

## Impact of Alternative Safety Programs to Fungicides on Management Squash Powdery Mildew

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**M**icroscopic examination of the conidial stage of squash (cv. Eskandrani) powdery mildew pathogen was performed. The parameters of conidia (24-40 X 16-26 $\mu$ ), length/width ratio (1.47) and characteristics of conidial germination confirmed that the pathogen belongs to the genus *Oidium* the conidial stage of *Sphaerotheca fuliginea* (Schlecht. Ex Fries) Poll. Three experiments were carried out under controlled greenhouse conditions to evaluate the efficacy of three plant oils, i.e. sesame oil, alovera oil and flax oil; three natural compounds, i.e. kaolin, potassium bicarbonate and whey in addition to four beneficial bacterial isolates, i.e. *B. subtilis*, *B. vallismortis*, *P. brasiliensis*, and *P. polymexa* against squash powdery mildew. Emulsified sesame oil at 0.3%, kaolin at 6% and *P. polymexa* ( $9 \times 10^8$  cfu/ml) were found to be more effective in reducing severity of powdery mildew to 7.42; 8.08 and 23.96%, respectively, against 35.71% in the untreated check. Under natural conditions, rotational application programs, including T1 (Prochloraz fungicide as single repeated treatment); T2 (Prochloraz alternative with kaolin) and T7 (Prochloraz alternative to a mixture of potassium bicarbonate, sesame oil and whey) were found to be more effective in reducing severity of powdery mildew to 4.59, 7.09 and 7.92%, respectively without significant differences against 41.25% in the untreated check. In conclusion, to fulfil effective control of powdery mildew, the use of fungicides must be integrated carefully with environmentally friendly materials.

**Keywords:** Bioagents, plant oils, kaolin, powdery mildew, potassium bicarbonate and whey.

Squash (*Cucurbita pepo* L.) is an immensely popular economic vegetable crop all over the world. According to last updated data published by the Food and Agricultural Organization of the United Nations (Anonymous, 2017), the world production of pumpkins, squash and gourds in 2014 reached 25,196,723 tons yielded from 2,004,058 ha. China is the biggest producer of pumpkins, squash and gourds in the world, while in Africa, Egypt is one of the 10 top producers that occupied the fifth rank. Squash is greatly challenged by different diseases especially powdery mildew. Symptoms usually start on the older leaves as small pale yellow spots. As the disease progresses, the spots enlarge, coalesce and turn to reddish-brown and necrotic. Severely infected leaves gradually dry and brittle, leaving the fruits to damage by sunburn. So, severe loss in yield and fruit quality was occurring.

Two main causative agents of cucurbit powdery mildew are reported in the literature, i.e., *Podosphaera xanthii* (Castag.) U. Braun & N. Shish (Synonym:

*Sphaerotheca fuliginea* (Schlecht ex Fr) Poll. and *Golovinomyces cichoracearum* (DC.) V.P. Heluta (Synonym: *Erysiphe cichoracearum* DC ex Merat.) (Vakalounakis *et al.*, 1994). The identification of cucurbit powdery mildew fungi has not always been accurate. The two major species, *i.e.* *Podosphaera xanthii* and *Golovinomyces cichoracearum* defined in the literature may overlap and were considered as synonymous (McCreight *et al.*, 1987). The causal agents of cucurbit powdery mildew produce identical symptoms and can be difficult to differentiate in the absence of the perfect stage (Block and Reitsma, 2005). However, morphological features of the asexual stage of *P. xanthii* differs from those of *G. cichoracearum* according to size and shape of conidia, presence of fibrosin bodies, immature conidia edge and germ tube morphology (Zaracovitis, 1965).

Control of powdery mildew with conventional chemicals is conducted by repeated applications of fungicides. However, frequent applications of chemical fungicides may cause potential negative effects on the environment and often result in the selection of pathogen strains that are resistant or tolerant to the fungicide. So, the use of fungicides must be carefully managed by searching for friendly non-chemical materials.

Different materials have been explored as alternative substances against powdery mildew such as biological control agents (Zhang *et al.*, 2011; Elkot and Derbalah, 2011); clay minerals (Marco *et al.*, 1994); plant oils (Hafez, 2008; Jee *et al.*, 2009 and Chee *et al.*, 2011) and whey (Bettiol, 1999; Bettiol *et al.*, 2008 and Chee *et al.*, 2011).

The overall goal of this research was to evaluate and investigate the effectiveness of some plant oils, kaolin, potassium bicarbonate, whey and some beneficial bacterial isolates as alternatives to synthetic fungicides. Also, to identify the causal pathogen of powdery mildew on squash (cv. Eskandrani) in Egypt, according to the anamorphic features.

## Materials and Methods

### 1. Morphological characterization of the causal pathogen

To visualize the conidiophores origin as well as the morphology of conidia, cuticle removal technique (CRT) was conducted by the application of colorless base nail varnish (nail base) composed of cellulose acetate dissolved in acetone (colorless, trade mark "Q Tock") onto the leaves abaxial surface followed by drying on a stand for 10 min. After drying, a transparent adhesive tape (Scotch tape, trade mark "Durex") was pasted over the enamelled leaf surface. After slight pressure between fingers, the tape was removed from the leaf containing the enamelled cuticle of the leaf with conidiophores of the fungus. The tape with the cuticle was then inverted as a coverslip on a microscope slide using lactophenol. For contrast, lactic acid and cotton blue (1g/L) was used (Moreira *et al.*, 2014).

For germination, conidia were secured by shaking the mildewed leaves over clean slides (Zaracovitis, 1965). Young conidia (preferably 24hr. old) were used in all tests, incubated at  $21\pm 1^{\circ}\text{C}$  in a saturated atmosphere in the dark. Percentage of

conidial germination was determined after 2.5, 5, 10 and 24 hr. The details of germ-tube and appressorial production, size and shape of conidia were recorded.

## 2. Pot experiments

### 2.1. Effect of different plant oils against squash powdery mildew

Alovera, sesame and flax oils were obtained from the Egyptian trade market. For emulsification, a test tube containing 5 ml oil, 4.5 ml distilled water and 0.5 ml Tween 20% was shaken vigorously 20 times by hand. The mixture was diluted with distilled water to make 0.1% oil emulsion. A mixture of water + 0.01% Tween 20 was used as a check. When 0.5% oil was used, the concentration of Tween 20 was also increased to 0.05% (Farid *et al.*, 2000 and Ko *et al.*, 2003). Pathogen inoculation was performed by shaking heavily infected squash leaves with powdery mildew onto healthy plants. Treatments were carried out twice, with an interval of one week, starting immediately after the appearance of the first symptoms of powdery mildew on the foliage. For check, plants were sprayed with water mixed with tween 20. Five plants with fully expanded leaves were used for each treatment.

### 2.2. Effect of kaolin, potassium bicarbonate and whey against squash powdery mildew

Application of the tested treatments, *i.e.* kaolin (3 and 6%), potassium bicarbonate (500 and 750 ppm) and whey (5 and 10%) were carried out twice, with an interval of one week, starting immediately after the appearance of the first symptoms of powdery mildew on the foliage. For check, plants were sprayed with water mixed with tween 20. Pathogen inoculation was performed by shaking heavily infected squash leaves with powdery mildew onto healthy plants. Five plants with fully expanded leaves were used for each treatment.

### 2.3. Effect of different bacterial strains against squash powdery mildew

Four different bacterial isolates, *i.e.* *Bacillus subtilis* sub sp. *subtilis* (co1-6), *Bacillus vallismortis* (wb2-1), *Paenibacillus brasiliensis* (Mc2Re-16) and *Paenibacillus polymyxa* (Mc5Re-14) were kindly obtained from Dr. Mohamed Adam, Zoology and Nematology Dep. Fac. of Agri. Cairo Univ., Giza, Egypt. The talc-based formulations of each bacterial isolate were prepared with some modifications of the method developed by Commare *et al.* (2002). The selected biocontrol formulations were inoculated into the King B broth medium and incubated in a rotary shaker at 150 rpm for 48 h at room temperature ( $28 \pm 2^\circ\text{C}$ ). One kg of talc powder was taken in a sterilized metal tray and then mixed with  $\text{CaCO}_3$  at the rate of 15 g/kg. 10 g of carboxymethyl cellulose (CMC) were added to each 1 kg of talc and mixed well and the mixture was autoclaved for 30 minutes. 400 ml of the 48-hour-grown suspension containing  $9 \times 10^8$  CFU/ml water were mixed with the carrier cellulose mixture under aseptic conditions. After drying (approximately to 35% moisture content) for overnight, it was packed in polypropylene bag, sealed and stored at room temperature ( $28 \pm 2^\circ\text{C}$ ). For check, plants were sprayed with water mixed with Tween 20. Pathogen inoculation was performed by shaking heavily infected squash leaves with powdery mildew onto healthy plants. Five plants with fully expanded leaves were used for each treatment.

### 3. Effect of different alternative programs on disease severity of squash powdery mildew under natural conditions

In the present experiments, eight different programs (Table 1) were designed to determine their efficacy against squash powdery mildew under natural infection. The experiment was conducted in plots (1 m<sup>2</sup>), located in the experimental unit of the Plant Pathol. Dept., Fac. of Agric., Cairo Univ., during 2014 and 2015 growing seasons. In both seasons, squash seeds (cv. Eskandrani) obtained from the Agricultural Research Institute, ARC, Giza, Egypt were planted on 17<sup>th</sup> March. All agricultural practices were carried out according to the recommendations of the Ministry of Agric., Egypt. Randomized complete block design with three replicates was performed. Treatments were carried out four times, with an interval of two weeks (Table 1), starting immediately after the appearance of the first symptoms of natural infection with powdery mildew on the foliage. For check, plants were sprayed with water mixed with Tween 20. Each treatment consisted of 15 plants and each replicate was represented by 5 plants. Disease severity was assessed on five randomly plants in each plot, one week after the last spray.

### 4. Disease assessment

The disease severity was estimated depending on the devised scale (0-4) described by Descalzo *et al.* (1990) with some modifications, where: 0 = no powdery mildew lesions; 1 = 25 or less; 2 = more than 25 to 50; 3 = more than 50 to 75 and 4 = more than 75-100% of the infected leaf area.

Disease severity was estimated using the following formula:

$$\text{Disease severity \%} = \frac{\sum (nxv)}{4 N} \times 100.$$

Where: n = number of infected leaves in each category; v = numerical values of each category and N = total number of the infected leaves.

### 5. Statistical analysis

The obtained data were statistically analyzed by the analysis of variance using MSTAT-C statistical software. Treatment means were compared using Duncan's multiple tests (Steel and Torrie, 1980). Probability levels lower than 0.05 were held to be significant.

## Results

### 1. Morphological characteristics of the causal pathogen

The microscopic examinations of the anamorphic stage of the causal pathogen showed that mycelium is septate and ectophytic. Conidiophores un-branched, have foot-cells associated with 3 shorter cells bearing conidia in long chains. Immature conidia are with crenate edge, hyaline, smooth and ellipsoid, 26-40 X 16-24 µm. Length/width ratio is 1.47. Germination of conidia requires more than 10 hr. on dry slide glass, and germinate to straight germ-tubes without well-differentiated appressoria. Accordingly, the pathogen was identified herein as *Oidium fuliginae* (Schlecht. Ex Fries) Poll the conidial stage of *Sphaerotheca fuliginae* (Schlecht. Ex Fries) Poll (Synonym: *Podosphaera xanthii* (Castag) Braun and Shish.

**Table 1. Alternative programs designed to study their effect on squash powdery mildew under natural conditions**

The tested programs	Time of spray/treatment			
	7 <sup>th</sup> May (1 <sup>st</sup> spray)	21 <sup>th</sup> May (2 <sup>nd</sup> spray)	4 <sup>th</sup> June (3 <sup>rd</sup> spray)	18 <sup>th</sup> June (4 <sup>th</sup> spray)
T1	Prochloraz fungicide	Prochloraz fungicide	Prochloraz fungicide	Prochloraz fungicide
T2	Prochloraz fungicide	Kaolin	Prochloraz fungicide	Kaolin
T3	Kaolin	Potassium bicarbonate + <i>P. polymexa</i>	Kaolin	Potassium bicarbonate + <i>P. polymexa</i>
T4	Kaolin	Potassium bicarbonate + Sesame oil	Kaolin	Potassium bicarbonate + Sesame oil
T5	Kaolin	Potassium bicarbonate + whey	Kaolin	Potassium bicarbonate + whey
T6	Kaolin	Potassium bicarbonate + Sesame oil + whey	Kaolin	Potassium bicarbonate + Sesame oil + whey
T7	Prochloraz fungicide	Potassium bicarbonate + Sesame oil + whey	Prochloraz fungicide	Potassium bicarbonate + Sesame oil + whey
T8	Check	Check	Check	Check

## 2. Pot experiments

### 2.1. Effect of different plant oils against squash powdery mildew.

Effect of three different oils, *i.e.* Alovera oil, sesame oil and flax oil was evaluated against squash powdery mildew under greenhouse conditions. Data presented in Table (2) show that all the tested oils at any of the tested concentrations decreased the percentage of disease severity in comparison to the untreated check. Disease severity was also decreased by increasing the concentration of the tested oils from 0.1 to 0.3%. The corresponding values were 7.42, 12.50 and 13.89% when the concentration 0.3% of sesame oil, flax oil and alovera oil was used, respectively. The lowest percentage value was 7.42% due to using sesame oil at 0.3% in comparison to the untreated check which recorded 35.71%.

**Table 2. Effect of the tested oils against squash powdery mildew under greenhouse conditions**

Treatments	Concentrations (%)	Disease severity (%)	Reduction in relation to check (%)
Alovera oil	0.1	16.00 <sup>b</sup>	55.19
	0.3	13.89 <sup>b</sup>	51.10
Sesame oil	0.1	8.13 <sup>cd</sup>	77.74
	0.3	7.42 <sup>d</sup>	79.22
Flax oil	0.1	14.58 <sup>b</sup>	59.17
	0.3	12.50 <sup>bc</sup>	65.0
Check		35.71 <sup>a</sup>	-

Values within the same column followed by the same letters are not significantly different ( $p < 0.05$ ).

### 2.2. Effect of kaolin, potassium bicarbonate and whey against squash powdery mildew

Three different treatments, *i.e.* kaolin, potassium bicarbonate and whey were evaluated against squash powdery mildew under greenhouse conditions. Data presented in Table (3) show that all treatments and their concentrations significantly decreased the severity of powdery mildew in comparison with the untreated check. It was noticed that increasing the concentration of the tested materials was accompanied by a noticeable decrease in disease severity. The lowest percentage value of disease severity was 8.08% due to using kaolin at 6% followed by kaolin at 3% concentration, being 14.67% in comparison with the untreated check that recorded 35.71%.

**Table 3. Effect of kaolin, potassium bicarbonate and whey against squash powdery mildew under greenhouse conditions**

Treatments	Concentrations	Disease severity (%)	Reduction in relation to check (%)
Kaolin	3%	14.67 <sup>d</sup>	58.92
	6%	8.08 <sup>e</sup>	77.37
Potassium bicarbonate	500 ppm	19.64 <sup>cd</sup>	45.0
	750 ppm	15.64 <sup>d</sup>	56.20
Whey	5%	29.33 <sup>b</sup>	17.87
	10%	22.54 <sup>c</sup>	36.88
Check		35.71 <sup>a</sup>	

Values within the same column followed by the same letters are not significantly different ( $p < 0.05$ ).

### 2.3. Effect of different bacterial strains against squash powdery mildew

Data presented in Table (4) show that all the tested bacterial strains were able to reduce the severity of infection with squash powdery mildew. In this respect, *P. polymexa* showed the lowest value of disease severity, being 23.96% followed by *B. subtilis*, being 27.71%, respectively, on the average without significant differences. Meanwhile, it is worth to note that no significant differences were found between *B. vallismortis* and the untreated check. The corresponding disease severity percentages were 32.47 and 35.71% on the average, respectively.

### 3. Effect of different alternative programs on disease severity of squash powdery mildew under natural conditions

Under natural infection, eight different programs were designed to control squash (cv. Eskandrani) powdery mildew during 2014 and 2015 growing seasons. Data presented in Table (5) show that all the tested programs significantly reduced the disease severity in comparison with the untreated check. T1, T2 and T7 tested programs reduced the severity of squash powdery mildew and showed the lowest values of disease severity, being 4.59, 7.09 and 7.92% on the average, respectively, without significant differences. On the other hand, programs, T6, T5 and T4, also reduced disease severity to intermediate values, being 10.00, 11.67 and 12.09% on the average, respectively. Meanwhile, T3 program recorded the highest disease severity value, being 15.00% in comparison with the untreated control, being 41.25%. The highest reduction percentage values, being 88.85, 82.78 and 80.77% were recorded due to using Prochloraz fungicide alone (T1) or alternative with kaolin (T2) or with a mixture of potassium bicarbonate, sesame oil and whey (T3), respectively.

**Table 4. Effect of different bacterial strains against squash powdery mildew under greenhouse conditions**

Treatments	Disease severity (%)	Reduction in relation to check (%)
<i>B. subtilis</i>	27.71 <sup>bc</sup>	22.40
<i>B. vallismortis</i>	32.47 <sup>ab</sup>	9.07
<i>P. brasiliensis</i>	30.08 <sup>b</sup>	15.77
<i>P. polymexa</i>	23.96 <sup>c</sup>	32.90
Check	35.71 <sup>a</sup>	-

Values within the same column followed by the same letters are not significantly different ( $p < 0.05$ ).

**Table 5. Effect of different programs against squash powdery mildew under natural conditions**

The tested programs		Disease severity %*	Reduction in relation to check (%)
T1	Prochloraz fungicide	4.59 <sup>d</sup>	88.85
T2	Prochloraz fungicide alternative with kaolin	7.09 <sup>d</sup>	82.78
T3	Kaolin alternative to a mixture of potassium bicarbonate and <i>P. polymexa</i>	15.00 <sup>b</sup>	63.55
T4	Kaolin alternative to a mixture of potassium bicarbonate and sesame oil	12.09 <sup>bc</sup>	70.53
T5	Kaolin alternative to a mixture of potassium bicarbonate and whey	11.67 <sup>c</sup>	71.82
T6	Kaolin alternative to a mixture of potassium bicarbonate, sesame oil and whey	10.00 <sup>c</sup>	75.68
T7	Prochloraz fungicide alternative to a mixture of potassium bicarbonate, sesame oil and whey	7.92 <sup>cd</sup>	80.77
T8	Check	41.25 <sup>a</sup>	-

\* Data of disease severity are means of two growing seasons (2014, 2015)

Values within the same column followed by the same letters are not significantly different ( $p < 0.05$ ).

### Discussion

Powdery mildew diseases are among the crucial factors affecting squash production all over the world. The disease affects all plant parts and causes severe losses in the total yield and its components. The utilization of fungicides is the most effective mean to reduce the disease incidence, but frequent application of fungicides leads to severe economic losses, emergence of resistant pathogen populations and potential ecological pollution (Ma and Michailides, 2005). So, Brent and Hollomon, (2007) has produced some guidelines to exclude the negative effects of fungicides, including: (1) not applying a one type of chemicals, (2) confining the quantity of chemical applications per season, (3) using the recommended dose of chemicals (4) applying chemicals as deterrent protectants instead of as eradicants and (5) empowering use of chemicals with different actions through the season.

The presented research was designed to reduce the number of fungicide applicant through designing programs containing different eco-friendly non-chemical compounds. Also, to determine the causal pathogen of powdery mildew on squash (cv. Eskandrani) in Egypt, according to the morphological and physiological features of the anamorphic stage.

The causal agents of cucurbit powdery mildew, *i.e.* *P. xanthii* and *G. cichoracearum* produce identical symptoms and can be difficult to differentiate in the absence of the perfect stage (Block and Reitsma, 2005). However,



morphological and physiological features of conidial germination are very prominent in this concern (Nour, 1959). According to Zaracovitis (1965) morphological features of the asexual stage of *P. xanthii* differ from those of *G. cichoracearum* according to size and shape of conidia, presence of fibrosin bodies, immature conidia edge and germ tube morphology

In respect to morphology of powdery mildew conidia, the slow germination of conidia on the dry slide and formation of forked germ-tube which was observed microscopically in this study agrees with Zaracovitis (1965) who reported that the powdery mildew of cucurbits has been shown to be *Sphaerotheca fuliginea* and not *Erysiphe cichoracearum*, where the conidia of *Erysiphe cichoracearum* germinate faster than *Sphaerotheca fuliginea* on dry slide and germinate within 5 hrs to germ-tube with distinct appressoria.

The effectiveness of plant oils in controlling powdery mildew on several plant hosts has been well verified (Hafez, 2008; Jee *et al.*, 2009 and Chee *et al.*, 2011). The superiority of the emulsified sesame oil, which noticed in this study agrees with Keinath and DuBose (2012). They reported that organocide, a fungicide containing 5.0% sesame oil and 92% edible fish oil, has a satisfactory protection against cucurbit powdery mildew.

Clay minerals, such as kaolin, have been reported to provide protection against several foliage plant diseases, including gray mold, leaf blight, anthracnose, rust, powdery mildew and fruit rots (Zakaria-Oren *et al.*, 1991, Marco *et al.* 1994; Hiseh and Huang, 1997 and Ehret *et al.*, 2001). The present research shows high efficiency of kaolin in reducing severity of squash powdery mildew under artificial conditions. These findings are in agreement with Marco *et al.* (1994) who found that weekly application of whitewash at 10% or inert clay containing kaolin resulted in 50-60% reduction in squash powdery mildew disease under field conditions. We can reveal this activity to its alumino-silicate mineral contents (Lamb *et al.*, 2002) which act as a physical barrier against pathogen penetration or by activating defense response in plants.

Another friendly non-chemical materials used to manage powdery mildew diseases are bicarbonate salts such as potassium bicarbonates. Bicarbonates are known for many years with their antifungal properties that act as a contact fungicide (Marku *et al.*, 2014). Data of the present study show that spraying squash with potassium bicarbonate at 1 and 3% concentrations resulted in significant reduction in powdery mildew severity under greenhouse conditions in comparison to the untreated check. It is worth to note that increasing the concentration of the tested material, the reduction in powdery mildew severity was increased as well. This finding is in coincide with those obtained by Zaki *et al.* (2011). They reported that application of potassium bicarbonate solution at 2% resulted in reduction in severity of powdery mildew of squash in comparison to the untreated check. The antifungal activity of bicarbonates against powdery mildew may reveal to the hypothesis of Ziv and Zitter (1992) who reported that bicarbonates collapse the cell walls of hyphae and shrink the conidia.

On the other hand, the beneficial effect of milk and whey as alternative means to synthetic fungicides in controlling powdery mildew on different hosts was reported (Bettiol *et al.*, 1999). Unfortunately, using milk in controlling diseases is very expensive. So, whey is considered a cheaper alternative to fresh milk. The reduction in severity of powdery mildew observed in this study due to applying whey as a foliar spray is in agreement with Bettiol *et al.* (2008). They reported that whey effectively controlled powdery mildew in cucumber and zucchini plants. The reduction in severity of powdery mildew caused by using whey could be revealed to the changes occurred in the populations of the beneficial organisms on leaf surfaces that lead to suppress the pathogen by the activity of these organisms (Medeiros *et al.*, 2006).

Biocontrol agents have attracted much attention as a friendly alternative to synthetic fungicides. They may act by different mechanisms such as antibiosis, mycoparasitism, competition and the induction of resistance in the host plant (Shoresh *et al.*, 2010), among these agents, many species belonging to *Bacillus* and *Paenibacillus* (Arrebola *et al.*, 2010). The great reduction in severity of powdery mildew obtained in this study was due to applying *P. polymyxa* and this agrees with Kim *et al.* (2003) who reported that treated tomato and red pepper plants infected with powdery mildew with *P. polymyxa* resulted in a severe reduction in disease incidence in comparison to the untreated check. *P. polymyxa* is known to produce two types of peptide compounds, one type is only active against bacteria and the other is active against fungi, gram positive bacteria and actinomycetes (Beatty and Jensen, 2002). This may explain the antifungal activity of this strain against powdery mildew disease in this research.

To improve the action of fungicides in controlling plant diseases, the present work suggested eight different programs, included rotational application materials rather than repeated spraying one to reduce the risk of intensive use of synthetic fungicides under natural conditions. The great reduction in severity of powdery mildew was observed in this work due to using the rotational application programs included, Prochloraz fungicide as a single repeated application program, Prochloraz alternative with Kaolin and Prochloraz alternative with a mixture of potassium bicarbonate, sesame oil and whey. The obtained results are in agreement with those reported by Yildirim and DaDeniz (2010); Savocchia *et al.* (2011) and Matheron and Porchas (2013). Concerning the work performed by Yildirim and DaDeniz (2010), they confirmed the efficacy of  $\text{NaHCO}_3/\text{K}_2\text{SiO}_3$  + Sulfur as well as  $\text{KH}_2\text{PO}_4$ /di-1-p-menthene as alternate spray programs against powdery mildew on grape. Also, Savocchia (2011) reported that sulfur, milk, whey, potassium bicarbonate (Ecocarb) and a mixture of potassium bicarbonate and botanical oils (Synertrol Horti Oil) alternated with whey are capable to reduce the severity of powdery mildew on grape bunches at harvest compared to the untreated check. The great activity of the programs contained Prochloraz, a fungicide known as inhibiting ergosterol biosynthesis could be explained by its insertion into the imidazole group, where imidazole could react with Kaolin and produce complex (Mirjalili and Akrami, 2015). This complex may be more effective than the original compound. On the other hand, the great activity in reducing severity of powdery mildew by programs

contained potassium bicarbonate and sesame oil may be due to its well distribution on the leaf surface (Sawant and Sawant, 2008). Also, the positive effect of incorporating potassium bicarbonate with oils was conducted at Cornell and focused on controlling fungal diseases on cucurbits (Ziv and Zitter, 1992). They used baking soda at 0.5% incorporated or not with SunSpray UFP<sup>®</sup> oil as a single spray application. They noticed a complete reduction in powdery mildew on heavily infected pumpkin foliage in comparison to plants treated with potassium bicarbonate without spray oil.

In conclusion, this research has shown that alternative treatments to fungicides have the potential to reduce powdery mildew on squash (cv. Eskandrani) and that these must be incorporated into rotational spray programs to reduce the use of synthetic fungicides. Other studies must be conducted to evaluate the effect of these programs on plant productivity.

### References

- Anonymous, 2017. Food and Agricultural Organization (FAO), Production. <http://www.fao.org/faostat/en/#data/QC/visualize>.
- Arrebola, E.; Jacobs, R. and Korsten, L. 2010. Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. *J. Appl. Microbiol.*, **108**:386–395
- Beatty, P.H. and Jensen, S.E. 2002. *Paenibacillus polymyxa* produces fusaricidin-type antifungal antibiotics active against *Leptosphaeria maculans*, the causative agent of blackleg disease of canola. *Can. J. Microbiol.*, **48**:159-69.
- Bettiol, W. 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. *Crop Prot.*, **18**:489-492.
- Bettiol, W.; Silva, H.S.A. and Reis, R.C. 2008. Effectiveness of whey against zucchini squash and cucumber powdery mildew. *Sci. Hortic.*, **117**:82–84.
- Block, C.C. and Reitsma, K.R. 2005. Powdery mildew resistance in the US National Plant Germplasm System cucumber collection. *Hortsci.*, **40**:416-420.
- Brent, K.J. and Hollomon, D.W. 2007. Fungicide Resistance in Crop Pathogens: How Can it be Managed? FRAC Monograph No. 1 (second, revised edition). Fungicide Resistance Action Committee, Brussels, Belgium, 57 pp.
- Chee, A.; Wurms, K.V. and George, M. 2011. Control of powdery mildew (*Sphaerotheca pannosa* var. *rosae*) on rose (*Rosa* sp.) using anhydrous milk fat and soybean oil emulsions. *NZ Plant Prot.*, **64**:195-200.
- Commare, R.R.; Nandakumara R.; Kandana A.; Suresh S.; Bharathi M.; Raguchander T. and Samiyappan, R. 2002. *Pseudomonas fluorescense* based bio-formulation for the management of sheath blight disease and leaf-folder insect in rice. *Crop Prot.*, **21**:671–677.

- Descalzo, R.C.; Rohe, J.E. and Mauza, B. 1990. Comparative efficacy of induced resistance for selected diseases of greenhouse cucumber. *Can. J. Plant Pathol.*, **12**:16-24.
- Ehret, D.L.; Koch, C.; Jim, M.J.; Sholberg, J. and Garland, T. 2001. Foliar sprays of clay, reduce the severity of powdery mildew on long English cucumber and wine grapes. *Hortsci.*, **365**:934-936.
- Elkot, G.A. and Derbalah, A.S.H. 2011. Use of cultural filtrates of certain microbial isolates for powdery mildew control in squash. *J. Plant Prot. Res.*, **51**:252-260.
- Farid, S.; Fathy, S.L.; Elmaghrabi, A. and El-Shazly, H.A.F. 2000. Controlling of powdery mildew and improving growth and yield of squash plant by using some natural essential oils, phytoextract and biological agents. *J. Agric. Sci., Mansoura Univ.*, **26**:4069-4087.
- Hafez, Y.M. 2008. Effectiveness of the antifungal black seed oil against powdery mildews of cucumber (*Podosphaera xanthii*) and barley (*Blumeria graminis* f. sp. *hordei*). *Acta Biol. Szeged.*, **52**:17-25.
- Hiseh, T.F. and Huang, J.W. 1997. Application of film-forming antitranspirants for control of plant diseases. *Plant Pathol. Bull.*, **6**:89-94.
- Jee, H.; Shim, C.; Ryu, K.; Park, J.; Lee, B.; Choi, D. and Ryu, G. 2009. Control of powdery and downy mildews of cucumber by using cooking oils and yolk mixture. *Plant Pathol. J.*, **25**:280-285.
- Keinath, A.P. and DuBose, V.B. 2012. Controlling powdery mildew on cucurbit rootstock seedlings in the greenhouse with fungicides and biofungicides. *Crop Prot.*, **42**:338-344.
- Kim, H.T.; Jang, K.S.; Choi, G.J.; Lee, S.W. and Cho, K.Y. 2003. Effect of Prochloraz on electrolytic leakage and spore germination of *Puccinia recondita* causing wheat leaf rust. *Plant Pathol. J.*, **19**:189-194.
- Ko, W.H.; Wang, W.; Hsieh, T.F. and Ann, P.J. 2003. Effects of sunflower oil on tomato powdery mildew caused by *Oidium neolycopersici*. *J. Phytopathol.*, **151**:144-148.
- Lamb, E.M.; Roskopf, E.N. and Koblebard, C. 2002. Use of kaolin clay for disease control in greenhouse cucumbers. *Proc. Fla. State Hort. Soc.*, **115**:180-182.
- Ma, Z. and Michailides, T.J. 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Prot.*, **24**:853-863.
- Marco, S.; Ziv, L.O. and Cohen, R. 1994. Suppression of powdery mildew in squash by applications of whitewash, clay and antitranspirant materials. *Phytoparasitica*, **22**:19-29.
- Marku, L.; Vrapu, H. and Hasani, M. 2014. Effect of potassium bicarbonate (Armicarb) on the control of apple scab (*Venturia inaequalis*) in the region of Puka in Albania. *Inter. Refer. J. Eng. and Sci. (IRJES)*, **3**:25-30.

- Matheron, M.E. and Porchas, M. 2013. Efficacy of fungicides and rotational programs for management of powdery mildew on cantaloupe. *Plant Dis.*, **97**:196-200.
- McCreight, J.D.; Pitrat, M.; Thomas, C.E.; Kishaba, A.N. and Bohn, G.W. 1987. Powdery mildew resistance genes in muskmelon. *J. Amer. Soc. Hort. Sci.*, **112**:156-160.
- Medeiros, F.H.V.; Pinto, Z.V.; Correa, E.B. and Bettiol, W. 2006. The lactoperoxidase system as a novel, natural fungicide for control of powdery mildew. In: Proceeding of the IOBC/WPRRS Working Group Fundamental and Practical Approach to Increase Biocontrol Efficacy, Spa, Belgium, September 6-10, 20 p.
- Mirjalili, B.F. and Akrami, H. 2015. Kaolin-SO<sub>3</sub>H as an efficient catalyst for one-pot synthesis of 1,2,4,5-tetrasubstituted imidazoles. *Iran. J. Catalysis*, **5**:129-134
- Moreira, L.S.; Carvalho, B.M.; Vivas, J.M.S.; Santos, P.H.D.; Vivas, M. and Silveira, S.F. 2014. Comparison of microscopy techniques to visualize powdery mildew (Erysiphales) conidiophores. *Cientifica, Jaboticabal*, **42**:46-60.
- Nour, M.A. 1959. Studies on the specialization of *Sphaerotheca fuliginea* (Schlecht.) Poll. and other powdery mildews. *Trans. Br. Mycol. Soc.*, **42**:90-94.
- Savocchia, S.; Mandel, R.; Crisp, P. and Scott, E.S. 2011. Evaluation of alternative materials to sulfur and synthetic fungicides for control of grapevine powdery mildew in a warm climate region of Australia. *Austr. Plant Pathol.*, **40**:20-27.
- Sawant, S.S.D. and Sawant, I.S. 2008. Use of potassium bi-carbonates for the control of powdery mildew in table grapes. Proc. IS on Grape Production and Processing. *Acta Hort.*, **785**:285 – 291.
- Shores, M.; Harman, G.E. and Mastouri, F. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu Rev Phytopathol.*, **48**:21-43.
- Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics, a Biometrical Approach, 2<sup>nd</sup> edition, McGraw-Hill, New York, USA, pp. 20-90.
- Vakalounakis, D.J.; Klironomou, E. and Papadakis, A. 1994. Species spectrum, host range and distribution of powdery mildews on Cucurbitaceae in Crete. *Plant Pathol.*, **43**:813-818.
- Yildirim, I. and DaDeniz, A. 2010. Effects of alternative spray programs and various combinations of green pruning on powdery mildew *Uncinula necator* (Schw.) Burr. in Karasakiz (Kuntra) grape cultivar. *Turk. J. Agric. For.*, **34**:2013–223.
- Zakaria-Oren, J., Eyal, Z. and Ziv., O. 1991. Effect of film-forming compounds on the development of leaf rust on wheat seedling. *Plant Dis.*, **75**:231-234.
- Zaki, K.I., Zayed, S. and Abd-Alraheim, A.M. 2011. Foliar application of compost-tea and bicarbonate salts for controlling powdery mildew disease on squash plants in north Sinai. *Egypt. J. Phytopathol.*, **39**:201-220.

- Zaracovitis, C. 1965. Attempts to identify powdery mildew fungi by conidial characters. *Trans. Brit. mycol. Soc.*, **48**:553-558.
- Zhang, S.; Vallad, G.E.; White, T.L. and Huang, C.H. 2011. Evaluation of microbial products for management of powdery mildew on summer squash and cantaloupe in Florida. *Plant Dis.*, **95**:461-468.
- Ziv, O. and T.A. Zitter. 1992. Effects of bicarbonates and film-forming polymers on cucurbit foliar diseases. *Plant Dis.*, **26**:513-517.

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## استخدام برامج آمنة للمبيدات في مكافحة مرض

## البياض الدقيقي في الكوسة

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أثبتت الدراسات المورفولوجية باستخدام الميكروسكوب الضوئي أن المسبب لمرض البياض الدقيقي في الكوسة (الصنف اسكندراني) يتبع الجنس أويديوم، الطور الكونيدي للفطر سفيروثيكا فاليجني وذلك من خلال دراسة أبعاد الجراثيم الكونيدية (26-40 × 16-24 ميكرون) ، كما بلغ معدل النسبة بين طول الجراثيم الكونيدية وعرضها حوالي 1.47، وأثبتت تجارب إنبات الجراثيم الكونيدية على الشرائح الزجاجية الجافة عند التحضين على درجة حرارة 21 درجة مئوية أن الجراثيم تنبت ببطئ وتعطي أنبوبة إنبات بدون تكوين عضو التصاق متميز. أجريت بعض التجارب تحت ظروف الصوبة لتقييم كفاءة ثلاثة زيوت نباتية هي زيت السمسم وزيت الصبار وزيت الكتان، ثلاثة مركبات طبيعية هي الكاؤولين وبيكربونات البوتاسيوم والشرش بالإضافة إلى أربعة عزلات بكتيرية كعوامل للمكافحة الحيوية وهم *B. subtilis*، *B. vallismortis*، *P. brasilensis* و *P. polymexa* ضد مرض البياض الدقيقي في الكوسة (الصنف اسكندراني). أظهرت النتائج أن كلا من زيت السمسم بتركيز 0.3% والكاؤولين بتركيز 3% والعزلة البكتيرية *P. polymexa* هم الأكثر كفاءة في زيادة نسبة الوقاية من المرض (79.22 ، 77.37 و 32.90% على التوالي).

تم تصميم عدة برامج بديلة للمبيد الفطري بينكونازول لرش أوراق الكوسة تحت ظروف العدوي الطبيعية وذلك خلال موسمين زراعيين هما 2014 و 2015. فوجد أن البرنامج المحتوي على مبيد البروكليوراز فقط يليه البرنامج المحتوي على مبيد البروكليوراز بالتبادل مع الكاؤولين يليه البرنامج المحتوي على مبيد البروكليوراز بالتبادل مع مخلوط من بيكربونات البوتاسيوم وزيت السمسم والشرش كانت الأكثر كفاءة في خفض شدة الإصابة بمرض البياض الدقيقي على الكوسة. توصي الدراسة الحالية باستخدام المبيدات والمواد الطبيعية الأخرى بالتبادل وذلك لخفض التأثيرات السلبية الناتجة عن الاستخدام المتزايد للمبيدات والعمل على زيادة كفاءتها في مكافحة مرض البياض الدقيقي.