EFFECT OF BIOTIC AND ABIOTIC AGENTS IN CONTROLLING CUCUMBER LEAF SPOT CAUSED BY *Alternaria cucumerina* UNDER PROTECTED CULTIVATION

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ABSTRACT

Alternaria cucumerina was isolated from blighted or spotted cucumber leaves. Pathogenicity test revealed that *A. cucumerina* was pathogenic to cotyledons and true leaves of cucumber plants. Matrix cv. gave the lowest infection with *A. cucumerina*, while Beta alpha recorded the highest infection. On the otherhand, *A. cucumerina* isolated from cucumber plants had ablility to infect other cucubit plants; i.e. melon; watermelon; pumpkins, Smooth Loofah and squash. Both *Bacillus* spp. and *Trichoderma* spp. inhibited the growth of *A. cucumerina* either *in vitro* or *in vivo. Trichoderma* sp., *T. harzianum, T. viride* and different *Bacillus* spp. (isolated from cucumber leaf surface) and their culture filtrates significantly inhibited the radial growth of *A. cucumerina* on PDA medium. Various concentrations of some plant oils and plant extracts reduced the radial growth and spore germination of *A. cucumerina*, especially clove oil. Also, Dithane M-45 was effective in reducing the radial growth of *A. cucumerina*. *Bacillus spp*. (isolate 4), *T. harzianum*, clove oil and Dithane M-45 were the most effective treatments in controlling cucumber leaf spot.

Keywords: *Alternaria cucumerina,* cucumber, biocontrol, plant extract, plant oils and dithane M-45.

INTRODUCTION

Alternaria leaf spot, incited by *Alternaria cucumerina* is an important foliar disease of cucrbits (**Thomas, 1990; Latin, 1992 and Atia, 2005 b**). *Alternaria* leaf spot is a perennial problem that must be controlled through the application of protective fungicides, for the presence of primary inoculum from previous crop (**Thomas, 1990**). **Latiny (1992**) mentioned that, A. cucumerina, infects plant leaves, fruits and reduce cucumber yield (**Parasada *et al*., 1972**). *Alternaria* leaf spot disease has a wide host range including Cucurbitaceae family, i.e., watermelons, muskmelons, pumpkins, and cantaloupes (**Thomas *et al*., 1990; Latin, 1992 and Atia, 2005 b**). Chemical control is a fast and effective method of controlling the fungal diseases (**Meena *et al*., 2004**). On the other hand, biological control of Alternaria leaf blight is considered much safer, for health and environmental considerations (**Atia, 2005 a,b; Atia and Esh, 2005 and Atia and Ahmed, Amal, 2001**). *Trichoderma* species have long been recognized as agents for the control of numerous plant diseases and for their ability to increase the plant growth and its development (**Harman, 2000, Atia, 2005 a and Atia and Ahmed, Amal 2011**).Conidia of *T. harzianum* at a concentration of 2.0 X 10^8 conidia/ml significantly suppressed the leaf spot on treated cucumber leaf-discs (**Batta, 2005**). *Bacillus* strains are examples of promising safe fungal biological control agents. *B. subtilis,* and *B. licheniformis*, showed antifungal activities against *Alternaria* spp. and other pathogenic fungi *in vitro* and *in vivo* (**Aly *et al*, 2002, and Atia and Ahmed, Amal, 2011, Atia *et al*., 2011, Esh *et al*., 2010**). Plant extract are a new

approache for controlling plant diseases. It can be used without any dangerous effects on human healthy (**Fawzi *et al.*, 2009 and Atia and Amal Ahmed 2011**). Chemical control still the faster factor can be used as a curative or preventive of disease control, but not safety for the human race in most cases. Thus, the use of chemical are restricted and apply them when there are great need. Several fungicides have been used to control alternaria disease i.e Amistar Both Dithane M-45 (mancozeb) and Rovral iprodione + carbendazim (**Mesta *et al.*, 2009**).The present study was designed to isolate, identify the causal of cucumber alternaria leaf spot, investigate the reaction of cucumber cvs., and cucurbit species as host range, effect of anti-fungal of bio-control agents, plant extract and oils as well as fungicides on the radial growth of *A. cucumerina* and its infection.

MATERIALS AND METHODS

Cucumber Plant:

Cucumber seedlings (cv. Matrix) were cultivated under greenhouse in pots (12 cm in diameter) filled with sand/peat mixture (1:3 v/v). The plants were fertilized weekly with nutrient solution (according to **Atia, 2005 b**). Seedlings at the cotyledon stage or 2-3 true leaves were used in this investigation.

Isolation, maintenance and inoculum preparation:

A. cucumerina, was isolated on potato dextrose agar (PDA) from infected cucumber leaves exhibited typical alternaria leaf spot or leaf blight symptoms and identified according to **Jakson (1958); and Barnett (1998**). Identification was carried out at Pl. Path. Laboratory, Agric. Bot. and Pl. Path. Dept., Fac. Agric., Zagazig Univ. Fungal isolates were kept under 4°C for the further studies. Inoculum of *A. cucumerina* was prepared from 7-12 day-old culture grown on PDA in Petri dishes using the technique described by **Atia, (2005 b)**. Conidial suspension was adjusted to $5x10^4$ spores /ml (**Latin *et al.*, 1994**).

Source of bioagents:

Bacteria spp. were isolated from leaf surface of cucumber using NA medium. The isolated bacteria were purified using dilution method and were identified according to their shape, pigmentation and culture characteristics based on Bergey's Manual of Determinative Bacteriology 9^{th} ed. (**Holt et al., 1994)**. *Trichoderma* spp., were obtained from Pl. Path. Laboratory, Agric. Bot. and Pl. Path. Dept., Fac. Agric., Zagazig Univ.

Pathogenicity tests:

Cucumber cotyledon and true leaves were detached and transferred to moist filter papers inside Petri dishes (15-cm diameter) withen wet filter paper. Each leaf was inoculated with 4 droplets, each 50µl of *A. cucumerina* spore suspension ($5x10^4$ spore /ml). The inoculated cotyledons were incubated at 25°C for 18 h., then, incubated under fluorescent light with an 11 h. photoperiod. The inoculated true leaves were incubated at 27°C for 18 h, then incubated under fluorescent light for 11 h photoperiod. Number of lesions, lesion diameter, lesion types and blighted area were recorded (**Atia, 2005 b**).

Susceptibility of cucumber cultivars to infect with *A. cucumerina*:

Three cultivars of parthenocarpic cucumber i.e. Matrix F1, Best F1 and Beita Alpha were infested with spore suspension ($5 x10^4$ spores/ml) of *A. cucumerina* and incubated at 27^oC., number of lesions; lesion diameter; and lesion type of blighted area were recorded.

Host range tests:

Six species of cucumbitaceae species i.e. Cantaloupe, Smooth Loofah, Muskmelon, Pumpkin, Squash and Watermelon were cultivated under greenhouse conditions. Inoculation with *A. cucumerina*, incubation and results were done as aforementioned in pathogenicity test.

***In vitro* studies**

Effect of selected bio-agents on the radial growth of *A. cucumerina.*

Isolates of *Bacillus* spp. were grown on nutrient agar while, isolates of *Trichoderma harzianum, T. viride and T.* spp. isolates were grown on PDA medium.The interaction between aforementioned microorganisms and *A. cucumerina* was tested. Petri dishes (9 cm in diam.) containing PDA medium were inoculated in the centre with disk (5 mm in diam.) taken from the edges of 7 day-old culture of *A. cucumerina*. Inoculation with the tested bacteria isolates was done by streaking on the surface of the media at the distance of 1.5 cm from the edge of the plates with aid of dual culture method. Discs (5 mm in diam.) of *Trichoderma* isolates were inoculated at the distance of 1.5 cm from the edge of the plates. Plates inoculated with *A. cucumerina* alone were used as a control. Three plates were used for each treatment. Radial growth of the different treatments was measured when one plate was full of the growth of *A. cucumerina*. The percentage of the radial growth inhibition of the tested fungus was calculated using the following formula (**Atia and Esh, 2005**):

$$\text{Inhibition \%} = \frac{X-Y}{X} \times 100$$

Where,

X=Average of the radial growth of the control (*A. cucumerina* alone) plate (cm).

Y= Average of the radial growth of the treated plate (cm).

Effect of bioagents culture filtrates on the radial growth of *Alternaria cucumerina*:

Isolates 1 and 4 of of *Bacillus* spp. were grown in nutrient broth medium at 28 °C for 36 h. *Trichoderma* isolates were grown on PD browth, at 28 °C for 7-10 days (**Aly *et al.*, 2002**). Cultures of *Bacillus* isolates were collected, filtered through filter papers, centrifuged at 3000 rpm for 15 minutes and sterilized using bacterial filter (filter syringe, 0.45 µm followed by 0.25 µm using Seitz apparatus) (**Aly *et al.*, 2002**). Culture filterate of *Trichoderma* spp. was filtered through filter paper and sterilized by using Seitz apparatus. The sterilized culture filtrates were added to flasks contained PDA medium before solidification at the rate of 5, 10 and 15 % of the medium and poured in Petri-dishes. Bioagents-free plates were used as control. All plates (3 plates /each treatment) were inoculated with an equal disc (5mm in diameter) from the edges of 7 day-old *A. cucumerina* culture at the centre of each plate and incubated at 28^{o}C until one plate was full of *A. cucumerina* growth. Results were recorded as aforementioned.

Effect of some plant oils on the radial growth of *A. cucumerina*:

Oils of ten plants, i.e. marjoram (*Majorana hortensis*), thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum*), onion (*Allium cepa*), garlic (*Allium sativum* L.), olibanum (*Baswellia scacra*), cress (*Eruca sativa Mill*.), basil (*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*) and ginger (*Zingiber officinale*), were obtained from El-Captain Company, Egypt were used in this study. Four concentrations; i.e. 0; 0.5; 0.1; and 2% of aforementioned plant oils were individually prepared by dissolving in 100 ml of autoclaved PDA before soildifing using 0.1ml of tween 20. Oils were

individually mixed with media and poured in Petri dish (9 cm in diam.). After solidification, plates were inoculated with *A. cucumerina*. Four replicates were used for each concentration. Oil- free PDA plates were used as a control. The inoculated plates were incubated at 28±2 ºC. Data were recorded after 7 days as aforementioned.

Effect of some plant extracts using cold and boiled distilled water on the radial growth of *Alternaria cucumerina:*

Three medicinal plants i.e. clove, basil and marjoram were dried, ground to fine powder. Ten gms powder was extracted by macerating in 100 ml of either sterilized cold distilled water (CDW) for 24 hr, or boiled distilled water (BDW) for 10 min. in water bath at 100 ºC. The extracts were filtered through filter paper, and sterilized through bacterial filter (filter syringe; 0.45µm followed with 0.25µm using Seitz apparatus). Three replicates of 4 concens; i.e. 0, 5, 10 , and 15 % were prepared from crude extract (100%) and were added to 100 ml of PDA medium before solidifying. Extracts-free medium was served as a control. Inoculation and incubation were done as aforementioned.

Effect of some fungicides on the radial growth of *A. cucumerina:*

Six concentrations; i.e. 0, 125, 250, 500, 1000, and 2000 ppm of 4 fungicides; i.e. Dithane M-45, Zineb, Ridomel and Topsin-M were tested to evaluate their effect on the radial growth of *A. cucumerina* using poison medium technique (**Atia, 2005** b). Four replicates of *A. cucumerina* were used for each particular concentration. Inoculation, incubation and growth reduction were carried out as aforementioned.

Effect of some plant oils and Dithane-M 45 on the spore germination of *A. cucumerina:*

Three concentrations; i.e. 0, 0.5, and 2 % of oils of 3 plants; i.e. basil, marjoram, clove as well as 4 concentrations; i.e. 0, 2000, 2500, and 3000 ppm of Dithane were individually added to 250 ml PDA before solidification and poured in Petri dishes.The plates were individually inoculated with 2 ml of spore suspension ($5x10^4$ spore/ ml) of *A. cucumerina* and spread with sterilized glass rod on the surface of the plates. Three replicates were used for each tested concentration. Oils or fungicide free plates were used as a control. All treatments were incubated at 28 $^oC \pm 2$. After 15 to 18 hr the germinated and non-germinated spores were counted in ten microscopic fields chosen at random for each slide (**Sharvelle,1961**). The percentage of spores germination was calculated.

***In vivo* studies**

Effect of selected bioagents on cucumber leaf spot disease.

The most effective bioagents; i.e. *Bacillus* spp. and *Trichoderma* spp. on reducing radial *A. cucumerium* were used. Fungal isolates (*Trichoderma* spp) were grown on 200 ml of sterilized PD broth in 500 ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 7 days at 28 $^oC \pm 2$ (**Aly *et al*., 2002**).The liquid culture were mixed in a blender and adjusted to contain 10^6 cfu/ml. Bacillus isolates were grown on NB (**Atia and Ahmed Amal, 2011**). Flasks (500 ml) each containing 100 ml of NB medium were inoculated with a lop full of 24 h old of Bacillus cultures. Flasks were incubated at 28 $^oC \pm 2$ on rotary shaker (100 rpm) for 24 h in case of bacterial bioagents (**Aly et al., 2002**). Cucumber plants Matrix cv. (45 day-old) were individually sprayed with 30 ml / plant of each tested organism. Control treatment were sprayed with water. The treated plants were left for two hours and then individually inoculated with *A. cucumerina* (4 drops , 20µl of 10^5 cfu/leaf) and covered with plastic socks and

kept under greenhouse conditions. In addition, detached leaves of cucumber were inoculated with 4 droplets (20µl) of a mixture of tested the bio-agents (*Bacillus* spp. cell and *Trichoderma* spp. spores) and *A. cucumerina* spore suspension (1:1 v/v). Detached leaves were transferred into moist Petri dishes. Inoculum-free plates were used as a control. Three Petri-dishes (15cm in diam.) were used as replicates for each treatment. Plates were incubated at 28 ^{o}C ± 2 and the disease incidence was calculated after 7-10 days. Number and diameter of necrotic lesions (mm) as well as blighted area (mm^2)/ leave were determined. Percentage of protection was calculated as follows (**Aly *et al*., 2002**):

Percentage of protection = 100 - A/B

Where:

A= Percentage of disease in treated (100 blighted area in treated/ blighted area in the untreated (control).

B= percentage of disease in untreated (control).

Effect of clove oil and Dithane-M45 on cucumber leaf spot disease:

Cucumber plants were treated with Dithane-M45 at the rate of 0.2 % (2 g/l) or clove oil at the rate of 0.5 % (5 ml/l) untill run off and left till plant were dryied. Treated leaves were transferred into moist filter papers in Petri dishes (15 cm in diam.) and inoculated with *A. cucumerina* spore suspension as mentioned before. Inoculum free plates were used as a control. Three Petri-dishes were used for each treatment. Incubation and results were recorded as mentioned above.

Statistical Analysis:

All of the obtained data were subjected to statistical analysis (**Snedecor and Cochran, 1980**) using SAS (**SAS, 1999**).

RESULTS AND DISCUSSION

Isolation and pathoginicity:

The isolated fungus, *A. cucumerina* seem to be the main causal organism of cucumber alternaria leaf blight. Isolation was carried out with PDA medium (**Latin *et al.*, 1994**). The isolated fungus was identified as *A. cucumerina* (Ellis and Everh J. A. Elliott). Identification was carried out according to disease symptoms, morphological characteristics of mycelia, hyphal conidia (**Latin *et al*., 1994 and Barnett, 1998**).

Results in Table (1) indicate that, *A. cucumerina* was pathogenic to cucumber cotyledon and true leaves. It also clear that, inoculation of cotyledons resulted in high significant of both lesion diameter (4.66 cm.) and blighted area (68.2 cm^2) than of the true leaves (6.42 cm²), lesion size/leaf and blighted for both respectively) after nine days of inoculation. Moreover, 9 days lesion size was expanded on cotyledon leaves more than on the true leaves. Lesions on cotyledon leaves were surrounded by a wide yellow margin, while it was narrow in the case of the true leaves. Simillar results on melon were obtained (**Atia, 2005 b**). Respecting the increases of infection with *A. cucumerina* on cotyledon leaves may be due to the low of sugars percentage on these leaves (**Atia, 2005 b**).

The fungi caused a cellular leakage and reduced chlorophyll content in susceptible cultivars (**Alabouvette *et al*., 1984**). In addition, necrotrophs i.e. Alternaria kills host cells prior to colonization, and that toxins are secreted to facilitate host cell death (**Lawrence et al., 2008**.

Table (1): Pathogenicity test of *Alternaria cucumerina* on cucumber cv. Matrix on cotyledon and true leaves.

Type of leaves	Disease parameters (9 days after inoculation)		
	Average of lesions/leaf	**Average of lesion (cm)**	**Blighted area (cm^2)**
Cotyledon leaves	4	4.66	68.20
True leaves	4	1.43	6.42
LSD (0.05)	N.s.	0.47	24.41

N.S. = Non significant

Susceptibility test of cucumber cultivars with *A. cucumerina*.

Results in Table (2) indicate that Matrix cv. gave the lowest infection with *A. cucumerina* (3 lesions / leaf ; 1.27 cm diameter of lesion ; and 3.8 cm^2 blighted area). While, Beta alpha was the highest infecion (3.02 cm diameter of lesion/leaf and 21.48 cm^2 blighted area), nine days after inoculation. After 9 days, lesion size was expanded. Lesions were recorded a circular, light brown with a narrow yellow margin. There were significant differences between the diameter of lesion and infected area of the tested cvs. of cucumber.

Table (2): Susceptibility of the tested cucumber cultivars to *Alternaria cucumerina*.

Cultivars	Disease parameters (nine days after inoculation on true leaves)		
	Average of lesion/ leaf	**Average diam. of lesion (cm)**	**Blighted area (cm^2)**
Matrix	**3**	**1.27**	**3.80**
Best	**3**	**2.85**	**19.13**
Beta alpha	**3**	**3.02**	**21.48**
LSD (0.05)	**N.s.**	**0.68**	**2.33**

N.s. = Non significant

Host range tests:

A. cucumerina isolated was able to infect melon, cucumber, watermelon, pumpkin, zucchini and Smooth Loofah. Squash gave the highest infection followed by pumpkins (6.04 and 5.21cm diameter of lesion/leaf, as well as 85.91 and 63.92 cm^2 blighted area, respectively) ten days after inoculation. While, muskmelon was the lowest infected host followed by watermelon (1.44 and 2.80 cm diameter of lesion/leaf and 4.88 18.46 cm^2 blighted area) (Table, 3 and Fig.1). Lesions were circular, light brown to black in colour with variation a wide yellow margin. The differences between the diameter of lesion and infected area of the host plants were significantly with some extent. The filtrates of pathogenic fungi containing their respective toxins, which caused a necrosis within 48 hrs and eventually mortality on susceptible cultivars.Many phytopathologist previously mentioned similar results. **Vakalounakis, (1990**) reported that, 27 species of *Cucurbitaceae* were found to be susceptible to infect with *A. alternata* artificially inoculated or exposed to natural infection in the greenhouse. Also *A. cucumerina* was known to infect different genotypes of melon i.e. muskmelon (**Latin, 1992**).

Table (3): Reaction of different cucurbit plants to *Alternaria cucumerina*.

Host plants	Disease parameters (10 days after inoculation)		
	Average number of lesion/leaf	Average of diam. of lesion (cm)	Blighted area (cm^2)
Squash	3	6.04	85.91
Watermelon	3	2.80	18.46
Muskmelon	3	1.44	4.88
Pumpkins	3	5.21	63.92
Cantaloupe	3	3.56	9.85
Loofa	3	3.07	22.20
L.S.D (0.05):	N.s.	0.97	3.71

N.s. = Non significant

Pumpkins leaves.

Cantaloupes leaves

Loofa leaves.

Watermelon leaves

Squash leaves

Muskmelon leaves

Fig: (1): Reaction of different cucurbit plants to *Alternaria cucumerina*, 10 days after inoculation.

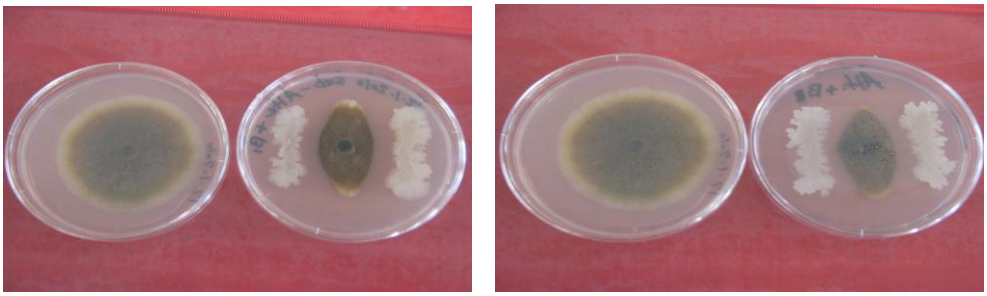
***In vitro* studies:**

Antagonistic activity of *Bacillus spp. and Trichoderma spp*. against *A. cucumerina*:

Data in Table (4) show that *Trichoderma* spp., *T. harzianum* and *T. viride* gave completely inhibiton of *A. cucumerina* growth (100 %) on PDA plates. *Bacillus* spp.(isolate 4) was the most effective which showing 64.94% inhibition of growth colony of *A. cucumerina* compared to the other tested isolates. Followed by *Bacillus* spp. (isolate 8), *Bacillus* spp. (isolate 1), and *Bacillus* spp. (isolate 7), respectively sex and ten days after incubation. Simellar results were obtained by (**Aly *et al.*, 2002; Batta, 2005; Esh *et al.*, 2010 Atia and Ahmed , Amal, 2011 and Atia *et al.*, 2011**).

Table (4): The inhibitory effect of the tested bacterial and fungal isolates against *Alternaria cucumerina.*

Bioagent	% Reduction after	
	6 days	10 days
Control	0.00	00.00
Bacillus spp.(I1)	60.77	72.78
B. spp.(I4)	64.94	79.44
B. spp.(I5)	46.58	61.11
B. spp.(I6)	34.89	52.78
B. spp.(I7)	57.43	70.56
B. spp.(I8)	61.60	73.33
T. harzianum	100.00	100.00
T. spp.	100.00	100.00
T. viride	100.00	100.00
Mean	62.62	71.00
L.S.D. (0.05):	3.18	1.42



Bacillus spp., (I 1) **Bacillus spp., (I 4)**

Fig. (2): Effect of *Bacillus spp*. isolates on the mycelial growth of *Alternaria cucumerina*

Bacillus spp. isolated from phyllosphere was used against several diseases; i.e. late blight of tomato (**Aly *et al*., 2002**). Bacilli are known to produce a wide range of antibiotic compounds that are inhibitory to fungi and its capacity to use chitin and β-glucan as substrates, (**Bargabus *et al.*, 2004**); potato late blight (**Atia, 2005 a**); gray mould of strawberries (**Ju, *et al*., 2007**); cucurbit powdery mildew (**Gilardi, *et al*., 2008**). Several bacterial genera have been successfully used for the biological control of other plant diseases **(Chen *et al*., 2008** and **Gilardi *et al.*, 2008**); and cercospora leaf spot of sugar beet (**Esh *et al.*, 2010**).

Trichoderma strains have highly effective antagonistic mechanisms to survive and colonize the competitive organisms of the rhizosphere, phyllosphere and spermosphere. A major part of Trichoderma antifungal system consists of a number of genes encoding for an astonishing variety of secreted lytic enzymes, including endochitinases, N-acetyl-β-glucosaminidases, chitin 1,4-β-chitobiosidases, proteases, endo- and exoglucan β-1,3-glucosidases, endoglucan β-1,6-glucosidases, lipases, xylanases, mannanases, pectinases, pectin lyases, amylases, phospholipases, RNAases, and DNases (**Lorito, 1998**).

Effect of *Bacillus* spp. and *Trichoderma* spp. filtrates on radial growth of *A. cucumerina*:

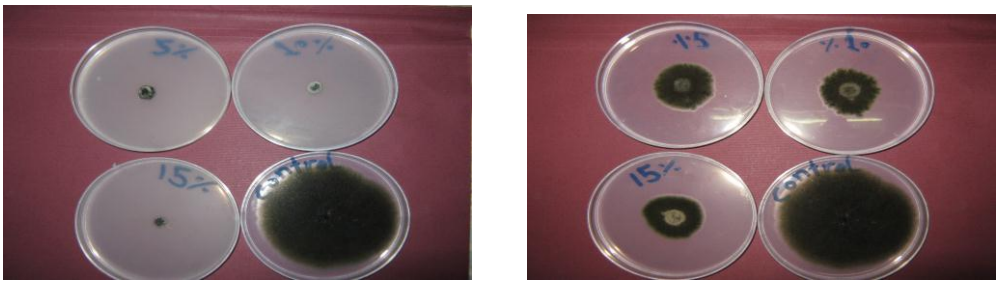
Data in Table (5) show that, culture filtrate (at 10% and 15% cocentrations) of *Bacillus* spp. (isolates 1 and 4) inhibited the radial growth of *A. cucumerina* and the effect increased by increasing the concentration. Culture filtrate of

Bacillus spp. (isolate 1), was the most effective, followed by *Bacillus* sp. (4) filtrate. On the otherhand, *Trichoderma* spp., resulted in the lowest effect (Table 5) and Figure (3). These results are agreement with those obtained by **Leifert *et al.*, (1995)** who found that, *B. polymyxa* KB-8 produced at least two antibiotics, KB-8A and KB-8B. However, these antibiotics produced *in vitro* can not provide a sufficient proof for the involvement of the antibiotics in the biocontrol activity *in vivo*, because *Bacillus* spp. produce other metabolites including biosurfactants, chitinase and other fungal cell wall-degrading enzymes, volatiles and compounds which elicit plant resistance mechanisms, and are involved in a number of mechanisms of biological control not only a antibiosis but also competition.

Table (5): Effect of bio-agent culture filtrates on the radial growth of *Alternaria cucumerin:*

Treatments	% Culture filtrate concentrations				
	0	5	10	15	Mean
	Growth reduction (%)				
***B.* spp. (I1)**	0	88.62	100.0	100.0	96.21
***B.* spp. (I4)**	0	54.11	56.9	56.13	55.71
***T.* spp.**	0	9.72	23.09	27.34	20.05
Mean		38.11	44.01	45.87	42.99

L.S.D. (0.05):
Treatments (T) = 0.08
Concentration (C) 0.08
T. X C. 0.13



***Bacillus* spp. (I 1)** ***Bacillus* spp. (I 4)**

Fig. (3): Effect of Bacillus spp. culture filtrates on the growth of *Alternaria cucumerina*

Effect of some plant oils on the radial growth of *Alternaria cucumerina*:

Data presented in Table (6) and Figure (4) indicate that clove oil at 4 concens was the most effective on reducing the radial growth of *A. cucumerina*, followed by the marjoram oil, basil oil cinnamon oil.While, cress oil was not effective, compared to control treatment. The obtained data are in agreement with those obtained by **Parajuli *et al.*, (2005); Mironescu and Georgescu, (2008); Sitara *et al.*, (2008); Fawzi *et al.*, (2009) and Atia, and Ahmed, Amal (2011).**

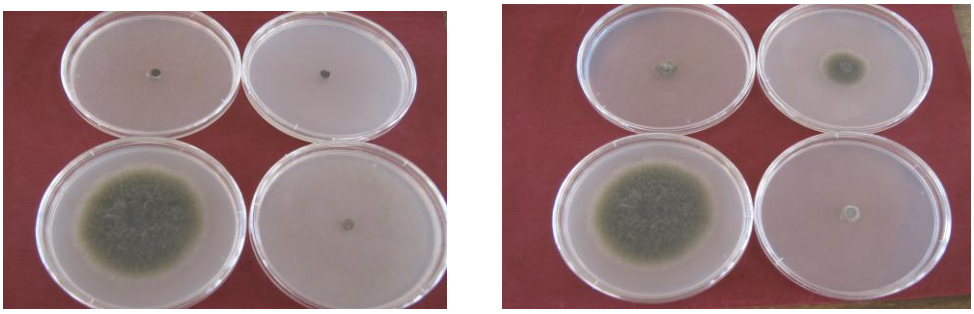
Clove oil was the most effective on inhibition growth of *A. cucumerina*, due to its content of eugenol, the major component of clove oil (**Chami *et al*, 2005**). The inhibitory effect of plant oils might be regarded to which act as cidal agent against fungal growth and showed abnormal conidia and malformations as swollen, often septated and pale color of hypha (**Suwitchayanon and Kunasakdaku, 2009**).

Some of plant oils can inhibit the conidial germination of cucumber and barley powdery mildews. Furthermore, mycelial growth of many pathogens was

severely restricted after application of oils. Levels of hydrogen peroxide (H_2O_2) and superoxide (O^{2-}), and some antioxidants were decreased such as dehydroascorbate reductase (DHAR), but the other enzymes were increased such as ascorbate peroxidase and glutathione S transferase (**Hafez, 2008**).

Table (6): Effect of different concentrations of essential oil on the growth reduction percentage of *Alternaria cucumerina.*

Treatments	0.5	1	2	Mean
	Growth reduction (%)			
Clove	100.00	100.00	100.00	100.0
Marjoram	57.40	84.76	85.95	76.04
Basil	38.31	41.27	78.55	52.71
Cinnamon	19.82	38.61	63.31	40.58
Ginger	19.82	22.04	38.91	26.92
Thyme	10.06	12.72	20.86	14.55
Onion	5.77	23.08	35.32	21.39
Garlic	3.85	10.95	17.16	10.65
Olbanum	1.18	5.33	6.51	4.34
Cress	-3.25	-0.59	-0.59	-1.48
Control	0.00	0.00	0.00	0.00
Mean	22.10	30.31	40.55	31.43
L.S.D (0.05):				
Treatments (T)	0.28			
Concentrations (c)	0.15			
T. X C. =	0.28			



Clove oil **Marjoram oil**

Fig. (4): Effect of different plant oils on the mycelial growth reduction of *Alternaria cucumerina.*

Effect of some plant extracts on the radial growth of *Alternaria cucumerina*

Data in Table (7) show that, clove extract at all concentrations tested was the most effective in reducing the radial growth of *A. cucumerina* followed by marjoram. On the otherhand basil was not effective as a boiling and cold distilled water. Significantly differences were detected between the tested plant extracts.These results are agree with those found by **Fawzi *et al*., (2009)** and **Suwitchayanon and Kunasakdakul, (2009)**.

Effect of some fungicide on the radial growth of *A. cucumerina*:

Dithane M-45 was the most effective on reducing the radial growth of *A. cucumerina*, followed by Topsin-M then Zineb. The inhibition effect was increased with increasing the concentrations. While, Ridomil recorded the lowest effect (Table, 8). The results of some researchers were agreement with our results (**Atia and Ahmed, Amal 2011**).

Table (7): Effect of different concentrations of some plant extracts using cold distilled water (CDW) and boiled distilled water (BDW) on the radial growth reduction of *Altrenaria cucumerina*:

Treatments	% Growth reduction of *Alternaria cucumerina*							
	Boiled distilled water				Cold distilled water			
	Plant extracts concentrations (%)							
	5	**10**	**15**	**Mean**	**5**	**10**	**15**	**Mean**
Clove	15.43	47.77	64.09	42.43	11.55	38.55	56.61	35.57
Marjoram	-0.59	6.53	15.13	7.02	8.38	10.99	14.34	11.24
Basil	-9.50	-6.38	-6.03	-7.30	-8.38	3.72	11.92	2.42
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	1.34	11.98	18.30	10.54	2.89	13.32	20.72	2.31
L.S.D. (0.05):								
Treatments (T)	0.06							
Concentrations (C)	0.07							
T. X C.	0.12							

Table (8): Effect of different fungicides on the radial growth reduction of *A. cucumerina*.

Fungicides	**% Growth reduction at concentrations (ppm)**					
	0	125	250	500	1000	2000
DithaneM- 45	0	77.21	82.76	85.47	100.00	100.00
Topsin-M	0	18.23	20.51	25.21	30.20	36.61
Zineb	0	-13.68	-9.69	-3.56	3.56	10.54
Ridomil	0	-17.09	-16.10	-14.39	-13.96	-11.25
Mean	0	12.93	15.50	18.69	23.96	27.18

Ridomil was noted as ineffective against of *A. cucumerina* (**Sitara *et al*., 2008**). Results (Table 8) indicated that, the tested fungicides were significantly differed in their action against the fungi. Differences in reaction might be due to selective action of the fungicide fungus (**Singh and Siradhana, 1990**).

Effect of oils and fungicides on the spore germination of *A. cucumerina*:

Dithane M-45 being the most effective in reducing spore germination of *A. cucumerina* at all concentrations, followed by clove oil and basil. While marjoram was the lowest effective (Table, 9). These results are in agreement with those reported by **Hafez, (2008)** and **Mesta *et al*., (2009)**.

Table (9): Effect of some plant oils and Dithane-M 45 on the percentage of spore germination of *A. cucumerina*

Treatments											
Plant oils								Fungicides		
Control (m/l)		Clove oil (m/l)		Marjoram oil (m/l)		Basil oil (m/l)		DithaneM-45 (ppm)		
0.5	2	0.5	2	0.5	2	0.5	2	0	1000	2000
0	0	**12.57**	**35.43**	**7.79**	**9.95**	**11.47**	34.12	0	0	0

***In vivo* studies:**

Diseases management with plant oil and DithaneM-45:

Results in Table (10) and Figure (5) indicate that, Dithane M-45 and clove oil significantly reduced cucumber leaf spot compared to untreated control at nine days after inoculation. The present results are in agreement with those reported by (**Batta, 2003 and Hafez, 2008**). Although *in vitro* screening

of plant extracts is an important of first step in identifying plants with potential application in agriculture, *in vivo* confirmation of this potential is essential in the search for plant derived preparations with the potential to be commercialized (**Tegegne and Pretorius, 2007**). The inhibitory effects of plant oils might be regarded to which act as cidal agent against fungal growth and showed abnormal conidia and malformations as swollen, often septated and pale color of hypha (**Suwitchayanon and Kunasakdaku, 2009**).

Table (10): Effect of clove oil and DithaneM-45 against alternaria leaf spot caused by *Alternaria cucumerina* of cucumber Matrix cv. 9 days after inoculation.

Treatments	Disease parameters				
	Number of lesion	Diameter of lesion (cm)	Blighted area (cm^2)	Blighted area (%)	Protection (%)
DithaneM-45	4	0.00	0.00	0.00	100.00
Clove oil	4	0.00	0.00	0.00	100.00
Control	4	2.91	26.60	100.00	0.00

Clove oil DithaneM-45 Control

Figs. (5): Effect of DithaneM-45 and clove oil on cucumber leaf spot caused by *Alternaria cucumerina*:

Diseases management with bioagents (*T. harzianum* and *Bacillus* spp):

Results in Table (11) and Figure (6) indicate that, cucumber plants treated with *Bacillus* spp. (isolate 4) as spray and as a mixture application effectivelly reduced lesion diameter (0.87 and 0.77 mm) and blighted area (2.38 and 1.86 mm^2), followed by *T. harzianum* (2.07 and 2.69 mm of lesion diameter) and blighted area (13.46 and 22.72 mm^2). While, control one recorded 4.66 cm and 68.19 mm^2. Similar results were obtained by Aly *et al.*, (2002), Esh et al., (2010) and Atia and Ahmed Amal (2011).

Table (11): Effect of bacterial and fungal bioagents isolates against alternaria leaf spot caused by *Alternaria cucumerina* of cucumber cv. Matrix- 9 days after inoculation.

Treatments	Disease parameters				
	Number of lesion	Diameter of lesion (cm)	Blighted area (cm^2)	Blighted area (%)	Protection (%)
***Bacillus* spp.** (4) spray application	4	0.87	2.38	3.49	96.51
***Bacillus* spp. (4)** mixture inoculation	4	0.77	1.86	2.73	97.27
T.harzianum application	4	2.07	13.46	19.74	80.26
T.harzianum mixture inoculation	4	2.69	22.72	33.32	66.68
Control	4	4.66	68.19	100	**0.00**
L.S.D. (0.05):		1.10	0.50		

Bacillus spp. (I 4) spray

Bacillus spp. (I 4) *mixture inoculation*

Control

T. harzianum spray

T. harzianum mixture inoculation

Fig. (6): Effect of different bioagents on cucumber leaf spot caused by *Alternaria cucumerina*

Bacillus spp. has been used to control a number of leaf spot diseases due to forming endospores facilitates long-term storage, relatively easy commercialization, capable of surviving desiccation, heat, oxidizing agents and UV and γ radiation, as well as, produce a wide range of antibiotic compounds that are inhibitory to fungi (**Bargabus *et al.*, 2004**). Antifungal factor includes siderophores, pterines, pyrroles (**Sarani *et al.*, 2008**), phloroglucinols (**Shanahan *et al.*, 1992**), proteases and chitinases (**Nielsen *et al.*, 1998**). Bacteria produce antifungal antibiotics; elicit induced systemic resistance in the host plant (**Aly, *et al.*, 2002 and Atia *et al.*, 2011**).

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تأثيربعض العوامل الحيوية وغير الحيوية في مقاومة تبقع أوراق الخيار المتسبب عن الفطرألترناريا كيوكاميرينم تحت ظروف الزراعة المحمية

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تم عزل الفطر ألترناريا كيوكاميرينا من أوراق الخيار التي ظهرت عليها أعراض المرض، وأثبتت اختبارات المرضية أن الفطر المعزول ممرض للأوراق الفلقية والأوراق الحقيقية لنباتات الخيار. سجا صنف الخيار ماتريكس أقل نسبة إصابة بالفطر ألترناريا كيوكاميرينا بينما سجل الصنف بيتا ألفا أعلى نسبة إصابة بنفس الفطر. وعلى الجانب الآخر فإن الفطر ألترناريا كيوكاميرينا المعزول من أوراق نباتات الخيار كان قادرا على إصابة نباتات أخري من العائلة القرعية مثل الشمام والبطيخ القاوون واللوف والكوسة. ثبطت كل من أنواع الباسيللس والتريكودرما النمو الميسليومي للفطر ألترناريا كيوكاميرينا تحت ظروف كل من المعمل والصوبة. وقد ثبط الفطر تريكودرما سبيشيز، تريكودرما هارزيانم، والفطر تريكودرما فيريدي وأنواع مختلفة من الباسيلس (المعزولة من علي سطح أوراق الخيار) ورواشح مزارعها ثبطت النمو الميسليومي للفطر ألترناريا كيوكاميرينا علي بيئة البطاطس والدكستروز والآجار بدرجة معنوية. ولقد ثبطت التركيزات المختلفة لبعض الزيوت النباتية وبعض المستخلصات النباتية ثبطت النمو الميسليومي وإنبات جراثيم الفطر ألترناريا كيوكاميرينا، وخاصة زيت القرنفل. أيضا فإن المبيد دياثين إم ٤٥ كان مؤثرا في خفض النمو الميسليومي للفطر ألترناريا كيوكاميرينا. وكانت معاملات كل من باسيللس سبيشيز (العزلة ٤) والفطر ترايكودرما هارزيانم وزيت القرنفل والمبيد دياثيم إم ٤٥ هي المعاملات الأكثر فاعلية لمكافحة تبقع أوراق الخيار الألترناري.