THE EFFICACY OF MILK FOR MANAGING POWDERY MILDEW IN HORTICULTURAL CROPS

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ABSTRACT

This study was conducted to investigate the efficacy of diluted milk in managing powdery mildew (*Oidium* sp.) in tomato (*Solanum lycopersicum*). Six greenhouse experiments were conducted, the results showed that milk, when applied to tomato plants weekly at a concentration of 0.1 was as effective as higher concentrations of milk and the fungicide control, Ecocarb® (3 g L-1). The efficacy of milk was improved by increasing the treatment frequency when milk was applied at concentration of 0.1 and 0.05. However, sunlight had no effect on the efficacy of milk to control powdery mildew when milk was applied biweekly at 0.05 concentrations to shaded tomatoes and tomatoes under ambient sunlight. Milk had protective and curative antifungal properties when milk 0.05 was sprayed before and after disease onset.

INTRODUCTION

Powdery mildew is a host-specific fungal disease that affects important economic crops, causing significant yield loss worldwide. In horticultural crops, for example, the genera of fungi causing powdery mildew include *Oidium* spp. in tomato, *Erysiphe polygoni* in pea and *Podosphaera xanthii* (previously known as *Sphaerotheca fuliginea*) in watermelon (1). Powdery mildew can cause severe damage to all aerial parts of infected plants. Fungal mycelium is clearly visible and appears as patches or spots of white or grey on the surface of the leaves, stems, buds, flowers and fruit of infected plants (17).

In conventional horticulture, management of powdery mildew relies mainly on the intensive use of sulfur and synthetic fungicides. The application of synthetic fungicides can lead to the development of fungicide resistance and phytotoxicity, and it poses a risk to the environment (15). Sulfur application can provide an acceptable control of powdery mildew but may cause undesirable side effects, including foliage burn of sensitive crops Gevens and Vallad (12). Sulfur also poses a risk to the health of agricultural workers (8). Moreover, there are concerns regarding the negative impact of sulfur on beneficial microorganisms and arthropods (19). There is a demand for the use of alternative, environmentally safe methods to avoid the undesirable effects of fungicides. A number of such options have been investigated for managing powdery mildew on various crops, including the use of inorganic salts such as soluble silicon (23), potassium silicate (K2SiO3) (25), potassium bicarbonate (available commercially as Ecocarb®) (5, 6, 14), plant extracts (26) and compost extracts (22).

The application of milk-based fungicides, including milk and whey, has been investigated for control of powdery mildew in fruit and vegetables such as strawberries (5, 6, 18), apples (24), zucchini (2), pumpkin (10) and cucumber (3), grains such as wheat (9) and ornamental crops such as roses (4). Milk and its various fractions (whey, lactoferrin, fat, lactose and anhydrous milk fat) at different concentrations and frequencies have been applied, though the results have been variable.

Whilst these studies have identified milk-based treatments as potential alternatives to sulfur and synthetic fungicides for the control of powdery mildew, there are different explanations for the mode of action of milk. Milk consists of

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amino acids, fat, proteins and salts (such as oxalate, sodium bicarbonate and tribasic or dibasic potassium phosphate), which induce systemic resistance to pathogens, contribute to antifungal activity and induce biological control Bettiol (2). Medeiros *et al*. (16) found high populations of bacteria, fungi and yeast on milk-treated leaves of zucchini squash compared with control plants (water and fungicide treatments). These microorganisms might be involved in the powdery mildew control as a result of the milk application. As the mode of action of milk is not well understood, complementary studies are warranted to identify these actions to obtain a better understanding for the mode of action of milk in relation to powdery mildew suppression. Milk has been suggested as an environmentally friendly option to control powdery mildew disease.

The objectives of this study were to investigate the efficacy and the optimum concentration of milk to control powdery mildew on tomatoes, peas and watermelons in order to select a model for horticultural crops. Tomato was then selected as a model for horticultural crop to examine the optimum milk-application frequency to control powdery mildew, the effect of sunlight on the efficacy of milk.

MATERIALS AND METHODS

Greenhouse experiments on the efficacy of milk for controlling powdery mildew Seeds of tomato, cv. Grosse Lisse (*Solanum lycopersicum*), pea, cv. Dwarf Peas (*Pisum sativum*) and watermelon, cv. Sugar Baby (*Citrullus lantatus*) (Yates, Australia) were sown in small containers of cocopeat potting mix, with several seeds per pot and then reduced to one plant per pot. All experimental seedlings were established in a powdery mildew-free greenhouse at 20–25ºC. The greenhouse was kept free of powdery mildew by using a sulfur burner (Smoltz garden supply, Adelaide). Growth was maintained throughout the experimental period by exposing plants to natural light in the greenhouse, which is located at the University of Adelaide Waite Campus in Urrbrae, South Australia.

A randomized block design was used for all greenhouse experiments. Between 3 and 4 weeks after germination, the plants were transferred to 15-cm diameter pots and they were moved to a greenhouse containing powdery mildew-affected plants. Plants were infected with powdery mildew naturally within the greenhouse. Healthy plants were inoculated by dusting infected leaves with conidia of powdery mildew and then maintained in a greenhouse as a source of inoculum. Sticky fly paper was used to minimise the possibility of insect infestation such as white fly. Plants were watered when required. No fertilizer was applied in any of the experiments.

Full cream powdered milk was provided by MG Nutritionals (Murray Goulburn Co-operative Co. Ltd., Victoria, Australia). Potassium bicarbonate ‘Ecocarb®’ was provided by Organic Crop Protectants Pty Ltd., South Australia. A hand-pressurised one-litre pump sprayer (Hozelock, England, UK) was used to apply treatments to run-off.

Selecting a model for a horticultural crop

A preliminary experiment was conducted with three horticultural crops (pea representing *Fabaceae,* tomato for *Solanaceae,* and watermelon for *Cucurbitaceae*) to select a horticultural model that was best suited for investigating the efficacy of milk for managing powdery mildew in the greenhouse. Three-week-old pea, tomato and watermelon plants were treated weekly, 3 treatments for pea and 4 treatments for tomato and watermelon with

either tap water (control) or milk diluted with tap water 0.1. Five replicate plants per treatment were used. Disease was assessed 2 days post-treatment. Disease severity was averaged across the five replicate plants in each treatment.

Optimal concentration of milk

The optimal concentration of milk to control powdery mildew in tomato, pea and watermelon was investigated by treating plants with four concentrations of diluted milk 0.02, 0.05, 0.1 and 0.2. Control plants were treated with water or potassium bicarbonate (Ecocarb®, 3 g L-1). Five replicate plants per treatment were used. Plants were treated weekly, 3 treatments for pea and 4 treatments for tomato and watermelon. Disease was assessed 2 days post-treatment. Disease severity was averaged across the five replicate plants in each treatment.

Optimal frequency of milk application

The optimal frequency of milk application was investigated by treating 3-week-old tomato plants with milk at four frequencies (3, 7, 10 and 21 days). This experiment was conducted twice with two concentrations of milk (0.5 and 0.1). Five replicate plants per treatment were used. Disease severity was assessed 2 days post-treatment every 3 weeks for 9 weeks. Disease severity was averaged across the five replicate plants in each treatment.

Effect of sunlight on the efficacy of milk

In winter 2012, an experiment was conducted to investigate the efficacy of milk for controlling powdery mildew in tomato under sunny conditions (plants under ambient sunlight) and overcast conditions (plants shaded with shade cloth). Five replicate plants per treatment were used. Plants were treated with milk (0.05) and tap water as controls applied on sunny days twice a week. Shade cloth tents (Gale Pacific Limited, Victoria, Australia) were used to cover plants (shaded plants) to mimic spraying during cloudy conditions by reducing exposure to sunlight for 3 hour immediately following treatment. The average light intensity, for 5 random days during the experimental period, measured using a light meter, (John Morris Scientific Pty Ltd., South Australia) was 24.4, 154.4 and 456.7 mmol quanta m-1 s-1 for shaded plants, unshaded plants and outside the greenhouse, respectively. Disease was assessed weekly, 2 days post-treatment for 4 weeks. After three treatments, one leaf from each plant was sampled 24 hour post-treatment for analysis in a scanning electron microscope (SEM).

SEM

Leaves were examined in a Philips XL30 field-emission SEM with microscope stage at the Adelaide Microscopy Laboratory. For each of the five leaves sampled per treatment, sections, approximately 0.5 cm square were sampled, mounted on microscope slides and frozen by placing them directly in liquid nitrogen. The samples were then dried in a vacuum chamber. After that, the samples were coated with platinum and were transferred to the microscope stage (at<-150ºC) to generate 20 digital images per section at ×1000–×2000 magnification. Using the following scale, the percentage of damaged conidia, based on the relative proportion of conidia that appeared undamaged on the images, was as follows: 0=0%, 1=10%, 2=20%, 3=30% to 10=100%. The same scale was used to estimate the damage to the hyphae. For each treatment, the mean damage score was calculated for conidia and hyphae. The method for SEM analysis was adapted from Crisp *et al.* (7), incubated the grape leaves *in vitro* in a

growth cabinet for 2 weeks with 12 hour light/dark rotation in order to allow the powdery mildew fungus to grow on the leaf surface.

Mode of action of milk

To investigate whether the antifungal activities of milk were protective and/or curative, milk (0.05) and water as control were applied to tomato plants either 2 days before inoculation with *Oidium* sp. (protective) or 2 days after inoculation (curative). This experiment was repeated except that milk was applied 3 days before inoculation with *Oidium* sp. or 3 days after inoculation. Plants were inoculated by shaking leaves infected with powdery mildew about 1 meter above a settling tower (of 1 m2) in order to obtain uniform coverage of conidia on plant leaves Reifschneider and Boiteux(20). Five replicate plants per treatment were used in this experiment. Disease severity was assessed at 7 days after inoculation. Then disease severity was averaged across the five replicate plants in each treatment.

Assessment of powdery mildew

The severity of powdery mildew was visually assessed on all leaves using the following assessment key for tomato and pea; 0=no obvious disease, 1=1%, 2=2%, 3=5%, 4=10%, 5=25%, 6=40%, 7=55, 8=70, 9=85%, 10=100%; (see appendices 1 and 2) (Godfrey D., University of Adelaide, personal communication). As no scale is available for assessing powdery mildew on watermelon, the following grapevine-assessment key was employed because watermelon and grapevine have leaves of a similar shape. The scale (developed by Emmett R. & Wicks T., South Australian Research and Development Institute, personal communication) is as follows: 0=no obvious disease, 1=0.8%, 2=2.3%, 3=4.7%, 4=9.4%, 5=18.8%, 6=37.5%, 7=62.5%, 8=81.3%, 9=89.5%, 10=100%; (see appendix 3). Disease severity was averaged for each plant and then averaged across the five replicate plants in each treatment.

Data analysis

Data were analyzed separately for each assessment date. Data were collected using one-way analysis of variance (ANOVA) using SPSS Statistics 19, IBM. Fisher's least significant difference (LSD) at $P<0.05$ and standard error of means were used to separate the means of the experimental data within each experiment. Microsoft Office Excel 2007 was used to prepare graphs.

RESULTS AND DISCUSSION

Selecting a model for a horticultural crop

Weekly applications of milk 0.1 reduced the severity of powdery mildew in tomato, pea and watermelon compared with the water treatment Figure (1). Treatments were applied for 4 weeks on tomato and watermelon, but only 3 weeks on pea because pea plants had begun to senesce and the experiment was terminated early. The conditions in the greenhouse were not found to be conducive to healthy growth of the pea plants and consequently this crop was not deemed suitable as a model crop for additional long-term studies. The mean severity of powdery mildew on milk-treated leaves of tomato, pea and watermelon was 11.8%, 11.0% and 2.1%, respectively. This was significantly lower ($p<0.05$) than on water-treated leaves, which were 32.9%, 50.1% and 20.4%, respectively Figures (1a, b and c). The most effective control was on watermelon, where the mean severity of powdery mildew on milk-treated-plants was reduced to approximately 10% that of the disease on water-treated plants Figure (1c). However, the watermelon plants required considerably more

greenhouse space than the tomato plants, rendering watermelon unsuitable as a model plant for long-term experiments to assess the effect of milk treatment on fruit quality. In addition, tomato belongs to the *Solanaceae,* a family for which no research has been conducted to investigate the effect of milk on powdery mildew. While the optimal concentration was investigated for all three crops, tomato was selected as the model for horticultural crops for all additional experiments.

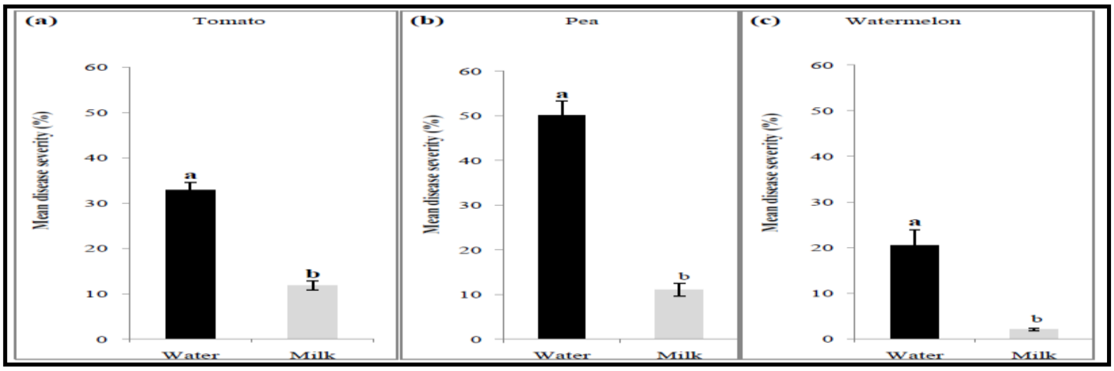

Figure 1: Severity of powdery mildew after weekly treatments for 4 weeks on (a) tomato, 3 weeks on (b) pea and 4 weeks on (c) watermelon. The treatments were tap water and milk (0.1=12.5g milk powder L^{-1}). Data represent means of five replicate plants. Error bars represent standard error of mean. Results are expressed as the mean ± of replicate plants $p<0.05$ by Fishers Least Significant Difference ($LSD_{5\%}$) for comparison of milk treatment versus water. Different letters correspond to significantly different means at $p<0.05$.

After 4 weeks of treatment, weekly application of milk 0.02, 0.05, 0.1 and 0.2 showed significant reduction in the severity of powdery mildew compared with water treatment. For these milk applications, the mean severity of tomato powdery mildew was 15.3%, 10.2%, 9.4% and 4.0%, respectively while it was 24.3% for water treatment Figure (2a). The efficacy of milk was concentration-dependent, with higher concentrations resulting in greater suppression of powdery mildew. However, there was no significant difference between the effects of milk 0.05 and milk 0.1. Also, there was no significant difference in disease suppression between the highest concentration of milk 0.2, which resulted in 4.0% mean disease severity, and the commercial organic fungicide Ecocarb®, which had a mean disease severity of 2.7%.

The severity of powdery mildew on pea was reduced significantly by applying milk 0.05, 0.1 and 0.2 weekly for 3 weeks compared with water treatment Figure (2b). The mean severity of powdery mildew for these milk treatments was 5.6%, 1.5% and 1.2%, respectively, and for water treatment it was 13.6%. There was no significant difference in disease severity between milk treatments and Ecocarb®, which was 1.6%, with the exception of the lowest concentration of milk 0.02, which resulted in 14.1% disease. Powdery mildew severity following treatment with milk 0.02 was not significantly different from water treatment.

After 4 weeks of treatment on watermelon, weekly applications of milk 0.02, 0.05, 0.1 and 0.2 resulted in significantly less powdery mildew than water treatment did Figure (2c). There were no significant differences between any of the milk treatments and Ecocarb®. The mean powdery mildew severity was 2.7%, 0.8%, 0.09% and 0.04% on milk treatments of 0.02, 0.05, 0.1 and 1:05,

respectively. Disease severity was significantly reduced in all milk treatments compared with water treatment, which resulted in a severity of 8.2%.

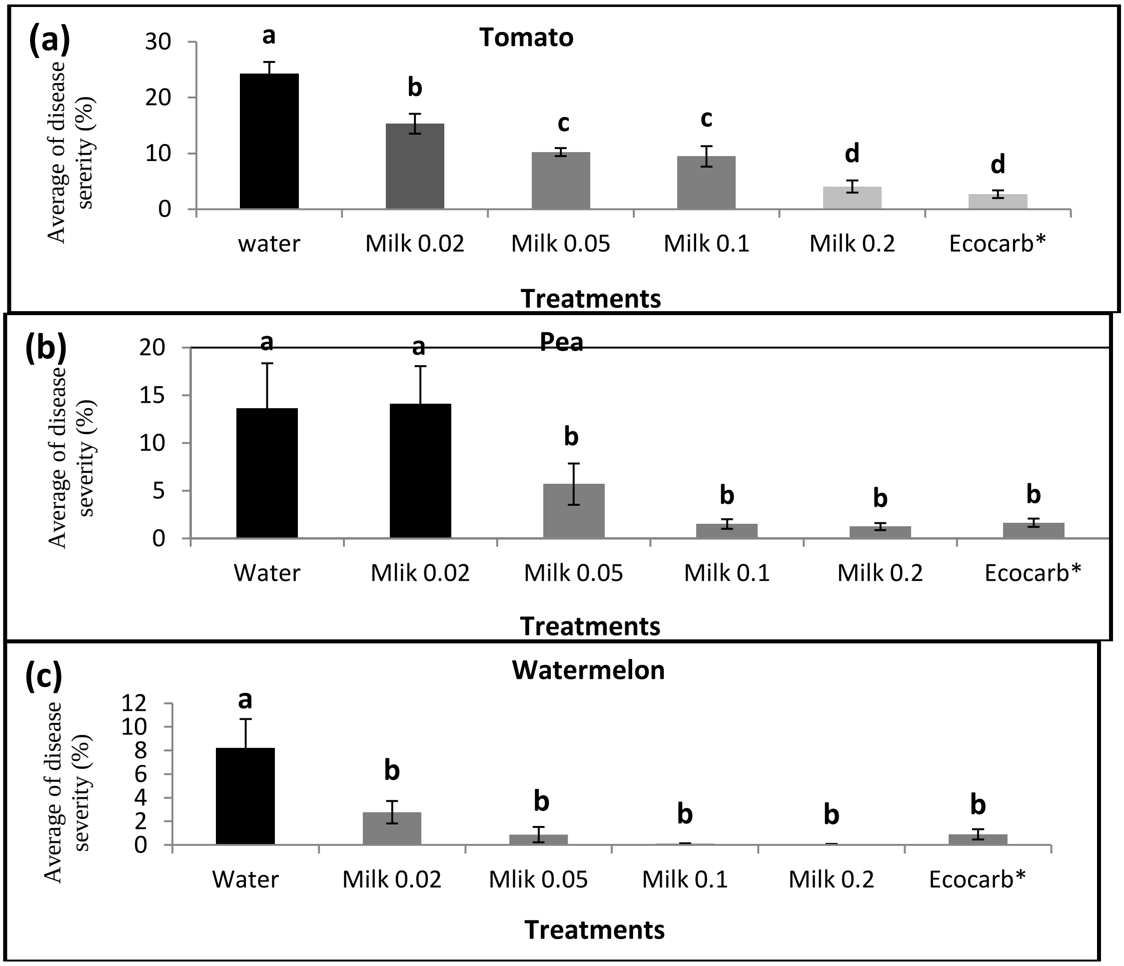

Figure 2: Severity of powdery mildew after weekly treatments for 4 weeks on (a) tomato, 3 weeks on (b) pea and 4 weeks on (c) watermelon with water, milk or Ecocarb®. Water=tap water, milk diluted with water (0.02=2.5 g milk powder L^{-1}, 0.05=6.25 g milk powder L^{-1}, 0.1=12.5 g milk powder L^{-1}, 0.2=25 g milk powder L^{-1}) and Ecocarb®= potassium carbonate 3 g L^{-1}. Data are means of five replicate plants. Error bars represent standard error of mean. Different letters correspond to significantly different means at $P<0.05$. Least Significant Difference (LSD5%) for (a) tomato=4.086, for (b) pea=7.447 and for (c) watermelon=3.095.

Optimal frequency of milk

Application of milk 0.05 at all four frequencies (3, 7, 10 and 21 days) resulted in significantly less powdery mildew than untreated control tomato plants, which experienced a disease severity of 26.0% Figure (3a). More frequent application resulted in decreased powdery mildew severity on infected leaves. Powdery mildew was not visible on tomato leaves when milk 0.05 was sprayed twice a week. There was no significant difference between spraying milk every 3, 7 and 10 days, the results being 0.00%, 0.32% and 3.49%, respectively. Plants treated every 21 days had significantly more disease (13.6%) than following other milk treatments.

Treatment with milk 0.1 significantly reduced powdery mildew severity at all frequencies compared with untreated control plants Figure (3b). Plants treated biweekly had significantly less powdery mildew than those with all other milk treatments. Disease severity was only 0.17% on the tomato leaves that were treated biweekly. There was no significant difference in disease suppression between frequencies of milk treatments every 7, 10 and 21 days, the results being 0.9%, 1.8% and 4.0%, respectively.

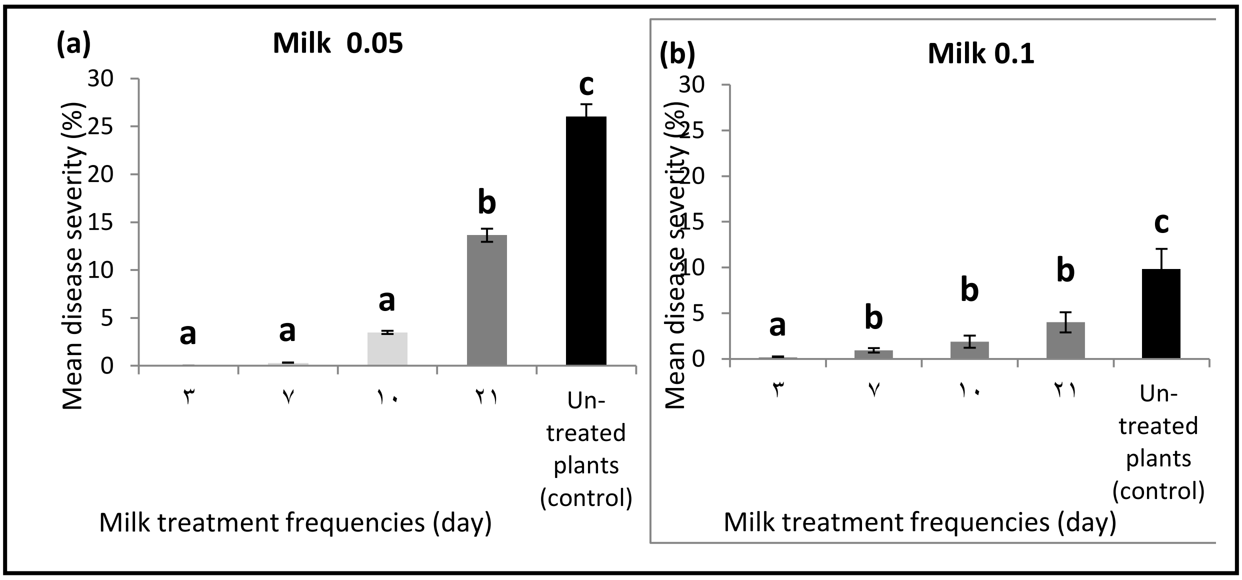

Figure 3: Severity of powdery mildew after three cycles of treatments in different frequencies on tomato by spraying milk (a) 0.05=6.25 g milk powder L^{-1} and (b) 0.1=12.5 g milk powder L^{-1}. Data are means of five replicate plants. Error bars represent the standard error of mean. Different letters correspond to significantly different means at P<0.05. Least Significant Difference (LSD5%) for (a) milk 0.05=5.135 and (b) milk 0.1=3.189.

Effect of sunlight on the efficacy of milk

Biweekly application of water to tomato plants exposed to ambient light inside a greenhouse resulted in significantly less disease severity than those of water-treated shaded plants Figure (4). The mean severity of powdery mildew was 13.6% for ambient sunlight plants and 26.5% for shaded plants.

For milk-treated plants, biweekly treatment significantly reduced the severity of powdery mildew compared with water treatment. However, there was no significant difference observed between the disease severity on ambient sunlight and shaded plants Figure (4).

Both levels (mean disease severity on shaded and ambient sunlight plants) were significantly lower than those of the water-treated plants, which were reduced by 26.5% (shaded) and 13.6% (under ambient sunlight).

After 3 weeks of biweekly treatment of milk 0.05 and water, fresh leaves from both shaded and ambient sunlight plants, which were at same age, were collected for analysis at SEM after 24 hour following the treatments. The result showed that there were significant differences in the percentage of collapsed conidia and hyphae on leaves between ambient sunlight tomato plants and shaded plants of water treatment Figures (5a, b, c). Approximately 80% of conidia and 50% of hyphae were collapsed on the samples from ambient sunlight

plants, while only 3% conidia and 7% hyphae were collapsed on leaves of shaded plants for the water treatment.

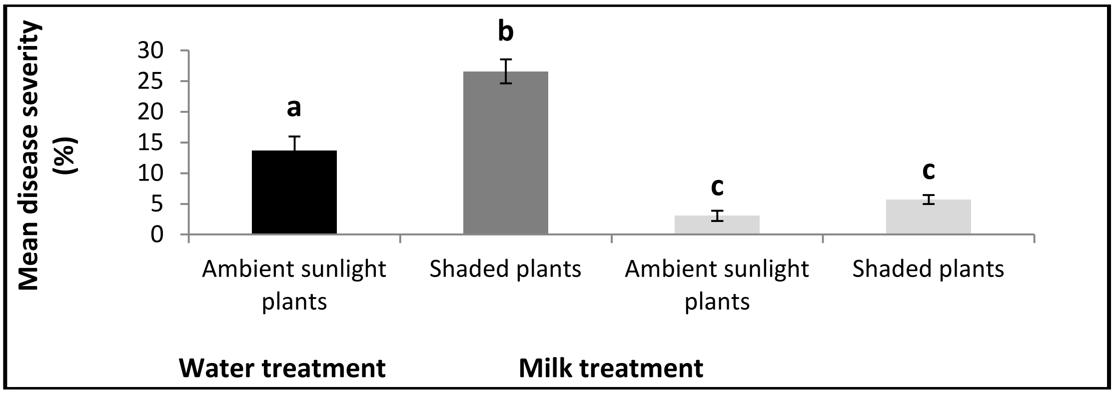

Figure 4: Severity of powdery mildew on tomato under ambient sunlight and under shaded conditions after four treatments with tap water or milk (0.05=6.25 g milk powder L^{-1}). Data are means of five replicate plants. Error bars represent the standard error of mean. Lower-case letters correspond to significantly different means at $p < 0.05$ level. Least Significant Difference (LSD5%) = 4.482

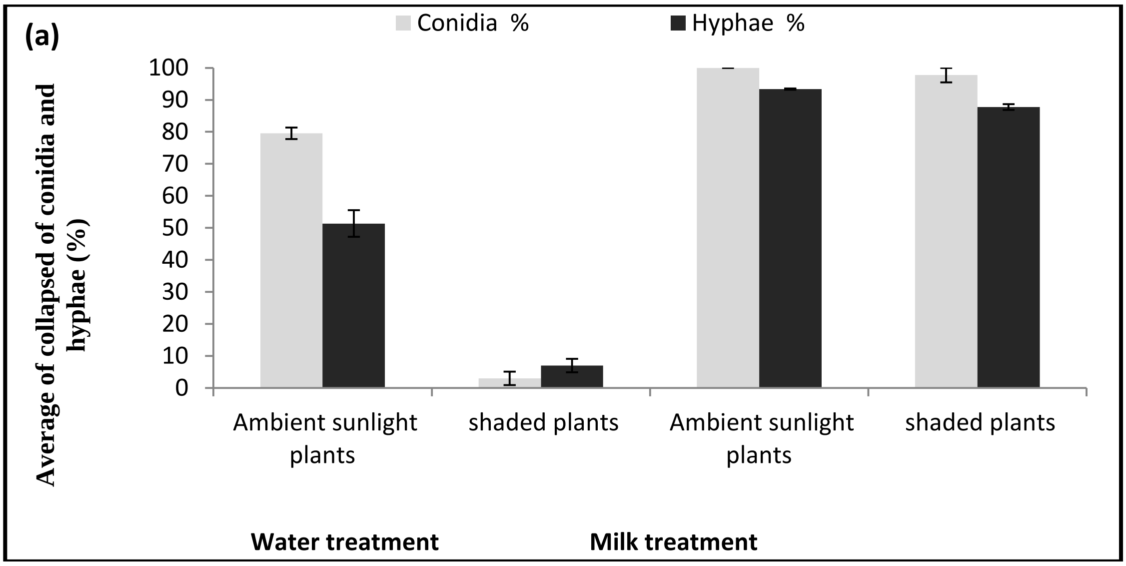

Figure 5a: The percentages of collapsed hyphae and conidia of *Odium* sp. 24 h after water and milk (0.05=6.25 g milk powder L^{-1}) treatments under ambient sunlight and shaded conditions (shaded for 3 h after treatment). Data are means of 20 images. Error bars represent the standard error of the mean.

The remaining hyphae and conidia were undamaged and turgid. In contrast, there were no significant difference in the percentage of collapsed conidia and hyphae on tomato leaves between ambient sunlight plants and shaded plants of the milk treatment Figures (5a, d, e). All the conidia (100%) and 93.3% of hyphae were collapsed on plants under ambient sunlight, and 97.7% conidia and 87.7% hyphae were collapsed following milk treatment on shaded plants.

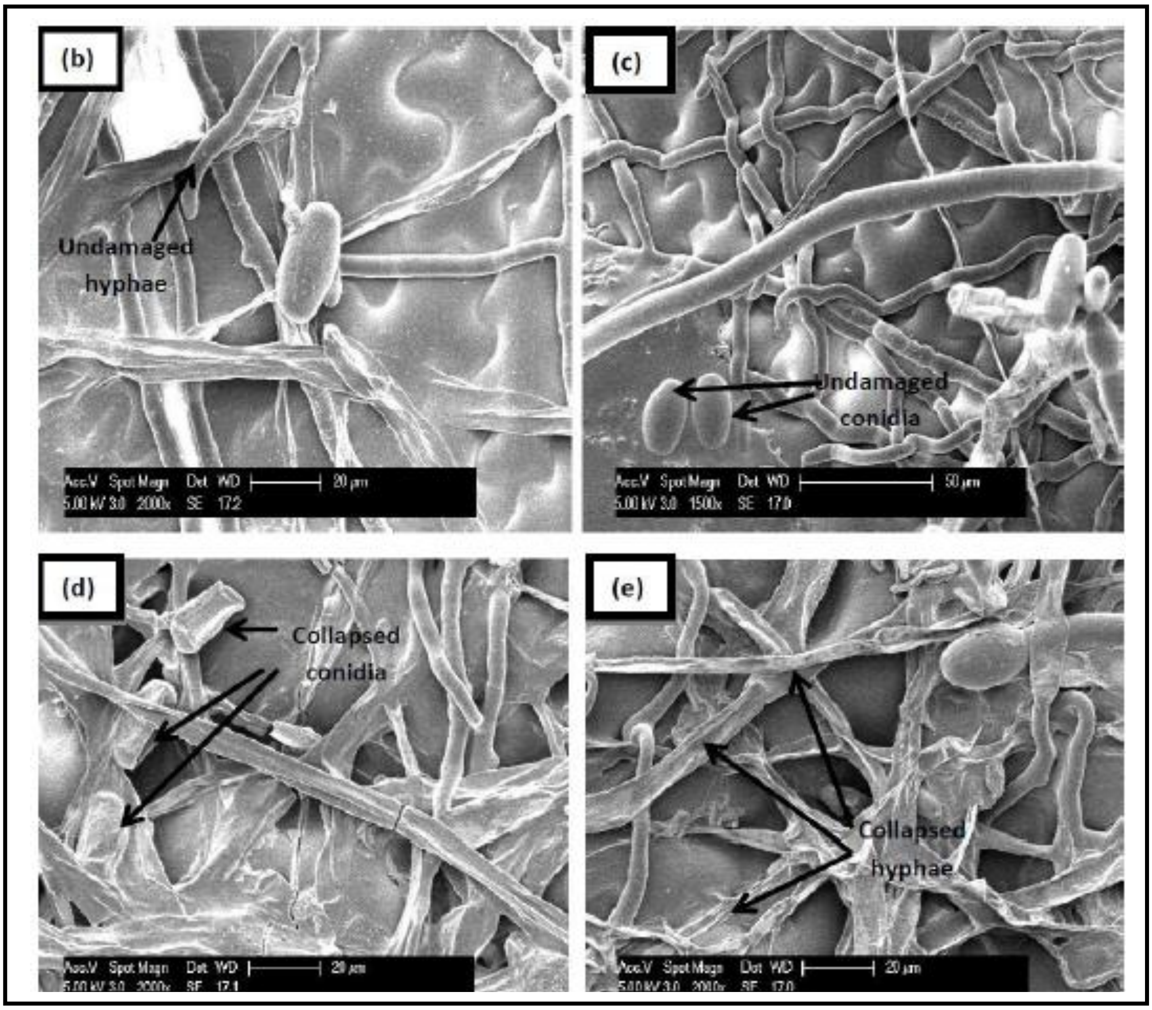

Figure 5b, c, d and e: Conidia and hyphae of *Odium* sp. on tomato leaf surface. (b) treated with tap water under ambient sunlight; (c) treated with tap water and shaded with cloth tents for 3 h after treatment; (d) treated with (0.05=6.25 g milk powder L^{-1}) under ambient sunlight; (e) treated with (0.05=6.25 g milk powder L^{-1}) and shaded with cloth tents 24 h prior to examination.

Mode of action of milk The mean powdery mildew severity was significantly lower on the leaves of plants that had been treated with a milk concentration of 0.05 than on water-treated leaves when treatments were applied either 2 days Figure (6a) or 3 days Figure (6b) before powdery mildew inoculation. Milk 0.05 treatment applied either 2 or 3 days following powdery mildew inoculation significantly reduced powdery mildew compared with water treatments. Powdery mildew was obvious first on the leaves of plants that were treated after inoculation. For the first trial, the disease severity was 4.6% in plants treated with milk 2 days before powdery mildew inoculation and 3.8% for those treated 2 days after Figure (6a). For the second trial, the mean powdery mildew severity in plants treated with milk was 12.6% three days before treatment and 15.7% three days after Figure (6b). For both trials, there was no significant difference in the disease severity when milk was applied before or after the inoculation.

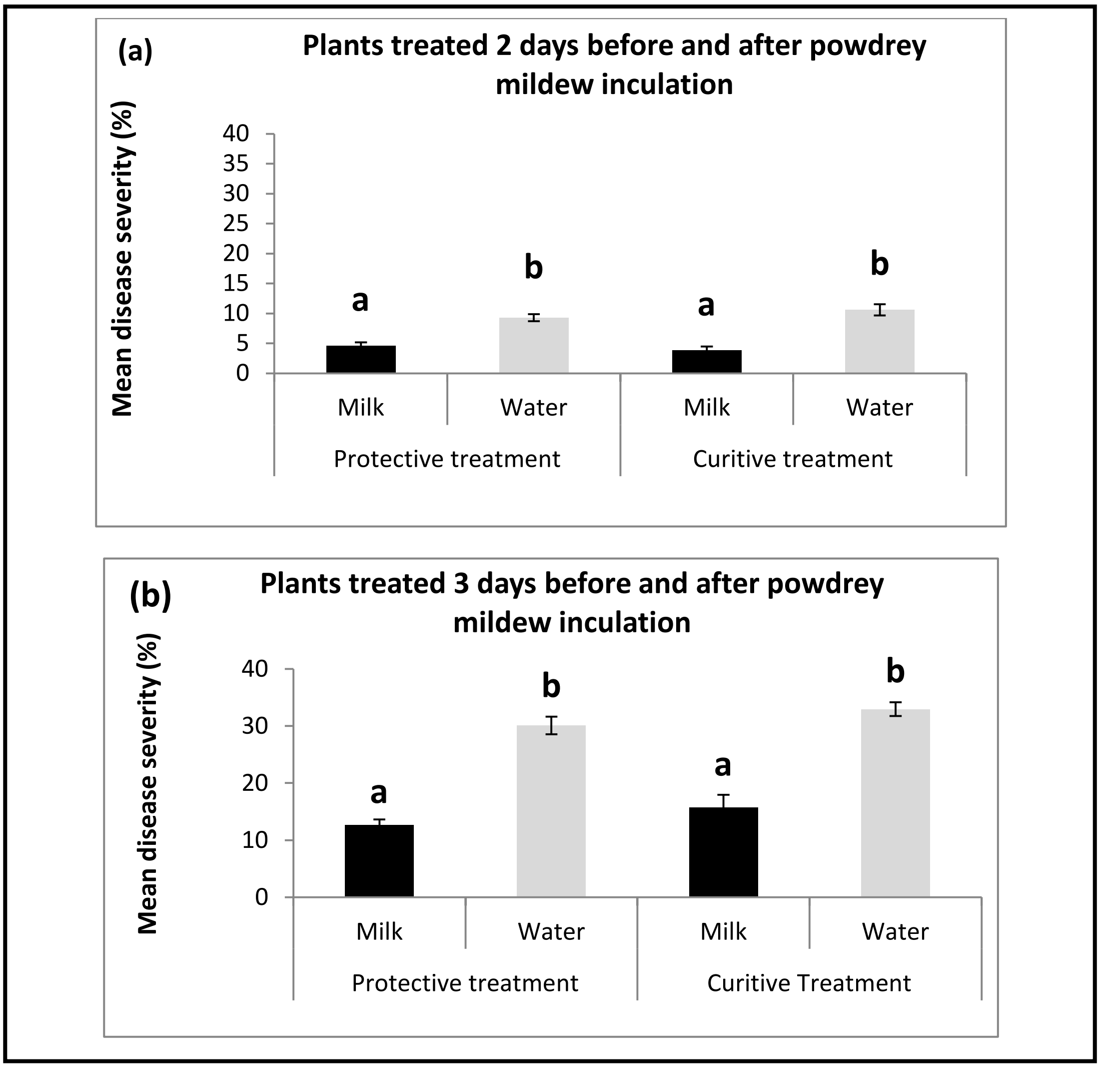

Figure 6 Severity of powdery mildew after one treatment on tomato by spraying tap water and 0.05=6.25 g milk powder L^{-1}. (a) either 2 days before inoculation with *Oidium* sp. (protective treatment) or 2 days after inoculation (curative treatment); (b) either 3 days before inoculation with *Oidium* sp. (protective treatment) or 3 days after inoculation (curative treatment). Data are means of five replicate plants. Error bars represent the standard error of mean. Different letters correspond to significantly different means at 0.05 level. Least Significant Difference (LSD5%) for (a) = 4.32 and for (b) = 9.59.

Weekly applications of milk significantly reduced powdery mildew severity on tomato, pea and watermelon, caused by *Oidium* sp., *E. polygoni* and *P. xanthii*, respectively. Milk had both protective and curative antifungal activity in reducing disease severity on tomato. Application of milk caused conidia and hyphae of *Oidium* sp. to collapse.

The higher concentrations of milk provided levels of disease control that were superior to the lower concentrations when milk was applied weekly to tomato, pea and watermelon plants. This result is in agreement with Bettiol (2), who reported that there was a negative correlation between powdery mildew severity and milk concentration when concentrations ranging from 10% to 50% were applied to zucchini squash either weekly or biweekly. In addition, Bettiol *et al.* (3) found that powdery mildew severity on zucchini squash and cucumber

correlated negatively with whey concentration ranging from 0% to 30%. When applied weekly to tomato, milk 0.2 provided control that was comparable to the commercially available organic fungicide Ecocarb® whereas, milk 0.05 on pea and milk 0.02 on watermelon for the same frequency provided control that was comparable to the same fungicide. This result is in accordance with previous studies that demonstrated that high concentrations of milk provided control as effective as conventional fungicides such as Fenarimol (0.1 ml L-1) and SupershieldTM (1% v/v) to control powdery mildew on zucchini and rose, respectively Bettiol, (2); Chee *et al.*(4). The results of the present study indicated that the three crops required different concentrations of milk to achieve control comparable to commercial fungicide. This study showed that milk in high concentrations offers an effective and non-toxic alternative to the use of commercial fungicides. In addition, plants treated with high milk concentration, like those treated with Ecocarb®, appeared healthy. However, high concentrations of milk may not be cost-effective. The lowest concentration n of milk 0.02 did not significantly reduce the development of pea powdery mildew compared with water treatment; however, this concentration significantly reduced powdery mildew on tomato and watermelon. This difference may be due to the variation in leaf characteristics in the three crops. For example, milk did not appear to stick on the smooth leaves of pea for long periods compared with tomato and watermelon. Residues from previous treatments were visible in the form of white deposits on the leaf surfaces of watermelon and tomato only, both of which are covered with hairs, a characteristic that could help to retain the milk and so inhibit disease development. Spraying diluted milk (0.05 or 0.1) on tomato plants at all of the frequencies examined here (3, 7, 10 and 21 days) reduced the severity of powdery mildew significantly when compared with untreated control plants efficacy was improved by increasing the treatment frequency. For instance, after 12 weeks of treatment, powdery mildew was not visible when milk was sprayed twice a week Figure (3a). Similarly, Bettiol *et al.* (3) found that powdery mildew development was lower on cucumber and zucchini squash in response to biweekly application of diluted whey than to weekly applications. However, biweekly application is likely to be uneconomical and unsustainable for long periods. Further cost/benefit analyses are needed to ascertain the optimum combination between the cost of milk application (which will be the lowest concentration with longest interval) and acceptable level of disease without compromising the crop quality.

The optimal conditions for powdery mildew include a temperature of 25°C, high relative humidity and relatively low light intensity Jacob *et al.*(13). The environment in the greenhouse provided these conditions. When water was applied biweekly on tomato plants, the level of disease on the plants treated under ambient sunlight conditions was significantly less than plants treated under shaded conditions Figure (4). For the ambient sunlight plants, most of the conidia and approximately half of the hyphae of *Odium* sp. collapsed compared with minimal collapse of conidia and hyphae on the shaded plants. The plants exposed to the ambient sunlight conditions may have experienced higher temperature and lower humidity conditions than the shaded plants, which may in turn have influenced disease severity.

There is some evidence from *in vitro* studies suggesting that sunlight increases the efficacy of milk for controlling powdery mildew Crisp *et al.*(7). This study is the first to investigate the effects of sunlight on the efficacy of milk in

greenhouse conditions. However, when milk was applied twice-weekly on the plants in the greenhouse under ambient sunlight or shaded conditions, the results did not provide any evidence that exposure to sunlight affected the efficacy of milk in controlling powdery mildew. For the milk-treated plants, almost all of the conidia and hyphae had collapsed and the proportion of collapse was similar on the leaves of treated plants exposed to ambient sunlight and shaded conditions. The different findings obtained from the *in vitro* and greenhouse studies may have resulted from altered UV light penetration in the greenhouse. For this reason, outdoor field trials may yield different results when milk is applied during sunny or cloudy conditions. Additional greenhouse and outdoor field trials are needed to understand better the role of sunlight in the efficacy of milk to control powdery mildew.

The application of milk before and after inoculation reduced powdery mildew severity, indicating that milk had both protective and curative properties for controlling powdery mildew. Reporting their work with wheat, Drury *et al.* (9) found that milk has protective and curative properties when applied before or after inoculation with *E. graminis*. Godfrey *et al.* (12) found that application of milk, either full cream or skim, showed both preventive and curative properties against *E. necator* in grapevine *in vitro*. When milk was applied before inoculation, it might act as a protective barrier on the leaf surface and prevent the pathogen from penetrating the leaf surface. SEM analysis revealed the collapse of hyphae and conidia of *Odium* sp. after milk treatment 2 or 3 days after inoculation, when the fungus was already actively growing on the leaf surface. Milk may cause the collapse of hyphae and conidia, resulting in reduced spore production.

In the present study, powdery mildew symptoms were obvious first in plants inoculated before being sprayed with milk (curative treatment), and disease development was slightly faster than in those of the protective treatment. This result agrees with Drury *et al.* (9), who found that powdery mildew development was slower on infected wheat when milk and whey were sprayed before inoculation than on those treated after inoculation. The present study indicated that spraying milk before the onset of disease was better because slower development resulted in better disease control.

In the concentration and frequency experiments, it was observed that growth of tomato plants was faster in milk-treated plants compared with water-treated plants or untreated plants (data not shown); the stems were weak and there were fewer leaves and branches in water-treated tomato. This result agrees with Wurms &Chee(24), who found that apple seedlings treated with anhydrous milk fat diluted with water 7 g L-1 grew significantly faster than water-treated plants. The differences in growth between milk-treated and water-treated plants may be related to the foliar application of milk which may provide additional nutrition. Therefore, future investigation is required to assess the nutritional effect of milk application on tomato and other crops.

In the present study, when leaf samples were analysed by SEM, no evidence of bacteria or fungi was observed other than powdery mildew. However, Scott *et al*. (21) reported that large microbial populations, such as bacteria, yeast and filamentous fungi, were detected on the surface of berries, grapes and in juice obtained from Shiraz and Verdelho treated with milk or whey compared with sulfur-treated or untreated plants. Bettiol (2) reported that mould grew on the upper surface of leaves treated with milk, especially at 30%

milk concentration and higher. Therefore, additional study is needed to examine the effect of milk on fruit quality after specific storage periods. If detrimental effects are observed, further research using microbial culturing or polymerase chain reaction (PCR)-based molecular techniques to diagnose and identify any potential spoilage microorganisms is warranted.

In conclusion, milk-based fungicides have the potential to be developed as safe alternatives to commercial fungicides for the control of powdery mildew in horticultural crops such as tomato, pea and watermelon. This research provides the basis for larger-scale trials in greenhouse and field settings in order to evaluate the use of milk-based treatments in other crops and other regions. Further study is necessary to compare the cost of milk application at an effective concentration and frequency with commercial fungicides and sustainability.

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كفاءة الحليب المخفف لمكافحة البياض الدقيقي على المحاصيل البستنية

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الملخص

أجريت هذه الدراسة للتحقق من كفاءة الحليب المخفف لمكافحة مرض البياض الدقيقي ***Oidium sp.*** على الطماطة صنف ***Solanum Alycopersicu***. ستة تجارب اجريت في البيت الزجاجي في كلية الزراعة، جامعة اديلايد/استراليا، إذ أظهرت النتائج ما يأتي: عند معاملة نبات الطماطة المصاب بمرض البياض الدقيقي بمادة الحليب أسبوعياً وبتركيز **0.1** كانت فعالية هذا التركيز مشابهة لفعالية الحليب ذات التراكيز العالية وكذلك لفعالية المبيد الفطري **Ecocarb®3g/L^{-1}** الذي استخدم للمقارنة. وجد أيضا إن كفاءة الحليب تزداد عند تكرار معاملة النبات المصاب عند تركيز **0.1** و**0.05**.

كذلك وجد أنه ليس هناك أي تأثير لأشعة الشمس في كفاءة الحليب لمكافحة البياض الدقيقي عندما رُش الحليب المخفف مرتين بالأسبوع وبتركيز **0.05** مقارنة بالنباتات المزروعة تحت الظل. عند التحقق من أن الحليب واقي ام شافي لمرض البياض الدقيقي، ووجد ان الحليب المخفف يحمل صفات المبيد الفطري الواقية والشافية لهذا المرض عندما تم رشه قبل حدوث الإصابة مرة وبعد حدوث الإصابة مرة أخرى وبتركيز **0.05**.

بحث مستل من رسالة الماجستير للباحث الاول.

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