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Pathological studies on alternaria leaf spot of cucumber under protected cultivation

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Abstract:

Alternaria leaf blight disease of cucumber (*Cucumis sativum* L.), is an important foliar disease worldwide, especially under the protected cultivations (plastis tunnel and greenhouses) conditions. The causal fungus was identified as *Alternaria cucumerina* Ellis & Everh J.A. Elliott(*Macrosprium cucumerinum*), and its pathogenicity was confirmed on cotyledon and true leaves of cucumber Matrix cv. using detached leaf assay technique. Cucumber cultivar Matrix, Best and Beta alpha were susceptible to the infection with *A. cucumerina*. Cantaloupes, watermelon, muskmelons, pumpkins, loofa and squash were infected with *A. cucumerina* as a host range. Squash and pumpkin were the most infected ones, while muskmelon and water melon were the lowest ones. *Trichoderma* sp., *T. harzianum, T. viride* and different *Bacillus* spp. (isolated from cucumber leaf surface) and its culture filtrates significantly inhibited growth of *A. cucumerina* on PDA medium. Various concentrations of some plant oils and plant extracts reduced growth and spores germination of *A. cucumerina*, especially clove oil. Also, dithane M-45 was the most effective fungicide in reducing growth of *A. cucumerina*. *Bacillus* sp. (isolate 4), *T. harzianum*, clove oil and Dithane M-45 were the most effect treatments in controlling cucumber leaf spot.

Keywords:*Alternaria cucumerina*, cucumber, biocontrol, plant extract, plant oils.

Introduction:

Alternaria leaf blight, incited by *Alternaria cucumerina* (Ellis & Everh J.A. Elliott) (*Macrosprium cucumerinum*) is an important foliar disease of cucrbits (Thomas, 1990; Latin, 1992 and Atia, 2005b). Alternaria leaf blight is a perennial problem that controlled through the application of protective fungicides, for the presence of primary inoculum from previous crop (Thomas, 1990). A more economical and environmental acceptable methods of disease control is the use of resistance cultivars (Thomas *et al.*, 1990). Latiny, 1992 mentioned that,

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A. cucumerina, infected plant leaves, fruits and reduce yield (Parasada *et al.*, 1972). This disease has a wide host range including member of Cucurbitaceae family, *i.e.*, watermelons, muskmelons, pumpkins, and cantaloupes (Thomas *et al.*, 1990; Latin, 1992and Atia, 2005b). Chemical control is a fast and effective method of fungal control. Two disadvantages of this practice are increasing consumer rejection to chemical residues and development of pathogen resistance to fungicides (Meena *et al.*, 2004). On the other hand, biological control of plant diseases is considered much safer, for health and environmental considerations (Atia, 2005a, b; Atia and Esh, 2005 and Atia and Ahmed,Amal, 2001).

Trichoderma species have long been recognized as agents for the control of plant diseases and for their ability to increase the plant growth and its development (Harman, 2000, Atia, 2005a and Atia and Ahmed, Amal 2011). formulated conidia of *T. harzianum* at a concentration of 2.0 $X10^8$ conidia/ml significantly suppressed the leaf spot on treated cucumber leaf-discs (Batta, 2005). *Bacillus* strains are an example of promising safe fungal biological control agents. *B. subtilis*, *B. licheniformis*, showed *in vitro* and *in vivo* antifungal activities against *Alternaria* spp. and other pathogens (Aly *et al*., 2002, Esh *et al*., 2010, Atia and Ahmed, Amal, 2011 and Atia *et al*., 2011,).

Plant extracts are new approaches for controlling plant diseases. It can be used without any dangerous effects on human health, on the other hand, resulting great levels in controlling plant diseases. Several investigators used water and ethanolic plant extract and oils *i.e. Mentha piperita*, *Coriandrum sativum*, *Piper nigrum*, *Carum carvi* and *Urtica dioica Thymus linearis*, *Artemisia gmelinii*, *A. dubia*, *Juniperus recurva*, *Nardostachys grandiflora* and *Zanthoxylum armatum Ocimum gratissimum* to control several plant pathogeeveral protease (Fawzi *et al.*, 2009 and Atia and Amal Ahmed 2011).

Chemical control still the faster factor can be used as a curative or preventive on 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). Theus, the present study was designed to isolate, identify the causal of cucumber alternaria leaf spot and to investigate the reaction of cucumber cvs., and cucrbites species as host range, the anti-fungal activity of bio-

control agents, plant extract and oils as well as fungicides on growth and the infection with *A. cucumerina* were undertaken.

2. Material and Methods

2.1. Plant:

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

2.2.Pathogen isolation, maintenance and inoculum preparation:

A. cucumerina, was isolated on potato dextrose agar (PDA) medium from infected cucumber leaves exhibited typical alternaria leaf blight symptoms and identified according to Jakson (1958); Latin *et al.* (1992) and Barnett (1998). Identification was carried out at Pl. Path. Laboratory, Agric. Bot. & Pl. Path. Dept., Fac. Agric., Zagazig Univ.Fungal isolates were kept under 4 °C to use at the further studies. Inoculum of *A. cucumerina* was prepared from 7-12 days old culture grown on PDA medium in Petri dishes using the technique described by Atia, (2005b). Conidial suspension was adjusted to $5x10^4$ cfu /ml (Latin *et al.*, 1992).

3. Sourse of bioagents:

Cucumber leaf surface bacteria Was isolated, purified using dilliution method. Bacteria was isolated using nutirant agar (NA) medium. Identification of bacteria was carried according to their shape, pigmentation and culture characteristics based on Bergey's Manual of Determinative Bacteriology 9th ed.(Holt *et al.*,1994). *Trichoderma* spp., were obtained from Pl. Path. Laboratory, Agric. Bot. & Pl. Path. Dept., Fac. Agric., Zagazig Univ.

2.3.Pathogenicity performance:

cucumber cotyledon and true leaves were detached and transferred into Petri dishes (15-cm diameter) withen wet filter paper. Each leaf was inoculated with 4 droplets, each 50µl of *A. cucumerina* spore suspension ($5x10^4$ cfu /ml). The inoculated cotyledons were incubated at 21 ± 2°C for 18 h., then, incubated under fluorescent light with an 11 h. photoperiod. The inoculated true leaves were incubated at 27 ± 2°C for 18 h and high, then incubated under fluorescent light with an 11 h photoperiod. The number of lesion, lesion diameter, lesion types and blighted area were recorded (Atia, 2005b).

Susceptibility of cucumber cultivars to *A. cucumerina*:

Three cultivars of parthenocarpic cucumber (Matrix F1, Best F1 and Beit alpha) were used. Inoculation, incubation and results were done as mentioned above in pathoginicity test using detached leaf assay.

Host range:

Six cultivars of different cucurbitaceae species *i.e.* cantaloupes, loofa, muskmelons, pumpkins, squash and watermelon were tested as host rang of *A. cucumerina*. Plants were cultivated under greenhouse

conditions. Inoculation, incubation and results were done as mentioned above.

***In vitro* studies:**

Effect of selected bio-agents on mycelia growth of *A. cucumerina*:

Bacterial isolates (*Bacillus* spp.) were grown in nutrient agar and *Trichoderma harzianum*, *T. viride* and *Trichoderm* spp. isolates were grown on PDA medium. The interaction between different aforementioned microorganisms and *A. cucumerina* growth was tested. Petri dishes (9 cm in diameter) containing PDA medium were inoculated at the center with disk (5 mm in diameter) taken from the edges of 7 days old culture. Inoculation with the tested isolated bacteria were 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. While, in case of fungi, plates were inoculated with agar disks (5 mm in diameter) of the tested fungi at the distance of 1.5 cm from the edge of the plates. Plates inoculated with *A. cucumerina* alone were used as a control. Then plates were incubated at 28 ± 2°C (Aly *et al*., 2002). Three plates were used for each treatment. After that, the mean diameter of the mycelial growth of different treatments was measured at 6 and 10 days after incubation. The percentage of growth reduction was calculated using the following formula (Atia and Esh, 2005):

Inhibition % = X-Y÷ X x 100

Where,:

X= Average radial growth of control plate (cm).

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

Effect of bio-agents culture filtrates on mycelial growth of *A.cucumerina*:

Bacterial isolates (*Bacillus* sp. 1 isolate and *Bacillus* sp. 4) were grown in nutrient broth medium at 28 ± 2°C for 36h. While fungal isolates wer grown on PD browth, at 28 °C for 7-10 days (Aly *et al.*, 2002). After that, cultures were collected, filtered through filter papers, centrifuged at 3000 rpm for 15 minutes then sterilized using bacterial filter (filter syringe) 0.45 µm followed by 0.25 µm (Seitz) according to (Aly *et al.*, 2002). The sterilized culture filtrate was added to flasks contained PDA medium before solidification at the rate of 5, 10 and 15 % of the medium and pured in Petri-dishes. Control treatment was don without filtrates. The plates were inoculated with an equal disc (5mm in diameter) from the edges of 7 days old *A. cucumerina* culture at the center of plat. Three replicates were used for each particular treatment. Incubation and date were carried out as mentioned above.

Effect of different plant oils on mycelial growth of *A. cucumerina*:

Ten plants oils *i.e.* marjoram oil (*Majorana hortensis*), thyme oil (*Thymus vulgaris* L.), clove oil (*Syzygium aromaticum*), onion oil (*Allium cepa*), garlic oil (*Allium sativum* L.), olbanum oil (*Baswellia scacra*), cress oil

(*Eruca sativa* Mill.), basil oil (*Ocimum basilicum*), cinnamon oil (*Cinnamomum zeylanicum*) and ginger oil (*Zingiber officinale*), were obtained from El-Captain Company, Egypt. The anti-fungal activity of the tested oils was evaluated on radial growth of *A. cucumerina*. Different oils concentrations (0.5, 1 and 2%) were tested. Oil concentrations were prepared by dissolving in 100 ml autoclaved warm PDA using 0.1ml of tween 20. Then oils were thoroughly mixed withen media and poured in 9 cm sterilized Petri dish. After solidification, plates were inoculated with *A. cucumerina*. Four replicates were used for each concentration. The inoculated plates were incubated at 28±2 ºC. Data were recorded after 7 days as mentioned above.

Effect of different plant extracts using cold and boiling distilled water on mycelial growth of *A. cucumerina*:

Medicinal plants (clove, basil and marjoram) were dried, ground to fine powder, then 10g of each one was extracted by macerating in 100 ml of either sterilized cold distilled water (CDW) for 24 hr, or boiling distilled water (BDW) for 10 min. in water bath at 100 ºC. The extracts were filtered through filter paper, filtered through muslin cloth and sterilized by passing it through bacterial filter (filter syringe) 0.45µm followed with 0.25µm (Seitz). The obtained extracts were set as original concentrations . Then different ratio (0, 5, 10 and 15 %) of the original concentrations were used. Three replicates were used. The plant extracted was thoroughly mixed with the medium after autoclaving. Medium without extracts was served as a control. Ioculation, incubation were done as mentioned above using the following formula as stated by Sundar *et al.*, (1995).

Effect of different fungicides concentration on mycelia growth of *A. cucumerina*.

Different concentrations (125, 250, 500, 1000, 2000 ppm of active ingredients) of Dithane M-45, Zineb, Redomel and Topsine-M were tested to evaluate their effect on radial mycelial growth of *A. cucumerina* using poison medium technique (Atia, 2005b). Four replicates of *A. cucumerina* were used for each particular concentration.Ioculation, incubation and growth reduction were carried out as mentioned above.

Effect of different plant oils and Diathan-M45 on spore germination of *A. cucumerina*:

The effect of basil oil, marjoram oil, clove oil, Dithane-M45 on spore germination were tested using sterilized distilled water. Fungicide (2000, 2500 and 3000 ppm) and oils (0.5 and 2%) were added to a 250 ml flasks containing 50 ml sterile distill water. Conidial suspension of *A. cucumerina* was obtained as mentioned in pathoginicity. Then 2ml of conidia suspension were

added into 2ml of each treatment alone and throully mixed. Then, 0.2 ml of this suspension was put on, then sild fitted on glass rods "U shape" in Petri dishes containing wetted filter paper. Three replicates were used for each tested concentration. All treatments were incubated at 28 ^{o}C ± 2. After 15 to 18hr the germinated and non-germinated spores were counted in ten microscopic fields chosen at random for each slide (Sharvelle,1961). The percentage of spore germination was calculated.

***In vivo* studies**

Effect of selected bioagents on cucumber leaf spot disease:

The most effective antagonistic bacteria and fungi in reducing growth of *A. cucumerium* were used. Fungal isolates (*Trichoderma* spp.) were grown on 200 ml of sterilized PD broth medium in 500 ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 7 days at 28±2°C. (Aly *et al.*, 2002). The liquid culture were mixed in a blender and adjusted to be 10^6 cfu/ml. Bacterial isolates were grown in 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 bacterial cultures. Flasks were incubated at 28°C on rotary shaker (100 rpm) for 24 h in case of bacterial (Aly *et al.*, 2002).

Cucumber plants Matrix cv. (45 days old) were sprayed with 30ml/ plant of each tested organism suspension alone. Control treatment were considered, then all treated plants left for two houres. Treated plant were inoculated with 4 drops (20µl of 10^5 cfu)/leaf of *A. cucumerina* inoculums as mentioned before and covered with plastic box 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 (bacterial cell and or fungal spores) with *A. cucumerina* spore suspension (1:1 v/v) to test the direct effect of bio-agents on the disease incidence. Detached leaves were transferred into 15 cm. ∅ Petri dishes with moisten filter papers, then inoculated at the lower surface. Three Petri-dishes were used as a replicates for each concentration. Plates were incubated at 28°C and the disease incidence was calculated after 7-10 days. Number and diameter of necrotic lesions (mm) as well as blighted area (mm^2)/ leaves were determined. Percentage of protection was calculated acording to Aly *et al.*, (2002) as follows: Percentage of protection = 100- A/B

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 selected plant oil and diathan-M45 on cucumber leaf spot disease:

Cucumber plants were treated with diathan-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. Then treated leaves were transferred into 15 cm. ∅ Petri dishes with moisten filter papers, and inoculated at the lower surface with *A. cucumerina* spore suspension as mentioned before. Three Petri-dishes were used as a replicates for each concentration. Data were recorded as mentioned above.

Statistical Analysis:

The obtained data were subjected to statistical analysis (Snedecor and Cochran, 1980) and SAS (SAS, 1999).

Result and Discussions

Isolation and pathoginicity:

A. cucumerina, was isolated from naturally infected cucumber collected from tunnle and greenhouses.

The isolated fungus same to be the causal pathogen of cucumber alternaria leaf blight. Isolation was carried out on PDA medium (Latin *et al.*, 1994). Isolated fungus was identified as *A. cucumerina* (Ellis & Everh J. A. Elliott). Identification was carried out by disease symptoms, morphological characteristics of mycelia and conidia. These characters were in agreement with those described by (Latin *et al.*, 1992 & Barnett, 1998).

Results in Table (1) indicate that,*A.cucumerinum* was pathogenic to cucumber cotyledon and true leaves. It also clear that, inoculation of cotyledons resulted in more significant lesion diameter and blighted area than of the true leaves (4.66, 1.43 cm 68.20 cm^2 and 6.42 cm^2, lesion size/leaf and blighted for both respectively) nine dayes after inoculation. After 9 days lesion size was expanded on cotyledon leaves more than on the true leaves. Type of lesion on cotyledon leaves appears with a wide yellow margin, while it is narrow in case of true leaf. Simillar results on melon were obtained(Atia,2005b). Respecting the increases of infection with *A. cucumerina* on cotyledon leaves may be due to the low of sugars percentage on these leaves (Atia, 2005b).

Susceptibility of cucumber cultivars to infection with *A. cucumerina*.

Results in Table (2) indicated that Matrix cv. was the lowest infected one with *A. cucumerina* followed by best cv. which exhibited 1.72 and 2.85 cm diameter of lesion leaf and 3.80 and 19.13 cm^2 blighted area respectively. While, Beta alpha was the highest infected one (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 are some significant differences between the diameter of lesion and infected area of the tested cvs. of cucumber. The differences between tested cucumber cultivars to *A. cucumerina* might be at-

tributed to differences in genetic make up of tested cvs., in addition to the environmental factors, that might affect host–pathogen interaction (Goodwin *et al*., 1995).

Table (1): Pathogenicity test of *Alternaria cucumerina* on cucumber cv. Matrix on cotyledon and true leaves 9 days after inoculation.

Type of leaves	Disease parameters		
	No. of lesions	Diameter of lesion (cm)	Blighted-area (cm^2)
Cotyledon leaves	4.00	4.66	68.20
True leaves	4.00	1.43	6.42
LSD (0.05)	Ns	0.47	24.41

Ns = not significant

Table (2): Susceptibility of tested cucumber cultivars to *Alternaria cucumerina* nine days after inoculation on true leaves.

Cultivars	Disease parameters		
	Number of lesion	Diameter of lesion (cm)	Blighted area (cm^2)
Matrix	3.00	1.27	3.80
Best	3.00	2.85	19.13
Beta alpha	3.00	3.02	21.48
LSD (0.05)	ns	0.68	2.33

Ns = not significant

Host rang of *A. cucumerina*.

A. cucumerina isolated able to infect melon, cucumber, watermelon, pumpkin, zucchini and lufa. Squash was the highes infected one 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 inoculatio. While, muskmelon was the lowest infected one followed by watermelon (1.44 and 2.80 cm diameter of lesion/leaf and 4.88 18.46 cm^2 blighted area) Table, (3). The filtrates of pathogenic fungi containing their respective toxins, which caused a necrosis within 48hr and eventually mortality on susceptible cultivars.Many phytopathologist previously mentioned similar results (Atia, 2005b). Vakalounakis, (1990) found that, 27 species of Cucurbitaceae were found to be susceptible to infect with *A. alternate* artificially inoculated or exposed to natural infection in greenhouse. Also *A. cucumerina* was known to infect different genotypes of melon *i.e.* muskmelon (Latin, 1992).

***In vitro* studies:**

Antagonistic activity of selected bio-agents isolates against *A.cucumerina*

Trichoderma sp., *T. harzianum* and *T. viride* completely inhibited

growth of *A. cucumerina* (100%) on PDA plates Table (4) and Figure (1). *Bacillus* sp. (isolate 4) was the most effective one which showed 64.94% inhibition of growth colony compared to the other tested isolates, followed by *Bacillus* sp. (4), *Bacillus* sp. (8), *Bacillus* (sp. 1), and *Bacillus* sp., (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).

Trichoderma isolates have highly effective antagonistic mechanisms to survive and colonize the competitive organisms of the rhizosphere, phyllosphere and spermosphere. A major part of the *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).

Table (3): Reaction of different cucurbits plants to *Alternaria cucumerina* 10 days after inoculation.

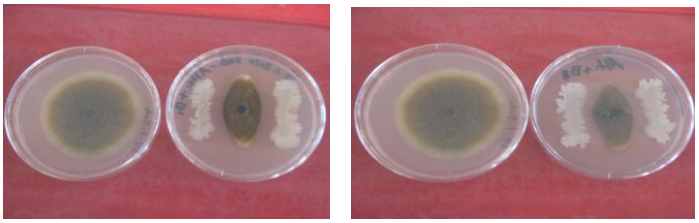
Host plants	Disease parameters		
	Number of lesion	Diameter of lesion (cm)	Blighted area (cm^2)
Squash	3.00	6.04	85.91
Watermelon	3.00	2.80	18.46
Muskmelon	3.00	1.44	4.88
Pumpkins	3.00	5.21	63.92
Cantaloupe	3.00	3.56	29.85
Loofa	3.00	3.07	22.20
LSD (0.05):	ns	0.97	3.71

Ns = not significant

Bacillus spp. isolated from phyllosphere was used against several fungal diseases. As well as, several bacterial genera have been successfully used for the biological control of other plant diseases (Chen *et al.*, 2008 and Gilardi *et al.*, 2008). 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).

Table (4): The inhibitory effects of tested bacterial and fungal isolates against *A. cucumerina* 6 and ten days after incubation.

Bioagent	Reduction (%)	
	6 days	**10 days**
Control	0.00	00.00
***Bacillus* sp.1**	60.77	72.78
***Bacillus* sp.4**	64.94	79.44
***Bacillus* sp.5**	46.58	61.11
***Bacillus* sp.6**	34.89	52.78
***Bacillus* sp.7**	57.43	70.56
***Bacillus* sp.8**	61.60	73.33
Trichoderm harzianum	100.00	100.00
Trichoderm viride	100.00	100.00
***Trichoderma* sp.**	100.00	100.00
Mean	62.62	71.00
LSD (0.05):	3.18	1.42

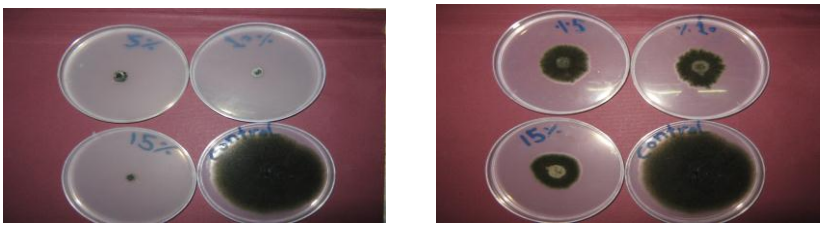


***Bacillus* sp. isolate (1)** ***Bacillus* sp. isolate (4)**

Fig. (1): Effect of *Bacillus* spp. isolates on growth of *Alternaria cucumerina*

Table (5): Effect of bio-agent culture filtrates on growth of *Alternaria cucumerina.*

Treatments	Culture filtrate concentrations (%)				
	Mean	**5**	**10**	**15**	Mean
Growth reduction (%)					
***Bacillus* sp. (1)**		54.11	56.9	56.13	55.71
***Bacillus* sp. (4)**		88.62	100.0	100.0	96.21
Trichoderma harzainum.		9.72	23.09	27.34	20.05
Control		0.00	0.00	0.00	0.00
Mean		38.11	44.01	45.87	42.99
LSD(0.05): Treatments (T) 0.08	0.08				
Concentrations (C)	0.13				
T. X C.					



***Bacillus* sp. (1)** ***Bacillus* sp. (4)**

Fig. (2): Effect of bio-agent culture filtrates on growth of *A. cucumerin*a

Effect of selected bio-agents culture filtrates on growth of *A. cucumerina.*

Results show that, culture filtrate of bacterial isolates tested at 10 and 15% concentration inhibited growth of *A. cucumerina*. The effect was increased by increasing concentration. *Bacillus* sp. (isolate 1), being the most effective ones, followed by *Bacillus* sp. (4) filtrate, respectively. *T.harzainum*., was the lowest one (Table 5) and Figure (2). These results are in agreement with those obtained by (Batta, 2005 and Esh *et al.*, 2010). *B. subtilis* may be inhibited pathogens by producing antibiotic. Leifert *et al.*, (1995) found that, *B. polymyxa* KB-8 produced at least two antibiotics, KB-8A and KB-8B. However, these antibiotics produced *in vitro* cannot 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.

Effect of different plant oils on mycelial growth of *A. cucumerina*:

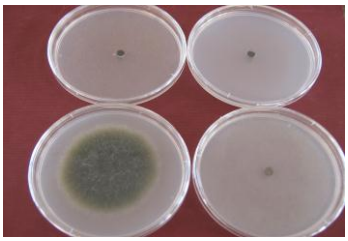
Data presented in Table (6) and Figure (3) indicated that clove oil at all concentrations tested was the most effective oil on reducing radial growth of *A. cucumerina*, followed by the marjoram oil, basil oil cinnamon oil. While, cress oil was not effective compared to control treatment. Obtained data are agree 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 antifungal activity of the essential oils is different, depending on the mould type (Mironescua & Georgescub, 2008). The inhibitory effects of plant oils might be regarded to which act as cidal agent against fungal growth and showed abnormal conidia and

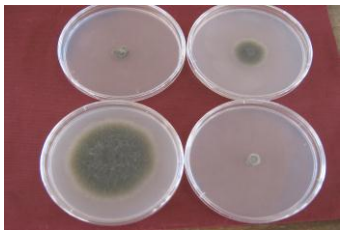
malformations as swollen, often septated and pale color of hypha (Suwitchayanon and kunasakdaku, 2009). Oils inhibited the conidial germination of cucumber and barley powdery mildews. Furthermore, mycelial growth of the pathogen was severely restricted after application of oils. Levels of hydrogen peroxide (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 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
LSD (0.05):				
Treatments (T) 0.28				
Concentrations (c) 0.15				
T. X C. = 0.28				



Clove oil



Marjoram oil

Fig. (3): Effect of different plant oils on mycelia growth reduction of *A. cucumerina*.

Effect of different plant extracts on mycelia growth of *A. cucumerina*

Data in Table (7) revealed that, clove extract at all concentrations tested was the most effective in reducing mycelial growth of *A. cucumerina* followed by marjoram respectively. On the other hand basil was not effective

as a boiling and cold distilled water. Significant 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 different fungicide concentrations on mycelial growth of *A. cucumerina*.

Dithane M-45 was the most effective one on reducing mycelial growth of *A. cucumerina*, followed by Topsin-M then Zineb. The inhibition effect was increased by raise concentrations. While, Redomil was less effective one (Table, 8). Similar results were obtained by Atia and Ahmed, Amal (2011). Redomil was noted as not effective against of *A. cucumerina* (Sitara *et al.*, 2008). In order to achieve successful control, it is necessary to use the effective active ingredient at the appropriate concentration, applied at the right time.Use of different class of chemicals in a rotational and/or repetitive programme, will prevent fungi from developing resistance to given active ingredient (Doster & Michailides, 1999). The above mentioned results indicated that, the tested fungicides were significantly differed in their action against the fungi. Differences in reaction might be due to selective active fungicide on fungus as reported by Singh and Siradhana (1990).

Table (7): Effect of different concentrations of some plant extracts using cold distilled water (CDW) and boil distilled water (BDW) on growth reduction of *A. cucumerina*

Growth reduction *Alternaria cucumerina* (%)

Treatments	Boil 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
LSD (0.05):								
Treatments (T)		0.06						
Concentrations (C)		0.07						
T. X C.		0.12						

Effect of oils and fungicides on spore germination:

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 lowest effective

one (Table, 9). These results are in agreement with those reported by Hafez, (2008) and Mesta *et al.*, (2009).

Table (8): Effect of different fungicide concentrations on growth reduction of *A.cucumerina*.

Fungicides	Concentrations (ppm)					Mean
	125	250	500	1000	2000	
	Growth reduction (%)					
Dithane M- 45	77.21	82.76	85.47	100.00	100.00	**89.09**
Topsin-M	18.23	20.51	25.21	30.20	36.61	**26.15**
Zineb	-13.68	-9.69	-3.56	3.56	10.54	**-2.57**
Redomil	-17.09	-16.10	-14.39	-13.96	-11.25	**-14.56**
Control	0.00	0.00	0.00	0.00	0.00	**0.00**
Mean	**12.93**	**15.50**	**18.69**	**23.96**	**27.18**	**19.62**

LSD (0.05)
Fungicides=0.10
Concentrations=0.10
Fungi. X Conc.=0.21

Table (9): Effect of different concentrations of plant oils and fungicide Dithane- M45 on spores germination of *A.cucumerina* (reducing %).

Treatments	Concentratios (ml/l)		M
	5	2	
Clove oil	12.57	35.43	24.00
Marjoram oil	7.79	9.95	8.87
Basil oil	11.47	34.12	22.80
Concentratios (ppm)			
	1000	2000	M
Dithane M-45	0.00	0.00	0.00

LSD (0.05)
Treatments (A) =6.25
Concentrations (B)=4.42
A x B =8.84

***In vivo* studies:**

Diseases management with certain plant oil and Dithane M-45:

Results in Table (10) and Figure (4) indicated that, Dithane M-45 and clove oil were significantly reduced cucumber leaf spot compared to untreated control at nine dayes 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 Dithane M-45 against alternaria leaf spot caused by *A. cucumerina* of cucumber Matrix cv. nine days after inoculation.

Treatments	Disease parameters				
	Number of lesion	Diameter of lesion (cm)	Blighted area (cm^2)	Blighted area (%)	Protection (%)
Dithane M-45	4.00	0.00	0.00	0.00	100.00
Clove oil	4.00	0.00	0.00	0.00	100.00
Control	4.00	2.91	26.60	100.00	0.00

Cove oil **Dithane M-45**

Control

Figs. (4): Effect of Dithane M-45 and clove oil on alternaria leaf spot cucumber.

Diseases management with bioagents (*Trichoderma harzianum* and *Bacillus* sp4 isolate).

Results in Table (10) and Figure (5) indicated that, treated cucumber plants with *Bacillus* sp (isolate 4) as spray and as a mixture application effectivelly reduced lesion diameter (0.87 and 0.77 cm) and blighted area (2.38 and 1.86 cm^2), followed by *T. harzianum* (2.07 and 2.69 cmof lesion diameter) and blighted area (13.46 and 22.72 cm^2). While, control one recorded 4.66 cm and 68.19 cm^2. Similar results were obtained by Aly *et al.*, (2002), Esh *et al.*, (2010) and Atia and Ahmed Amal (2011).Pyllospheric and rhizospheric microorganisms have an inhibitory effect and stimulate the induction of systemic resistance mechanisms within the plant (Bargabus *et al.*, 2004 and Aly *et al.*, 2002). Foliar biological control agents including: both Gram negative (*Erwinia* spp. and *Pseudomonas*

spp.) and Gram positive (*Bacillus* spp.) and streptomyces (Esh *et al.*, 2010 and Atia, *et al.*, 2010).

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).

Table (11): Effect of bacterial and fungal bioagents isolates against alternaria leaf spot of cucumber

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

***Bacillus* sp. isolate (4) as spray application**

***Bacillus* sp. isolate (4) mixture inoculation**

Control

***T.harzianum* spray as spray application**

***T. harzianum* mixture inoculation**

Fig. (5): Effect of different bioagents on alternaria leaf blight cucumber

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دراسات مرضية على تبقع اوراق الخيار الالترناري في الزراعات المحمية

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يعتبر مرض تبقع الأوراق الالترناري واحداً من أهم أمراض المجموع الخضرى فى الخيار على مستوى العالم. وقد وجد أن هذا المرض منتشر بصورة وبائية تحت ظروف الزراعات المحمية بالانقاق والصوبات البلاستيكية. تم تعريف الفطر المعزول على انه الترناريا كيكوميرينا (ماكروسبوريم كيكوميرينم) و قد اختبرت قدرته المرضية على الأوراق الفلقية والأوراق الحقيقية للخيار، وذلك باستخدام طريقة الأوراق المنزوعة. وقد كانت أصناف الخيار الثلاثة المختبرة (ماتركس –بست- بيتا الفا) حساسة للمرض بدرجات مختلفة، وكان صنف ماتركس أقلهم عرضه للإصابة. كانت نباتات الشمام والكنتالوب والبطيخ والكوسة والقرع واللوف حساسة للمرض بدرجات مختلفة، حيث تم اختبارها بطريقة الأوراق المنزوعة. وقد خفض فطر تريكودرما وتريكودرما هاريزيانم وفيريدي والعديد من عزلات البكتيريا من جنس باسيلس المعزولة من سطح أوراق الخيار. كما ثبط راشح نمو عزلات البكتيريا من جنس باسيلس معنويا نمو الفطر المسبب تحت ظروف المعمل. وقد خفضت بعض تركيزات الزيوت ومستخلصات النباتات المختبرة من النمو الميسليومي وإنبات جراثيم الفطر الترناريا كيوكوميرينا، وكان زيت القرنفل أكثرها فعالية في ذلك. وكذلك كان المبيد الفطري دياثين–م 45 أكثر المبيدات المختبرة تأثيراً على نمو الفطر وإنبات جراثيمه معملياً. وقد خفضت عزلة الباسيلس رقم 4 الإصابة بالفطر الترناريا كيوكوميرينم على أوراق الخيار رشا أو حقناً تلاها فطر تريكودرما هرزيانم. كما خفض زيت القرنفل والمبيد الفطري دياثين–م 45 من الإصابة بالفطر الترناريا كيوكوميرينا لأوراق الخيار بصورة معنوية.