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Application of epidermal coating antitranspirants for controlling cucumber downy mildew in greenhouse

Wafaa M. Haggag

Department of Plant Pathology, National Research Centre, Dokki, Egypt.
Corresponding author, E-mail: whaggag@link.net ; Fax no:002023832317
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ABSTRACT

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Downy mildew in cucumber plants, caused by *Pseudoperonospora cubensis*, was reduced by foliar spraying of antitranspirants film Kaolin, Nu-Film, Bio-Film, Folicote and Polyacrylamide Anti-Stress 550. In pots experiments, antitranpirants were proved to be effective in reducing disease incidence, severity and pathogen sporulation whatever prior or post inoculated. Among these compounds, Kaolin and Nu-Film (1.0%) were more effective in reducing spores counts germination, infected area and lesions number of downy mildew. Scanning electron microscopic examination showed that Kaolin antitranspirant inhibited spores germination and made the sporangia becoming collapsed and lost its turgidity when applied either pre or post inoculation. Under protected cultivation and natural infection conditions, all antitranspirants showed a remarkable effectiveness on the reduction of disease severity when applied twice, 45 and 75 days after sowing in cucumber leaves. Furthermore, all tested antitranspirants, significantly increased the cucumber plant height and yield. Kaolin strongly protected cucumber against downy mildew and increased yield. Conclusively, antitranspirants film can be used as effective treatments for the control of downy mildew disease in cucumber plants under plastic houses.

Key words : Film forming antitranspirants, cucumber, downy mildew, *Pseudoperonospora cubensis*

INTRODUCTION

In Egypt, cucumber (*Cucumis sativus* L.) is one of the most important vegetable crop grown under protected cultivation. Downy mildew of cucumber, caused by *Pseudoperonospora cubensis* (Berk and Curtis) Rostovzev, is one of the most prevalent and distributed foliar diseases of protected cultivation, that reduce the production considerable from early spring until autumn seasons [1,2,3,15,16] and greatly affects both yield and quality [17]. The successive production of sporagia and zoospores during the growing season of the crop ensures a high coefficient of reproduction and rapid epiphytotic spread. Biflagellate zoospores are thought to playa major role as infective propagules of these fungus [4]. This pathogen is present and develop in a high humidity and often causes sever epidemics [8,15]. There are many chemical, but in view of their undesired secondary effects and the fact that cucumber require continuous prolonged harvesting, for finding and using new natural products which are more reliable and less dangerous for the environment are high desired. Epidermis-coating polymers, such as film forming antitranspirants, have been reported to provide protection against several foliar plant diseases included grey mould, leaf blight, anthracnose, rust, powdery mildew and fruit rots [5,11,12,19,20]. Han (1990) used antitranspirant Masbrane and controlled 12 foliar diseases on nine verious hosts. Most of these film forming antitranspirants are non phytotoxic and resistant to weathering [5]. Although the effects of antitranspirants on disease control appear similar to those of the natural cuticle layer in defencing plant pathogens [6,7], physical effects should be also considered [10]. When applied to leaves as a pre-inoculation treatment to prevent a pathogen from becoming established in plant tissue, protective coating such as antitransprants oils, wax and various surfactant adhesives seem to be promising alternative to chemical fungicides [6,12]. The objective of the present study was to evaluate the efficacy of five film forming antitranspirants applied to cucumber leaves for the control of cucumber downy mildew in protected cultivation and their possible mechanisms involved in the reduction of the disease.

MATERIALS AND METHODS

Pathogen

Infected leaves of cucumber plants were collected from local commercial plastic houses. *P. cubensis* was maintained

on cucumber plants in pots (25 cm in diameter) and incubated at 20℃, 18 hr. light and 90% relative humidity (RH). To inoculate cucumber plants, the procedure described by Okuno *et al*. (1991) was used. Sporangial spores were harvested from infected leaves using a brush and water containing 0.01% tween 80 (Poly oxyethylene) as sufficient. The Sporangia spores suspension was filtered through fine nylon mesh to remove large hyphal aggregates, then washed twice with sterilized water, using Whatman No. 3 filter paper to retain the sporangia. The sporangia concentration was adjusted to 10^5 spores per ml with a hemaicytometer and lightly atomized onto cucumber leaves.

Film-forming antitranspirants

The following film forming antitranspirants were tested on cucumber plants, Kaolin (aluminum silicate; Miller Chemical and Fertilizer Co., Hanover, PA), Nu-Film (di-1-P-menthene, low viscosity; Miller Chemical and Fertilizer Co., Hanover, PA), Bio-Film (an ionic-nonionic blend; Callo Agricultural Chemical Inc., Overland Park, KS), Folicote (wax emulsion; Crystal Soap and Chemical Co., Inc., Lansdale, PA) and Polyacrylamide Anti-Stress 550 (cross-linked carbon acrylic latex polymer; Miller Chemical and Fertilizer Co., Hanover, PA). The film forming antitranspirants were applied at two concentrations of 1.0 and 3.0% (W/V).

Leaf disk assay

Cucumber plants of the susceptible cultivars, Delta Star (Samtrade company; 1.5 month growth) were used to prepare leaf disks. Tenth leaves below the apex were surface sterilized with 0.1% sodium hypochlorite and rinsed three times in sterile water. Ten leaf disks (15 mm in diameter) were sprayed with a thin layer of film forming antitranspirants. Sterile water was used as control treatment. After 24 hr., 2 ml of sporangia suspension (10^5 spores per ml) was sprayed over the disks. The disks were plased inside petri dishes (90 mm. in diameter) containing wettable filter paper. All experiments involved six leaves as replicates. The disks then incubated at 20℃ in darknessfor 24 hr. Spores germination as well as its infecting were determined under an Olympus microscope light/fluorescent (BX50+BX-FLA, Olympus Optical).

Greenhouse assay

The effect of five film forming antitranspirants on the incidence of downy mildew disease was measured under greenhouse conditions. Five cucumber plants cv. Delta Star were grown in plastic pots (25 cm in diameter) and incubated at 20℃ and 90% RH.

Seedlings were divided into two treatments; the first pre inoculation treatment, in which antitranspirants coating film were sprayed four days before inoculation. The second treatment, cucumber plants were sprayed with antitranspirants film coating four days after inoculation. Inoculum suspension was sprayed onto adaxial (upper) or abaxial (lower) leaf surfaces of cucumber plants using a glass atomizer at about 10 ml/plant. Inoculated plants were placed at previous conditions for 10 days before assaying the disease severity. Five replicates were used for each treatments. Disease severity was measured according to Reuveni (1983) using color index and infected area. The color index was calculated as follows; 0, no symptoms;1, greenish; 2, yellowish; 3, yellow; 4, brown. The infected area index was as follow:1= indicates that symptoms on 25% or less; 2=26-50; 3=51-100% of leaf area. Multiplication of the color and infected area indexes for each leaf yields a value of disease severity. Spores of *P. cubensis* were collected from infected lesions 10 days after inoculation. Leaves were taken at the early morning and then immersed in a jar containing 10 ml of distilled water. Spores were released from lesion using a brush, and were counted by using heamocytometer slide. Means of six replicates, each containing 10 leaves, were calculated.

Scanning electron microscopy (SEM)

Infested leaves were treated with Kaolin antitranspirants (1.0%) was sampled 10 days after application for scanning electron microscope (SEM). Samples 2-3 mm. in diameters were immersed in 5% glutareldehyde in 0.1M phosphate buffer, pH 7.2, washed in the same phosphate buffer, dehydrated by passages through graded aqueous ethyl alcohol series (10, 30, 50, 75 and 95%), then placed in 100% ethanol at room temperature for a few minutes. Critical point dried specimens were coated with gold palladium in a polaron E500 sputter coater in National Research Center, Center Lab, Egypt. Micrographs were taken on Polaroid type positive film with UV-haze and 0.2 orange filters. Two samples per treatment were examined.

Protected field experiments

An experiment was carried out under natural infection condition in protected cultivation of plastic houses at Gezerit El Dahab, Giza govarnorate during the two successive seasons of spring and autumn of 2000 and 2001, to study the effect of film forming antitranspirants on the control of downy mildew disease. Cucumber plants of Premo, (c.v. Slous and groots company) hybrid were sprayed with 1.0% of film antitranspirants twice at 45 and 75 days after sowing. The control treatment was treated with water. A complete randomized block design with six replicates was used. Each replicate consisted of 50 plants spaced at 50 cm apart. The plants were grown on both sides of row. Disease severity and sporangia counts of *P. cubensis* were measured after 45, 60, 75, 90, 100 and 120 days of sowing as previous above. At harvest stage, cucumber yield was determined per m^2 (Kg). Data was statistically analyzed using New Least Significant Difference (New LSD) and (LSD). All treatments in experiments described consisted of three or more replications.

RESULTS

Leaf disk assay

Spores germination and infection of *P. cubensis* on cucumber leaf disks treated with film forming antitranspirants, 24 hr pre inoculation are exhibited in Fig. 1. Film forming antitranspirants were effective in reducing the percentage of *P. cubensis* germination ranged from 1.0 to 29.3% in all treatments in comparing with untreated control (85.0%). Only, one percent of spores were germinated when leaf disk were sprayed with Kaolin at both concentrations. Moreover, when leaf disks were sprayed with the Nu-Film and Bio-Film at both concentrations, spore germination was significantly decreased compared with untreated control. At the same time, all antitranspirants showed a high efficacy in reducing direct penetration of *P. cubensis* (0.0-40%) as compared with 93% of untreated control. Kaolin was the most effective in preventing penetration. Moreover, Nu-Film and Bio-Film proved highly effective in reducing pentration.

Greenhouse assay

All film forming antitranspirants reduced the severity of downy mildew (Table 1). Among the tested film forming antitranspirants, good results were obtained by both of Kaolin and Nu-Film at both concentrations when sprayed either pre or post inoculation.

In cucumber plants sprayed with antitranspirants 4 days before or after inoculation, a significant reduction in sporulation of *P. cubensis* was observed in infected lesion. Whereas, the sporangia counts were reduced by 97.6-1643.6

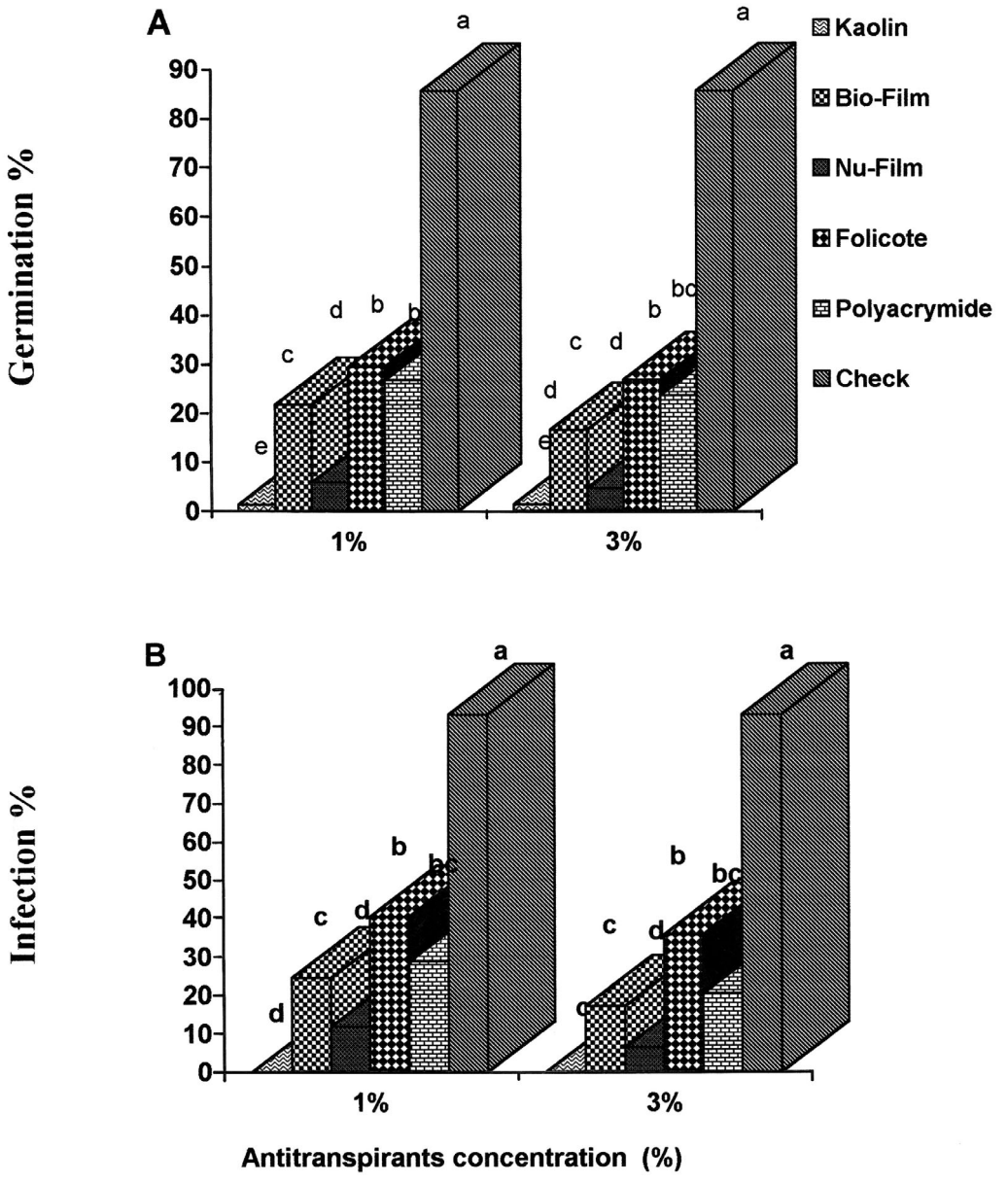

Fig. 1. Assessment of germination (A) and infection (B) of *Pseudopernospora cubensis* on cucumber leaf disks (15 mm) sprayed with antitranspirants, 24 h pre inoculation and incubation at 20℃.

Table 1. Efficacy of film forming antitranspirants applied to cucumber leaves on development of downy mildew disease when applied pre or post inoculation of *Pseudopernospora cubensis*

Antitranspirant	Disease severity [1]				*P. cubensis* spores counts/cm^2 of infected lesion [1]			
	Pre-inoculation		Post-inoculation		Pre-inoculation		Post inoculation	
	1%	3%	1%	3%	1%	3%	1%	3%
Kaolin	0.1f [2]	0.1e	0.8f	0.5e	97.6f	81.0f	392.1f	298.4f
Bio-Film	1.7d	1.0d	2.3d	2.1cd	625.2d	482.1d	934.6d	702.7d
Nu-Film	0.8ef	0.5e	1.6e	1.3d	343.4e	281.3e	767.8e	624.8e
Folicote	3.6b	3.3b	4.3b	4.0b	1643.6b	1336.4b	1989.4b	1831.1b
Polyacrylamide	2.7c	2.1c	3.6b	3.0c	1187.1c	981.7c	1464.3c	1231.6c
Check	8.1a	8.1a	8.1a	8.1a	4173.6a	4173.6a	4693.2a	4693.2a

[1.] Disease severity and sporulation were recorded 10 days after inoculation and incubation at 20℃ in greenhouse.
[2.] Data followed by the same letter in each column do not differ significantly (P<0.05).

and 298.4-1989.4 spores/cm^2 pre and post inoculation with antitranspirants compared with 4173.6 and 4693.2 spores/cm^2, respectively.Among those antitranspirants, Kaolin was best in suppressing sporulation of *P. cubensis* on infected lesions. However, Folicate was less effective in reducing the sporulation.

SEM examination

The effect of Kaolin antitranspirant on *P. cubensis* development was examined by using SEM as shown in Fig. 2. Closer examination showed that growth of *P. cubensis* was extensive onto cucumber leaves 10 days after inoculation (Fig.2 A). Since, sporangia was formed, growth and developed. Spraying of Kaolin at 1% pre or post inoculation, affected the growth and sporulation of *P. cubensis* on cucumber leaves. Leaves surface coating with Kaolin pre-inoculation process completely inhibited spore germination and growth development (Fig.2 B). Also, spores were affected and collapsed. The fungus from the cucumber leaves sprayed post inoculation with Kaolin was much less profile and growth than the control and had become collapsed and loss of turgor of the outermost rind spores cell (Fig.2 C).

Protected field experiments

Effect of film forming antitranspirants on downy mildew disease incidence

Figure 3 showed downy mildew disease development on cucumber plants grown in commercial plastic houses of autumn and spring seasons of 2000 and 2001. In natural infested plants, downy mildew disease severity was increased during the growth period up to 120 days after sowing ranging from 1.3 to 4.8 and 1.9 to 4.3 in spring season and from 1.8 to 7.8, 1.8 to 7.6 in autumn season of 2000 and 2001, respectively. Spraying plants with film forming antitranspirants, resulted in reduction of downy mildew disease severity, whereas the average disease severity, 120 days after planting, abridged by 0.0 and 4.3. Clearly, Kaolin was considered to be the best film forming antitranspirant for controlling downy disease incidence which proved to control the disease almost completely in spring season and reduced the incidence of disease to minimum level (0.6) in autumn season after 75 days of sowing as observed on comparison with untreated control (4.8-4.3) and (7.8-7.6) for 2000 and 2001, respectively (Fig. 4). Still, Nu-Film was the most effective in controlling disease incidence in spring season (0.3) and autumn season (0.6) of both years. Bio-Film and Polyacrylamide were moderate effective in controlling downy mildew disease incidence.

Effect of film forming antitranspirants on *P. cubensis* sporulation

There were prominent differences between population curve of the pathogen in untreated control and antitranspirants film treatments. Since, in untreated plants, population counts of sporangia spores of *P. cubensis* increased during 120 days of growth from 34.3 to 1263.2 and from 35.2 to 1301.2 x 10^3 spore per cm^2 in spring season and from 92.2 to 6234.2 and 87.3 to 6124.3 x 10^3 spore per cm^2 in autumn season of 2000 and 2001, respectively (Fig.5). Film forming antitranspirants sprayed cucumber plants, resulted in a reduction in *P. cubensis* sporulation in infected lesions, whereas the average spores counts, 120 days after planting, abridged by 0.0 to 1987.3 x 10^3 spore per cm^2. Application of Kaolin (1%), led to a slight decreased in *P. cubensis* sporulation ranging from 33.5 to 21.5 and from 33.5 to 19.3 x 10^3 spore per cm^2 in spring season and from 76.3 to 2016.2 and 63.3 to 198.5 x 10^3 spore per cm^2 in autumn season of 2000 and 2001, respectively. Data also espied that Nu-Film and Bio-Film motivated reduction in pathogen level, whereas, Folicate showed the lowest effect in pathogen reduction.

Effect of film forming antitranspirants on plant height and yield

Plant height and yield of cucumber plants under all film forming antitranspirants were highly increased than of

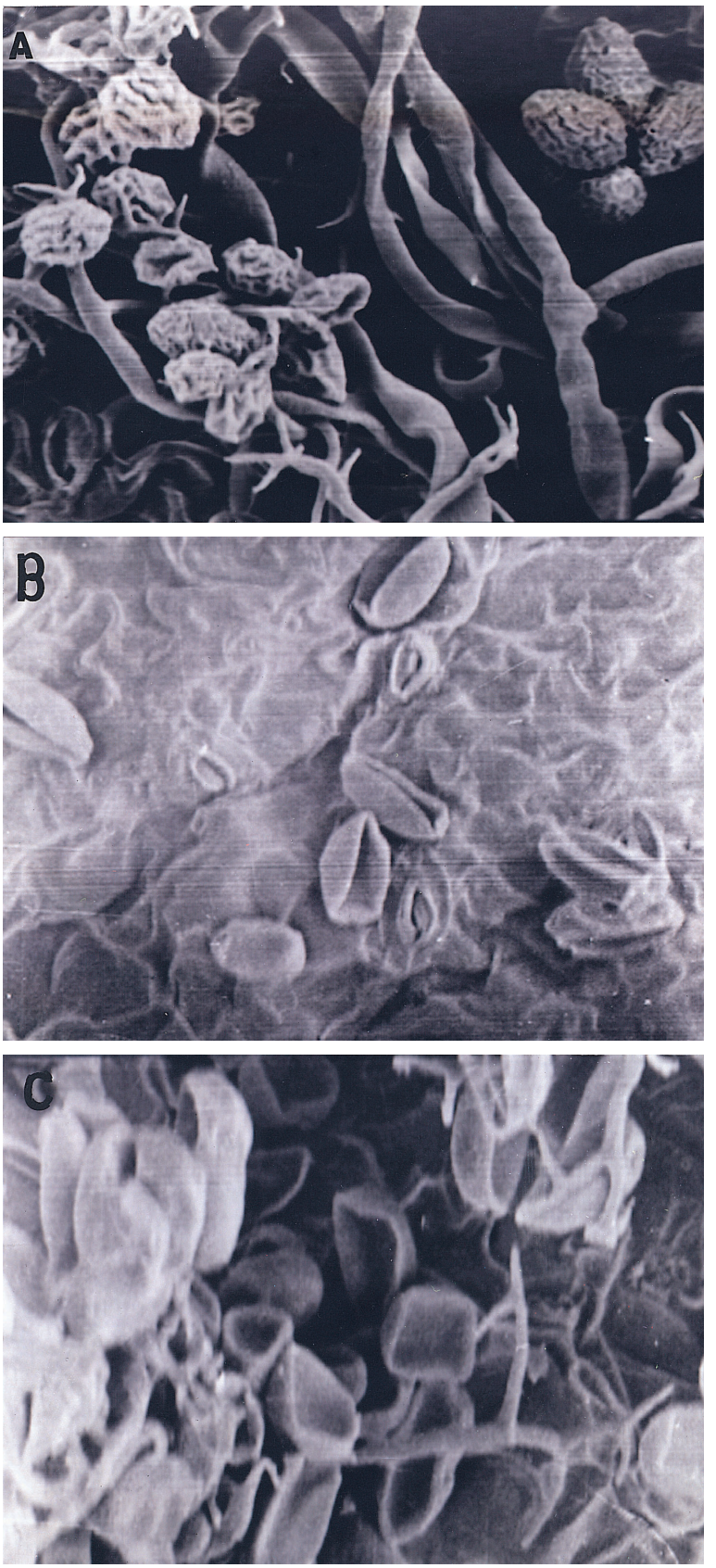

Fig. 2. SEM micrographs of *Pseudopernospora cubensis* on cucumber leaves sprayed with Kaolin at 1%, 1000X. (A) Infected, untreated showed heavily *P. cubensis* growth callose encasements of sporangia and branched hyphae. (B) Collapsed sporangia in cucumber plant sprayed with Kaolin pre inoculation. (C) Collapsed and turgor loss sporangia and hyphae in cucumber plant sprayed with Kaolin post inoculation.

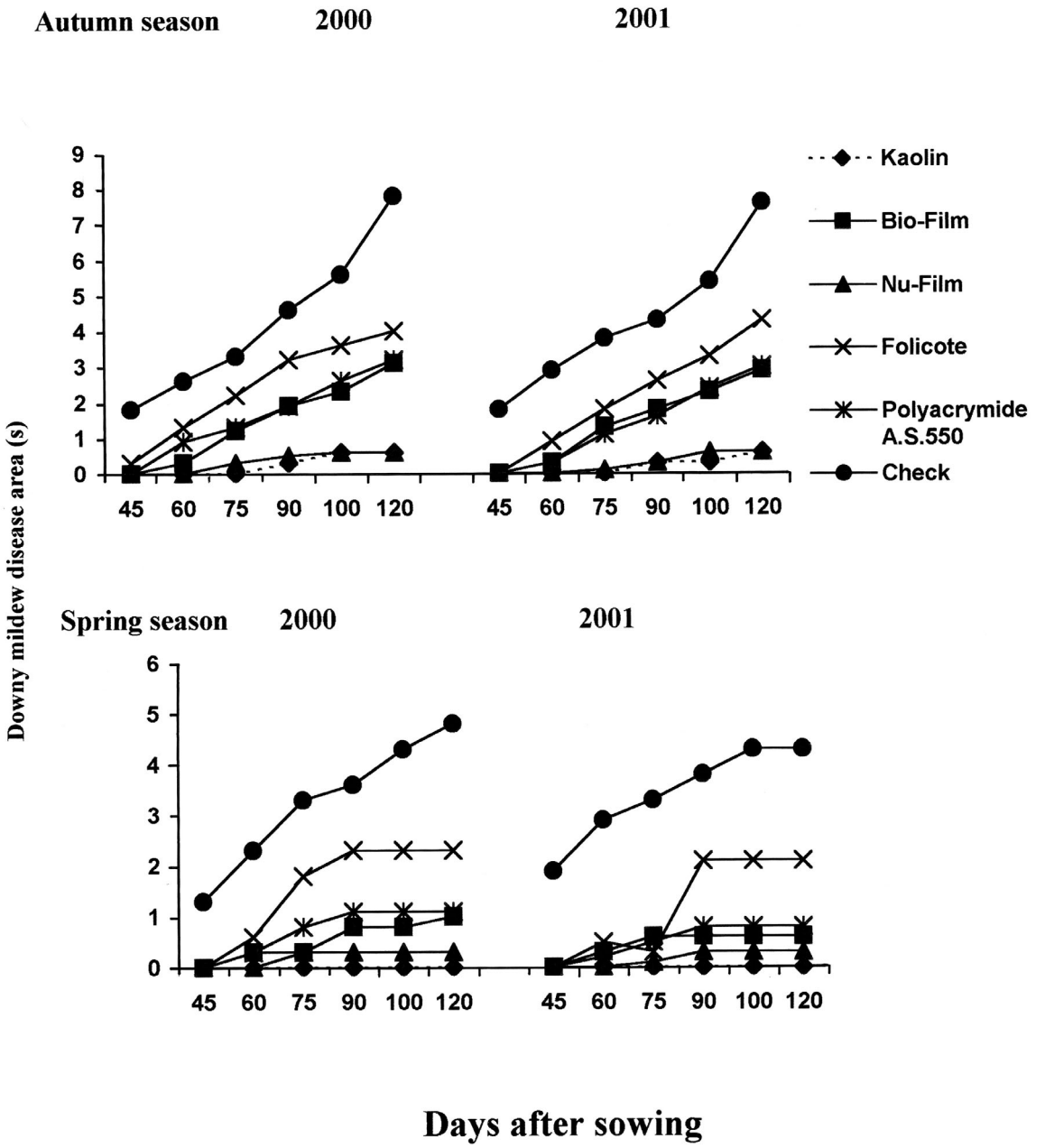

Fig. 3. Efficacy of film forming antitranspirants on the control of downy mildew disease caused by *Pseudopernospora cubensis* in cucumber plants grown in the protected cultivation.

untreated plants in both seasons of 2000 and 2001 (Table 2). The highest record of plant height and yield was obtained from Kaolin antitranspirant. Also, plants treated with Nu-Film and Polyacrylamide antitranspirants gave highest plant height and yield in both seasons.

DISCUSSION

Downy mildew is a severe disease in cucumber plants during the low temperature and high humidity season in Egypt. The causal agent, *P. cubensis* has developed resistance to fungicides [9]. Therefore, there is an urgent need to find an alternative mean to control the disease. The results obtained in this work showed that polymers of film-forming antitranspirants as foliar spray serve as an alternative mean to protect cucumber plants against *P. cubensis* and the best control is achieved by sprays carried out soon after and before infection as well as reduce environmental pollution. The disease was effectively controlled by all the tested polymers antitranspirants. The preliminary experiments of this study showed that, under greenhouse conditions the film forming antitranspirants were effective in suppressing pathogen germination, development and disease incidence when applied pre or post inoculation at 1.0%. Increasing concentration at 3%, resulted in a progressive reduction in the

Fig. 4. Cucumber plants infected with *Pseudopernospora cubensis* under protected cultivations. (A) ; Infected, not treated. (B) ; Sprayed with Kaolin at 1%.

lesion number, area and pathogen population. Clearly, Kaolin at 1% had an excellent effect on reduction downy mildew disease incidence and pathogens development compared with control treatment. Scanning electron microscopy revealed that coating leaves surfaces with Kaolin either pre or post infection process was active in preventing pathogen germination, development and its penetration. Collapsed hyphae and spores were also observed. The film forming antitranspirants, Nu-Film and Bio-Film and Polyacrylamide Anti-Stress 550 at 1.0% reduced downy mildew disease when applied to cucumber plants pre or post inoculation treatment. Also, this study shows that, the film forming antitranspirants at low concentration (1.0%) are effective in suppressing disease incidence and pathogen sporulation on leaves under protected cultivation and natural infected conditions with pathogen. Moreover, antitranspirant Kaolin was more effective in complete controlling of disease incidence in spring season as well as reduced and delayed disease incidence by 75 days in autumn season.

Various mechanisms for the protection of plants with coating polymers have been suggested [5,18,10,12,11]. The effect of film forming antitranspirants may be similar to those of the natural cuticle layer in defense against pathogens. In this respect, Zekaria *et al*. (1991) and Hsieh and Huang (1997) obtained that polymers of film forming antitranspirants provide either an impenetrable surface associated with their thickness or are resistance to enzymatic degradatin. Kamp (1985) demonstrated that film antitranspirants are hydrophobic, thus creating a low water potential at infection sites. Hsief and Huang (1999) used epidermis-coating antitranspirants such as film forming polymers and polyelectrolytes against *Botrytis elliptica* on lily and suggested that the disease control achieved with polyelectrolytes is due to the reduction of spore germination and the reduction of estrease secretion by pathogen.

The antitranspirants used in this study were hydrophobic, thus creating a low water potential on the leaves surface. The fact that the polymers reduced downy mildew disease incidence suggests that the fungistatic effect of these compounds also play a role in disease reduction, whereas it prevented spores germination, infection and growth of fungus when applied pre inoculation. Inhibition of growth and its development of fungal spores was also observed when Kaolin was applied four days post inoculation. In addition, collapsed and turgor loss of hyphae and spores were observed. Overall, polymers holds great promise for protection and enhancing the plant freshness, yield and quality. The effectiveness of polymers in plant protection, reducing desiccation and

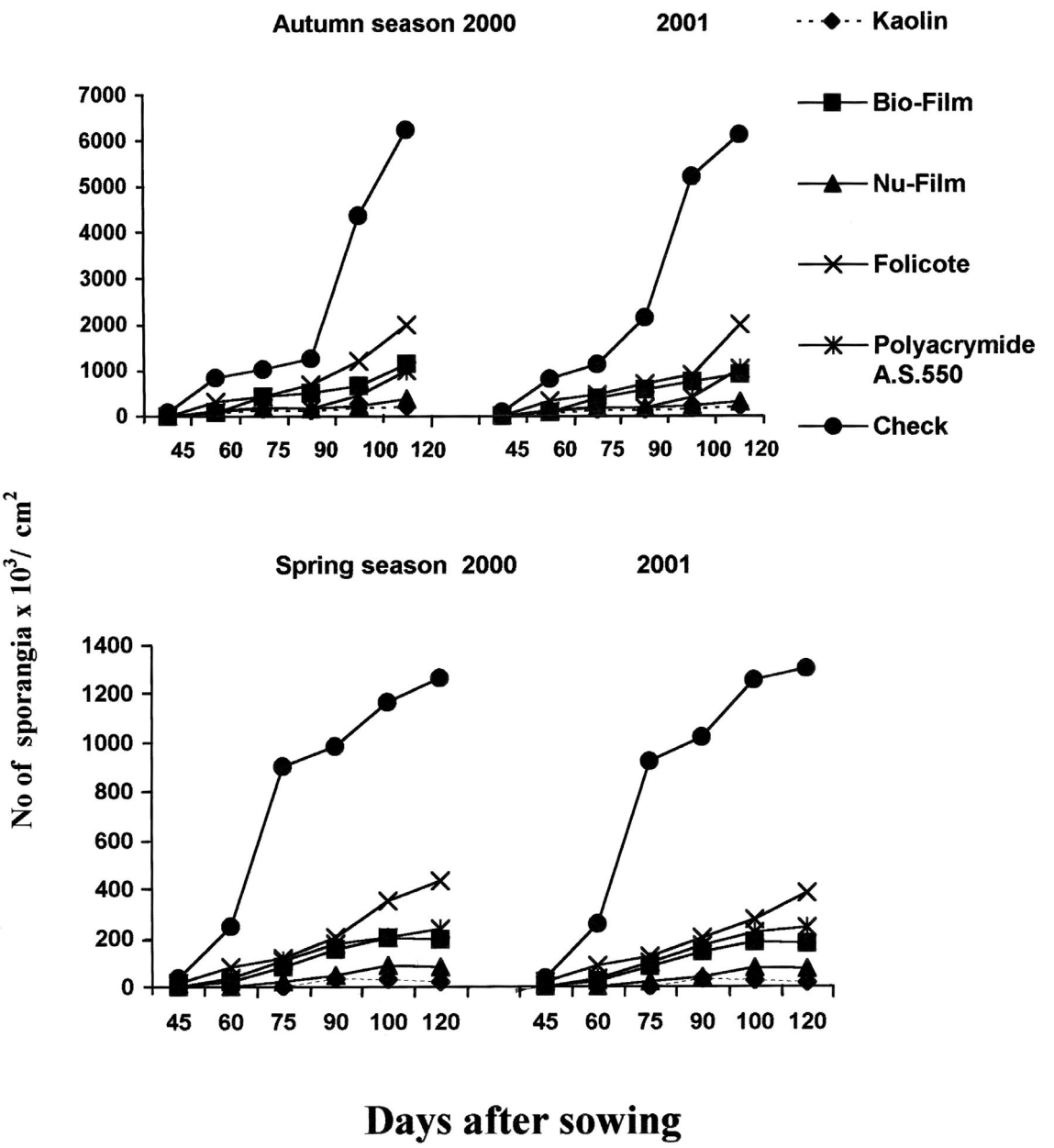

Fig. 5. Sporulation of *Pseudopernospora cubensis* on cucumber plants sprayed with film forming antitranspirants and grown in the protected cultivations.

Table 2. Plant height and yield of cucumber plants sprayed with film forming antitranspirants under protected cultivations

Antitranspirant	Spring season				Autumn season			
	2000		2001		2000		2001	
	Plant height (cm)	Yield (Kg/m^2)	Plant height (cm)	Yield (Kg/m^2)	Plant height (cm)	Yield (Kg/m^2)	Plant height (cm)	Yield (Kg/m^2)
Kaolin	214.4a [1]	10.9a	219.4a	12.0a	198.2a	7.14a	198.3a	7.34a
Bio-Film	181.3c	8.55bc	186.4c	9.01cd	171.5c	5.56bc	175.3c	5.82bc
Nu-Film	199.3b	8.91b	201.3b	9.75c	186.3b	6.88b	186.5b	6.23b
Folicote	168.3d	7.80c	170.6d	8.26d	154.6d	5.14c	158.4d	5.27c
Polyacrylamide	194.5b	9.2a	198.4b	10.4b	181.1b	6.55b	183.3b	6.02b
Check	148.9d	5.72d	150.4e	7.21e	128.0f	3.89d	130.0e	4.4d

[1.] Data followed by the same letter in each column do not differ significantly ($P<0.05$).

preserving plant freshness due to the substances properties. The results of this study suggested it may be possible to replace conventional chemical fungicides with polymer coatings antitranspirants, it is safe for human, environment and thus provided both economical and ecological efficacy.

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摘 要

Haggag, W. M. 2002. 在溫室利用抗蒸散劑防治胡瓜露菌病. 植病會刊11:69-78 (埃及國家研究中心植病系；聯絡作者，電子郵件: Whaggag@link.net；傳眞機: 002203832317)

由 *Pseudoperonospora cubensis* 引起的胡瓜露菌病，可經由葉部噴佈 Kaolin、Nu-Film、Bio-Film、Folicote 及 Polyacrylamide Anti-Stress 550 等抗蒸散劑，降低病害發生率。盆栽試驗證明不管在病原菌接種前或接種後，噴佈抗蒸散劑均可有效的減輕病害發生百分率外，尚可降低病原菌的產孢作用。在這些參試的化合物中，施行1% Kaolin與Nu-Film兩者是較有效的處理組，它們可以減少露菌的產孢量、孢子發芽率及減輕受害的面積。電子顯微鏡檢查發現Kaolin抗蒸散劑具有抑制孢子發芽及促使孢囊崩解與喪失膨壓的功效，在自然的感染條件下，播種胡瓜後第45及75天，噴佈兩次抗蒸散劑，可顯著減少病害的罹病度外，尚可增進胡瓜株高及提高產量。綜合研究成果證明在設施中施用抗蒸散劑，尤其是Kaolin，可以有效防治胡瓜露菌病。

關鍵詞：胡瓜、露菌病、抗蒸散劑、*Pseudoperonospora cubensis*