared with the leaves of un-treated infected plants.

Moharam (2013) mentioned that plants infected with biotrophic fungal pathogens, including powdery mildew, exhibit reduced rate of photosynthesis. It seems that powdery mildew can inhibit the photosynthetic processes by various mechanisms, including lower supply of light energy due to covering of the leaf surface by mycelium and inhibition of CO_2 influx due to stomata closure. Powdery mildew can also affect photosynthesis indirectly, via pathogen-induced changes in source-sink relations and nutrient remobilization towards infection sites This can result in the inhibition of the Calvin cycle and in the inhibition of photosynthetic light reactions in thylakoid membranes (Prokopováet al., 2010 and Tian *et al.,* 2024).

It has been found that camel′s whey milk, salicylic acid, *T.harzianum* resulted in significant increase in the phenolic compounds content in the leaves of pepper plants with low variation in their values compared with the two controls. In addition, the infected plants (without any treatments the highest figure of total phenolic compounds followed by Byleton fungicides. Both camel′s whey milk and

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T.harzianum recorded, to somewhat, similar values and the lowest value of the phenolic compounds content was found in the leaves of the un-infected control.
The tested items of disease management resulted in significant increase in the phenolic compounds content in the leaves of pepper plants with low variation in their values compared with the two controls. In addition, salicylic acid was the most efficient one in this regard followed by Byleton fungicide. Both camel′s whey milk and *T.harzianum* recorded , to somewhat, similar values. Higher accumulation of phenolic compounds was, also noticed in plants pretreated with salicylic acid and able to enhance the resistance against invasion of the pathogens to the plants (Vimala and Suriachandraselvan, 2008 and Kanday *et al.,* 2024)

Phenolic compounds act as a vital chemical defense mechanism in plants. When attacked by a pathogen, plants significantly increase the production of these secondary metabolites. Phenolic compounds content are compounds whose quantity raised when a plant comes under attack by a pathogen (van Peer *et al.,*1991, Waterman and Mole ,1995 and Abada *et al., ,* 2018). They added that pathogen alone was able to induce phenolic formation in plants but with slightly increased levels.

Phenolic compounds are produced by the plants mainly for their growth, development, and protection. These aromatic benzene ring compounds are very much essential during the plant's biotic and abiotic stress interactions. They constitute an essential part of plant's secondary metabolites and play a vital role in various physiological and mechanical activities. These diverse plant phenolic compounds act both as attractants and repellents toward various organisms in the environment. They could act as attractants toward the beneficial organisms and as toxicants against the invading pests and pathogens. These metabolite compounds often enhance during a plethora of stress conditions and act as the first line of defense to provide plant disease resistance. While the presence of a pathogen alone can trigger some phenolic formation, the levels produced are generally only slightly elevated without a targeted defense response. Phenolic compounds serves as a strong indicator of plant defense mechanisms, playing a crucial role in disease resistance and pathogen inhibition (Pratyusha, 2022).

Phenolic compounds play a vital role in the natural disease resistance of plants against powdery mildew. When the plants attacked by fungi, resistant varieties naturally accumulate higher concentrations of these secondary metabolites to form physical and chemical barriers against infection. Also, it was noticed that phenolic compounds and resistance to powdery mildew were positively correlated (Kanday *et al.,* 2024).

5. Conclusion

Since plants cannot "cure" existing powdery mildew once established, the goal is to trigger Systemic Acquired Resistance (SAR) before or immediately at the onset of disease. Also, beneficial soil and foliar fungi such as *T.harzianum* can trigger metabolic pathways that boost phenol and flavonoid contents in the plant's aerial parts.
Since pepper is harvested (green pods) weekly, the use of pesticides would be unsafe and inappropriate, as there would be residues of these pesticides in the pepper pods, which is unacceptable as it has a harmful effect on human health. In addition, camel, cow, goat, and sheep milk whey is available in the local environment and inexpensive because it is a by-product of these animals, it can be used to powdery mildew in pepper to an acceptable degree to produce pepper pods free of pesticide residues and therefore safe for human consumption. . Based on the present investigation a new strategy must be developed for managing pepper powdery mildew *in vivo*.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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