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Control of Powdery and Downy Mildews of Cucumber by Using Cooking Oils and Yolk Mixture

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Powdery and downy mildews caused by *Sphaerotheca fusca* and *Pseudoperonospora cubensis* are the most common and serious diseases of cucumber worldwide. In spite of the introduction of highly effective systemic fungicides, control of these diseases remains elusive. Hence, this study aimed to develop an alternative method to chemicals in controlling the diseases by using different types of cooking oil. Egg yolk, which contains a natural emulsifier, lecithin, was selected as a surfactant to emulsify the oils. Among the different cooking oils used, soybean, canola (rape seed), safflower, sunflower, olive, and corn oils showed over 95% control values against powdery mildew of cucumber in a greenhouse test. In particular, 0.3% canola oil emulsified with 0.08% yolk (1 yolk and 60 ml canola in 20 *l* spray) was found to be the most effective. The treatment resulted in 98.9% and 96.3% control efficacies on powdery and downy mildews, respectively, of cucumber in the field. Canola oil exhibited direct and systemic effect, wherein powdery mildew of cucumber was suppressed only on treated leaves but not on non-treated leaves in a plant, while mycelia and conidia of the pathogen were severely distorted or destroyed by the treatment. The prospect of using the canola oil and yolk mixture as a natural fungicide is highly promising because of its effectiveness, availability, low cost, simple preparation, and safety to humans and the environment. The use of the canola oil and yolk mixture is expected to be an effective fungicide for use in organic farming and home gardening.

Keywords : cooking oil, cucumber, downy mildew, egg yolk, powdery mildew

In recent years, Korean consumers have been very much concerned about food safety and clean environments in harmony with their health and well-being. Accordingly, environment-friendly agriculture, particularly organic farming, has become a popular trend in ensuring sustainable agriculture and sound environments. In 1997, the Ministry of Agriculture and Forestry (MAF) of Korea enacted the "Environment-friendly Agriculture Supporting Law" to sustain agricultural productivity, conserve soil and water, reduce pollutions, recycle organic resources, and produce safe foods in the country. Consequently, the MAF aimed to reduce chemical fertilizers and pesticides used in agriculture by 40% in 2013 compared with the 2003 level (Anonymous, 2005; Jee, 2007).

Management of various diseases and insect pests, however, has been reported to be the major obstacle to the growth and success of organic agriculture in Korea (Jee, 2007). Because of intensive agricultural conditions in the country, basic standards for pest management in organic agriculture such as crop rotation and other physical, ecological, biological, and cultural practices are not easily met (Codex, 2004; Foguelman, 2003). Therefore, organic farmers largely rely on substances which are expected to provide pesticidal actions and are allowed to be used in organic farming. Some commercial products originating from plants or microbes, such as neem, pyrethrum, or *Bacillus* spp., are widely used as substitute to chemical pesticides in the country (Anonymous, 2005; Jee, 2007).

Oils are considered as one of the oldest natural pesticides as documented by a Roman scholar in the 1st century (Grossman, 1990). Among the oils obtained from various sources such as petroleum, coal mineral, plants and animals, oils from petroleum were considered to be the most important in terms of their insecticidal and acaricidal activities (Calpouzos, 1966; Grossman, 1990). Mineral oils have long been used as dormant sprays to reduce overwintering populations of insects on fruit trees in many countries. Vegetable cooking oils are also believed to have the same pest killing physical modes of action as mineral oils. Commercial brands of cooking oils such as coconut, corn, cottonseed, palm, peanut, safflower, soybean, and sunflower have been proven to have similar effect in controlling aphids, whiteflies, and spider mites on plants (Grossman, 1990). However, there have been relatively few studies on plant oils and extracts as a pesticide although their usage

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extends back to more than 150 years (Isman, 2006).

Researches on oils for the control of plant diseases did not receive much attention, although Martin and Salmon (1930) reported that vegetable oils showed marked toxic properties against powdery mildew of hop caused by *Sphaerotheca humili* as early as the 1930s. Calpouzos (1966) reviewed the pesticidal actions of oils in the control of plant diseases, pointing out that mineral oil spray was the chief method in controlling the banana leaf spot called 'Sigatoka' disease caused by *Mycosphaerella musicola* in major banana producing regions of Central America, West Indies, and Africa. However, the study on the control of plant diseases by using oils seemed to have ceased since then.

The interest on the efficacy of oils in controlling plant diseases was revived in the 1990s, triggered by the studies of Northover and Schneider (1993, 1996) on the use of vegetable oils in controlling powdery mildew of apple caused by *Podosphaera leucotricha*. Ko et al. (2003) revealed that food grade plant oils from such sources as canola, corn, grape seed, peanut, safflower, soybean, and sunflower greatly reduced the severity of tomato powdery mildew caused by *Oidium neolycopersici*, suggesting that cooking oils are ideal substitute for chemical fungicides in controlling powdery mildew of cucumber in organic farming. However, one constraint in using cooking oil in previous studies was the use of chemical surfactants, such as Agral or Tween, to emulsify the oil. These chemical surfactants are strictly prohibited in organic farming, according to the international and domestic standards of organic agriculture (Codex, 2004; Lee et al., 2007).

Therefore, this study sought to determine the effectiveness of egg yolk as a natural emulsifier of cooking oils, and to evaluate the combined efficacy of the egg yolk and cooking oil mixture in the control of powdery and downy mildews of cucumber. The study also attempted to understand the modes of action of the yolk-oil combination in controlling the diseases, and to develop a practical application method of the mixture for appropriate use in organic farming.

Materials and Methods

Preparation of cooking oil and egg yolk mixture. Among the various cooking oils used in baked goods, salad dressings, and deep-frying in Korea, seven most popular commercal vegetable oils were chosen. These include: soybean oil, caster oil, canola (rape seed oil), safflower oil, sunflower oil, olive oil, and corn oil. The oils and eggs were purchased from a local market in Suwon, Korea. To prepare 20 *l* spray solution of cooking oil and yolk (COY) mixture, 1 egg yolk (ca. 15 ml) was added in 100 ml water and vigorously macerated with a motor-mixer for 3-4 min. Each aliquot amount of cooking oil was supplemented into the yolk solution and homogenized for over 5 min with the mixer. The COY mixture was added in distilled water to bring 20 *l* spray solution. Final concentrations of cooking oil in the spray were adjusted to 0.05, 0.1, 0.3, 0.5, 1% and final concentration of egg yolk was ca. 0.08%. A commercial microbial product 'Pangjaba' used for the control of powdery mildew was used to compare the efficacy with the COY mixtures.

Bioassay in a greenhouse. Cucumber seedlings (cv. Baegbongdadagi) grown in pots for 4-8 weeks in a greenhouse were used for the bioassay. Powdery mildew of cucumber caused by *Sphaerotheca fusca* occurred readily without inoculation under the condition. When the disease symptom started to appear on leaves, different concentrations of the COY solutions were sprayed enough to cover the upper and lower side leaves. After the treatment, the infected leaf area was recorded every 2 days until 14 days. Three plants were used as replicates for each treatment and the experiment was repeated once. In another experiment, glycerin 0.1% was mixed with canola COY 0.3% to increase and prolong the efficacy of COY in controlling powdery mildew of cucumber.

Field experiment. The farmer's field made of plastic film house sized ca. 540 m^2 (6×90 m) was located in Suwon Ipbukdong. The field was divided into 15 plots, each plot with a size of ca. 33 m^2 for five treatments with three replicates. Cucumber seeds were planted on July 1, 2006 for harvesting on August 10, 2006. The symptoms of powdery and downy mildews of cucumber started to appear on July 28. Four treatments namely, canola COY 0.3%, an antifungal isolate of actinomycete CA23 and caster COY 0.3% mixture, CA23 and wood vinegar 500x mixture, and a microbial product 'Pangjaba' were applied two times on August 1 and 5. The severity of the diseases was evaluated based on the infected leaf area on August 8, three days after the 2nd treatment. The infected leaf area was calculated by dividing the sum of the infected leaf areas by the total number of leaves. Data were analyzed by using the SAS 2004 program (SAS Inc., Cary, NC).

Mode of action. Canola COY 0.3% was sprayed onto the lower three leaves of the cucumber plant infected by powdery mildew at 7-day intervals. The controls were the three newly growing and expanding upper leaves on the same plant, which were not treated with the COY. On the 7th day after the 2nd treatment, disease infected areas of the plant were evaluated as described above. Features of the fungal mycelia of the cucumber powdery mildew pathogen on leaves were examined under a scanning electron microscope (SEM) at 3 days after treatment with canola COY 0.3%.

Results

Effects of cooking oil and yolk mixture. All cooking oils emulsified with egg yolk (cooking oil and yolk, COY) were highly effective in controlling powdery mildew of cucumber caused by *Sphaerotheca fusca* in the greenhouse test. Formulation of 0.3% COY using soybean, canola, safflower, sunflower, olive, and corn oils showed over 95% control values, while caster COY and commercial microbial product showed 87.5% and 75.6% control values, respectively (Table 1). Among the different cooking oils, canola oil showed the highest control values followed by sunflower oil (Table 1). The most effective concentrations of COY ranged from 0.3 to 0.5% (Fig. 1). Although the canola COY 1% provided 100% control of the disease, cucumber leaves remained oily for 2 days. The effect of canola COY 0.3% on suppression of cucumber powdery mildew lasted only for 5 days, and after that, disease symptoms developed again (Fig. 2). Glycerin 0.1% supplemented into the COY mixture slightly slowed down the disease development. The severity of the disease was not much different between those with and without glyceride until 10 days. However, after 14 days, a marked difference in disease severity was observed (Fig. 2).

Table 1. Effect of cooking oil in controlling powdery mildew of cucumber in a greenhouse test

Cooking oils[a]	Powdery mildew	
	Diseased leaf area (%)	Control value (%)[b]
Soybean oil	0.80	95.2 a
Caster oil	5.67	87.5 b
Canola oil	0.67	98.5 a
Safflower oil	2.23	95.1 a
Sunflower oil	0.90	98.0 a
Olive oil	1.10	97.6 a
Corn oil	1.47	96.8 a
Commercial microbial product 'Pangjaba'	11.10	75.6 c
Control	45.43	0 d

[a] Cooking oil was emulsified with egg yolk. Concentrations of oil and yolk in the spray solution was 0.3% and 0.08% (Oil 60 ml and a yolk comprising ca. 16 ml/20 *l*).
[b] Values followed by the same letter are not significantly different at P=0.05 according to Duncan's new multiple range test.

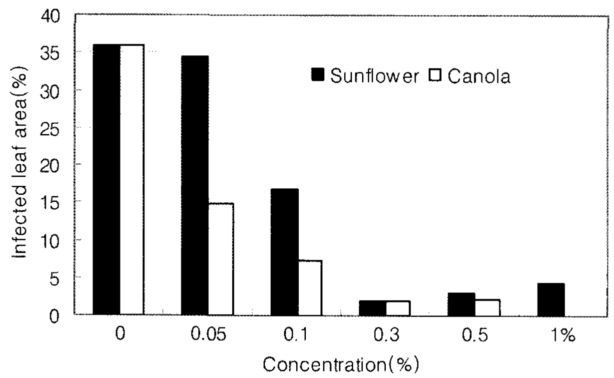

Fig. 1. Effects of concentration of cooking oil on control of powdery mildew of cucumber in a greenhouse.

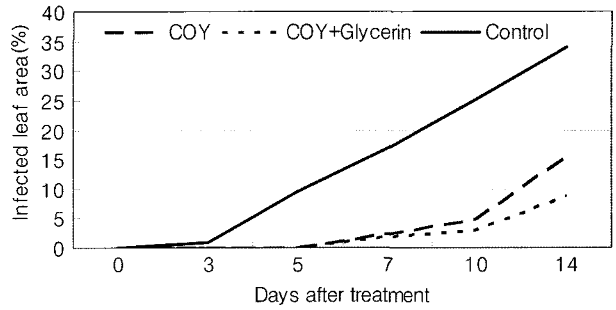

Fig. 2. Effect of glycerin 0.1% supplemented to canola COY 0.3% on suppression of powdery mildew of cucumber.

Field experiments. 0.3% Canola COY was superior to other oils with the same concentration in controlling powdery and downy mildews of cucumber in a farmer's field. The treatment showed 98.9% control efficacy in controlling powdery mildew, while caster 0.3% COY and CA23 mixture, wood vinegar and CA23 mixture, and a microbial product showed only 63.7%-72.5% control values. Similarly, canola 0.3% COY was highly effective in controlling downy mildew of cucumber caused by *Pseudoperonospora cubensis*. The treatment revealed 96.3% control efficacy, while other treatments only showed 66.4%-88.8% control values (Table

Table 2. Control of powdery and downy mildews of cucumber in a farmer's field by canola and yolk mixture

Treatment[a]	Powdery mildew (%)		Downy mildew (%)	
	Infected leaf area	Control value[b]	Infected leaf area	Control value[b]
Canola COY	0.1	98.9 a	0.4	96.3 a
Caster COY + CA23	3.3	63.7 b	1.2	88.8 b
Wood vinegar + CA23	2.5	72.5 b	2.5	76.6 c
Commercial product 'Pangjaba'	2.8	69.2 b	3.6	66.4 c
Non-treated control	9.1	–	10.7	–

[a] COY stands for cooking oil and yolk mixture. Concentrations of canola and yolk in the spray were 0.3% and 0.08%, respectively. CA23 was an antifungal isolate of actinomycetes.
[b] Values followed by the same letter are not significantly different at P=0.05 according to Duncan's new multiple range test.

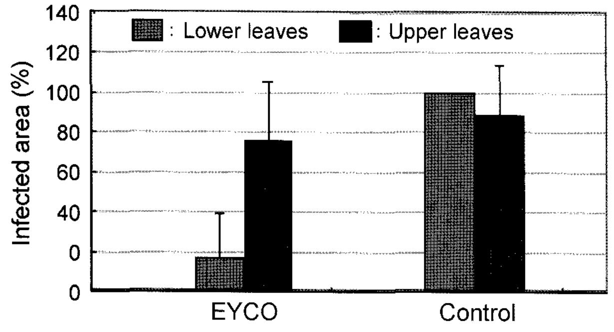

Fig. 3. Severity of powdery mildew of cucumber on treated lower leaves and non-treated upper leaves with canola 0.3% COY.

2, Fig. 4).

Mode of action. The powdery mildew symptom was more severe on the lower old leaves than on the upper new leaves as shown in Fig. 3. However, canola 0.3% COY effectively suppressed the disease only on the treated lower leaves, but not on the non-treated upper leaves. The severity of the disease on the non-treated upper leaves was not much different from the treated and non-treated plants (Fig. 3). Mycelia and conidia of *Sphaerotheca fusca* on a cucumber leaf surface appeared to be dense and vigor, but were destroyed and distorted severely after treatment with canola 0.3% COY (Fig. 4).

Discussion

Powdery mildews caused by *Erysiphaceae* are the most common and widespread problems of cucumber worldwide, especially in warm and dry climates. Systemic fungicides are commonly used to control the diseases in Korea. However, the disease problems remain unsolved and the use of systemic fungicides generated various negative side effects. Other control methods such as elemental sulfur, dinocap, phosphate salt solutions, detergents, and ultrafine oils have been tested as alternative methods for the control of the diseases (Foguelman, 2003; Pasini et al., 1997; Trdan et al., 2004).

Several oils from minerals and plants have been reported to control powdery mildews of many plants such as rose, hop, apple, and tomato caused by various genera of *Erysiphaceae* (Grossman, 1990; Ko et al., 2003; Martin and

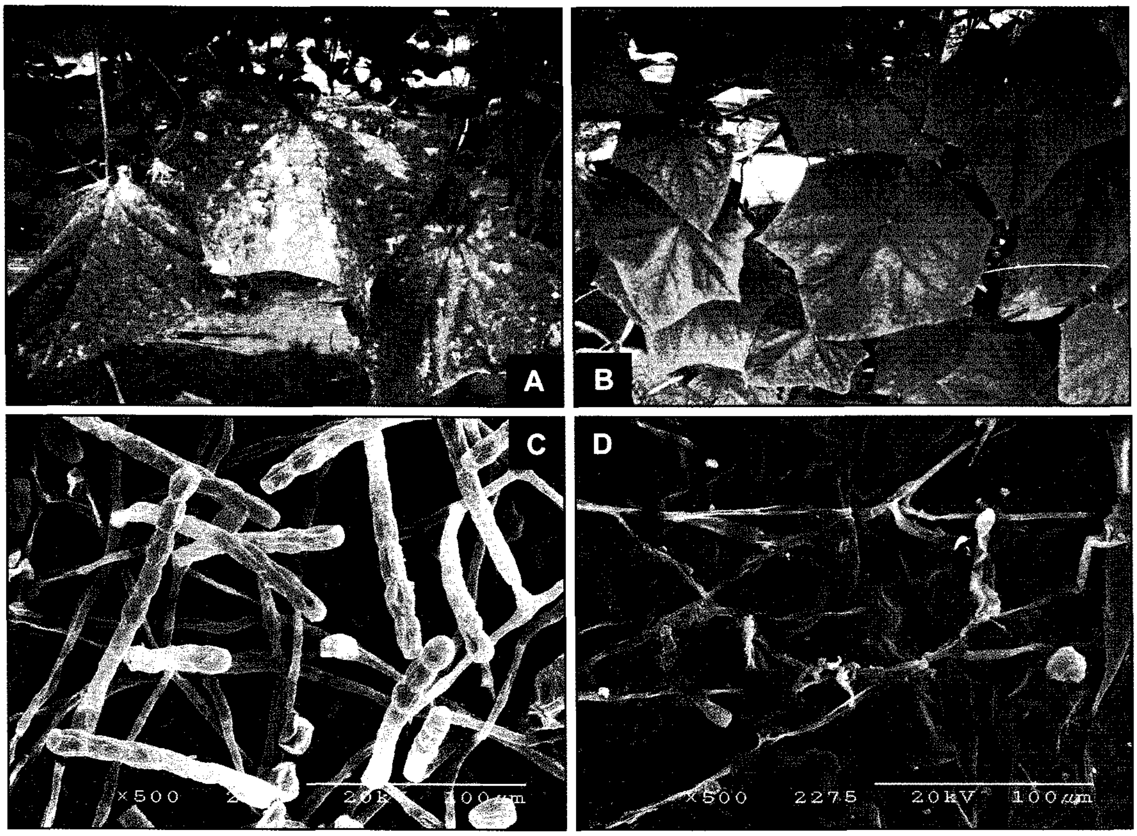

Fig. 4. Comparisons of symptoms and the fungal structures of *Sphaerotheca fusca*. A: Severely infected cucumber leaves by powdery and downy mildews in the control plot. B: Healthy cucumber leaves in the treated plot with canola COY 0.3%. C: Vigorous mycelia and conidia of *S. fusca* on a cucumber leaf before treatment. D: Distorted and destroyed structures of the fungus after treatment with canola COY 0.3%.

Salmon, 1930; Northover and Schneider, 1993). Ohtsuka and Nakazawa (1991) reported that mineral oil could control satisfactorily even resistant strains of powdery mildew pathogen of cucumber because of its physical mode of action. However, the oils used in previous studies were emulsified with the same amount of soft soap or chemical surfactants which are not allowed to be used in organic production (Anonymous, 2005; Codex, 2004; Lee et al., 2007).

In this study, egg yolk was selected as emulsifier for cooking oil. Yolk is a rich nutrient source containing ca. 0.9% of lecithin that is well known to be a natural emulsifier and a ubiquitous component in living organisms. Its safety as a food additive emulsifier for mayonnaise, margarine, chocolate, ice cream, caramel, etc. is well validated (Homma et al., 1977; Levene and Rolf, 1921). In addition, lecithin is listed as among the materials allowed for use in disease control in organic agriculture standards (Anonymous, 2005; Codex, 2004). In our experiments, 1 yolk comprising ca. 15 ml was sufficient to emulsify up to 200 ml of cooking oil. This shows that using yolk as emulsifier can be a cheap, but a very safe and efficient method. Moreover, the oils emulsified with yolk dispersed smoothly in spray solution because of both hydrophilic and lipophilic properties and dispersibility of lecithin (Homma et al., 1977; Levene and Rolf, 1921).

All cooking oils except caster oil proved highly effective in controlling powdery mildew of cucumber when emulsified by yolk. These results are consistent with those of Northovar and Schneider (1993). They reported that sunflower, olive, canola, corn, soybean, and grape seed oil emulsified with a chemical surfactant Agral 90 were equally effective in providing over 99% control of powdery mildew of apple caused by *Podosphaera leucotricha*. Ko et al. (2003) also reported that plant oils emulsified with Tween 80 greatly reduced powdery mildew of tomato, and that sunflower oil was the most effective. In our experiments, canola COY showed the best control of powdery mildew of cucumber in terms of effectiveness, physical property, and cost.

The effectiveness of plant oils in controlling powdery mildew has been well verified, while few reports are available on their effects in controlling other diseases. Northovar and Schneider (1996) reported that canola and soybean oils were effective in controlling powdery mildew of grape, but not against downy mildew of grapevine caused by *Plasmopara viticolar*. The oils showed only slight prophylactic activity against *Venturia inaequalis*, and their level of control was not much different from that of a surfactant, Agral 90, used to emulsify the oils (Northover and Schneider, 1993). In contrast, canola oil emulsified with egg yolk in this study provided an excellent control effect on downy mildew of cucumber caused by *Pseudoperonospora cubensis*. While this study did not clarify how the oil controlled the downy mildew of cucumber, the most plausible explanation could be the physical modes of action such as direct killing of the pathogen or coating of the leaf surface to prevent germination and penetration of the pathogen resulting in the control of the disease.

While concentrations of cooking oil in the final spray solution are important in terms of effectivity, phytotoxic effects must also be avoided such as plant growth retardation or yellowing of leaves. Previous reports showed that the optimum concentrations of sunflower oil were 0.5% in the study of Ko et al. (2003) and 0.5% to 2.0% concentrations of various oils were in other studies (Fernandez et al., 2006; Grossman, 1990; Martin and Salmon, 1930; Northover and Schneider, 1993; Ohtsuka and Nakazawa, 1991). In this study, 0.3% of COY was optimal to control powdery and downy mildews of cucumber. High concentrations of the oil, over 0.5%, resulted in the leaves remaining oily for two or more days, and caused plant growth retardation when continuously sprayed. Meanwhile, canola or sunflower oil at 0.3% provided similar control values with 0.5% or 1.0% and never induced phytotoxicity on cucumber.

The modes of action of the various oils on the control of powdery mildew have been discussed by many researchers. While some controversies exist, physical and direct fungicidal activities of oil are generally accepted as the mode of action causing the disease control (Ko et al., 2003; Grossman, 1990; Calpouzos, 1966; Northover and Schneider, 1996; Ohtsuka and Nakazawa, 1991). Ko et al. (2003) demonstrated that sunflower oil inhibited conidial germination and mycelial growth on tomato leaf surface but never induced host defense mechanisms. In this study, powdery mildew of cucumber was controlled only on treated leaves but not on non-treated leaves in the same plant. Mycelia and conidia of the pathogen were severely distorted and contracted by the treatment. Results were consistent with previous reports that the mode of action of canola oil is direct and indirect mechanisms.

The prospect of using cooking oils as fungicides is highly appealing because of its effectiveness, availability, low cost, simple preparation, and safety to humans and the environment. In particular, the cooking oil and yolk mixture, referred to as COY in this study, is suitable not only for organic agriculture but also for home gardening. The only limitation in using COY is in ensuring proper preparation such as achieving very small oil particles that can be suspended evenly in the spray solution, and the right treatment to cover the entire surface of the plants to obtain optimum control. Further researches on increasing the effects of COY and broadening the spectrum of target diseases by using other substances as supplements are in progress to pave the way for the success and growth of organic farming in Korea.

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