

**STUDIES ON CUCUMBER POWDERY MILDEW DISEASE
CAUSED BY *Sphaerotheca fuliginea* UNDER COMMERCIAL
PLASTIC HOUSE CONDITIONS IN EGYPT
2- NOVEL CONTROL METHODS BY USING SYSTEMIC
RESISTANCE INDUCERS, NATURAL AND MINERAL OILS AND
FUNGICIDES**

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ABSTRACT: As a true attempt to control cucumber powdery mildew disease under commercial plastic greenhouses by novel control methods using ten systemic resistance inducers, three natural oils, three mineral oils and three recommended fungicides were compared for their antifungal effects on cucumber powdery mildew disease before and after infection with *Sphaerotheca fuliginea*.

All systemic resistance inducer treatments obviously proved the best results in controlling the disease . Cobalt sulphate, Cali-Green, Potassium silicate, Lithium chloride and Agri - fos significantly reduced the disease (determined by five different parameters) especially Potassium silicate, Cobalt sulphate and Cali-green. In this regard, monobasic potassium phosphate (high dose) only succeeded in reducing disease percentage, and disease severity percent whereas dibasic Sodium phosphate gave satisfactory results only in reducing disease severity parameters. In addition, light effect of the systemic resistance inducers, Vapour-guard was noticed .On the other hand, all the other tested substances, three natural oils,three mineral oils and three fungicides, significantly controlled the disease before and after infection.

The tested fungicides (Afugan, Sumi-eight and micronized Surel), the mineral oil Super masrona and the natural oils Trilogy and Naterolow gave excellent results in reducing the disease severity percent , followed by the natural oil Neemix then the Kz mineral oil. Moreover, all tested substances strongly decreased the average number of colonies / leaf comparing with the check .Acute decreasing was markedly resulted when the fungicides were used followed by mineral oils and natural oils. The results of this research suggest that the promising systemic resistance inducers or mineral oils could be replaced the fungicides to control *S. fuliginea*, the causal pathogen of cucumber powdery mildew, in greenhouses.

Key Words: Cucumber, powdery mildew, *Sphaerotheca fuliginea* , control.

During relatively dry warm seasons, the fungus aggressively attacks cucumber (*Cucumis sativus* L.) and causes severe damage to these plants and acute losses on fruit quantity and quality (Arimoto and Homma, 1995).

As a true attempt to control cucumber powdery mildew disease under commercial plastic house conditions, there are new manners proved satisfied results in controlling this disease all over the world such as: (1) Using systemic resistance inducers as Lithium chloride (Abood *et al*, 1992 and Losel *et al*, 1994); Potassium silicate (Samuels *et al*, 1991 a,b & 1993 and Menzies *et al*, 1992); Sodium and Potassium phosphates Reuveni *et al*, 1993, 1995 a,b and 1996). (2) Using natural oils (Steck and Schneider, 1993 and Dik *et al*, 1994; Daayf *et al*, 1995 and Konstantinidou-Doltsinis, 1998). (3) Using mineral oils (Ontsuka *et al*, 1991). Consequently, this study aimed to research about the ideal, standard, suitable and economical methods to control this dangerous disease under commercial plastic greenhouses far away polluting the environment and maintenance of human health using systemic resistance inducers, natural oils and mineral oils comparing with the effect of the most common recommended fungicides. The experimental results were confirmed using five different disease assessment parameters.

MATERIALS AND METHODS

1- Effect of ten systemic resistance inducers on the disease incidence:

Ten chemical systemic resistance inducers (12 treatments); Cobalt sulphate, dibasic Sodium phosphate, monobasic Sodium phosphate, dibasic Potassium phosphate (high and low doses), monobasic Potassium phosphate (high and low doses), Cali-green, Agri-fofos, Potassium silicate, Vapour-guard and Lithium chloride; were tested for their ability to control cucumber powdery mildew disease under greenhouse conditions (Table-1)

Four seeds of the susceptible Anas cucumber hyb. were planted in 20 cm in diameter pot containing sandy-clay soil (1:1). Plants, at the two or three true leaf stage, were sprayed with each of the resistance inducers according to their rate of application with constant volume for each plant. Four pots were devoted for each resistance inducer. Set of pots receiving no resistance inducer but sprayed with distilled water served as a check.

Inoculation

Diseased cucumber (Beit-Alpha, hyb.), showing typical symptoms of powdery mildew disease, were collected from commercial plastic houses of Bostan region, Beheira governorate. The leaves were shaken 24 h before conidial harvest to dislodge the old conidia and ensure a high viability of inoculum. Powdery mildewed colonies were kindly brushed with water and immediately were used in inoculating the plants. The leaves of all plants

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Table (1) :Chemical name, active ingredients and rate of application of different resistance inducers.

Chemical and trade name	Active ingredients	Rate of application
1-Cobalt sulphate (CO_{SO}4-7 H₂O)	Cobalt (Co)	Equal to: 0.1 mM
2-Dibasic sodium phosphate (Na₂H₂PO₄)	Sodium and phosphate	Equal to: 100 mM
3-Mono-basic sodium phosphate (NaH₂PO₄)	Sodium and phosphate	Equal to: 100 mM
4-Dibasic potassium phosphate (K₂HPO₄)	Potassium and phosphate	Equal to: 100 mM (high dose) and 6 mM (low dose)
5-Mono-basic potassium phosphate (KH₂PO₄)	Potassium and phosphate	Equal to: 100 mM (high dose) and 6 mM (low dose)
6-Cali – Green	80% potassium bicarbonate in the form of ester of fatty acid	150g/ 100 litre water
7-Agri. – Fos	Mono and di potassium phosphate	400ml/ 100 litre water
8-Potassium silicate (K₂SiO₃)	Silicon (Si)	Equal to: 17 mM
9-Vapour Guard	96% di-l- menthene	2% concentration
10-Lithium chloride (LiCl)	Lithium (Li)	Equal to: 1 mM

were inoculated with the optimum concentration of *Sphaerotheca fuliginea* conidia (3×10^4 conidia / ml⁴) one day after conidial harvest (Reuveni *et al*,1995).

Disease measurements

Five different assessment parameters were followed to assay cucumber powdery mildew disease ,seven days after inoculation:

- Disease severity which depends on the infected leaf area using the formula suggested by Townsend and Huberger (1943)
- Average counts of colonies / leaf .
- Average diameter of colonies .
- Average counts of conidia / leaf .
- Average of disease percentage .

(Gyongyver , 1987 ; Floris and Alvarez , 1991 ; Menzies *et al* ,1991 ; Haberie and Schlosser,1993 ;Boiteux *et al* ,1995 and Awad , 2000) .

2-Effect of some natural and mineral oils in comparison with the common recommended fungicides:

Three natural oils (Naterolow,Neemix and Trilogy) ,three mineral oils (Super –masrona, Kz- oil and Chemisol) and three common recommended fungicides (Afugan , Sumi-eight and micronized Sural) were tested against cucumber powdery mildew disease before and after inoculation with *S. fuliginea* (Table-2) .

Cucumber Anas hyb., grown under greenhouse conditions , at 26C+2 in 20 cm in diameter sandy – clay soil (1:1) pots was used in this trial. Four pots (each was sown by four 21 day-old cucumber seedlings) were devoted for each substance . The substances were applied before or after inoculation.

A- Befor inoculation:

Set of the pots were sprayed with the substances, four pots for each, at the rates shown in (Table-2). Aceton was used as a solvent in the case of natural oils at the rate of 1ml / liter .Two days later from application, the seedlings were inoculated with *S. fuliginea* .

B- after inoculation:

Other set of pots were inoculated with the pathogen and 2 days later the inoculated seedlings were sprayed with the tested substances, four pots also for each .Untreated but inoculated seedlings were used as check .

RESULTS

1-Effect of ten systemic resistance inducers on the disease incidence:

It is clear from data in (Table-3) that Cobalt sulphate,Cali-green, Potassium silicate , monobasic Potassium phosphate(high dose),Lithium chloride, Agri-

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Table (2) :Trade name, Chemical name, active Ingredients and rate of application of natural and mineral oils and fungicides.

Trade name	Chemical name and active ingredients	Rate of application
1-Naterolow	A mixture of fatty acid, triglycerids and vegetable oil 93% inert material 5%	7 ml/ 1 litre water
2-Neemix (Neem oil)	Azadirachtin 4.5%	3ml/1 litre water
3-Trilogy (Neem oil)	90% clarified hydrophobic extract of neem oil	10 ml/1 litre water
4-Super masrona	94% summer mineral oil	15 ml/1 litre water
5-KZ oil	Mineral oil 95% EC	15 ml/1 litre water
6-Chemisol	95% summer mineral oil	15 ml/1 litre water
7-Afugan 30%	Ethyl 2-Diethoxythiophosphoryloxy-5-methylpyrozolo (1, 5-a) pyrimidine 6- carboxylate (pyrazophos)	1 ml/1 litre water
8-Sumi eight	(E) 1- (2, 4-dichlophenyl)-4, 4-dimethyl 2- (1, 2, 4, trizole 1 y 1) 1- penten 3- o1 (18% Diniconazol)	0.35 ml/1 litre water
9-Micronized surel 70% W.P.	70% Micronized sulphur	2.5 g/1 litre water

Table (3): Effect of some resistaneinducers on the cucumber powdery mildew disease incidence by five disease assessment parameters.

Resistant	Average disease %	Disease severity %	Average diameter (cm)	Average number/ leaf	Average conidia/ leaf (million)
Cobalt sulphate	37.50	19.70	0.3	16	13.0
Dibasic sodium phosphate	100.00	27.20	0.5	38	14.0
Monobasic sodium phosphate (high dose)	100.00	37.50	0.3	36	13.0
Dibasic Potassium phosphate (high dose)	100.00	31.50	0.4	58	18.0
Dibasic Potassium phosphate (low dose)	100.00	50.00	.4	39	12.5
Monobasic potassium phosphate (high dose)	50.00	30.00	0.4	44	21.0
Monobasic potassium phosphate (low dose)	100.00	50.00	0.3	24	16.0
Cali-Green	37.50	28.70	0.3	30	8.0
Agri-Fos	81.25	15.98	0.3	17	5.0
Potassium silicate	31.25	21.10	0.2	12	2.0
Vapour-Guard	100.00	40.00	0.5	50	28.0
Lithium chloride	56.30	35.20	0.3	37	12.0
Control	100.00	73.70	0.7	140	71.0
LSD at 0.05	17.21	5.07	0.14	6.71	2.93

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fos systemic resistance inducers significantly reduced the disease incidence than others .

As for disease severity , Cobalt sulphate , Agri-fos , Potassium silicate , dibasic Sodium phosphate , Cali-green , monobasic Potassium phosphate (high dose) and Lithium chloride proved significant high effect. Similar effects of these systemic resistance inducers were recorded when the average diameter of colonies and counts of colonies were calculated . Meantime, the least counts of conidia /leaf (In millions) resulted in the case of spraying Potassium silicate, Agri-fos,Cali-green, Lithium chloride and Cobalt sulphate .

2-Effect of some natural and mineral oils in comparison with the common recommended fungicides:

It is clear from data in (Table-4) that all tested substances, natural and mineral oils and fungicides markedly reduced the disease percentage and disease severity percentage either before or after inoculation ,but with not significant differences between the application before and after inoculation in the case of disease severity. Continuance, data elucidated also that all fungicides especially Afugan, the mineral oil Super masrona and the natural oils Trilogy and Naterolow gave the best results in reducing the disease severity.

In point all substances strongly decreased the average number of colonies /leaf either before or after inoculation. Acute decreasing was recorded in the case of fungicides followed by mineral then natural oils.

DISCUSSION

Some systemic resistance Inducers had been tested in this work as an attempt to reduce the cucumber powdery mildew disease incidence far away polluting the environment conditions. Actually, some of these inducers proved their ability in reducing cucumber powdery mildew (*Sphaerotheca fuliginea*) as Cobalt sulphate, monobasic Potassium phosphate (high dose), Cali-green, Agri-fos, Potassium silicate and Lithium chloride, These inducers significantly reduced all used disease assessment parameters (disease percentage, percentage of disease severity, average colony's diameter on leaf blade, average colony s counts/ leaf and average conidia counts / leaf). Other inducers as, dibasic Sodium phosphate, monobasic Sodium phosphate, dibasic Potassium phosphate (high and low doses) and monobasic Potassium phosphate (low dose) showed intermediate results. These results confirmed the results of many Investigators. Abood and Losel (1991), Abood et al (1992) and Losel et al (1994). The first and second authors demonstrated that Lithium chloride treatment to inoculated cucumber plants with *S. fullginea* reduced the number of conidia produced by pathogen colonies grown on leaves, or these treatments inhibited the development of the fungus

Table (4): Effect of nine different substances related to natural and mineral oils chemical fungicides on the powdery mildew disease incidence.

Treatments	Disease Measures						General mean
	Before Inoculation			After Inoculation			
	Disease %	Disease severity %	Av.No. of colonies/ leaf	Disease %	Disease severity %	Av. No. of colonies/ leaf	
A-Natural oils							
Naterolow	56.25	23.80	72.00	37.50	9.50	74.80	21.65
Neemix	50.00	16.30	80.00	100.00	32.50	87.00	24.40
Trilogy	50.00	15.00	88.50	50.00	24.30	60.00	19.65
B-Mineral oils							
Super masrona	31.25	8.80	17.00	31.25	6.80	22.00	7.65
KZ – oil	50.00	36.30	33.00	43.75	19.00	31.50	27.65
Chemisol	50.00	33.80	26.00	37.50	32.50	27.00	33.15
Fungicides							
Afugen	6.25	2.50	4.50	0.00	0.00	0.00	1.25
Sumi-Eight	0.00	0.00	0.00	12.50	7.50	7.50	3.75
Micronized Surel	12.50	3.75	7.75	25.00	20.00	12.80	11.88
Control	100.00	77.50	313.8	100.00	77.50	313.80	77.50
General Menan		21.55			21.90		

Time of application (M) = N.S.

Treatments (T) = 2.32

Time of application x Treatments (M x T) = 9.01

structures and lowered the efficacy of conidia. The second authors also confirmed these results and attributed the effectiveness of Lithium chloride in reducing the infection to the interference with phosphatidyl inositol turnover.

The results proved also the excellent activity of Potassium silicate in reducing the disease incidence. However, Menzies *et al* (1991) found negative correlation between Silicon content of plant tissues and cucumber powdery mildew severity. Samuels *et al* (1991 and 1993) explained the mobility and deposition of Silicon in cucumber plants. They found that when the cucumber plants, grown in Silicon insufficient medium and transferred to Silicon containing media showed rapid silification of leaf tissue primarily trichome bases and exhibited increasing in disease resistance to *S.fuliginea* and high concentration of Silicon in the leaf epidermis surrounding the invading

pathogen's hyphae. However, plants grown in Silicon-supplemented media and transferred into Silicon-deficient media contained residual Silicon in the leaf trichome bases, but failed to display disease resistance or silification of the host tissues surrounding the invading pathogen's hyphae. Likewise, Powen *et al.* (1992) found that when potted grape plants fed with 1.7mM, Silicon didn't exhibit resistance against powdery mildew (*Uncinula necator*) but foliar sprays at 17 mM Silicon substantially reduced the number of mildew colonies. Scanning electron micrographs showed that on Silicon sprayed leaves, hyphae didn't develop where thick Silicon deposited and where surface deposits were not present, Silicon translocated laterally through the leaf and surrounded the appressoria. The authors suggested that the reducing in severity of grape mildew by Silicon sprays may partly be due to a physical barrier to hyphal penetration and to a resistance response involving the lateral movement of Silicon and its deposition within the leaf at fungal penetration sites. Also, in that regard Jim Menzies *et al* (1992) concluded that cucumber plant sprays of > 17 mM Silicon developed fewer colonies. They also concluded that the active ingredient of Potassium silicate sprays was Silicon and 17mM Silicon spray, applied 7 days before inoculation with *S.fuliginea* reduced mildew colony formation.

In view of the effect of Sodium and Potassium phosphates in this investigation, these salts proved good to moderate results at their used doses. Reuveni *et al* (1993) found that a single spray of 100 mM solutions of dibasic Potassium phosphate, monobasic Potassium phosphate, Sodium phosphate & Sodium pyrophosphate on the upper surface of the first true cucumber leaf, 2 h before inoculation with *S.fuliginea* conidia, induced systemic protection against the disease to the second and third leaves. However, dibasic Sodium phosphate had little or no effect. Moreover, spraying of dibasic Potassium phosphate at the same conditions actively induced systemic protection and reduced the number of pustules/

plant. However the induction of systemic protection by mono & dibasic Potassium phosphate were consistently the most effective treatments. The same authors (1995) recorded that a single spray by 0.1 M mono or dibasic Potassium phosphate on the upper surface of the first cucumber true leaf before inoculation with *S.fuliginea* induced highly protection to the second to fifth leaves up to 25 days after inoculation. The same authors (1996) added that *S.guliginea* was successfully controlled on greenhouse grown cucumber by a foliar spray (pre-inoculation) with 20 mM of mono or dibasic Potassium phosphate. They also found that 25 mM of mono or dibasic Potassium phosphate was protective against *S.fullnigea* natural infection under greenhouses. These salt solutions are not phytotoxic to plant foliage, continuance, it is suggested the inhibitory effect of phosphate and Potassium salts makes them useful biocompatible fungicides and possibly ideal foliar fertilizers. Moreover, Cobalt sulphate is considered the most important inducer in this research. It proved excellent control against cucumber powdery mildew. These results are in harmony with Gamil (1995).

The natural oils (Naterolow, Neemix and Trilogy), mineral oils (Supermasrona, Kz oil and Chemisol and common recommended fungicides (Afugan, Sumi-eight and micronized Sural) proved satisfactory control to cucumber powdery mildew under artificial inoculation and greenhouse conditions particularly Afugan, Sumi-eight, Super masrona Trilogy and Naterolow which clearly reduced disease severity followed by Neemix and Kz oil. In point, all substances strongly decreased the number of colonies / leaf (fungicides, mineral oils and natural oils respectively). Costache, et al (1984), Qvarnstrom (1989), Iqbal et al (1994) and Mustafa et al (1994) concluded that Afugan showed good control against cucumber powdery mildew disease under both field and greenhouse conditions. Similar results were obtained in the case of Sulfur application and are in complete agreement with Charifi-Tehrani (1984 & 1987), Bhatia and Thakur (1989). As for the role of mineral and natural oils in reducing the disease, Ohtsuka et al (1991) demonstrated that ultrastructural alterations induced by spraying the inoculated cucumber seedlings with an oil suspension included deformation of the hyphae, separation of the hyphal plasma membrane and the degeneration of the cytoplasm, led to death of the treated hyphae. The machine oil didn't obviously affect the morphology of the haustorium in the epidermal cells, suggesting that control of the mildew is happened by direct action on the hyphae. Also natural oils, in this research, showed good effect against the disease. Similar results are consistence with that of Herger, et al (1988), Steck and Schneider (1993), Dik et al (1994) and Daayf et al (1995). They found that applications of aqueous and ethanolic leaf extracts of *Reynoutria sachalinesis* (Milsana) controlled *Sphaerothica fuliginea* and reduced disease severity on cucumber under greenhouse conditions.

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A rapid and distinct accumulation of fungitoxic phenolic compounds occurred in leaves treated with Milsana especially in infected leaves. A slight inhibition of conidial germination was the only direct effect of Milsana on *S.fuliginia*. These results support the hypothesis that Milsana may act indirectly by inducing plant defence reactions and that may be useful in the integrated management of cucumber powdery mildew. These results are in agreement with those obtained by Konstantinidou Doltsinis (1998). They added that extract treated cucumbers yielded as many or more fruit as fungicide treated plants. Qvarnstrom (1992) and Reimers *et al* (1993) observed good prevention on glasshouse cucumber against powdery mildew infection by using 1-5% emulsion of garlic extract (ajoene compound).

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دراسات على مرض البياض الدقيقى على الخيار المتسبب عن الفطر

Sphaerotheca fuliginea تحت ظروف الصوب فى مصر

٢- الطرق الحديثة فى مقاومة المرض باستخدام مستحضات المقاومة

الجهازية وبعض الزيوت الطبيعية والمعدنية والمبيدات الفطرية

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الملخص العربى

كمحاولة حقيقية وجادة للحد من خطورة مرض البياض الدقيقى على الخيار ومقاومته والذي يعد بالفعل واحدا من اهم واخطر امراض الخيار داخل صوب الانتاج البلاستيكية وذلك باستخدام أبسط وايسر الطرق الحديثة فى مقاومة المرض بعيدا عن ملوثات البيئة وخطوة للأمام من اجل اعادة التوازن البيئى المقتد من جديد اختبر لهذا الغرض عشرة مواد كيميائية (اثنى عشر معاملة) لاستحضات المقاومة الجهازية داخل نباتات الخيار كما تم اختبار ستة زيوت طبيعية ومعدنية (ثلاثة لكل منها) قبل وبعد العدوى بالفطر المسبب *Sphaerotheca fuliginea* مقارنة بأفضل ثلاثة مبيدات فطرية موصى بها .

حققت النتائج الغرض المرجو من هذا البحث واشادت بقوة الى فعالية المواد المختبرة بدرجات مختلفة فى استحضات مقاومة نباتات الخيار مقارنة بالنباتات الغير معاملة. أظهرت النتائج تفوقا ملحوظا للمواد المستحثة كبريتات الكوبلت ، الكاليجرين ، سيليكات البوتاسيوم ، كلوريد الليثيوم ، والأجريفوس مقارنة بباقي المواد فى خفض كل مقاييس المرض (النسبة المئوية للمرض - النسبة المئوية لشدة المرض- متوسط عدد المستعمرات لكل ورقة- متوسط قطر المستعمرات -متوسط عدد الجراثيم الكونيدية بالمليون لكل ورقة) الا ان التفوق كان لصالح سيليكات البوتاسيوم ، كبريتات الكوبلت ، الكاليجرين . كما بينت النتائج ايضا ان المادة المستحثة فوسفات البوتاسيوم احادية القاعدة بالجرعة العالية قللت فقط نسبة وشدة الإصابة بالمرض بينما حققت فوسفات الصوديوم ثنائية القاعدة نتائج طيبة فى خفض النسبة المئوية

لشدة المرض فقط في حين احتلت المادة المستحثة فابور-جارڤ المرتبة الأخيرة من حيث الكفاءة في مقاومة المرض. وهكذا يمكن استخدام مستحاثات المقاومة الجهازية التي اثبتت كفاءة في الحد من خطورة هذا المرض.

على الجانب الآخر اكدت النتائج فعالية كل من الزيوت الطبيعية(تريولوجي-ناترولو ونيميكس) والزيوت المعدنية (سوبر مصرونا - ك زد- كيميوسول) والمبيدات الفطرية (افيوجان-سومي ايت- كبريت ميكروني) المختبرة ضد المرض سواء عوملت بها النباتات قبل او بعد العدوى بالفطر المسبب للمرض(سفيروثيكا فلاجينا) حيث حققت المبيدات الفطرية افويوجان ، سومي ايت والكبريت الميكروني والزيوت المعدنية سوبر مصرونا والزيوتين الطبيعيين تريولوجي و ناترولو افضل النتائج في خفض النسبة المنوية لشدة المرض يليها في الفعالية الزيت الطبيعي نيميكس والزيت المعدني ك-زد .

اوضحت النتائج ايضا ان كل المواد المختبرة قللت بوضوح متوسطات اعداد المستعمرات الفطرية لكل ورقة مقارنة بالنباتات الغير معاملة . وقد تفوقت في هذا الغرض المبيدات الفطرية يليها الزيوت المعدنية ثم الزيوت الطبيعية وهكذا تؤكد النتائج فعالية مقنعة للزيوت المعدنية وخاصة سوبر مصرونا ، ك-زد حيث تأتي في المرتبة الثانية بعد المبيدات الفطرية. وهكذا يمكن استخدامها في مقاومة هذا المرض سواء قبل الاصابة(وقائيا) او بعد الاصابة (علاجيا)، كما يمكن استخدام الزيوتين الطبيعيين تريولوجي وناترولو لنفس الغرض بدلا من استخدام المبيدات الفطرية