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Efficiency of the Antagonist *Tilletiopsis pallescens* Formulated with Some Natural Oils for Biocontrol of the Powdery Mildew in Greenhouse Cucumber

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Abstract: The efficacy of *Tilletiopsis pallescens*, formulated with natural oils on the inhibition of cucumber powdery mildew in greenhouses was investigated. Two foliar sprays of cotton, maize, sunflower or paraffin oil formulations (0.05 %) of *T. pallescens* were sprayed on cucumber plants, 30 and 75 days after planting. The oils formulated with *T. pallescens* improved the biocontrol potential. The most effective treatments were cotton, paraffin and maize oil formulations which enhanced the *T. pallescens* survival and the plant protection against the powdery mildew disease incidence. Where, the disease colonies, disease severity and infected leaf areas as well as *Sphaerotheca fuliginea* sporulation were greatly suppressed. Furthermore, cotton oil formulation of *Tilletiopsis* isolate exhibited high quantities of chitinase , ß-1,3- glucanase, cellulase and protease isozyme bands of cucumber leaves surface after 10 extended to 40 days of application. On the contrast, unamended oil treatments showed moderate isozyme bands activity after 10 days of application and then began to decline after 40 days of application. Whereas no ß -1,3- glucanase or cellulase were detected. At the same time, yield of cucumber plants treated with oil formulations of *T. pallescens* was significantly higher.

Key words: Cucumber greenhouses, isozyme, oil formulations, powdery mildew, *Sphaerotheca fuliginea* , *Tilletiopsis pallescens*.

Introduction

Cucumber powdery mildew caused by *Sphaerotheca fuliginea* (Schechtend Fr) Pollacci, is a prevalent and dangerous foliar disease, attacking cucumber plants, growing in greenhouses in Egypt and other countries (Harfoush and Salama, 1992; Mosa, 1997; Reuveni *et al.*, 1997 and Verhaar *et al.*, 1997). Methods for disease control are generally achieved by the use of fungicides and utilization of mildew tolerant cultivars. However, these methods provide an adequate level of disease control and each has its limitations (McGrath, 1991). Thus, in spite of much efforts, powdery mildews remain a major problem for greenhouse producers worldwide. As a consequence, recent efforts have been directed towards new control methods that could be effective, reliable and safe for environment as well. Even through the natural microflora present on the leaves, antagonist yeast cells include genus *Tilletiopsis* were able to reduce the development of pathogen by competition for the space, nutrients and extra cellular enzyme production (Muhsin *et al.*, 1997; Begerow *et al.*, 2000 and Hamamoto *et al.*, 2000). *Tilletiopsis pallescens* Gokhale, a yeast like fungi, which occurs naturally on mildew-infected leaves of plant species was shown to provide excellent control of powdery mildew on cucumber plants (Urquhart *et al.*, 1994; Urquhart and Punja, 1997 and Mohamed- Karima, 1999) , rose (Ng *et al.*, 1997) and barley (Klecan *et al.*, 1990).

Knowledge of the mechanism of antagonism operative in biocontrol may be helpful in enhancing the efficacy of control. The antagonism might reduce mildew sporulation and hyphal growth by competition and extra cellular enzymes production markedly reduced disease incidence (Urquhart *et al.*, 1994). For biological control agents to be effective against pathogen growth, survival in the phyllosphere is an important requirement. Potential and survival depend on nutrient and moisture available on the leaf surface (Knudsen and Skou, 1993; Verhaar *et al.*, 1998 and 1999 b). To enhance the survival of phyllosphere yeast, formulation of inoculum with nutrients, mineral or vegetable oils can be attempted to reduced humidity requirements and possibly also nutrients for mycoparasite to germinate (Hijwegen, 1992 and Verhaar *et al.*, 1999a).

For the foregoing reasons, the objective of this research was to select some natural oil formulations which can improve the growth, survival, extracellular enzymes and potential activity of *Tilletiopsis pallescens* on cucumber leaves against the powdery mildew under greenhouse conditions.

Materials and Methods

Cucumber: Cucumber plants (*Cucumis sativus* L.) of the susceptible cultivars premo were grown in a plastic house with 90-95% RH and 15-20 °C (autumn season) and 80-85% RH and 25-40 °C (spring season). The plants were fertilized and irrigated with a commercial mineral fertilizer solution.

The mycoparasite: *Tilletiopsis pallescens* Gokhale was isolated from cucumber leaves and identified in Microbiology Department, National research Center, Egypt. Culture was grown in a medium containing 2.5% D-glucose, 0.1% yeast extract and 1.0% peptone (GYP) and incubated at 25 °C for 10 days. For inoculum production, blastopores from GYP broth medium were harvested, filtered through cotton wall and adjusted at $2x10^4$ spore/ml. In addition, recovery of the *T. pallescens* in cultured and inoculated cucumber leaves, the blastospores suspensions were filtrated, diluted to $2x10^4$ ml with sterilized water and plated onto a semi selective medium composed of 17g corn meal agar, 10 μg ml^{-1} ,dichloran and 100 mg l^{-1} ampicillin (Urquhart *et al.*, 1994).

Oil formulations: The natural oils i.e. cotton, maize, sunflower and paraffin were used. Formulations were prepared by straining 1 ml of each oil with 0.05 ml Tween 80 in 1 L water on a magnetic stirrer for one hr. Spore suspension containing 10^7spores/ml was added to bring the spore density as $5x10^6$ in 0.05% oil and 0.025% Tween 80 formulation.

In vitro*, effect of oil formulations on *Tilletiopsis pallescens

growth and sporulation: GYP broth medium amended with 0.05 % of cotton, maize, sunflower and paraffin oil sterilized by autoclaving for 15 min, then inoculated with 0.1 ml of 72-hr *T. pallescens* suspension culture and incubated on a rotary shaker (150 rpm) at 25 °C. *Tilletiopsis* colonies were determined by a serial dilution and planting on semi selective medium as colony forming units (cfu x 10^4) . After 7 and 10 days, the extent of mycelial growth was measured. Biomass and blastospore production was measured by filtrating the mycelium from 72 and 144 hr old liquid culture and dried at 40 °C for 72 hr. To quantify the blastospore production, a 0.2 ml sample was with drawn from the flask, and the blastospore density was estimated with a heamocytometer.

Greenhouse experimental design: A greenhouse experiment on cucumber plants, premo cv. was conducted in 1999/2000 and 2000/2001, during the autumn and spring seasons in a commercial plastic house at Gezerit El-Dahab , Giza Egypt . Cucumber plants were sprayed twice (30 and 75 days after sowing) with different oil formulations, using a hand- held automizer onto expanded leaves (total volume applied per leaf =0.5 ml). Three replicates were used for each treatment with 50 plants per each. A randomized complete block design was used. Fifteen plants were selected from each replicate of each treatment, and ten leaves of each plant were examined as follows:

***In vivo*, survival of *Tilletiopsis pallescens* on leaves:** Population counts of *Tilletiopsis* on cucumber leaf surface after application was determined. A suspension of blastospore from the leaf surface was placed in sterile glass bottle containing distilled sterilized water. The number of *Tilletiopsis* colonies were estimated by serial dilution onto semi selective medium (cfu x 10^4) as described previously.

Assessment of powdery mildew incidence on leaves: Colonies number of powdery mildew per naturally infected cucumber leaf was measured periodically after the oil formulations application. A 0-5 scale, modified from Descalzo *et al.* (1990) was used for rating disease severity on leaves. Percentage of mildewed leaf area (s) in different treatments compared with the control treatment were assessed. Sporulation on infected tissue was assessed per cm^2 using heamocytometer.

Effect on extra cellular enzymes activity of *Tilletiopsis pallescens* on leaves: Healthy leaf samples were taken 10 and 40 days after the 1st application of 2001 autumn season, treated with cotton oil formulation (the best treatment according to the results of 1999/2000 seasons). These were examined for extra cellular enzymes activity of *Tilletiopsis* on leaves surface by activity staining on sodium dodecylsulfate polyacrylamid electrophoresis (SDS-PAGE). Ten leaves were aseptically transferred to 15 cm petri- dishes containing 100 ml water for few minutes. The extract water was divided into two parts, the first one was subsequently serial diluted in GYP medium to determine the presence of any inoculum. The second part, 10 ml of water extract with 1 ml cold 0.05 M sodium buffer was used for detecting the enzymes activity. Isozyme patterns were detected using staining in SDS-PAGE with 4 % acrylamide in separating gel. Chitinase activity was assayed with N-Acetylglucosamine substrate according to the Kang *et al.* (1989) methods. ß -1,3-glucanase and cellulase isozymes were separated on gels according to Bertheou *et al.* (1984) and Chernoglazov *et al.* (1989), respectively. Where, protease was separated on 15 % (w/v) polyacrylamide slab gel according to Stegemann (1979).

Cucumber yield: Cucumber fruit yield was also recorded during the harvesting period (kg/ m^2).

Treatments were replicated at least three times. Data were statistically analyzed using the analysis of variance for completely [illegible] ns were compared using the protected least significant differences (L.S.D) values at 5% according to Daniel (1987).

Results

Effect of oils on *Tilletiopsis pallescens* growth and sporulation: Fig.(1) shows that use of oils at low concentration in growth medium can stimulate the *Tilletiopsis* growth development expressed as colonies recovery on semi selective medium, biomass and blastospores production in broth medium. Cotton oil improved the number of colonies recovery by 7.6 and 13.6 x10^4 as compared with 2.3 and 6.6 x 10^4 of unamended oil after 7 and 10 days of incubation, respectively. At the same time, cotton oil enhanced biomass and blastospores production of *Tilletiopsis* by [illegible] and 13.6 and 8.8 spore/ml) in comparison with (25.3 and 72.6 mg) and (7.6 and 14.3 spores/ml) for unamended oil after 72 and 144 hr respectively. Data also showed that paraffin and maize oils had positive effects on *Tilletiopsis* growth development. Sunflower oil had less effect on enhancement of the *Tilletiopsis* growth.

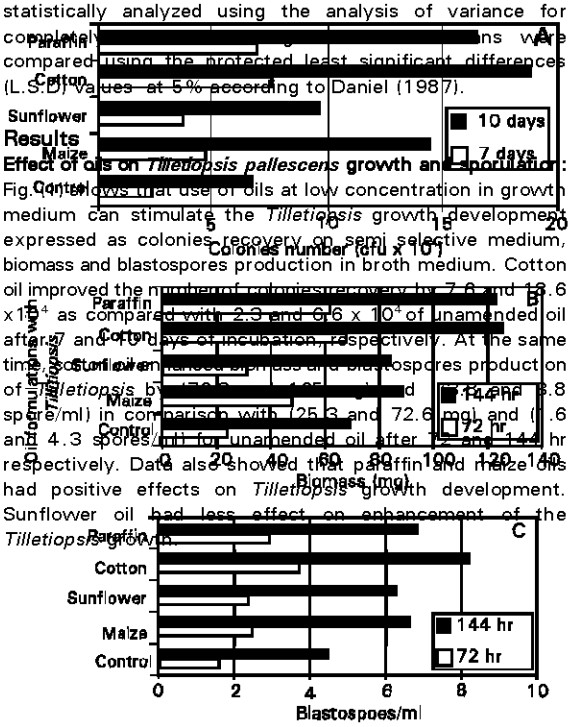

Fig. 1: *In virto*, colony growth (A), biomass (B) and sporulation production (c) of *Tilletiopsis pallescens* in growth medium amended with oils

Survival of *Tilletiopsis pallescens* on cucumber leaves: Growth and survival of *Tilletiopsis* colonies after recovering from the leaf surface indicated that, population density of *T. pallescens* was high in autumn season than in spring one (Fig. 2). At the same time, population counts of *T. pallescen* was increased immediately after application, where they declined in successive weeks. *Tilletiopsis* survival on the surface of cucumber leaves was affected by oil amendments. Cotton oil formulation with *T. pallescens* sustained at higher population during autumn and spring seasons. Also, paraffin and maize oil formulations had the modest effect on *Tilletiopsis* survival on cucumber leaf surface during the two seasons.

Assessment of powdery mildew incidence on leaves: Under the protected conditions, twice applications of *T. pallescens* oil formulations on cucumber plants after 30 and 75 days of planting, had resulted a significant reduction in powdery mildew disease incidence, expressed as disease severity, infected area and conidia density during autumn and spring seasons of 1999/2000 and 2000/2001 years (Tables 1,2 and 3). In general, the pattern of results was similar across two seasons and years. Results given in Table (1) show that, statistically significant differences in powdery colony numbers were found among oil formulations with *T. pallescens* and unamended oils. A comparison of means calculated at each formulation treatment revealed that, the

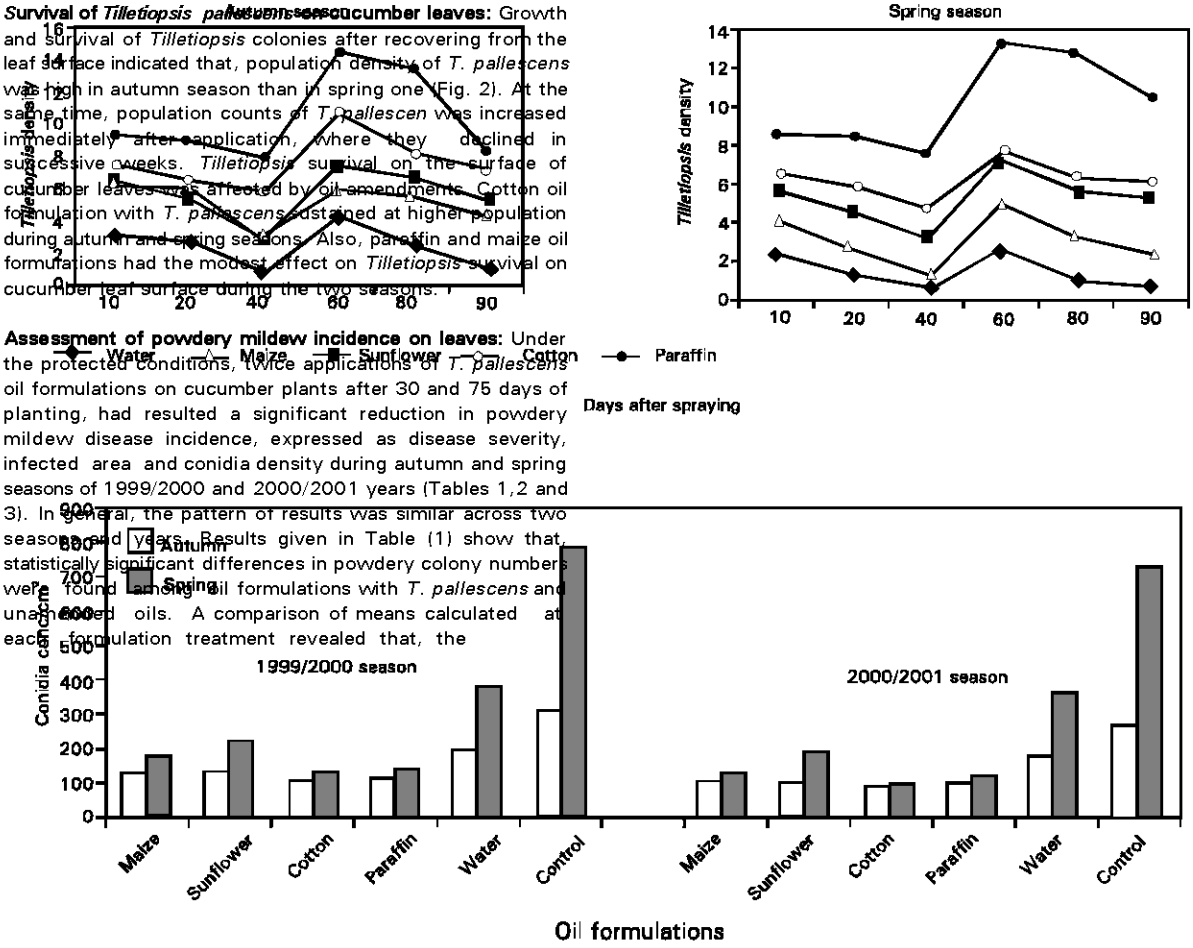

Fig. 2: Recovery of *Tilletiopsis pallescens* blastospores on cucumber leaf Surface after spraying at 30 and 70 days of planting with oil formulations under greenhouse conditions. (cfux10^4)

Fig. 3: Effect of spraying *Tilletiopsis pallescens* oil formulations on the sporulation of *Sphaerotheca fuliginea* incidence on cucumber leaves surface, 120 days old, under greenhouse conditions.

Table 1: Spots numbers of powdery mildew on cucumber leaf surface treated at 30 and 70 days of planting with the *Tilletiopsis pallescens* oil formulation, under greenhouse conditions

Oil formulations	Mean number of powdery spots/leaf at various intervals											
	1999/2000 season						2000/2001 season					
	Autumn season			Spring season			Autumn season			Spring season		
	Days after spraying						Days after spraying					
	40	60	90	40	60	90	40	60	90	40	60	90
Maize	0.0 d	4.5 d	18.4 d	8.0 d	21.1 d	28.7 d	0.0 d	10.3 de	15.4 c	5.3 c	8.8e	26.3 d
Sunflower	5.2 c	7.5 c	28.6 c	21.6 c	39.6 c	42.1 c	3.6 c	13.4 cd	16.3 c	9.6 c	29.4c	28.4 c
Cotton	0.0 d	3.6 d	10.3 e	3.2 e	7.0 e	21.0 e	0.0 d	1.6 f	10.5 d	0.0 d	11.6de	16.4
Paraffin	4.6 c	7.0 c	17.5 d	6.3 de	10.3 e	28.7 e	0.0 d	6.4 e	18.7 c	8.9 c	18.4d	24.8 d
Water	12.0 b	16.3 b	32.3 b	27.6 b	34.5 b	63.3 b	13.2 b	18.6 b	29.6 b	19.4 b	39.6b	52.1 b
Control	26.3 a	45.2 a	65.3 a	62.3 a	86.3 a	164.5 a	28.9 a	46.7 a	68.7 a	36.6 a	85.4a	126.4a

Means in the same column followed by the same letter are not significantly different at $P<0.05$.

different oil formulations of *T . pallescens*, reduced the number of powdery colonies up to 90 days. The spots number on cucumber leaves had a significant reduction for cotton oil formulation after 90 days of application as (10.3 and 21.0) in the 1999/2000 and (10.5 and 16.4) in the 2000/2001 compared with unamended oil (32.3 and 63.3) and (29.6 and 52.1) and untreated plants (65.2 and 164.3) and (68.7 and 126.4) in autumn and spring seasons , respectively. However, the decrease in the number of powdery mildew spots was significant under paraffin and maize oil formulations.

Evidently, oil formulations with *Tilletiopsis* were more effective in reducing the powdery lesion areas on cucumber leaves compared to the unamended oil treatment or water- as control in autumn and spring seasons (Table 2). This activity was consistently high with cotton oil formulation in both seasons and years. Also, the infected areas were much reduced in both seasons

Fig. 4: Comparison of powdery growth on cucumber leaves surface sprayed at 30 and 70 days of planting with (A) sterilized water, (B) *Tilletiopsis pallescens* suspension and © cotton oil formulation with *Tilletiopsis pallescens*, 120 days old, under greenhouse conditions

Table 2: Powdery mildew disease area (s) on cucumber leaf surface treated at 30 and 70 days of planting with the *Tilletiopsis pallescens* oil formulations, under greenhouse conditions.

Oil formulations	Disease area (s)											
	1999/2000 season						2000/2001 season					
	Autumn season			Spring season			Autumn season			Spring season		
	Days after spraying						Days after spraying					
	40	60	90	40	60	90	40	60	90	40	60	90
Maize	0.0 c	0.6 c	1.6 d	1.6 d	3.2 d	5.8 d	0.0 c	0.3 c	1.3 c	0.3 d	0.6 e	4.8 d
Sunflower	0.6 c	1.6 c	3.3 c	3.6 c	7.6 c	11.4 c	0.4 c	0.6 c	1.6 c	2.6 c	6.7 c	9.6 c
Cotton	0.0 c	0.6 c	0.8 d	0.4 e	0.6 e	1.3 e	0.0 c	0.3 c	0.6 c	0.0 e	0.3 e	2.1 e
Paraffin	0.3 c	0.6 c	1.4 d	2.3 d	2.3 d	3.4 de	0.0 c	0.3 c	0.6 c	1.3 d	2.4 d	3.2 de
Water	8.6 b	2.7 b	19.9 b	25.4 b	25.4 b	39.8 b	6.5 b	10.4 b	15.4 b	11.2 b	20.3 b	28.4 b
Control	13.6 a	18.3 a	29.7 a	76.3 a	76.3 a	104.6 a	11.1 a	21.3 a	32.5 a	24.6 a	64.8 a	94.6 a

Mean in the same column followed by the same letter are not significantly different at $P<0.05$.

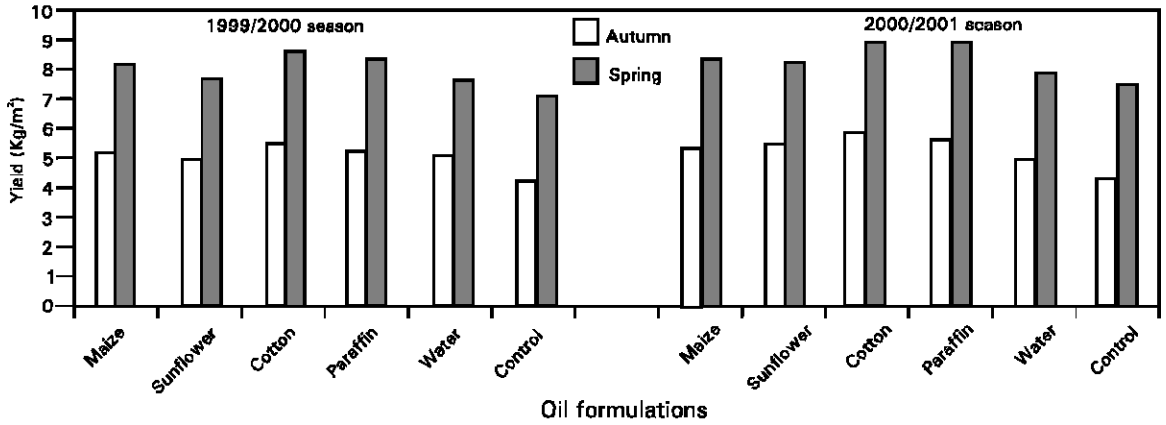

Fig. 5: Sodium Dodecyl sulfate Polyacrylamide Gel Electroghoresis of isozyme bands of chitinanse (A), β-1,3-glucanase (B), Cellulase © and protease (D) produced by *Tilletiopsis pallescens* on the cucumber leaves surface, 10 (I) and 40 (II) days after application, a=cotton oil formulation with *Tilletiopsis*, b= *Tilletiopsis* suspension.

Table 3: Powdery mildew disease severity on cucumber leaf surface treated at 30 and 70 days with the *Tilletipsis pallescens* oil formulations, under greenhouse conditions.

Oil formulations	Disease severity*											
	1999/2000 season						2000/2001 season					
	Autumn season			Spring season			Autumn season			Spring season		
	Days after spraying						Days after spraying					
	40	60	90	40	60	90	40	60	90	40	60	90
Maize	0.0 d*	0.3 c	0.8 c	0.7 cd	0.8 d	1.3 c	0.0 c	0.6 bc	0.8 c	0.2 c	0.3 d	1.1 d
Sunflower	0.3 cd	0.3 c	1.3 b	1.0 bc	1.6 c	1.8 c	0.5 b	0.8 b	1.0 bc	0.6 b	1.3 c	1.8 c
Cotton	0.0 d	0.3 c	0.3 d	0.4 d	0.6 d	0.6 d	0.0 c	0.3 c	0.5 d	0.0 c	0.6 d	1.1 d
Paraffin	0.3 cd	0.6 bc	0.8 c	0.4 d	0.8 d	1.3 c	0.0 c	0.3 c	0.0 e	0.2 c	0.6 d	1.2 d
Water	0.6 bd	0.9 b	1.6 b	1.3 b	2.3 b	2.6 b	0.6 b	0.8 b	1.3 b	0.8 b	2.6 b	3.0 b
Control	1.6 a	2.8 a	3.3 a	2.3 a	5.0 a	5.0 a	1.3 a	2.3 a	3.6 a	2.1 a	4.3 a	5.0 a

* Disease severity was rated on 0-5 scale; 1=1-25, 2=26-50, 3=51-75, 4=76-100, 5>100 colonies per leaf
Mean in the same column followed by the same letter are not significantly different at $P<0.05$.

Fig. 6: Effect of *Tilletiopsis pallescens* oil formulations on the cucumber yield (kg/m^2), under greenhouse conditions

and years under cases of paraffin oil formulated with *Tilletiopsis* (Table 2).

In 1999/2000 and 2000/2001 years, significant differences in disease severity attributed to treatments were detected at both seasons. Foliar spray of *T. pallescens* suspension on cucumber leaves markedly suppressed disease severity of powdery mildew as shown in Table (3). Since, after 90 days of application, the disease severity in *T. pallescens* was recorded as (1.6 and 2.6) in 1999/2000 and (1.3 and 3.0) in 2000/2001 compared with untreated plants (3.3 and 5.0) and (3.6 and 5.0) in autumn and spring seasons, respectively. Clearly, application of oil formulations of *T. pallescens*, suppress the disease severity up to 90 days in both seasons. Cotton oil formulation was significantly the best treatment in delaying and decreasing the disease severity in 1999/2000 (0.3 and 0.6) and 2000/2001 (0.5 and 1.1) after 90 days of application in autumn and spring seasons, respectively. The second best disease control was obtained on leaves treated with paraffin oil formulation with *Tilletiopsis* than the others. At the same time, in all oil formulation treatments, powdery mildew density declined as powdery spots compared to a sole liquid suspension of the *T. pallescens* (Fig. 3). Plants treated with oil formulations of *T. pallescens* reduced spores forming of *S. fuliginea* on the powdery spots. Again, cotton and paraffin oil formulations of *T. pallescens* reduced the *S. fuliginea* conidia forming 90 days after application of treatments. However, maize and sunflower oil formulations reduced the spores density on powdery spots in both seasons and years.

Disease incidence, as with disease severity was consistently lower at the cotton oil formulation of *T. pallescens* than on those treated with other oils (Fig. 4)

Effect on extra cellular enzymes activity of *Tilletiopsis pallescens* growing on cucumber leaves: Results in Fig. 5 depicts that *T. pallescens* produced chitinase , β -1,3-glucanase, cellulase and protease on the cucumber leaves surface. *T. pallescens* formulated with cotton oil displayed higher isozyme potency. Since, optimal quantity of isozymes bands activity were observed initially 10 days after the first spray of *T. pallescens*, whereas no powdery mildew symptoms were seen during this stage of plant age. However, a noticeable enzymes were decreased of chitinase and protease after 40 days of where no detection of cellulase and β-1,3-glucanase bands were also observed. On contrast, quantity of isozyme bands of each enzyme was enhanced and greatly increased on the cucumber leaves surface 10 days after application with cotton oil formulation with *T. pallescens*. Moreover, after 40 days of application, isozyme bands activity of different enzymes remained very high.

Cucumber yield: The produced cucumber yield (kg/m^2) differed according to the spraying treatment (Fig. 6). In general, foliar application of oil formulations with *T. pallescens* resulted in the highest cucumber yield. The highest significant yield was recorded in plants treated with cotton oil formulation in 1999/2000 (5.4 and 8.3 kg) and in the 2000/2001 (5.9 and 9.0 kg) compared with (5.0 and 7.7 kg) and (5.9 and 8.0 kg) of unamended oil and (4.2 and 7.0 kg) and (4.2 and 7.4 k) in untreated control at autumn and spring seasons, respectively. A moderate increase, however , in yield was attained by maize oil formulation.

Discussion

Powdery mildew caused by *Sphaerotheca fuliginea* is a severe disease of cucumber plants under greenhouse conditions, especially during spring season. The utilization of antagonistic microorganisms is a promising technology for the reduction or even replacement of fungicides in the control of powdery mildew of cucumber plants growing under greenhouses. Success of a biological disease control method in a conventionally managed agricultural production system requires that the biocotrol agent (s) that will establish adequate populations consistently. This study demonstrated that application of *Tilletiopsis*, to reduce the incidence of the powdery mildew. In previous studies, species of *Tilletiopsis* were demonstrated to be isolated at a higher frequency from powdery mildew infected leaves compared with healthy ones (Klecan *et al.*, 1990; Urquhart *et al.*, 1994; Ng *et al.*, 1997 and Urquhart and Punja, 1997). *Tilletiopsis* cause retraction of cytoplasm in *Sphaerotheca fuliginea* cells and hyphal plasmolysis and breakdown occurred, leaving only the cell wall after 24 hr by cell wall degrading enzymes such as ß-1,3-glucanase (Urquhart *et al.*, 1994).

However, humidity regimes of alternating high and relative have an enormous influence of the development of *Tilletiopsis* (Urquhart and Punja, 1997 and Verhaar *et al.*, 1999b). Urquhart and Punja (1997) stated that the extent of colonization of *S. fuliginea* by *T. pallescens* on leaf disks was reduced at 70% RH and found to be better at 90 %. Knudsen and Skou (1993) also found a good control of cucumber powdery mildew with *Tilletiopsis* but they stressed again the importance of the lack of high humidity to maintain efficacy. Formulation of biocontrol agents can be liquid, powder or granular. For biocontrol of powdery mildew, liquid oil formulations are the most interesting. Formulation with oils could reduce the humidity requirements of biocontrol agents on leaves surface and possibly also nutrients for the mycoparasite on germination, survival and infecting the powdery mildew. Cotton and paraffin oils as formulation of *T. pallescens* increased the biomass, spore production and their survival on growth media. Evidently, large numbers of *T. pallescens* were recovered from leaves to which oil formulation had been applied than from *T. pallescens* control treatment in autumn season as well as at low humidity on spring season. In addition, the two foliar sprays of the used oil formulations interval by 45 days also offered protection against disease and inhibited the conidia production on the cucumber leaves either in autumn or spring seasons. In two years and seasons, lesions were smaller and less frequent on oil formulation of *T. pallescens* treated leaves than on negative control. The data on disease control confirm previous finding on the anti-fungal activity of *Tilletiopsis* against *S. fuliginea* on cucumber (Hijwegen, 1992; Urquhart *et al.*, 1994 ; Ng, 1997 and Mohamed, 1999).

The treatment with oil formulations for post- infection activity offers more possibilities for disease control. Therefore, antifungal metabolites produced by *T. pallescens* were examined on the leaves surface of cucumber plants treated with cotton oil formulation the best treatment and fractionation by staining on SDS-PAGE. The data showed that when plants treated with cotton oil formulation of *T. pallescens*, extracellular enzymes produced on leaves surface had high activity . Since, the relative quantities of isozyme bands of chitinase, ß,1-3, glucanase, cellulase and protease production were increased within 10 days and remained high

up to 40 days on leaves surface, compared with unamended oil, where chitinase and protease detected only at low quantities after 40 days . Based on the isozyme bands activity of *T. pallescens* on the leaves, corresponded to better disease control. The amount of *T. pallescens* growth achieved should positively correlate with the levels of biocontrol, since there was more enzyme production on cucumber leaf surface. Therefore, proving amendments to inoculum which would moderate the effect of low humidity in spring season could provide a mean of improving the biological control activity against *S. fuliginea*. Results of several investigations were found to declare the positive effect of oil formulation and improved biological control activity (Reuveni *et al.*, 1997 and Verhaar *et al.*, 1998).

In conclusion, cotton or paraffin oils has potential as formulation of *T. pallescens* for biocontrol on cucumber powdery mildew under greenhouse conditions. It is safe for human, consumption, biodegradable, non- pollutant and increase the effectiveness of *T. pallescens* to control powdery mildew.

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