Plant Pathol. J. 21(3) : 270-274 (2005)

The Plant Pathology Journal

Control Efficacy of Milk Concentration Against Powdery Mildew of Strawberry

Myeong Hyeon Nam[1], Won Keun Lee[1], Sok Su Lee[1], Nam Gyu Kim[2] and Hong Gi Kim[2]*

[1]*Nonsan Strawberry Experiment Station, Chungnam ARES, Nonsan 320-862, Korea*

[2]*Department of Applied Biology, Chungnam National University, Daejeon 305-764, Korea*

(Received on April 4, 2005; Accepted on August 22, 2005)

The aim of this study was to determine the effect of milk as one of the environmental friendly materials that substitute chemical fungicides for control powdery mildew (*Sphaerotheca aphanis*) of strawberries (*Fragaria* × *ananassa* Duch.). 'Maehyang' and 'Akihime' varieties planted in greenhouses were evaluated for the control of powdery mildew. Applications of 5%, 10% and 20% milk had much better effects on controlling powdery mildew. In particular, 10% milk showed a higher efficacy than other concentrations applied onto strawberry in greenhouse experiments. Foliar spray application of 10% milk was effective for powdery mildew, whereas drench application was not. Also, foliar spray of 10% milk was able to accelerate more firmness and calcium contents of strawberry fruits than the non-treated. The 10% milk applied with fertilizer salts ($Ca(H_2PO_4)_2 \cdot H_2O$ and KH_2PO_4) showed there was a similar efficacy to 10% milk alone in greenhouse experiments. White crystals and cracks on strawberry fruits appeared by 20% milk. This result indicated that 10% milk was a useful substitute for fungicides to control powdery mildew of strawberry.

Keywords : disease control, milk, powdery mildew, strawberry, substitute

Strawberry (*Fragaria* × *ananassa* Duch.) contained high natural vitamine C and favored for winter cultivation when the production of other vegetables and fruits is relatively low. The total cultivation area of strawberries were 7,503 ha containing 7,172 ha of greenhouses and the total production of strawberries for fresh market was 205,427 M/T in Korea at 2003 (Anon., 2004). The cultivating method is increasing of forcing use among forcing, semi-forcing and open field culture. The main cultivars used for forcing are 'Maehyang', 'Akihime' and 'Redpearl', but the farmers are in difficulties for the susceptibility of the cultivars against powdery mildew (Nam et al., 2005).

Powdery mildew of strawberry caused by *Sphaerotheca aphanis* (Wallr.) U. Braun var. *aphanis* (Nakazawa and Uchida, 1998; Shin, 2000) is the most serious disease for strawberry production (Nam et al., 2005). This disease occurred on leaves, flowers and fruits, and appeared severely under cool and dry conditions. The conidia on transplants were prominent inoculum sources in the planting field and were spread to healthy plants by air, wind and bees (Kanaiso, 1995). Fungicide application is the major method used to control powdery mildew of strawberry but it is not always successful due to decreased sensitivity of the fungus to fungicides (Okayama et al., 1995). Moreover, the increasing concern for the environment and food safety against fungicide application during harvesting period motivates farmers to optimize powdery mildew control by reducing fungicide application. Therefore, there has been a demand to develop the substitutes for chemicals, which are more environment-friendly materials.

Alternative of fungicidal control was use of soluble silicon, surfactant (Cho et al., 1998) and sodium bicarbonate. Sodium bicarbonate has been found to be effective about powdery mildew of strawberry (Nam et al., 2003), rose (Horst et al., 1992), Euonymus (Ziv and Hagiladi, 1993), cucumber (Ziv and Zitter, 1992), and green mold (Smilanick et al., 1999), *Botrytis cinerea* (Palmer et al., 1997) of citrus. Other studies have reported that milk had some preventive effects on powdery mildew on greenhouse growing cucumber (Bettiol, 1999). Fresh milk may have a direct effect against *S. fuliginea* due to its germicidal properties (Salle, 1954) and the substances such as several salts, and amino acids have been proved to be effective in controlling powdery mildew (Reuveni et al., 1995).

The objective of this investigation was to determine the effectiveness of milk alone and in mixture with surfactant and fertilizer salts to accelerate safety and to develop effective materials against powdery mildew on strawberry.

Materials and Methods

Cultural practices. Field experiments were conducted on the loam soil (clay 16%; sand 38%; silt 46%) in greenhouses located at Nonsan Strawberry Experiment Station during 2002-2004. The experimental design was a randomized block plot with 3 replications and virus free

*Corresponding author.
Phone) +82-42-821-5768, FAX) +82-42-823-8679
E-mail) hgkim@cnu.ac.kr

plants cvs. "Maehyang" and "Akihime" grew with the pot (∅ 16 cm) was transplanted on Sept. 25, 2002 and 2003. Irrigation was conducted as drip irrigation every 7 days. To control insect pests in 2002 and 2003, acetamiprid WP at 0.5 g/L and mibemectin E at 1 ml/L were applied on Oct. 20 and 28, respectively.

Milk treatments. Selected milk for experiments was sterilized milk that was currently on the market, and treatments were applied with milk at 5%, 10% and 20% in water (w/v) and fenarimol E (0.3 ml/L) and sanyol E (DBEDC = complex of bis (ethylenediamine) copper-bis-(dodecylbenzenesulfonic acid, 2 ml/L) as fungicides, which are registered on strawberry in Korea. The milk concentrations were sprayed on strawberry plant cv. "Maehyang" until run off three times at 1 week intervals from Jan. 20, 2004 when powdery mildew occurrence was begun. Disease assessment was calculated for diseased fruits rate (the number of diseased fruits/the number of total fruits x 100) and area under the disease progress curve (AUDPC) from Jan. 27 to Feb. 13, 2004. The AUDPC was calculated according to the equation of Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{i}^{n-1} [(y_i + y_{i+1})/2](t_{i+1} - t_i)$$

where n is the number of evaluations, y the diseased fruits rate, and t is the number of days after harvesting where at each evaluation. $(t, y) = (0, 0)$ is included as the first evaluation.

Control efficacy of surfactants and fertilizer salts with milk. To accelerate the effect of milk on the control of powdery mildew, the 10% milk was sprayed in mixture of surfactant; tween 20® (polyoxyethylene sorbitanmonolaurate) at 0.5%, Reitron® (spreader-sticker, Kyeongnong Co., Korea) at 4,000 dilution and fertilizer salts; 0.01 M Ca $(H_2PO_4)_2 \cdot H_2O$, 0.01 M KH_2PO_4. The surfactant milk solutions was sprayed three times to strawberry plant cv. "Maehyang" until run off with one week intervals from Feb. 18, 2003 and Jan. 20, 2004 and fertilizer salt milk solutions were sprayed 3 times from Mar. 8, 2004 when powdery mildew occurred weakly. Disease assessment was calculated for diseased fruits rate on Mar. 11, 2003 and Feb. 13, 2004 and from Mar. 26-Apr. 6, 2004, respectively. Phytotoxicity test was assessed on leaves and fruits visually. The AUDPC was calculated for severity from Mar. 22-Apr. 6, 2004.

Application methods and fruit characteristics analysis of strawberry. To determine control effects of the milk treatment on the powdery mildew, the milk was sprayed on leaves and dripped to strawberry plant cv. 'Akihime" and applied 3 times with one week intervals from Dec. 24, 2003. Disease assessment was calculated for diseased fruits rate from Dec. 30-Jan. 26, 2004. Firmness of strawberry was measured at the center of fruit using SD-700 (Rheo Tex, Japan) with Ø 0.5 mm tip for 30 fruit per plot on 5 days after treatment. Then, the soluble solidity of these fruits was measured with a refractometer PR-100 (ATago Co., Ltd., Japan). The concentration of peroxidase (mg/L), ascorbic acid (mg/L) and calcium (mg/L) was measured followed by descriptions in a test kit of RQflex2® (Merck Co., LTD, Germany).

Data analysis. Data were analyzed with the analysis of variance (ANOVA) and treatment mean comparisons were carried out using the Fisher's protected least significant difference test at $P \leq 0.05$ (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

Milk treatments. Milk at 5%, 10%, 20% and fenarimol were highly significant at Fisher's protected LSD ($P \leq 0.05$) and AUDPC analysis on diseased fruits rate also significant compared with the control. Disease severity of 10% milk was significantly lower than other concentrations and showed a similar control effect as fenarimol (Table 1).

From review of previous research, the drip treatment with 10% nonfat dry milk applied during seeding of watermelon transplant to control the cucumber green mottle mosaic virus (CGMMV) of watermelon showed a high control effect (Lee, 1997). In addition, milk affected the control of

Table 1. Control efficacy of milk-treated strawberry (cv. 'Maehyang') on the powdery mildew in 2004

Treatment	Application dates[z]	Diseased fruits (%)			AUDPC[x]
		1/27	2/3	2/13	
Control	NA	27.8a[y]	54.0b	59.2b	1,835.7b
5% milk	A, B, C	27.8a	12.5a	8.8a	465.8a
10% milk	A, B, C	5.1a	6.3a	5.9a	160.1a
20% milk	A, B, C	38.5a	16.8a	17.2a	615.4a
Fenarimol (0.3 ml/L)	A, B, C	13.6a	16.4a	8.8a	388.7a

[z] Application date: A = Jan. 20, B = Jan. 27, and C = Feb. 3. NA = not applicable.

[y] Treatment means followed by different letters vary significantly (Fisher's protected LSD, $P \leq 0.05$).

[x] Area under the disease progress curve (AUDPC) is an estimation of diseased fruits in a long period and includes diseased fruits data collected from Jan. 27 to Feb. 13, 2004.

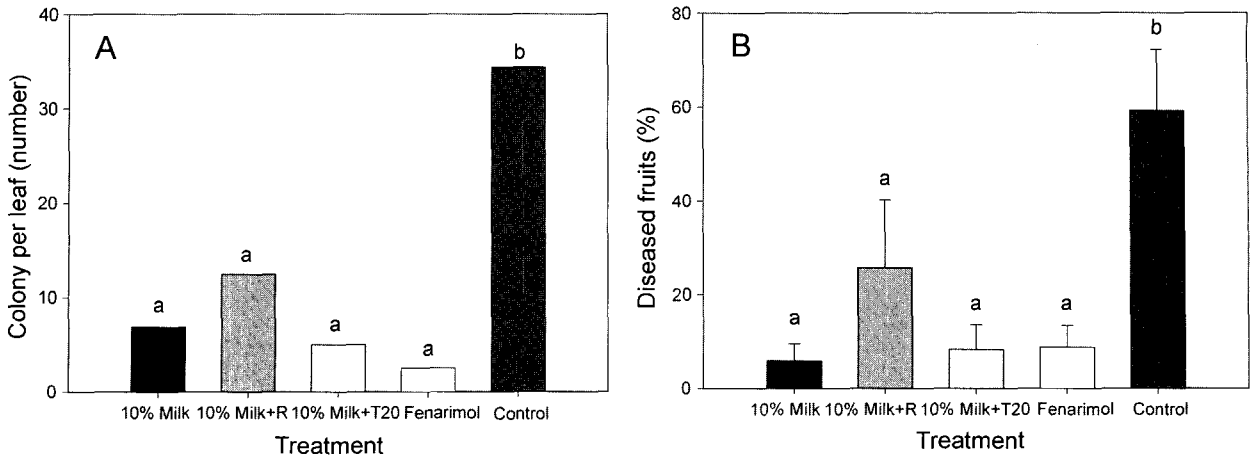

Fig. 1. Effect of milk, surfactant alone, and mixture of both on the powdery mildew on strawberry cultivar 'Maehyang' according to milk concentration. A was applied on Feb. 18, 25, and Mar. 4, 2003, and B was applied on Jan. 20, 27, and Feb. 3, 2004 when powdery mildew occurred weakly. Examination dates were Mar. 11 and Feb. 13, respectively. Lines on each bar represent standard deviations in each treatment. T: polyoxyethylene sorbitanmonolaurate. R: spreader-sticker. Numbers followed by the same letter within each column are not significantly different from each other according to Fisher's protected LSD ($P \leq 0.05$).

powdery mildew in cucumber and the highest concentration of 50% milk showed the best effect (Bittiol, 1999). From results of this research, however, 20% milk treatment was significantly lower than 10% milk in controlling, and suppressed plant growth forming white crystals on leaves and fruits.

Control efficacy of surfactants and fertilizer salts with milk. When leaves were sprayed with 10% milk alone and in mixture with tween 20® and Reitron® before powdery mildew occurred less than 10% in 2002-2003, treatment with mixture was not significant at Fisher's protected LSD ($P \leq 0.05$) compared with 10% milk treatment alone (Fig. 1A). This treatment showed also similar results for the diseased fruits rate in 2003-2004 (Fig. 1B).

Sodium bicarbonate mixed with mineral oil and surfactant was a more effective treatment for powdery mildew control than any of a material treated alone (Horst et al., 1992; Nam et al., 2003; Ziv and Zitter, 1992). However, the treatment of milk with surfactant obtained similar result as the treatment of milk alone. It was apparent that milk had a similar role of surfactant because it emulsifying by itself.

When leaves were sprayed with 10% milk alone and with mixture with 0.01 M $Ca(H_2PO_4)_2 \cdot H_2O$ and 0.01 M KH_2PO_4 before the powdery mildew appeared by 10% or less, the treatment with mixture was not significant at Fisher's protected LSD ($P \leq 0.05$) compared with 10% milk alone. Whereas 10% milk with 0.01 M KH_2PO_4 obtained a continuous effect up to 18 days after treatment (Table 2).

A previous study demonstrated the effectiveness of phosphate salts in inducing local and systemic protection against powdery mildew (Reuveni et al., 1995). Treatment of milk with fertilizer salts, however, obtained similar result to those of milk alone. Bittiol (1999) was not yet sure why it worked so well, but he speculated that milk contained the

Table 2. Control efficacy on the powdery mildew on strawberry (cv. 'Akihime') treated with 10% milk alone and with mixture of fertilizer salts

Treatment	Application dates[a]	Diseased fruits (%)			AUDPC[c]
		3/26	3/29	4/6	
Control	NA	16.3b[b]	28.1c	35.7b	789.0c
10% milk	A, B, C	0.0a	19.1bc	33.9b	397.2b
10% milk + 0.01 M $Ca(H_2PO_4)_2 \cdot H_2O$	A, B, C	3.9a	10.4ab	32.8b	353.0b
10% milk + 0.01 M KH_2PO_4	A, B, C	7.8ab	19.7bc	13.1a	316.9b
Sanyol (2 ml/L)	A, B, C	0.0a	1.7a	5.3a	52.5a

[a] Application date: A = Mar. 8, B = Mar. 13, and C = Mar. 19. NA = not applicable.
[b] Treatment means followed by different letters vary significantly (Fisher's protected LSD, $P \leq 0.05$).
[c] Area under the disease progress curve (AUDPC) is an estimation of diseased fruits in a long period and includes diseased fruits data collected from Mar. 22 to Apr. 6 in 2004.

Table 3. Characteristics of strawberry (cv. 'Akihime') fruits treated with milk on 5 days after application

Treatment	Firmness (Ø 0.5 mm)	Sugar content (Brix°)	Peroxidase (mg/L)	Ascorbic acid (mg/L)	Calcium (mg/L)
Control	213.9±2.9	10.7±0.6	1.1±0.3	94.7±2.5	7.8±0.6
10% milk spray	226.7± 2.9	11.3±0.4	0.7±0.1	100 ±3.2	8.5±1.6
10% milk drench	214.4± 2.3	11.5±0.8	1.2±0.5	90.2±4.3	7.3±0.5
Fenarimol (0.3 ml/L)	214.6± 2.5	11.4±0.6	0.9±0.3	98.4±7.8	7.1±0.5

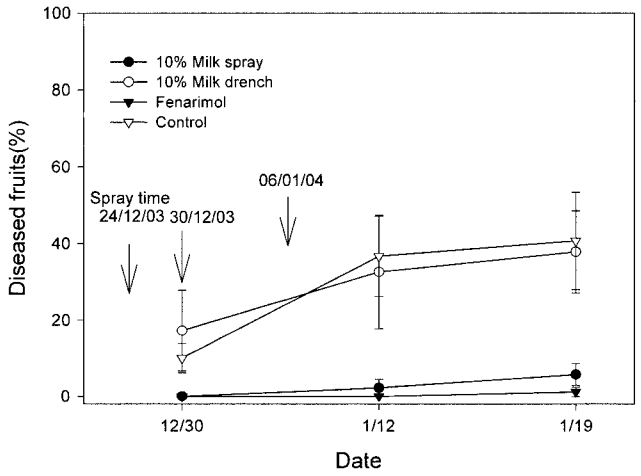

Fig. 2. Average diseased fruits of the powdery mildew on strawberry cultivar 'Akihime' according to various methods of milk application. Treatment was applied on Dec. 24, 30, 2003 and Jan. 6, 2004 when the powdery mildew appeared weakly. Lines on each bar represent the standard deviations on each date.

potassium phosphate, which boosted plants' immune system so may help it control the powdery mildew's growth. Our results showed that the milk was more effective in controlling the powdery mildew in strawberry. In the future, this research will continue to study the role of milk to control in and around powdery mildew-affected area in detail.

Application methods and fruits analysis of strawberry. When 10% milk was sprayed and dripped on plants, the spray method of 10% milk did not make a significant difference with fenarimol on 5% of diseased fruits rate. However, the drip method of 10% milk was not effective (Fig. 2). The spray treatment of 10% milk showed to accelerate firmness, concentration of calcium and ascorbic acid, but concentration of peroxidase was reduced compared with non-treated fruits (Table 3). The powdery mildew of strawberry, therefore, can be controlled effectively with 10% milk applied in the spray method based on the results obtained from this study and the field experiment.

References

Anonymous. 2004. 2003 vegetable production results. *Ministry of Agriculture and Forestry of Korea.*

Bettiol, W. 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. *Crop Protection* 18:489-492.

Campbell, C. L. and Madden, L. V. 1990. Introduction to plant disease epidemiology. Wiley, New York.

Cho, I. C., Lee, S. H. and Cha, B. J. 1998. Effects of soluble silicon and several surfactants on the development of powdery mildew of cucumber. *Kor. J. Environ. Agric.* 17:306-311.

Horst, R. K., Kawamoto, S. O. and Porter, L. L. 1992. Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot of roses. *Plant Dis.* 76:247-251.

Kanaiso, Y. 1995. Problems on occurrence of powdery mildew of strawberry: relationship between increase of disease and cultural method. *Plant Protection* 49:237-240.

Lee, K. W. 1997. Control and occurrence of cucumber green mottle mosaic virus. *Plant Dis. Agric.* 3:5-11.

Nakazawa, Y. and Uchida, K. 1998. First record of cleistothecial stage of powdery mildew fungus on strawberry in Japan. *Ann. Phytopathol. Soc. Jpn.* 64:121-124.

Nam, M. H., Kim, H. G. and Lee, Y. B. 2005. Control and ecology of strawberry diseases and pests. Chungnam Natl. Univ. & Nonsan Strawberry Experiment Station, Korea. page 10.

Nam, M. H., Jung, S. K., Ra, S. W. and Kim, H. G. 2003. Control efficacy of sodium bicarbonate alone and in mixture with polyoxyethylene sorbitanmonolaurate on powdery mildew of strawberry. *Kor. J. Hort. Sci. Tech.* 21:98-101.

Okayama, K., Nakano, T., Matsutani, S. and Sugimura, T. 1995. A simple and reliable method for evaluating the effectiveness of fungicides for control of powdery mildew (*Sphaerotheca macularis*) on strawberry. *Ann. Phytopathol. Soc. Jpn.* 61: 536-540.

Palmer, C. L., Horst, R. K. and Langhans, R. W. 1997. Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. *Plant Dis.* 81:1432-1438.

Reuveni, M., Agapov, V. and Reuveni, R. 1995. Suppression of cucumber powdery mildew (*Sphaerotheca fuliginea*) by foliar sprays of phosphate and potassium salts. *Plant Pathol.* 44:31-39.

Salle, A. J. 1954. Fundamental principles of bacteriology. McGraw-Hill Book, New York.

Shin, H. D. 2000. *Erysiphaceae* of Korea. National Institute of Agricultural Science and Technology, Suwon, Korea. pages

215-220.

Smilanick, J. L., Margosan, D. A., Mlikota, F., Usall, J. and Michael, I. F. 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. *Plant Dis.* 83:139-145.

Ziv, O. and Zitter, T. A. 1992. Effects of bicarbonates and film-forming polymers on cucurbit foliar diseases. *Plant Dis.* 76: 513-517.

Ziv, O. and Hagiladi, A. 1993. Controlling powdery mildew in Euonymus with polymer coatings and bicarbonate solutions. *HortScience* 28:124-126.