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# CONTROL OF GREY MOULD ON SOME HORTICULTURAL CROPS WITH FILM FORMING POLYMERS

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## ABSTRACT

Grey mould caused by *Botrytis cinerea* was controlled on some horticultural crops included, tomato, bean, onion and geranium , by applying film forming polymers coating to plant foliage. *In vitro*, the polymers, Wilt Pruf, Vapor Gard, Bio-Film, Nu-Film17 , Masbrane and Kaolin reduced germination , growth and sclerotial viability of *B. cinerea*. In addition, the hydrolytic enzymes produced by *B. cinerea*, Exo-PolyGalacturonase (ExoPG) and Caroboxymethyl Cellulase (CMCase) were also suppressed by the polymers. The most inhibition effect of the pathogen development was obtained by Kaolin , Nu-Film17 and Bio-Film at 1.0% concentration. The application of the polymers on leaf disks as bioassay reduced disease incidence and conidiospores density of *B. cinerea* . Under artificial and natural infection conditions, all film forming polymers significantly reduced grey mould disease severity on leaves, stems and fruits of various crop plants when sprayed three times at three weeks intervals. Furthermore, the application of polymers on plant foliage reduced conidiospores densities of *B. cinerea*. Kaolin and Nu-Film17 were strongly suppressed grey mould and the pathogen sporulation at all tested plants under field conditions.
**Keywords**: *Botrytis cinerea*, film forming polymers, Grey mould, tomato, bean, onion and geranium.

## INTRODUCTION

Grey mould caused by *Botrytis cinerea* , inflicts serious disease in many horticultural crops. *Botrytis cinerea* , occurs in many countries all over the world and attacks more than 200 hosts through effects on leaves, flowers, stems and fruits .( Sutton , 1990, Dik and Elad, 1999, Dik *et al.*, 1999 ,Pappas, 2000 and Vallejo *et al.*, 2001). At low temperature and high humidity or when free moisture is present on the plant surface, the pathogen can infect tissues ( Kuzniak and Sklodowska, 2001). Lesions often girdle the stems and cause the death of the plant above the lesion, thus creating substantial yield losses (Baptista *et al.*, 2000). *B. cinerea* frequently becomes resistant to chemical fungicides (Katan *et al.*, 1989). Furthermore, for environmental reasons research is aimed at limiting the input of fungicides. Therefore, new strategies for grey mould control are necessary.

Epidermis –coating antitranspirants, such as film forming polymers, have been used to provide protection against several plant diseases included rust, powdery mildew, downy mildew, anthracnose, leaf blight and spots (Elad and Ayish 1990, Ziv and Zitter, 1992, Ziv and Hagiladi, 1993, Hsieh and Huang, 1997, Triki and Priou, 1997, Nasraoui *et al.*, 1999, Puterka *et al.*, 2000 and Sutherland and Walters, 2001). Han (1990) used of epidermal coating and reduced downy mildew disease on cucumber plants, anthracnose on watermelon, early blight on tomato and controlled the black rot on grapes.

The effect of polymers on the control of diseases appear similar to those of the natural cuticle layer in defending plants against pathogens (Nasraoui 1993 and Hsieh and Huang ,1999).Coating polymers, such as antitranspirants, mineral oils, surfactants and other products, have been used as artificial barriers on leaf surface to inhibit foliar pathogens development on various host plants (Ziv and Zitter, 1992). Haggag, Wafaa (2002) found that film forming antitranspirants were most effective in reducing downy mildew incidence and of its sporulation on cucumber plants by inhibition of germination and growth development. Moreover, scanning electron microscope examination showed that Kaolin inhibited growth development and made the sporangia and zoospores becoming collapsed and lost of turgidity when applied pre or post inoculation.

The objective of the present study was to evaluate the effect of some film forming polymers i.e. Wilt Pruf, Vapor Gard, Bio-Film, Nu-Film17 , Masbrane and Kaolin on the control of grey mould disease in some horticultural crops include tomato, bean, onion and geranium.

## MATERIAL AND METHODS

### Isolation of *Botrytis cinerea*

*Botrytis cinerea* Pers. Fr. was isolated from diseased tomato, bean, onion and geranium plants and maintained on Potato Carrot agar medium. Pure fungus was transfered to Potato Dextrose agar (PDA) plates incubated at 20 $^0$C under continuous light were made at regular intervals to provide conidia. The spores were harvested after 4 days by washing with 5 ml sterile water, containing 0.01% Tween 80, filtratered through a 30 µm nylon filter and centrifuged for 5 min at 2600 x *g*. The resulting conidial pellet was adjusted to a concentration of $10^4$ conidia /$ml^{-1}$ as determined by heamocytometer slide.

### Film -forming polymers

Six film forming polymers were used, Wilt Pruf (ρ-pinene plymer Nursery Speciality Products, Green Wich, CT, USA) ,Vapor Gard (96%-1- ρ -menthene, Miller Chemical and Fertilizer Co., Hanover, PA), , Nu-Film17 ( di-1- ρ -menthene, low viscosity; Miller Chemical and Fertilizer Co., Hanover, PA) , Bio-Film ( an ionic- nonionic blend; Callo Agricultural Chemical Inc., Overland Park, KS ) Kaolin (aluminum silicate; Miller Chemical and Fertilizer Co., Hanover, PA) and Masbrane (Acrylic, Anti-Stress; AG, Fresno Calif.). The film forming polymers were applied at concentrations of 0.25, 0.50 and 1 .0 % ( W/V).

### Laboratory experiments

### Effect of polymers on *B. cinerea* growth

The effect of the various film forming polymers on inhibition of conidial germination and growth of *B. cinerea* was examined on PDA medium amended with various concentrations of polymers. 50µl of conidial suspension ($10^4$) were sprayed on to the medium in petri dishes (90 mm).

The percentage of reduction of conidial germination was examined after 24h of incubation under alternating 12h light: 12h dark at 20 ±1$^0$C with light microscope. The percent of growth reduction was measured on PDA inoculated with 5 mm. disk of *B. cinerea.* Sclerotia viability of *B. cinerea* were also measured by rinsed in various concentrations of polymers for five minutes and then sowned on PDA. After 10 days of incubation at 20°C, the percent of reduction of viable sclerotia was recorded.

**Effect of polymers on the hydrolytic enzymes production by *B. cinerea***

Conidia suspension was placed aseptically into 250 ml Erlenmeyer flasks containing 50 ml liquid of synthetic medium (Kapat *et al.*, 1998) and amended with various concentrations of polymers. The medium contained ($g1^{-1}$): $KH_2PO_4$, 1.36; $Na_2CO_3$, 0.5; $MgSO_4$, 0.5; asparagines, 1.0; $FeSO_4.7H_2O$, 0.005; $MnSO_4$, 0.0016; $ZnSO_4$. 7H2O, 0.0014 and $CaCL_2$, $2H_2O$, 0.002. After four days of incubation at 20 ±1$^0$C, the culture filtrate was collected, dialyzed and stored at-20 $^0$C until used.

**Exo-PolyGalacturonase (ExoPG)** activity was determined by measuring the rate of increase of galacturonic acid concentrations using dinitro salicylic (DNS) acid reagent (Miller, 1959).The reaction mixture contained 0.4 ml of 0.25% polygalacturonic acid dissolved in sodium acetate buffer ( 0.05 M, pH 5.2) and 0.1 ml of culture filtrate The net increase of reducing sugar in the reaction mixture was determined by comparing the measured optical densities with those on a standard curve prepared with galacturonic acid. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 µmol of galacturonic acid per minute per ml of the culture filtrate under the assay conditions.

**Caroboxymethyl Cellulase (CMCase)** activity was measured in terms of the reducing sugar released as a results of enzyme- substrate reaction by Miller, (1959) method. The reaction mixture contained 0.3ml of substrate (1.0% carboxymethyl cellulase dissolved in 50 mM acetate buffer, pH 5.2 ) and 0.2ml of culture filtrate. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 µmol of glucose per minute per ml of the culture filtrate under the assay conditions.

**Bioassays test**

Leaf disks - 20 mm in diameter from tomato (*Lycopersicum esculentum* L. cv. Peto 86), Bean (*Phaseolus vulgaris* L. cv. Bronco), Onion (*Allium cepa* L. cv. Giza 20) and geranium (*Pelargonium graveolens* Ait. cv. Ben Franklin) were surface disinfected with 10% (v/v) sodium hypochlorite for 3 min, washed four times with sterilized water, and dried between filter papers, One leaf disc was placed on each petri dish containing Whatman paper wetted with sterile water. Leaves were sprayed with film forming polymer at 1.0% concentration. Then, one ml of conidia suspension of *B. cinerea* ($10^4$) containing 0.02 M glucose and 0.02 M $KH_2Po_4$ to promote infection (Leone and Tonneijick, 1990) was sprayed on each leaf disc, and incubated at 20°C under alternating 12h light: 12h dark. Water was used as control. Five replicates were used for each treatment. The diameter of the necrotic lesions on the leaf disc was measured after 7 days of incubation. Spores concentrations were counted using a heamocytometer slide and expressed as no of spores per $cm^2$.

**Pots experiments**

Seedlings of tomato (*Lycopersicum esculentum* L. cv. Peto 86), Bean (*Phaseolus vulgaris* L. cv. Bronco) and Onion (*Allium cepa* L. cv. Giza 20) as well as geranium cuttings (*Pelargonium graveolens* Ait. cv. Ben Franklin) were sowned in 1-1 plastic pots containing peat, vermiculite and sand (1:1:1) and grown in a glasshouse at 22 $\pm 2^0$C to left and grow to the 2 $^{nd}$ leaf stage. Leaves were sprayed with film forming polymers at 1.0% concentration. Then, leaves were inoculated with $10^4$ $ml^{-1}$ conidial suspension of *B. cinerea* containing 0.02 M glucose and 0.02 M $KH_2Po_4$. Grey mould disease severity was assessed after 10 days as disease index (0-7) (Elad *et al.*,1994), whereas 0= symptomless leaf tissue, 1= 1-12% of the area under the droplet is necrotic, 2= 13-25% ; 3= 26- 50% ; 4= 51-100% ; 5 = necrotic area diameter exceeds the droplet diameter by up to 1 mm ; 6= necrotic area diameter exceeds droplet diameter by 1-3 mm; 7= necrotic area diameter exceeds droplet diameter by more than 3 mm.

**Field experiments**

During 2001 and 2002 seasons of autumn and spring seasons, four experiments were carried out against *B. cinerea* on tomato (cv. Peto 86), bean (cv. Bronco), onion (cv. Giza 20) and geranium (cv. Ben Franklin ) under natural field infection conditions at El Kalubia Govarnorate. Plants were planted in a clay soil with 50 cm distance between plants and 100 cm between rows for tomato plants and 50 cm for the others. Each plot equal about 5 $m^2$ area. Plots with five replicates were arranged in a randomized block design. Plants were grown up to the 2 $^{nd}$ leaf stage, and the film forming polymers then were sprayed at 1.0% concentration. The polymers were mixed with distilled water, with 0.01 % surfactant and sprayed to run- off on all leaves. The plants were sprayed three times at three weeks intervals. Control plots were treated with water . Disease severity of each treatment was measured and classified on a scale of 1-7 (Elad *et al.*,1994) as previously mentioned . Disease assessments on leaves, stem and fruits were done at 6-15 day intervals. The average diseased area of lesions was calculated for all experiment data's. The average diseased area was calculated per plant. The natural population of *B. cinerea* was measured on infected lesions. Incidence of *B. cinerea* sporulation was measured in lesions after incubation for 3 days in a moist chamber at 22 $^0$C. Spores concentrations were counted using a haemocytometer slide and expressed as No of spores per $cm^2$.

The obtained data were statistically analyzed by using Duncan's multiple range test at $P < 0.05$.

## RESULTS

### Effect of polymers on *B. cinerea* growth

Germination of *Botrytis cinerea* was affected by film forming polymers treatments compared with untreated control (Fig. 1). The efficacy of the six polymers was affected at concentration of 1.0 % , but it declined with decreasing concentration to0.2%. Kaolin, Nu-Film 17 and Bio-Film were considered to be the best polymers for completely reducing conidial germination at 1.0%. Germination of *B. cinerea* was also reduced by Wilt Pruf and Vapor Gard.

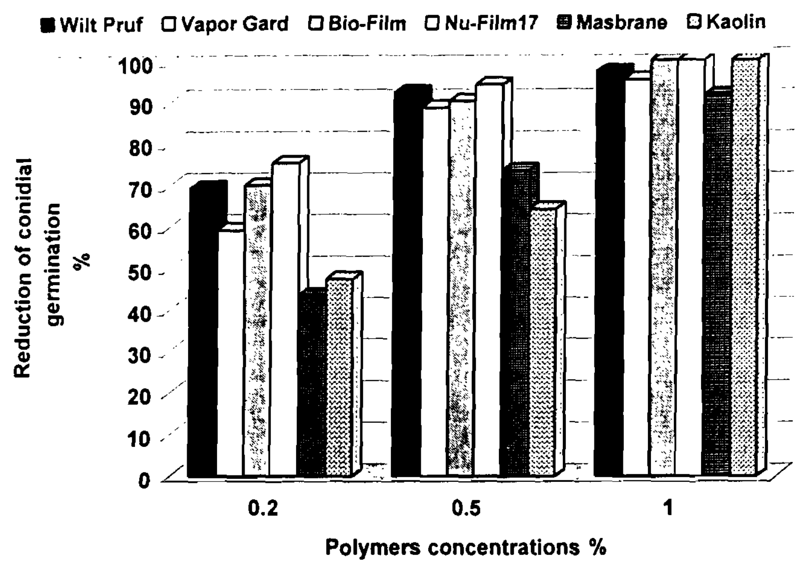



**Fig. (1): Reduction of conidial germination (%) of *Botrytis cinerea* by film forming polymers.**

The efficiency of film forming polymers on inhibition of *B. cinerea* growth was assessed (Fig. 2). Also, the fungal linear growth reduction was decreased with increasing concentration to 1.0%. The best inhibition of *B. cinerea* growth using polymer was achieved with Kaolin, Nu-Film17 and Bio-Film. Wilt Pruf and Vapor Gard reduced *Botrytis* growth in comparison with control treatment.

In addition, film forming polymers were effective to reduce sclerotial viability of *B. cinerea* (Fig. 3). There was corresponded between the polymers concentration and reduction of sclerotia viability , whereas reduction was increased by increasing concentration to 1.0%. A higher percentage of sclerotia viability reduction was recorded with Kaolin, Nu-Film 17 and Bio-Film, respectively .However, Masbrane had the less effect than the others.

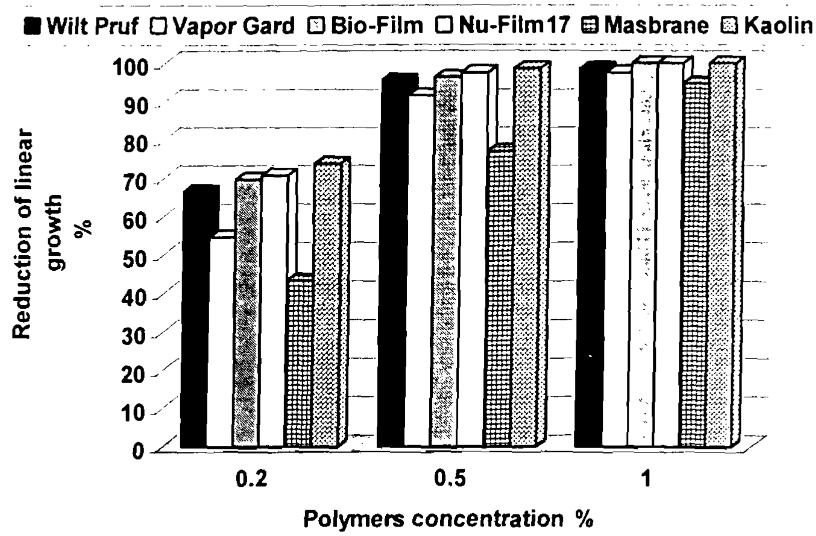



Fig. (2): Reduction of liner growth (%) of *Botrytis cinerea* by film forming polymers.

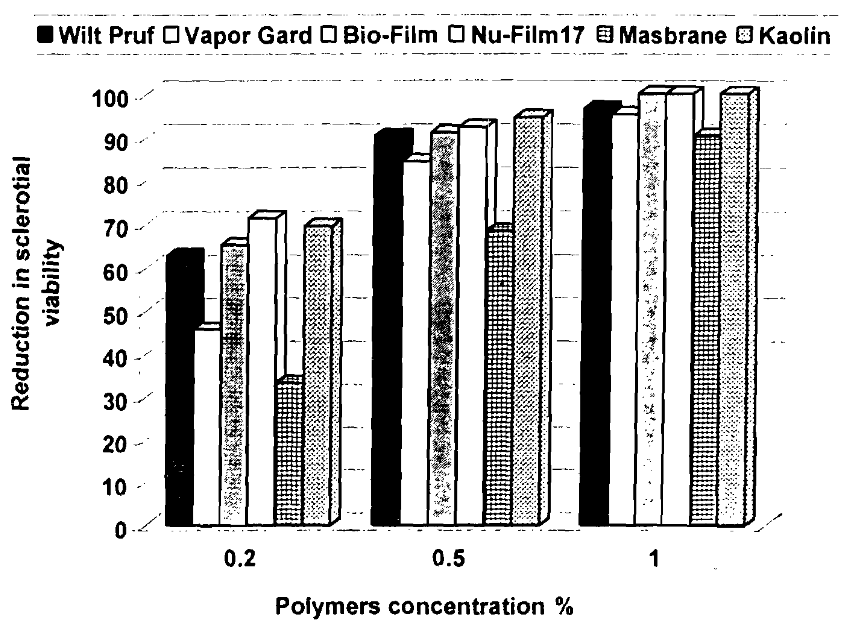



Fig. (3): Reduction of sclerotial viability (%) of *Botrytis cinerea* by film forming polymers.

**Effect of polymers on the hydrolytic enzymes production by *B. cinerea***

The effect of film forming polymers on the secretion of ExoPG and CMCase was assayed (Fig. 4). Polymers were more effective in reducing ExoPG and CMCase activities produced by *B. cinerea*. Increasing concentration of polymers from 0.2 to 1.0% , had a corresponding effect on reducing the ExoPG and CMCase activities. ExoPG was completely reduced 100% by 1.0% concentration of Kaolin, Nu-Film17 and Bio-Film until the end of incubation period. At 1.0% concentration , Wilt Pruf reduced ExoPG by 95.0%, whereas Vapor Gard reduced by 86.0%. Similar data were observed with CMCase activities in the presence of film forming polymers. The effectiveness increased remarkably at 1.0%, but it decline with the decreasing the concentrations up to 02%. CMCase activity was completely inhibited by 1.0% of Kaolin, Nu-Film17 and Bio-Film, whereas 97.6 and 95.0% reduction were observed by Wilt Pruf and Vapor Gard, respectively. Cleary, Masbrance had the lowest effect of enzymes reduction.

**Bioassays test**

The influence of different polymers on disease incidence of grey mould on leaf disks bioassay was investigated (Fig. 5). The poly film polymers had higher effect on reduction the disease incidence and conidiospores densities of *B. cinerea* on various plants in comparison with untreated leaves. The disease incidence and conidiospores of *B. cinerea* on different leaf disks were reduced at Kaolin and Nu-Film 17 treatments. However, Bio-Film treatment, gave the better protection and more reduction of *Botrytis* disease incidence .

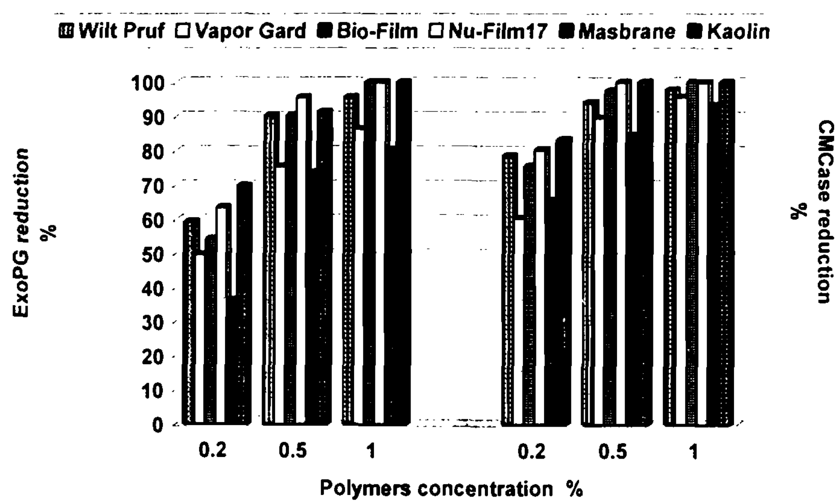
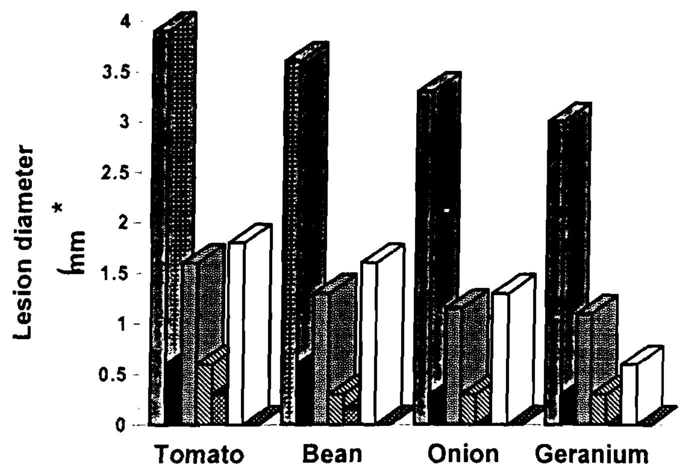



**Fig. ( 4 ): Reduction of ExoPolyGalacturonase ( ExoPG ) and CarboxyMethyl Cellulase (CMCase) (%) activities of *Botrytis cinerea* by film forming polymers.**



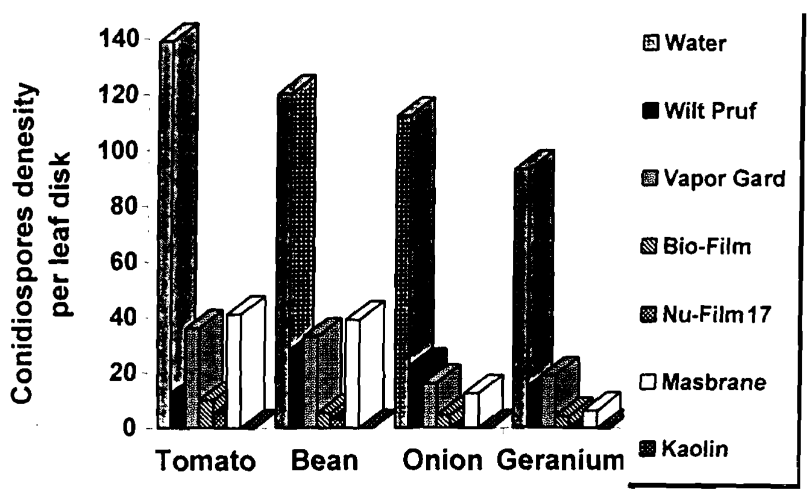




**Fig. (5): Bioassay test for influenced of different poly film polymers on grey mould disease incidence and conidiospores density (per cm²) of *Botrytis cinerea* on tomato, bean, onion and geranium leaf disks.**

**Pot experiments**

Disease severity of grey mould on tomato, bean, onion and geranium leaves inoculated with *B. cinerea* spores reached to 6.8, 6.1, 6.0 and 5.4, respectively (Fig. 6). Grey mould was reduced by using all film forming polymers in comparison with untreated control. Kaolin polymer was the most effective , which showed completely inhibition the disease on different tested plants. Nu-Film 17 and Bio-Film polymers reduced disease severity on tomato (0.3), bean (0.3 and 0.6), onion (0.0 and 0.08) and geranium (0.13 and 0.3), respectively.

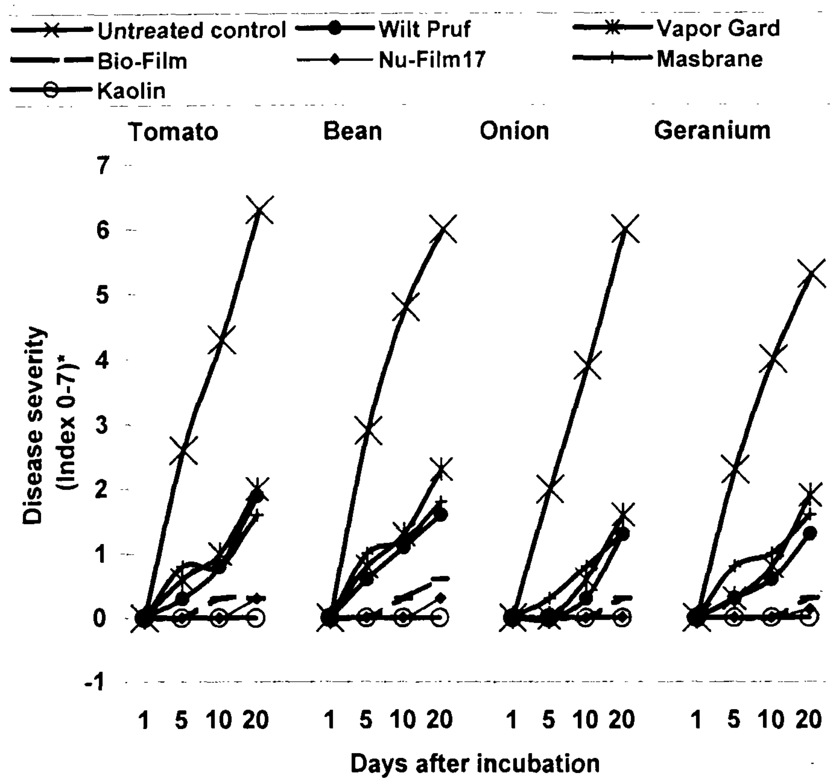



**Fig. (6): Disease severity of gery mould pathogen (*B. cinerea*)on some different hosts after spraying with film forming polymers .* Disease index (0-7),whereas 0= symptomless leaf tissue, 1= 1-12% of the area under the droplet is necrotic, 2= 13-25% ; 3= 26- 50% ; 4= 51-100% ; 5 = necrotic area diameter exceeds the droplet diameter by up to 1 mm ; 6= necrotic area diameter exceeds droplet diameter by 1-3 mm; 7= necrotic area diameter exceeds droplet diameter by more than 3 mm.**

**Field experiments**

In order to find out the effect of film forming polymers on grey mould disease severity, four experiments were conducted with polymers using 1.0% concentration on tomato, bean, onion and geranium plants. Disease severity and conidiospores counts were deterimined weekly by visually estimating the fungal mycelia of the covered grey mould.

**First experiment**: The effect of film forming polymers on the suppression of *B. cinerea* on leaves and fruits of tomato plants was tested in autumn and spring seasons of 2001 (Table 1). In all treatments, disease incidence was increased on the fruits and leaves during the autumn season than in spring one. Complete suppression and higher reduction of grey mould and *B. cinerea* sporulation were observed with Kaolin at autumn and spring seasons , respectively. When tomato plants sprayed with Nu-Film 17 and Bio-Film polymers, disease incidence and *B. cinerea* sporulation significantly suppressed in both seasons.

**Table (1): Effect of spraying film forming polymers on grey mould incidence on tomato plants under field conditions during 2001 seasons.**

| Treatment | Disease severity (Disease index 0-7)* | | | | Conidiospores density (no. / $cm^2$)* | | | |
|---|---|---|---|---|---|---|---|---|
| | Autumn | | Spring | | Autumn | | Spring | |
| | Leaf | fruits | Leaf | fruits | Leaf | fruits | Leaf | fruits |
| Untreated control | 5.3a [x] | 6.6a | 4.0a | 5.9a | 208.3a | 340.3a | 188.3a | 198.3a |
| Wilt Pruf | 1.8b | 2.0bc | 0.9c | 1.8c | 31.6c | 43.6c | 25.6c | 35.6c |
| Vapor Gard | 2.0b | 3.6b | 1.8b | 2.4bc | 55.6b | 71.2b | 45.5b | 55.2b |
| Bio-Film | 1.6bc | 1.8c | 0.5c | 0.9d | 21.3d | 34.3c | 18.3cd | 24.5cd |
| Nu-Film 17 | 0.9cd | 1.3c | 0.15cd | 0.5d | 18.5d | 20.7d | 8.6de | 18.7d |
| Masbrane | 2.1b | 3.8b | 1.95b | 3.6b | 58.0b | 87.8b | 42.5b | 61.6b |
| Kaolin | 0.0d | 0.15d | 0.0d | 0.0d | 0.0e | 16.0e | 0.0e | 0.0e |

***Disease index (0-7),whereas 0= symptomless leaf tissue, 1= 1-12% of the area under the droplet is necrotic, 2= 13-25% ; 3= 26- 50% ; 4= 51-100% ; 5 = necrotic area diameter exceeds the droplet diameter by up to 1 mm ; 6= necrotic area diameter exceeds droplet diameter by 1-3 mm; 7= necrotic area diameter exceeds droplet diameter by more than 3 mm.Means in the same column followed by the same letter are not significant differed at P<0.05 according to Duncan's multiple range test.[x] Data were the mean of five replicated and recorded weekly after treatment.**

**Second experiment**: the effect of film forming polymers on the suppression of *B. cinerea* infection on leaves of bean plants was tested in autumn and spring of 2001 and 2002 seasons (Table 2). At different treatments, disease incidence was increased during autumn season . Significant suppression of grey mould was obtained with all film forming polymers compared with untreated control. Kaolin caused complete disease control of grey mould as well as sporulation on leaves at both seasons. Nu-Film 17, Bio-Film and Wilt Pruf significant suppressed disease incidence and *B. cinerea* sporulation . Masbrane had the lethal effect .

**Table (2): Effect of spraying film forming polymers on grey mould incidence on bean plants under field conditions during 2001 and 2002 seasons.**

| Treatment | Disease severity (Disease index 0-7)* | | Conidiospores density (no. / cm²)* | |
|---|---|---|---|---|
| | Autumn | Spring | Autumn | Spring |
| Untreated control | 5.3a[x] | 4.6a | 163.2a | 121.3a |
| Wilt Pruf | 0.9c | 1.8b | 20.3c | 14.3c |
| Vapor Gard | 1.2b | 1.9b | 43.3b | 34.3b |
| Bio-Film | 0.3d | 0.3c | 12.3cd | 8.6cd |
| Nu-Film 17 | 0.15 | 0.15c | 9.4d | 5.5de |
| Masbrane | 1.3b | 1.9b | 49.6b | 39.3b |
| Kaolin | 0.0d | 0.0c | 0.0e | 0.0e |

*Disease index (0-7),whereas 0= symptomless leaf tissue, 1= 1-12% of the area under the droplet is necrotic, 2= 13-25% ; 3= 26- 50% ; 4= 51-100% ; 5 = necrotic area diameter exceeds the droplet diameter by up to 1 mm ; 6= necrotic area diameter exceeds droplet diameter by 1-3 mm; 7= necrotic area diameter exceeds droplet diameter by more than 3 mm.Means in the same column followed by the same letter are not significant differed at $P<0.05$ according to Duncan's multiple range test.[x] Data were the mean of five replicated and recorded weekly after treatment.

**Third experiments**: The study evaluated film forming polymers to control grey mould disease on onion leaves during 2001 and 2002 seasons (Table 3). The six film forming polymers were reduced the grey mould on onion plants compared with untreated ones in both years. Kaolin had the super effect than the other polymers, whereas complete inhibition of disease severity as well as *B. cinerea* sporulation on onion leaves were obtained. Also, Nu-Film ,17 Bio-Film and Wilt Pruf were effective on controlling grey mould.

**Table (3): Effect of spraying film forming polymers on grey mould incidence on onion leaves under field conditions during 2001 and 2002 seasons.**

| Treatment | Disease severity (Disease index 0-7)* | | Conidiospores density (no. / cm²)* | |
|---|---|---|---|---|
| | 2001 | 2002 | 2001 | 2002 |
| Untreated control | 4.61a[x] | 4.43a | 136.8a | 128.3a |
| Wilt Pruf | 1.33c | 1.30c | 23.6d | 21.6d |
| Vapor Gard | 1.66bc | 1.56bc | 36.4c | 33.3c |
| Bio-Film | 0.35d | 0.33d | 9.6e | 8.3e |
| Nu-Film 17 | 0.33d | 0.36d | 3.3ef | 2.6ef |
| Masbrane | 1.85b | 1.65b | 50.3b | 45.6b |
| Kaolin | 0.0d | 0.0c | 0.0f | 0.0f |

*Disease index (0-7),whereas 0= symptomless leaf tissue, 1= 1-12% of the area under the droplet is necrotic, 2= 13-25% ; 3= 26- 50% ; 4= 51-100% ; 5 = necrotic area diameter exceeds the droplet diameter by up to 1 mm ; 6= necrotic area diameter exceeds droplet diameter by 1-3 mm; 7= necrotic area diameter exceeds droplet diameter by more than 3 mm.Means in the same column followed by the same letter are not significant differed at $P<0.05$ according to Duncan's multiple range test.[x] Data were the mean of five replicated and recorded weekly after treatment.

**Fourth experiment**: The film forming polymers evaluation on the control of grey mould on geranium leaves and stems, similar results were obtained (Table 4). Treatments were significantly differ than the control one. The incidence of grey mould and *B. cinerea* sporulation on leaves and stems were greatest at autumn than in spring season . Clearly, Kaolin had more effect ascomplete controlling the disease on leaves and stems. Nu-Film 17and Bio-Film had significantly effect on reducing disease incidence on geranium plants.

**Table (4): Effect of spraying film forming polymers on grey mould incidence on geranium plants under field conditions during 2001 and 2002 seasons.**

| Treatment | Disease severity (Disease index 0-7)* | | | | Conidiospores density (no. / cm$^2$)* | | | |
|---|---|---|---|---|---|---|---|---|
| | Autumn | | Spring | | Autumn | | Spring | |
| | Leaf | Stem | Leaf | Stem | Leaf | Stem | Leaf | Stem |
| Untreated control | 5.6a$^x$ | 4.3a | 4.6a | 3.9a | 233.3a | 106.5a | 187.4a | 98.3a |
| Wilt Pruf | 1.6c | 0.6cd | 1.3c | 0.3c | 42.3c | 24.6c | 29.2d | 18.5c |
| Vapor Gard | 2.4b | 1.6b | 2.0b | 1.1b | 54.8bc | 12.4d | 38.3c | 21.3c |
| Bio-Film | 0.3d | 0.3d | 0.0d | 0.0c | 19.8d | 8.6de | 0.0e | 0.0d |
| Nu-Film 17 | 0.0d | 0.0d | 0.0d | 0.0c | 0.0e | 0.0e | 0.0e | 0.0d |
| Masbrane | 1.9bc | 0.9c | 1.6bc | 0.6bc | 67.2b | 43.5b | 45.3b | 35.4b |
| Kaolin | 0.0d | 0.0d | 0.0d | 0.0c | 0.0e | 0.0e | 0.0e | 0.0d |

***Disease index (0-7),whereas 0= symptomless leaf tissue, 1= 1-12% of the area under the droplet is necrotic, 2= 13-25% ; 3= 26- 50% ; 4= 51-100% ; 5 = necrotic area diameter exceeds the droplet diameter by up to 1 mm ; 6= necrotic area diameter exceeds droplet diameter by 1-3 mm; 7= necrotic area diameter exceeds droplet diameter by more than 3 mm.Means in the same column followed by the same letter are not significant differed at P<0.05 according to Duncan's multiple range test.$^x$ Data were the mean of five replicated and recorded weekly after treatment.**

## DISSCUSION

Grey mould is a sever disease during the low temperature season in many Mediterranean countries (Sutton, 1990, Elad *et al.*, 1994. Pappas, 2000). The causal agent, *B. cinerea* , had been reported to develop resistance to chemical fungicides (Katan *et al.*, 1989). Therefore, there is an urgent need to find an alternative means for controlling the disease. This study has demonstrated that film forming polymers may serve as an alternative method to protect some horticultural crops against grey mould disease infection and , in so doing, reduce environment pollution. Polymers have been reported to provide additional protection against various foliar pathogens under field conditions (Han 1990 , Hsieh and Huang, 1997, Triki and Priou, 1997, Nasraoui *et al.*, 1999 and Sutherland and Walters, 2001). Our results of the present study agree, therefore, with this previous work and show marked reduction in grey mould incidence on tomato, bean, onion and geranium plants grown under artificial and natural infected conditions. The different film forming polymers coating significantly reduced grey mould severity as well as *B. cinerea* sporulation on leaves, stems and fruits of various crops when sprayed three times at three weeks intervals. Clearly, Kaolin , Nu-Film17 and Bio-Film were strongly suppressed grey mould and pathogen sporulation on all tested plants.

Various mechanisms for the protected plants with coating film forming polymers have been suggested (Han, 1990, and Nasraoui *et al.*, 1999 ). The effect of film forming polymers may be similar to those of the natural cuticle layer in defense against pathogens (Hsieh and Huang, 1999). This layer has following properties: 1. Increasing water repellency; 2. Producing a mechanical barrier; 3. Misdirecting the pathogen germ tubes from penetration; 4. Cationic polyelectronic properties.

In the present study, *in vitro*, film forming polymers were found to inhibit the conidial germination , mycelial growth and sclerotial viability of *B. cinerea*. Moreover, polymers were more effective in reducing hydrolytic enzymes activity included ExoPG and CMCase produced by *B. cinerea*. Increasing concentration to 1.0% of polymers, resulted in a progressive reduction in the pathogen development and enzymes production. Elad and Ayish (1990) has shown that film forming polymers controlled *Botrytis cinerea* on different crops by inhibition of the conidia germination and linear growth of mycelium , suggests that the fungistatic effect of these compounds plays a role in disease reduction. Kapat *et al.* (1998) reported that hydrolytic enzymes i.e. ExoPG and CMCase play an important role in the infection process of *B. cinerea* on different crops. Kaolin , Nu-Film 17 and Bio-Film polymers were more effective under *in vivo* and *in vitro* conditions on reduction of symptoms of grey mould and pathogen development in most tested crops. Nasraoui, (1993) reported that film antitranspirants can be act as effective as fungicide treatment against foliage diseases. The fact that the polymers reduced grey mould suggested that the fungistatic effect of these polymers play a role in disease reduction, whereas it prevented pathogen development. These results were based on the percentage of inhibition of pathogen growth, which produced very few conidia and did not produced sclerotia , inability to synthesis of hydrolysis enzymes essential for the penetration host tissue and were found to be some of less aggressive . Furthermore, alternation of the mycelial morphology was also showed in the presents of polymers (unpublished data). So, these components had fungistatic effect on *B. cinerea*, they could be considered for control of the fungus.

The results of this study suggest that it may be possible to replace conventional chemical fungicides with film forming polymers, it is safe for human , environment and thus provided both economical and ecological efficacy.

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## مقاومة العفن الرمادى فى بعض المحاصيل البستانية بأستخدام غشاء رقيق من البوليمرات

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تم مقاومة مرض العفن الرمادى والمتسبب عن الفطر *Botrytis cinerea* فى بعض المحاصيل البستانية وهى الطماطم، الفاصوليا، البصل والعتر وذلك بأستخدام الغشاء الرقيق من البوليمرات . وأظهرت هذة البوليمرات وهى , Nu-Film17, Bio-Film, Vapor Gard, Wilt Pruf, Masbrane و Kaolin مقدرة على خفض نسبة أنبات الجراثيم، النمو وحيوية الأجسام الحجرية للفطر *B. cinerea*. كما ثبطت هذة البوليمرات انتاج الأنزيمات المحللة الناتجة من الفطر *B. cinerea* وهى PolyGalacturonase (ExoPG) و Caroboxymethyl Cellulase (CMCase) . وكانت أفضل هذة البوليمرات تأثيرا على تثبيط النمو وتطورة هى Kaolin , Bio-Film و Nu-Film17 عند تركيز ١% على الترتيب . كما اظهرت الأختبارات الحيوية بأستخدام قطع ورقية من النباتات المختلفة , أن لأستخدام هذة البوليمرات تأثير على تثبيط حدوث العفن الرمادى وتجرثم الفطر. وتحت ظروف العدوى الصناعية والطبيعية بالفطر *B. cinerea* . ادى رش النباتات ثلاثة مرات بالبوليمرات الى تقليل معنوى فى حدوث مرض العفن الرمادى وتجرثم الفطر *B. cinerea* على أوراق، سيقان، وثمار نباتات الطماطم، الفاصوليا، البصل والعتر. وأن Nu-Film17 و Kaolin كانت أفضل المركبات على تثبيط حدوث الأصابة بمرض العفن الرمادى فى المحاصيل المختلفة.