

EFFECT OF CERTAIN AQUEOUS INDIGENOUS PLANT EXTRACTS ON SUSCEPTIBILITY OF CUCUMBER PLANTS TO POWDERY MILDEW (*SPHEROTHECA FULIGINEA*) INFECTION

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ABSTRACT

Concerning effects of the tested extracts on controlling the powdery mildew disease caused by *Sphaerotheca fuliginea* on cucumber plants results indicate that, the most effective extract for controlling the disease incidence was *Narcissus* sp. which exerted efficacy of 37.7% against the disease incidence followed by *Chenopodium album* which resulted in 18.17% efficacy. The rest extracts ranged between 0.0 to 8.5%, while the fungicide (Rubigan) reduced the disease incidence with 62.61% efficacy. The results showed that germination of the fungal conidial spores was partially inhibited by contacting to the tested extracts after 24 hours of incubation, treatments with *C. album*, *Narcissus* sp. and the fungicide (Rubigan) were the most effective against germination of the fungal conidial spores and decreased the number of the germinated spores with 90% comparing to the control. Meanwhile, the least effective extract was *Conyza dioscoroides* which exerted 50% efficacy. After 48 hours of incubation, the most suppressive treatments were with (كاسيا), *Narcissus* sp. and *C. album* which decreased the germinated spores with efficacy (90.42, 84.92 and 79.45%), respectively. However, the least effective extract on spore germination was *C. dioscoroides*, which exerted only 15.05% efficacy.

Keywords : Plant extract, mode of action, powdery mildew, cucumber plants

INTRODUCTION

Controlling plant parasitic fungi by using certain plant extracts:

Cucurbit powdery mildew, caused by *Sphaerotheca fuliginea* (Schlecht) Pollacci, is a serious disease on cucurbits grown worldwide. Powdery mildew occurs on leaves, stems and fruits. Control methods currently available under commercial conditions include the use of repeated applications of elemental sulphur (Kimati et al. 1980) and other fungicides (Kimati et al. 1997). The constant use of fungicides, however, can result in environmental contamination and selection of resistant populations of *S. fuliginea* (McGrath & Shiskoff, 1996 and McGrath et al., 1996). However, many of pesticides are phytotoxic, harmful to natural enemies and hazardous to higher animals (Johnson and Feldmesser, 1987). Besides, in many developing countries, the use of pesticides is impractical because they are either not available or very expensive. For these reasons greenhouse growers have pursued alternative methods of controlling powdery mildew on cucumber, including some resistant cultivars, compost extracts, plant extracts, salts and detergents, surfactants, oils and biological control agents (Jorvis, 1992). Also, Dik, et al. (1994) reported that Milsana (a product containing leaf extracts from *Reynoutria sachalinensis*) decreased infection by *Sphaerotheca*

fuliginea on cucumber plants. They were applied as a 2% solution at 1500 and 3000 liter/ha to susceptible and partially resistant cultivars. All treatments reduced disease severity, although a significant yield increase was only obtained when the susceptible cultivar was sprayed at 3000 liters/ha. Recently, Daayf, *et al.* (1997a) proved that Milsana, a commercial formulation of extracts from leaves of the giant knotweed *Reynoutria sachalinensis* significantly reduced the incidence of powdery mildew (*Sphaerotheca fuliginea*) on cucumber plants under both small and large area of plantation. The active ingredients are believed to be natural elicitors that increase the plant's natural defense mechanisms. Therefore, the objectives of this study , were to assess efficacy of plant extracts of certain indigenous herbaceous and aromatic plants on disease severity and spore germination of *S. fuliginea* on cucumber plants (*Cucumis sativus* L.).

MATERIALS AND METHODS

Sowing and inoculation the host plants : Seeds of cucumber (cv., Beit alpha) were sown four seeds/pot in 25 cm diam. pots filled, with sterile loamy soil (2 kg/pot), under greenhouse conditions. After 10 days, the emergent seedlings were thinned at 3 seedlings/pot. Plants with 3 true leaves were artificially inoculated with conidial spores of *Sphaerotheca fuliginea*. Powdery mildew infected cucumber leaves covered with new-formed conidial spores were regularly shaken over the cucumber plants. Thereafter, the pots were irrigated and put in a polyethylene tent for 24 h and then the polyethylene tent was removed and the plants were put on bench of an ordinary greenhouse.

Preparation and application of the plant aqueous extracts :

The plant aqueous extracts of certain plants indicated in Table (1) were prepared by soaking 50g of each of the previous air dry plant materials in 1000 ml distilled water at 60°C. After 72 hrs., water extracts were filtered through whatman filter paper No. 1. The filtration was kept at 4°C in a refrigerator until using according to (Korayem, *et al.* 1993).

The cucumber plants were sprayed with the crude aqueous extracts at either the 2nd day before or after as well as on the same day of the artificially inoculation. In the same time, some plants were sprayed with the fungicide "Rupigan". The plants of water control were inoculated and kept without spray.

Table (1): The tested plants which used for preparing their extracts used throughout this work.

Family	Scientific name	Common name	Arabic name	Part
Compositae	<i>Conyza dioscoroides</i>	Fleabane	بلز نوف	Leaves
Chenopodiaceae	<i>Chenopodium album</i>	Lambsquarters	زربيح	Leaves*
Jabiteae	<i>Mentha longifolia</i>	Hierba-buena	رعراع	Leaves
Salicaceae	<i>Salix purpurea</i>	Purpurea willow	صفصاف عريض	Leaves
Myrtaceae	<i>Eucalyptus globules</i>	L. bluegume	كافور	Leaves
Punicaceae	<i>Punica granatum</i>	Pomegranate	قشر الرمان	Fruits
Amaryllidaceae	<i>Narcissus spp.</i>	Narcissus	الترجس	Bulb-peel

* = Development inhibitor

Determination of incidence and severity of powdery mildew disease :

Fifteen days after inoculation, number of the infected plants was recorded. Plant showed any number of mildewed leaves was considered as an infected plant. Disease severity was calculated by examining leaves of 8 randomized plants and dividing the examined leaves into 5 classes according to their mildewed leaf area as in the following scale :

Class	The mildewed area	The numirecal rate
First	Leaves with no powdery mildew symptoms	= 0
Second	1-25% of the leaf area was mildewed	= 1
Third	25-50% of the leaf area was mildewed	= 2
Fourth	51-75% of the leaf area was mildewed	= 3
Fifth	More than 75% of the leaf area was mildewed	= 4

$$\text{Disease severity} = \frac{\sum[\text{number of leaves in each class} \times \text{the numerical rate}] \times 100}{\text{Total number of the examined leaves} \times 4}$$

Effect of the aqueous plant extracts on the conidial spore germination:

One ml of each aqueous extract was smeared on one side of a glass slide and left to dry. This process was repeated 3 times to take each glass slide 3 ml. of an extract. The conidia of *S. fuliginea* were detached by shaking the mildewed leaves over the slides. The glass slides were held in a saturated atmosphere in the dark at 21±2°C for 48 hr. The percentage of conidial spore germination was recorded on each slide at 24 and 48 hr. Germination was completed if the conidia formed appresoria at the examination period.

RESULTS

Effect of the aqueous plant extracts on disease incidence and severity of powdery mildew on cucumber plants:

The percentages of the powdery mildew infected plants were significantly different due to the different treatments. The most effective plant extract in controlling powdery mildew incidence was *Narcissus* sp, which exerted efficacy on disease incidence reached 37.7%, and the subsequent effective extract was *C. album*, which resulted in 18.17% efficacy. It seemed that the other extracts had no obvious influence on powdery mildew incidence, as they resulted in efficacy which did not exceed 8.5%. (Table 2 and Fig.1). In contrast, the fungicide (Rubigan) was more effective than any plant extract, as it reduced the disease incidence by efficacy of 62.61% comparing to the control treatment. Concerning disease severity of powdery mildew on cucumber plants, treatments with Rubigan and *Narcissus* sp. Were still the most effective in controlling the powdery mildew disease development, as they kept the disease severity at 13.13 and 20.83%, respectively. It appeared that, *M. longifolia* and *C. album* had intermediate influence on powdery mildew severity, among the other treatment. As disease severity did not exceed 36.05 and 40.6% on cucumber plants, respectively.

Table (2): Effect of the aqueous plant extracts on disease incidence and severity of powdery mildew on cucumber plants.

Treatments	Infected plants %					Severity %				
	Plant extracts 2d before inoculation	Concomitantly with inoculation	Plant extracts 2d after inoculation	Mean	% Effect	Plant extracts 2d before inoculation	Concomitantly with inoculation	Plant extracts 2d after inoculation	Mean	% Effect
<i>Conyza discolorodes</i>	100.0 a	100.0 a	100.0 a	100.00	0.00	41.08 cd	54.25 d	54.38 d	51.90	39.53
<i>Mentha longifolia</i>	83.0 c	91.5 b	100.0 a	91.50	8.50	31.68 e	50.83 d	25.63 e	36.05	57.98
<i>Chenopodium album</i>	100.0 a	54.0 c	91.5 b	81.83	18.17	45.63 d	17.50 g	58.68 c	40.60	52.70
<i>Salix purpurea</i>	83.0 c	91.5 b	100.0 a	91.50	8.50	50.00 bc	46.88 e	58.60 c	51.83	39.61
<i>Eucalyptus globulus</i>	91.5 b	100.0 a	100.0 a	97.17	2.83	53.5 b	61.68 c	75.63 b	63.60	25.90
<i>Punica granatum</i>	100.0 a	100.0 a	100.0 a	100.00	0.00	32.75 e	72.88 b	54.38 d	53.34	37.85
<i>Narcissus sp.</i>	70.0 d	62.0 d	55.0 c	62.30	37.70	25.00 f	22.90 f	14.59 g	20.83	75.73
Mean	89.64	85.57	92.36	89.19	10.81	40.81	46.70	48.84	45.45	47.05
Rubigan (Fungicide)	50.0 e	16.68 e	45.5 d	31.39	62.61	13.75 j	8.13	17.50 f	13.13	84.70
Untreated Control	100.0 a	100.0 a	100.0 a	100.00	0.00	85.83 a	85.83 a	85.83 a	85.83	0.00

Means followed by a letter (s) in common are not significantly different at the 5% level according to (DMRT)

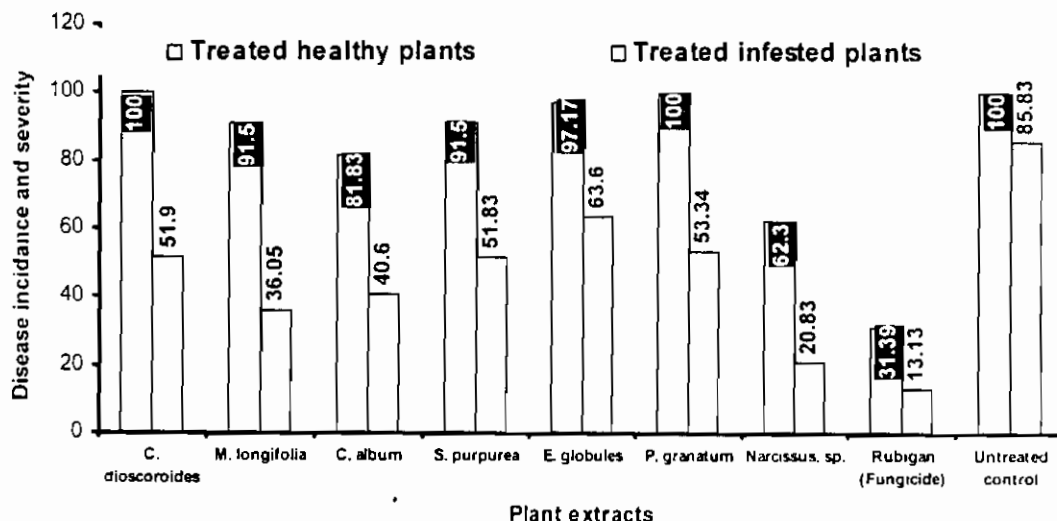


Fig. (1): Effect of certain aqueous plant extracts on disease incidence and severity of powdery mildew on cucumber plants.

In contrast, the fungicide "Rubigan" was more effective than any plant extract, as it reduced the disease incidence by 62.61% (its efficacy) comparing to the control treatment. Concerning disease severity of powdery mildew on cucumber plants; treatments with the fungicide Rubigan and *Narcissus. sp.* were still the most effective in controlling the powdery mildew disease development, as they kept the disease severity at 13.13% and 20.83%, respectively. It appeared that, *M. longifolia* and *C. album* had intermediate influence on powdery mildew severity, among the other treatment. As disease severity did not exceed 36.05 and 40.6% on cucumber plants, respectively. The other, plant extracts showed disease severity ranged between 51.83% and 63.6%, while the control plant maintained disease severity of 85.83%.

Effect of the aqueous plant extracts on conidial spore germination of *S. fuliginea* :

Conidial spore germination was microscopically examined after 24 and 48 h from exposing them to the plant extracts. Data in (Table 3 and Fig. 2) show that, although germination of the *S. fuliginea* conidial spores was very poor on a glass-slide, the aqueous plant extracts tested exerted different inhibition rates on the conidial germination. At 24 hours, all *C. album*, *Narcissus. sp.* and the fungicide Rubigan were superior in inhibiting germination of the conidial spores, as the germination did not exceed 4% with efficacy 90% comparing with the control treatment which recorded 40% germination. *M. longifolia*, *S. purpurea*, *E. globules* and *P. granatum* exerted intermediate effect on spore germination, where the percentages of conidia germination were 8, 8, 12 and 12%, and efficacy 80, 80, 70 and 70%, respectively. The least effective plant extract tested among them, was *C. dioscoriodes* which induce 20% germinated spores with efficacy 50%

Table(3):Effect of some plant extracts on percentage of disease incidence and disease severity of cucumber plants infestation

Plant extracts	24 h.		48 h.	
	germination %*	Efficacy % **	germination %*	Efficacy % **
<i>Conyza disscoriodes</i>	20.00	50.00	82.68	15.04
<i>Mentha longifolia</i>	8.00	80.00	33.32	65.76
<i>Chenopodium album</i>	4.00	90.00	20.00	79.45
<i>Salix purpura</i>	8.00	80.00	37.32	61.65
<i>Eucalyptus globulus</i>	12.00	70.00	61.32	36.99
<i>Punica granatum</i>	12.00	70.00	52.00	46.57
<i>Narcissus sp.</i>	4.00	90.00	14.68	84.92
Mean	9.71	75.73	43.05	55.76
Rubigan (Fungicide)	4.00	90.00	9.32	90.42
Untreated Control	40.00	00.00	97.32	00.00

* The percentage of germinated spores in 50 conidial spores
Control treatment – extract treatment

** Efficacy % = $\frac{\text{Control treatment} - \text{extract treatment}}{\text{Control treatment}} \times 100$

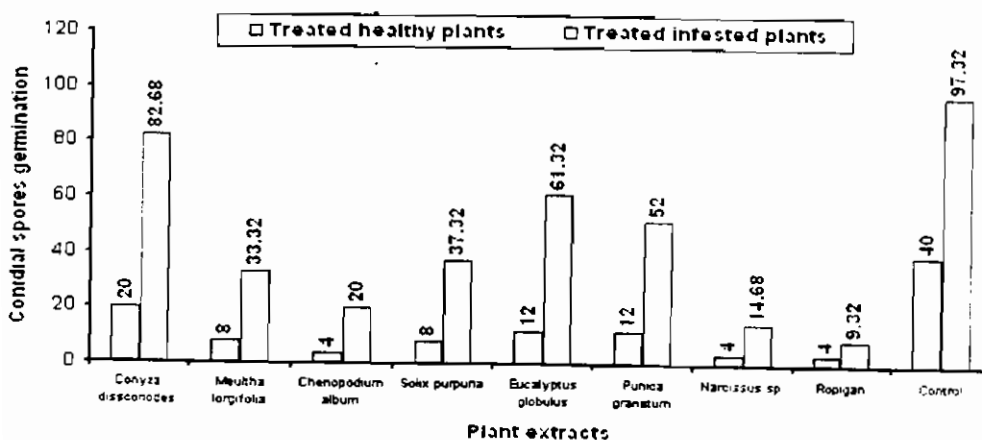


Fig. (2): Effect of the aqueous plant extracts on conidial spore germination of *S. fuliginea* at 24 and 48 h after treatment

At 48h, spore germination was gradually increased to reach 97.32% germinated spores in the control treatment. On the other side, the most suppressive treatments were the fungicide, Rubigan, plant extracts *Narcissus sp.* and *C. album*, which resulted in (9.32, 14.68 and 20.00%) spore germination, with efficacy 90.42, 84.92 and 97.45%, respectively. However, the least effective extract against spore germination was *C. dioscoriodes*, which recorded 82.68% spore germination with efficacy 15.04%. The rest plant extracts resulted in averages of spore germination ranged from 33.32% to 61.32% and efficacy ranged from 65.76% to 36.99%, respectively.

DISCUSSION

Effect of the plant aqueous extracts on powdery mildew disease "*S. fuliginea*" on cucumber plants :

With current restriction in pesticide usage in greenhouse, plant extracts as biological products are used as an alternative promising approach in powdery mildew disease management on cucumber plants.

In the present study, aqueous extracts of certain indigenous plants were used to protect the susceptible cucumber cultivar "Beit-alpha" against powdery mildew disease. Percentage of the infected plants or percentage of disease incidence and disease severity were used for understanding and judging influence of the aqueous plant extracts tested on the powdery mildew disease development. Large infection rates on the water control ensured sufficient infection occurred. Very little infection on the chemically treated control shows that the fungicide Rubigan controlled powdery mildew successfully as expected. Thus, when the effects of the aqueous plant extracts were analyzed, a preventative and curative effect of some plant extracts on the powdery mildew was apparent. Spraying the herb solution onto the seedlings 2 days before inoculating the seedling with powdery mildew fungus tested the preventative ability of herb extracts. Some herb extracts could be used as a preventative spray to prevent infection by powdery mildew. Only, *Narcissus* sp. extract was the most effective among the other aqueous extracts, as it reduced the number of infected plants by efficacy of 37.7%, comparing to the control treatment (its efficacy = 0). However, the fungicide "Rubigan" was superior as it exhibited 62.61% efficacy.

On contrast, effect of the aqueous indigenous plant extracts was very pronounced on disease severity of *S. fuliginea* on cucumber plants. Their efficacies on disease severity ranged between 25.9 to 75.73%, while the Rubigan induced 84.70% efficacy. The aqueous extracts of *Narcissus* sp., *M. longifolia* and *C. album* recorded efficacies reached 75.73, 57.98 and 52.70%, respectively, against the disease severity on the cucumber plants. This result could be encouraging in light of results of other studies. Qvarnstrom (1992) found that, applying 1-5% emulsion of garlic extract at 7 d intervals on cucumber leaves reduced the powdery mildew infected leaf area to 2 and 10% in spring and summer cucumber crops, comparing to 83 and 85% of the respective leaf areas of untreated plants in each season. Tyuterev, *et al.* (1994), found that two applications of a tomato leaf extracts and mycelial sonicates of *Monilia fructigena* and *Fusarium oxysporum* at 0.5% decreased powdery mildew (*Erysiphe cichoracearum*) development on cucumber by 71.3-77.6%. Moreover, weekly application of an aqueous formulation of extract (Milsana flussig) from leaves of the giant knotweed (*Reynoutria sachalinensis*) at conc. of 2%, provided control of powdery mildew (*S. fuliginea*) on the long English cucumber that was effective as the fungicide benomy L., lot fruit yield was not affected by this treatment (Daayf, *et al.* 1995).

In the present study, application of the aqueous plant extracts was occurred at either 2 d before, or 2d. after inoculation with powdery mildew. However, application of the extracts 2d. before inoculation gave prophylactic effect, which resulted in less disease severity of powdery mildew on cucumber plants (40.81%), comparing to application of the aqueous extracts at inoculation (46.70%) or 2 d. after inoculation (48.84%). The prophylactic property of these aqueous plant extracts may be due to presence of some phenolic compounds produced in the cucumber plants in response to an eliciting treatment with the aqueous plant extracts. Application of the aqueous plant extracts may exact a direct influence on the powdery mildew disease severity through their effect on germination of *S. fuliginea* conidial spores (Daayf, *et al.* 1995). Therefore, the main experiment was conducted to investigate these hypotheses. Germination of the conidial spores was tested on glass-slides smeared previously with three layers of the plant extracts. After 48h, the efficacy of *Narcissus* sp., *C. album*, *M. longifolia* and *S. purpurea* in inhibition of the conidia spore germination were 84.9, 79.45, 65.76 and 61.65%, while extracts of *P. granatum*, *E. globulus* and *C. dioscoroides* exhibited efficacy of 46.56, 36.99 and 15.04%, respectively. In the same time, the fungicide "Rubigan" induced the highest efficacy (90.42%) against germination of *S. fuliginea* conidial spores. From these results, it appears that the aqueous plant extracts had an inhibitor effect "direct effect" against the conidial spore germination on glass-slides. However, under a greenhouse condition in pot experiment, where the phyllosphere is full of conidial spores of powdery mildew, the effect of the aqueous extracts would be abolished because of presence of a heavy inoculum. Therefore, it seems that the effect of the plant extracts, in the present study, against severity of the powdery mildew development on the cucumber plants would be indirectly through increasing the host disease resistance. This conclusion is similar to that reported by Daayf, *et al.* (1995) who found that a slight inhibition of conidial germination was the only direct effect of Milsana on *S. fuliginea*, and a rapid and distinct accumulation of fungitoxic phenolic compounds occurred in leaves treated with Milsana, specially in the infected leaves. In addition, several defense enzymes such as peroxidase, polyphenoloxidase and phenylalanine, ammonia-lyase were stimulated in plants as a result of treatment with Milsana (Kowalewski and Herger 1992). Recently, it appears that plant extracts may act indirectly by inducing phytoalexins in the powdery mildew infected cucumber plants. Daayf, *et al.* (1997a&b) reported that phytoalexins was induced in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *R. sachalinensis*. At least eight separate phenolic compounds with antifungal activity were extracted from cucumber cv. Mustang leaves infected with powdery mildew (*S. fuliginea*). They supposed that cucumber plants might be produce elevated levels of phytoalexins in response to an eliciting treatment after infection.

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تأثير المستخلصات المائية لبعض النباتات المحلية على قابلية إصابة الخيار
بالبياض الدقيقي
الشافعي إبراهيم على الشافعي
قسم النبات الزراعي - كلية الزراعة - جامعة كفر الشيخ - ج. م. ع

إتضح من دراسة تأثير المستخلصات النباتية على إصابة نباتات الخيار بالبياض الدقيقي الذي يسببه الفطر *Sphaerotheca fuliginea* أن أكثر المستخلصات تأثيراً على مقاومة انتشار المرض هو النرجس البري ثم الزربيع، حيث كانت كفاءتهما في منع انتشار المرض ١٧، ١٨، ٧٠، ٣٧% على الترتيب بينما تراوحت كفاءة باقي المستخلصات (البارنوف، الزعراع، الصنصاف، الكافور وقشر الرمان) بين صفر الى ٨,٥% . أما المبيد الفطري "روبيجان" فقد أعطى كفاءة ٦٢,٦١% في منع انتشار المرض.

أما بالنسبة لتأثير تلك المستخلصات على إنبات الجراثيم الكونيدية للفطر، فقد وجد بعد ٢٤ ساعة من التحضين أنمستخلص نبات الزربيع والنرجس والمبيد الفطري "روبيجان" كانت افضل المعاملات في تثبيط إنبات الجراثيم حيث لم تزيد نسبة الإنبات عن ٤% بكفاءة ٩٠% ، بالمقارنة بالمعاملة الكنترول والتي أعطت نسبة إنبات ٤٠% . و كان أقل المستخلصات تأثيراً على نسبة إنبات للجراثيم هو مستخلص البارنوف حيث كانت نسبة الإنبات ٢٠% بكفاءة ٥٠% .

وبعد ٤٨ ساعة من التحضين زادت نسبة الإنبات تدريجياً لتصل الى ٩٧,٣٢% في معاملة الكنترول . وكانت أحسن المعاملات في تثبيط الإنبات هو المعاملة بالمبيد الفطري الروبيجان ومستخلصات نباتات النرجس والزربيع حيث كانت نسبة التثبيط ٩٠,٤٢ ، ٨٤,٩٢ و ٧٩,٤٥% على الترتيب. بينما كان أقل المعاملات تثبيطاً هو مستخلص نبات البارنوف ، حيث كانت نسبة تثبيط الإنبات هي ١٥,٠٤% .