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Control of Downy Mildew on Protected Cucumber Plants With Film Forming Antitranspirants

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Abstract: 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 greenhouse test, antitranpirants proved to be effective treatments in reducing disease incidence, severity and pathogen sporulation when applied prior or post to inoculation. Among these compounds, Kaolin and Nu-Film (1.0 %) were more effective in reducing spores counts, germination and infection as well as downy infected area and lesions number. Scanning electron microscope examination showed that, Kaolin antitranspirant inhibited zoospores germination, growth and development as well as had become collapsed and lost its turgidity when applied either pre or post inoculation. Although, spraying of Bio-Film and Polyacrylamide Anti-Stress 550 antitranspirants, at 1 and 3%, resulted also in better disease control. Under protected cultivation and natural infection conditions, all antitranspirants showed a remarkable effectiveness on the reduction of disease severity and pathogen density when applied twice at 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 yield increased. 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 cultivations. 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 (Reuveni and Raviv, 1997; Abd-El-Kereem, 1998; Shama *et al.*, 1998; Chaban and Okhrimchukv, 2000; Ahamed *et al.*, 2000) and greatly affects both yield and quality (Thomas, 1996). 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 have a major role as infective propagules of these fungus (Cohen *et al.*, 1995). This pathogen is present and develop in a high humidity and often causes sever epidemics (Jarvis, 1992; Reuveni and Raviv, 1997). For effective control of this disease there are many chemical products, but in view of their undesired secondary effects and the fact that cucumber require continuous prolonged harvesting, there is increasing interest in finding and using new natural products which are more reliable and less dangerous for the environment. 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 (Han, 1990; Zekaria-Oren *et al.*, 1991; Ziv and Zitter, 1992; Nasraoui *et al.*, 1996; Ziv and Hagiladi, 1993; Nasraoui *et al.*, 1999). Han (1990) used antitranspirant Masbnene and controlled 12 foliar diseases on nine diverse hosts. Most of these coatings are non phytotoxic and resistant to weathering (Han, 1990). Although the effects of antitranspirants on disease control appear similar to those of the natural cuticle layer in defencing plant pathogens (Hsieh and Huang, 1997; Hsieh and Huang, 1999), physical effects should be also considered (Nasraoui, 1993). 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 (Nasraoui *et al.*, 1996; Hsieh and Huang, 1997). The objective of this study was to evaluate the efficacy of five film forming antitranspirants applied on cucumber leaves for the control of cucumber downy mildew disease incidence under protected cultivation and possible mechanisms involved in the reduction of the disease.

Materials and Methods

Mildew: Infected leaves of cucumber plants were collected from local commercial plastic hoses and natural inoculation from these infected leaves was used. *P. cubensis* was maintained on cucumber plants in a separate growth in pots 25 cm in diameter and incubated at 20 ^{o}C, 18 hr. light and 90% relative humidity (RH). To inoculate cucumber plants, the procedure described by Okuno *et al.* (1991) was used. Spores were harvested from infected leaves using a brush and water containing 0.01% tween 80 (Poly oxyethylene) as sufficient. The 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 spores concentration was adjusted to 10^5 spore per ml with a hemacytometer and lightly atomized onto cucumber leaves.

Antitranspirants: The following antitranspirants were tested on cucumber plants, Kaolin (aluminum silicate), Nu-Film (di-1-P-menthene, low viscosity), bio-film(an ionic- nonionic blend), Folicote (wax emulsion) and Polyacrylamide Anti-Stress 550 (cross-linked carbon acrylic latex polymer). The film forming antitranspirants were applied at two concentrations of 1 and 3% (W/V).

Leaf disk assay: Cucumber plants of the susceptible cultivars, Delta Star (Samtrade company)-1.5 month old- were used to prepare leaf disks. The tenth leaf below the apex was surface sterilized with 0.1% sodium hypo-chlorit and rinsed three times in sterile water. Fifteen leaf disks (15 mm in diameter) were sprayed with a thin layer of antitranspirants and with sterile water used as control. After 24 h, 2 ml of spores suspension was sprayed over the disks. The disks were put inside petri dishes (90 mm. in diameter) containing wettable filter paper, with six replectes for each treatment. The disks then incubated at 20 ^{o}C in darkness. After 24 h spore germination as well as its infected were determined under an light microscope.

Pot experiments: The effect of five antitranspirants on the incidence of downy mildew disease was measured under greenhouse conditions. Cucumber plants cv. Delta Star were grown in plastic pots, 25 cm in diameter and incubated at 20 ^{o}C and 90% RH.

Pre and post inoculation: Seedlings were divided into two treatments; the first pre inoculation treatment, in which coating antitranspirants film were sprayed four days before inoculation. The second treatment, cucumber plants were sprayed with coating antitranspirants film 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. Disease severity was measured according to Reuveni (1983) using colour index and infected area. The colour index was calculated as follows:

0 = no symptoms 1 = greenish 2 = yellowish
3 = yellow 4 = brown

The infected area index was as follow:

1 = 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.

Spore of *P. cubensis* were collected in 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. Spore are released from lesion using a brush, then they were counted by using hemacytometer slide. Means of six replicates, each contained 15 leaves, were calculated.

Scanning electron microscopy (SEM): Leaves were treated with antitranspirants film, Kaolin (1%)- the best treatment- was taken 10 days after application for scanning electron microscope (SEM). Leaf lesions (0.5 cm^2) were rapidly freezed in the vapour phase above liquid nitrogen and placed in a glass vacuum container prior to lypophilization. The dried frozen leaf segments were mounted on aluminum stubs and coated with gold using vacuum evaporation prior to SEM examination in National Research Centere, Centeral Lab., Dokki, Giza.

Field experiments: An experiment was carried out under naturally infection under commercial plastic greenhouses at Gezerit El Dahab site, Giza govarnorate during the two successive seasons of spring and autumn of 2000 and 2001, for studying the effect of antitranspirants on the disease incidence of downy mildew. Cucumber plants of Peremo, C.V. (Slous and groots company) hybrid was sprayed with 1% of antitranspirants twice at 45 and 75 days after sowing. The control treatment was sprayed 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 *P. cubensis* counts were measured after 45, 60, 75, 90, 100 and 120 days of sowing as previous above. At harvest stage, cucumber yield was determined as Kgm^{-2}.

The obtained data were statistically analyzed by using T. Test according to Neler *et al.* (1985).

Results

Leaf disk assay: The different antitranspirants affected the spore germination of mildew with highly significantly under 1 and 3 % concentrations. Only, 1.0 % of spore were germinated and produced germ tubes when leaf disk were sprayed with Kaolin at both concentrations (Fig. 1). Nu- Film was considered to be the best treatment for reducing spore germination. Polyacrylamide and Bio- Film at both concentrations compounds were more less effect the antitranspirants used against downy mildew. At the same time, all antitranspirants showed a high efficacy in reducing direct penetration and *P. cubensis* infection abridge by (0.0- 40%) in compared with 93% of untreated control. Kaolin was the most effective in preventing infection. Moreover, Nu-Film and Bio-Film proved highly effectiveness in reducing infection.

Pot experiments

Pre and post inoculation: The trials showed that all antitranspirants application @1.0 and 3.0% were critical on reducing the severity of downy mildew incidence compared to control treatment (Table 1). Reduction in disease severity was highly increased on cucumber leaves sprayed either pre or post inoculation with film antitranspirants by 98.7-55.5 and 93.8 - 46.9%, respectively compared with untreated plants. Among the tested film forming antitranspirants, good results were achieved on both Kaolin and Nu-Film at both concentrations when used either pre or post inoculation. However, Bio-Film and Polyacrylamide at 3% were the most effective in reduced the disease severity.

In cucumber plants sprayed with antitranspirants 4 days before or after inoculation, a significant reduction in *P. cubensis* sporulation was found in infected lesion. Whereas, the percent of spore counts were reduced by 97.6-60.6 and 93.6 and 57.0% by pre and post inoculation with antitranspirants, respectively in compared with control treatment. Still, Kaolin strongly suppressed sporulations of *P. cubensis* on infected lesions by 97.6 and 92.4% in pre and post inoculation with antitranspirants, respectively compared with the other film antitranspirants. However, Folicate film antitranspirant was less effective.

SEM examination: Closer examination showed that growth of *P. cubensis* was extensive onto cucumber leaves,10 days after inoculation (Fig. 2A). Since, sporangia and zoospores were formed, growth as well as developed and infected leaf tissue. Spraying of Kaolin at 1% pre or post inoculation, affected the growth and sporulation of *P. cubensis* on cucumber leaves. Coating leaves surface with Kaolin pre-inoculation process was the most effective in completely inhibition of spore germination, growth development and penetration (Fig. 2B). 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. 2C). Moreover, these treatment was the most effective in reducing sporangia formation as well as zoospores germination and penetration.

Field experiments

Effect of antitranspirants on disease incidence of downy mildew: Fig. 3 showed that downy mildew development on cucumber plants grown under plastic houses of autumn and spring seasons of 2000 and 2001. Under natural infested plants, downy mildew disease severity was increased during the growth period up to 120 days after sowing ranged 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 antitranspirants, resulted in reducing of downy mildew disease, 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 almost complete disease control in spring season and minimum disease (0.6) in autumn season after 75 days of sowing was observed in compared 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. Compared with the control treatment, the incidence of downy mildew was reduced by Folicate.

Effect of film forming antitranspirants on *P. cubensis* sporulation: There were prominent differences between sporulation counts curve of the pathogen in untreated control and antitranspirants treatments (Fig. 5). Since, in untreated plants, population counts 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 per cm^2 in spring season and from 92.2 to 6234.2 and 87.3 to 6124.3 x 10^3 per cm^2 in autumn season of 2000 and 2001, respectively. Antitranspirants compounds were sprayed in cucumber plants, resulted a reduction in *P. cubensis* sporulation in infected lesions, whereas the average spore counts, 120 days after planting, abridged by 0.0 to

Table 1: Efficacy of antitranspirants applied to cucumber leaves on disease development of downy mildew when applied as pre or post inoculation of *P.cubensis* in the plastic house

		Pre–inoculation		Post-inoculation		Pre–inoculation		Post-inoculation	
Antitran-spirants	Conc.%	Disease severity	Percent Reduction%	Disease severity	Percent Reduction%	*Spore counts/cm² of infected lesion	Percent Reduction%	*Spore counts/cm² of infected lesion	Percent Reduction%
Kaolin	1	0.1f	98.7a	0.8f	90.1a	97.6g	97.6a	392.1e	92.4a
	3	0.1f	98.7a	0.5f	93.8a	81.0g	98.0a	298.4e	93.6a
Bio-Film	1	1.7d	81.4bc	2.3cd	71.6c	625.2f	85.0b	934.6d	80.0bc
	3	1.0e	83.9b	2.1d	74.0bc	482.1f	88.4b	702.7d	85.0b
Nu-Film	1	0.8ef	90.1ab	1.6e	80.2b	343.4f	91.7ab	767.8d	86.6b
	3	0.5f	93.8a	1.3e	83.9ab	281.3f	93.2ab	624.8d	89.6ab
Folicote	1	3.6b	55.5e	4.3b	46.9f	1643.6b	60.6e	1989.4b	57.6g
	3	3.3b	57.2e	4.0b	50.6ef	1336.4c	67.9de	1831.1b	60.9efg
Polyacry-lamide	1	2.7c	66.6d	3.6b	55.5e	1187.1d	71.5cd	1464.3c	64.7e
	3	2.1cd	76.0c	3.0bc	62.9d	981.7e	76.4c	1231.6c	73.7d
Check		8.1a		8.1a		4173.6a		4693.2a	

*Disease severity and sporulation were recorded 10 days after inoculation and incubation at 20°C in greenhouse. Spore were released into 10 ml of distilled water and counts with a hemacytometer. x Percent reduction was calculated compared to check treatment. y Data followed by the same letters in each column do not differ significantly ($P < 0.05$).

Table 2: Plant height and yield of cucumber plants sprayed with film forming antitranspirants at 1.0% under the plastic house.

	Spring season				Autumn season			
	2000		20001		2000		2001	
Antitranspirants	Plant height (cm)	Yield (Kg/m²)	Plant height (cm)	Yield (Kg/m²)	Plant height (cm)	Yield (Kg/m²)	Plant height (cm)	Yield (Kg/m²)
Kaolin	214.4a[y]	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

y Data followed by the same letters in each column do not differ significantly ($P < 0.05$).

1987.3 x 10^3 per cm². Application of Kaolin (1%), led to a slight decrease in *P. cubensis* sporulation ranged from 33.5 to 21.5 and from 33.5 to 19.3 x 10^3 per cm² in spring season and from 76.3 to 2016.2 and 63.3 to 198.5 x 10^3 per cm² in autumn season of 2000 and 2001, respectively. Data also espied that Nu-Film and Bio-Film motive a pathogen reduction. 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 untreated plants in both seasons of 2000 and 2001 (Table 2). The highest records of plant height and yield were obtained from Kaolin antitranspirant. Also, treated plants with Nu-Film and Polyacrylamide antitranspirants gave highest plant height and yield in different seasons, compared with the control treatment. Where, Folicate led to a slight increase compared with untreated control.

Discussion

Downy mildew is a severe disease in cucumber plants during the low temperature and high humidity seasons in Egypt. The causal agent, *P. cubensis* has development resistance to fungicides (Klinkenberg *et al.*, 1998). 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 the spore germination, development and disease incidence when applied pre or post inoculation at 1%. Increasing concentration at 3%, resulted in a progressive reduction in the 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 spore germination, development and its penetration. Collapsed hyphae and spore were also observed. The film forming antitranspirants, Nu-Film and Bio-Film and Polyacrylamide Anti-Stress 550 at 1% 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%) 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 for 75 days in autumn season.

Various mechanisms for the protected plants with coating polymers have been suggested (Han, 1990; Zekaria-Oren *et al.*, 1991; Nasraoui, 1993; Reuveni, 1983; Nasraoui *et al.*, 1999). 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 degrading. Kamp (1985) demonstrated

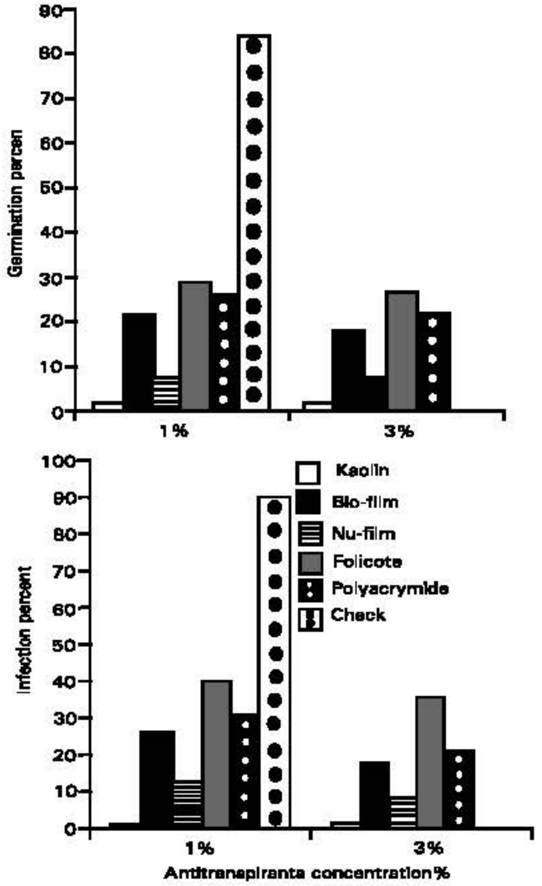
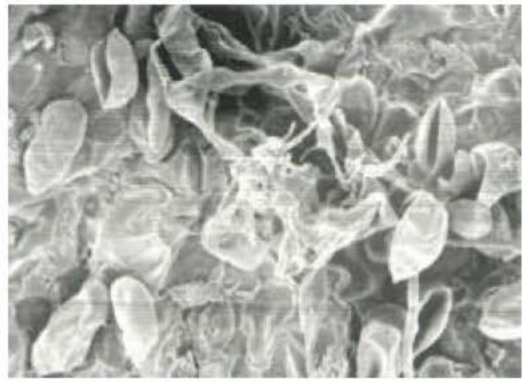


Fig.1: Assessment of spore germination and infection of *P. cubensis* on cucumber leaf disks (15mm) sprayed with antitanspirants, 24 h after inoculation and incubation at 20°C



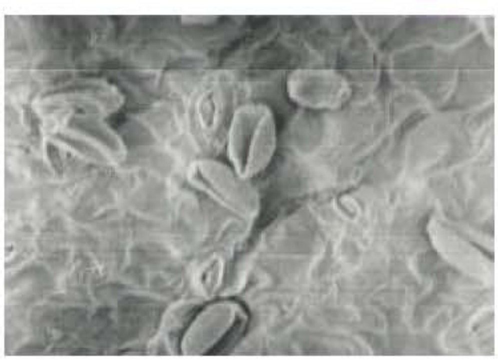


Fig. 2: SEM micrographs of *P. cubensis* on cucumber leaves sprayed with Kaolim at 1%, 100X, A) Infection, not treated showed heavily *P. cubensis* growth callose encasements of sporangia, zoospores and branched hyphae, B) Collapsed zoospores on cucumber leaf sprayed with Kaolim pre inoculation, C) Collapsed and turgor loss zoospores and hyphae on cucmber leaf spreyed with Kaolim post inoculation

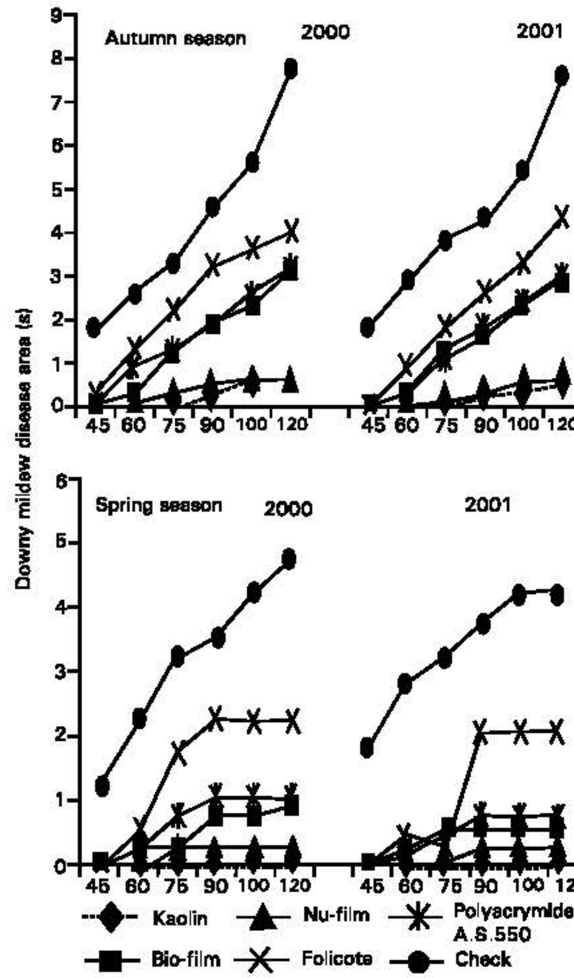


Fig. 3: Efficacy of film forming antitranspirants on the control of downy mildew disease caused by *P. cubensis* in cucumber plants grown in the protected cultivation

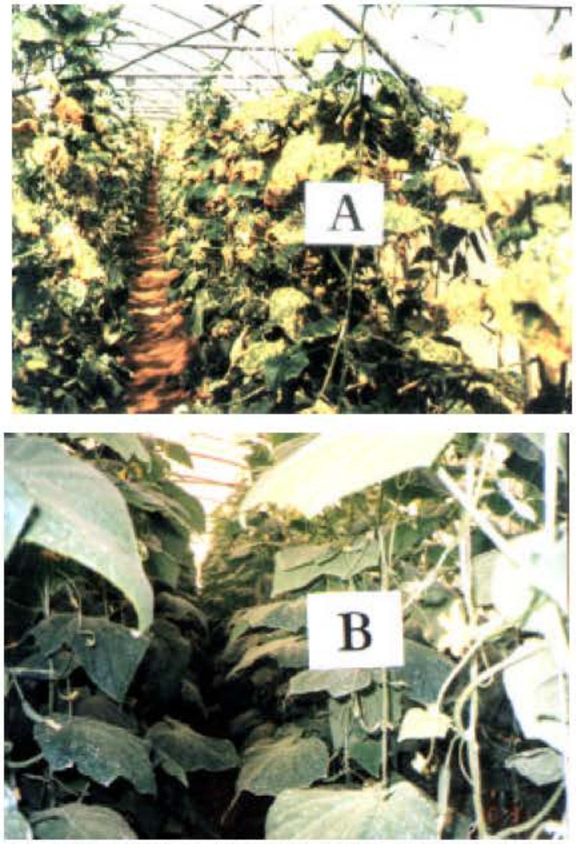


Fig. 4: Micrographs of cucumber leaves infection with P. cubensis under protected cultuvation, A) Infection, not treated, B) Cucumber plants sprayed with Kaolin at 1%

that film antitranspirants that coating substance 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 cinearia* 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 surfaces. The fact that the polymers reduced downy mildew disease incidence suggested that the fungistatic effect of these compounds also play a role in disease reduction, whereas it prevented spore germination, infected and growth when applied pre inoculation. Inhibition of growth and its development 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 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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