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Controlling Of Cumin Blossom Blight Disease in Egyptian Organic Plantations.

El-Deeb H. M.[1], Lashin S. M.[1], and Arab Y. A.[2]

[1]Plant Pathology Department, National Research Centre, (Affiliation ID: 60014618), Giza, Egypt.
[2]Faculty of Agriculture, Department of Botany, Al-Azhar University, Cairo, Egypt.

ABSTRACT

Exhibiting antibiosis between the pathogen, *Alternaria burnsii* and the antagonists, *Trichoderma album* and *T. harzianum*. Dithane M-45 was better than Topsin-M 70 in reducing *A.burnsii* linear growth. Foliar application of the biocide Bio-Zeid *(Trichoderma album)* followed by compost tea (1.5 L from each tested product / plot ≡ 600 L / fed.) significantly lowered the severity of cumin blight disease as compared to untreated control. Although foliar application of Dithane M-54 performed best in reducing the severity of cumin blight. However, it was not performed effect in increasing plant growth and yield parameters of cumin as compared to compost tea.

Keywords: Cumin (*Cuminumcyminum*), blossom blight disease, organic plantations, biological control, compost tea.

Corresponding author

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INTRODUCTION

Cumin (*Cuminumcyminum*, family *Apiaceae*) is a winter crop, grown mainly in Upper Egypt in October and November. High humidity in cumin fields in Gharbyiagovernorate, Egypt, during flowering and set fruit times causes a number of diseases, but heavy losses are sustained due to blossom blight incited by *Altenariaburnsii*. It causes a burning problem to the cumin growing areas in Egypt [22, 1]. The pathogen mainly attacks the flowers and some- times leaves. The primary inoculum source is likely wind or water borne spores from infected crop residues [2].

In the present study, we are trying to control the blight in cumin organic farming by using two alternative organic management approaches.

Organic farming is a form of agriculture that relies on techniques such as crop rotation, green manure, compost and biological pest control. Depending on whose definition is used, organic farming uses fertilizers and pesticides if they are considered natural, but it excludes or strictly limits the use of other methods , including synthetic petrochemical fertilizers and pesticides; plant growth regulators and human sewage sludge [19].

Trichoderma spp. are fungi present in substantial numbers in nearly all agricultural soils and in other environments. Among their other activities, they grow tropically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi. This process (mycoparastitism) limits growth and activity of plant pathogenic fungi. In addition, sometimes in conjunction with mycoparasitism, individual strains may produce antibiotics. However, numbers and the physiological attributes of wild strains are not sufficient only for highly effective control of plant diseases [12].

Compost tea, in modern terminology, is a compost extract brewed with a microbial food source like molasses under forced aeration and grows population of microbial community [8]. Compost tea ensures diverse and healthy food web communities which provide protection of plants from diseases. A modest to major control of several plant diseases were reported by the use of compost tea. Compost tea offered more measurable benefits in stimulating crop growth, yield and its quality than in suppressing disease [7]. Using compost tea instead of solid compost application may be the best use of technology to improve crop productivity and crop health.

In the present work, the efficacy of biocides and compost tea in controlling blossom disease of cumin were evaluated as compared to chemical means.

MATERIALS AND METHODS

Disease survey

Blossom blight disease of cumin was surveyed on the commercial cultivar grown during the growing seasons of 2009/2010 and 2010/2011 in open fields of four locations at Gharbyia governorate, *i.e.* Kafr Salem, Qureshiyah and Tokh Maziad (El-Santa county) and Nifia (Tanta county).Within each cumin field, five stripes were examined, while walking in zigzag pattern pathway into the field. Plants were inspected twice at each growing season. The number of blighted plants was recorded. The percent of cumin blossom blight incidence was calculated using the following formula:

$$\text{Disease incidence \%} = \text{No. of blighted plants} \div \text{Total examined plants} \times 100$$

Isolation of the causal pathogen

During the survey of cumin blight disease, fresh necrotic fruits (Seeds) having different levels of coloration were sampled, put in paper bags and transferred to the laboratory for isolation the pathogen(s). The samples were washed with running tap water. All seed samples were surface sterilized with 1% sodium hypochlorite solution for 2 min., washed several times with sterilized water and dried between sterilized filter papers. The sterilized samples were placed onto surface of PDA plates supplemented with streptomycin-

sulfate (100µg/ml) according to Rain *et al.* [20]. Some surface sterilized cumin seeds were placed onto wetted Whatman filter paper No.1 inside Petri plates. All plates were incubated at 27± 2 ºC for 5 days.

Single spores according to the procedure of Choi *et.al*. [4] or hyphal tips according to the procedure of [13] were taken from the developed fungi colonies and transferred to PDA slants, then kept in a refrigerator at 5 ºC for further studies. The fungal isolates were identified up to the genus level. As for *Alternaria burnsii*, the causal agent of cumin blossom blight the identification was made on the base of cultural characters and conidium and conidiophores morphology according to Green *et al.* [11].

***In vitro* experiments**

Detached leaf bioassay

The detached leaf bioassay was performed according to Vawdrey *et al*. [26] to assess the virulence of the obtained isolates. The second- or third-oldest leaves of cv. Balady cumin plants at least 30 days old were removed just below the blade and the upper surface gently wiped with a paper tissue. The detached leaves were inoculated with spore suspension of the *A. burnsii* virulent isolate. The spore suspension concentration should be around 10^5 spores /ml.

The leaves were long enough for 2 drops to be placed along the upper leaf. The leaves in a closed Petri dish (with a wet Whatman no. 1 filter paper underneath) were then incubated in a growth chamber for 2 - 6 days at 25˚C under continuous white light. Image analysis software according to [18] was used to measure the percentage of each inoculated leaf section showing spotting symptoms over time.

Effect of fungal antagonists on the growth of the causal fungus

Two antagonists, *i.e. Trichoderma album* and *T. harzianum* were obtained from culture collection of Department of Plant Pathology, Al-Azhar Univ., Nasr City, Cairo. and used in this trial to evaluatetheir activities against *Altenaria burnsii* using the dual culture method. Culture discs of both *A.burnsii* and each of *Trichoderma album* and *T. harzianum* were placed on opposite end of PDA plate, and in control plates only *A.burnsii* was placed. Each treatment was replicated three times. The plates were incubated at room temperature (27 ±6 °C) and the colony interactions were measured as percentage of inhibition of radial growth of *A. burnsii* by following formula suggested by [24] as follows:

$$\text{Percentage of inhibition} = (R_1 - R_2 \div R_1) \times 100$$

Where: R_1= Radius of the radial growth of the pathogen towards opposite side in control plate, R_2= Radius of the radial growth of the pathogen towards the opponent antagonist in test plate.

Effect of fungicides on the growth of the causal fungus

The relative efficacy of two fungicides was studied *in vitro* based on the radial growth inhibition of the test isolate of *A. burnsii* using the poisoned food technique of Lakshmanan *et al.*[17]. The test fungicides were one non-systemic (Dithane M-45 as Indofil M-45and the other systemic (Thiophanate methyl 70% WP as Topsin-M 70). The test concentrations of each fungicide, *i.e.* 500, 250 and 125 ppm a.i. /Lwere prepared. The calculated amounts of each fungicide were added to flasks containing sterilized PDA medium and mixed well, then they poured into 90 mm sterilized Petri plates. In control plates, no fungicide was placed. After complete solidification of the medium, 4 mm diameter disc of 5-7 days old culture of the targeted fungus, *A. burnsii* was taken and inoculated into the center of Petri plate in complete aseptic conditions. Each treatment was replicated three times.

Controlling of the causal pathogen under field conditions

The effect of some alternative organic management approaches on cumin natural infection with blossom blight disease and the effect of these treatments on cumin yield parameters were studied. The field was prepared at Qureshiyah, a village in El-Santa center, Gharbyia governorate, Egypt for the two consecutive years 2011 and 2012.All organic agricultural practices were carried out according to the recommendations of

the Min. of Agric., Egypt. The soil type was silt loam, previously cropped to cumin. In 2011, early chicken manure compost was applied at rate of 70 pounds of nitrogen per feddan according to the procedure of [3] and was thoroughly incorporated into the soil prior to bed formation. The soil was ploughed. After plough, weeds and rubbish were removed. In both years, the experiment was laid out in the randomized complete block design, with three replications. Cumin (cv. Balady) was seeded in a 4 m long plot with 4 rows / plot. The rows were spaced 30 cm apart with 10 cm between plants within the row.

The seeds were planted on the 5th and the 10th of October 2011 and 2012 seasons, respectively. The seeds were prose at the rate of 5 kg / feddan. The two alternative organic management approaches applied as foliar sprays, two prays of Bio-Zeid (The biocide which contains *Trichoderma album*) at 2.5 gm /L, followed by two sprays of compost tea preparation at the intervals of 15 days. First spray was given just flowering plants.

Compost tea was prepared by mixing compost with tap water at a ratio 1:5(w/v) followed by fermentation for at least one week. A cup of molasses was added in the mixture during fermentation to enhance the microbial growth in the compost tea. Then it was stirred once every day and allowed to ferment outdoors around at 25°C. After 7 days, the solution was filtered through cheesecloth and diluted (1:1). Cumin plants were sprayed with 1.5 L from each test product /plot (600 L /fed.).A total of 3 treatments were used, *viz.* T_1 (Control with no spray), T_2 (Control with DithaneM-45 at 2 g /L) and T_3 (Compost tea as foliar spray).The number of cumin flowers per plant are in nearly 16 umbels and nearly 12 flowers per umbel.

Disease severity was determined by using 0-10 rating scale according to [14] based on the average number of blighted flowers per plant, where: 1 = 0 %, 2 = 1 to 3 %, 3 = 4 to 6 %, 4 = 7 to 12 %, 5 = 13 to 25 %, 6 = 26 to 50 %, 7 = 51 to 75 %, 8 = 76 to 87 %, 9 = 88 to 94 %, 10 = 95 to 100 % of flowers. The disease severity percentage was calculated as follows:

$$D.S.\% = [Sum.\ (n \times v) \div N\ V] \times 100$$

Where: n = degree of infection according to the scale, v= Number of blighted flowers per plant, V= Total number of screened flowers and N= Highest degree of infection. Cumin yield parameters, *i.e.* No. umbels / Plant, No. seeds / Umbel, 1000 seeds weigh (g) and Seed yield (g/m^2) were determined after harvesting.

Data collection and analysis

The data were collected on the various growth and yield parameters of cumin at the time of harvesting. The data were analyzed statistically using analysis of variance to find out the variations resulting from experimental treatments and the treatment means were compared by Duncan's multiple range test [9].

RESULTS AND DISCUSSION

Disease survey

Data presented in Table (1) show that the mean percentage of cumin blight incidence in 2010/1011 was higher than that in 2009/2010 season.

Table 1: Cumin blight incidence percentage in some open fields of four villages at Gharbyia governorate during the growing seasons 2009/2010 and 2010/2011.

Gharbyia villages	% Cumin blight incidence during :	
	2009/2010	2010/2011
Kafr Salem	33.2	34.0
Nifia	32.4	33.2
Qureshiyah	34.7	35.1
TokhMaziad	31.3	32.3
Mean	32.9	33.7

Qureshiyah village exhibited 34.7 and 35.1 % disease incidence in 2009/2010 and 2010/2011 seasons on the average, respectively where the humidity in these fields was increased during January, the cumin

flowering time. These results are in agreement with those recorded by [27] who reported that cumin plants grow well in hot dry climate and not in a humid atmosphere.

Isolation of the associated fungi

Data presented in Table (2) indicate that the blotting paper method yielded high number of fungal isolates (15 isolates) belonged to six genera, *i.e. Alternaria*, *Aspergillus*, *Cladosporium*, *Drechslera*, *Fusarium* and *Penicillium*, while the agar plate method yielded 9 isolatesbelonged to fourgenera, *i.e. Alternaria*, *Chaetomium*, *Drechslera*, *Fusarium*.

Table 2: Number of the fungal isolates obtained from naturally infected cumin seeds using two isolating methods

Isolating method	Fungal genera	No. fungal isolates
Agar plate	*Alternaria*	2
	Chaetomium	2
	Drechslera	2
	Fusarium	3
Blotter paper	*Alternaria*	3
	Aspergillus	2
	Cladosporium	1
	Drechslera	3
	Fusarium	4
	Penicillium	2

The obtained results are in agreement with the findings of Rathore *et al*. [21] who isolated *Chaetomium sulphureum* as a new causal agent of cumin leaf spot disease in India. Also, Chohan *et al*. [5] isolated twenty three species of fungi from cumin seed samples. They found that *Alternaria* spp., *Fusariumoxysporum*, *Fusariumequesti*, *Cladosporium* spp. and *Drechslera* spp. were predominating. In the same respect, [10] used the blotter method in their study to countthe mycoflora of cumin seeds.

***In vitro* experiments**

Detached leaf bioassay

The five isolates of *Alernaria* were tested for their pathogenicity by the detached leaf bioassay. One *Alternaria*isolate from Qureshiyah samples was selected as the fierce isolate and identified as *A. burnsii*.

Effect of fungal antagonists on the growth of the causal fungus

The results in Table (3) of the dual-culture assay on potato dextrose agar (PDA) demonstrated that a clear zone of inhibition was observed, exhibiting antibiosis between the pathogen, *A.burnsii* and the antagonists *Trichoderma* spp. *Trichoderma album* reduced the growth of *A. burnsii* by 70.0%. In this respect, [25] and Deepak *et al*. [6] stated that *Trichoderma* spp. effectively inhibited the growth of *A. burnsii* under *in vitro* conditions.

Table 3: Antagonistic activity of two species of *Trichoderma* spp. Against *A. burnsii*.

Trichoderma	% Growth inhibition of *A. burnsii* *
T. album	70.0
T.harzianum	60.0

* Each value is the mean of three replicates

Effect of fungicides on the growth of the causal fungus

The efficacy of the tested fungicides against *A. burnsii* linear growth is presented in Table (4). The data proved that the two fungicides were effective against the pathogen.

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Table 4: Effect of some chemical fungicides on the linear growth of *A.burnsiiin vitro*.

Fungicide	Conc. (ppm)	Linear growth in cm.	% Reduction in the linear growth*
Dithane M-45	125	2.6	70
	250	0.0	100
	500	0.0	100
Topsin-M-70	125	4.3	50
	250	2.6	70
	500	0.0	100
Control	0.0	8.5	0.0

* Each value is the mean of three replicates

The inhibition zone was increased by increasing the fungicide concentration. Dithane M-45 was better than Topsin-M-70 in reduction the growth of *A. burnsii*. In this respect, Sankhla *et al*. [23] found that Dithane M-45 was the most effective out of six fungicides against *A. burnsii* under *in vitro* conditions.

Controlling cumin blossom blight under field conditions

Data presented in Table (5) show that the mean percentage of cumin blight disease severity during 2011/2012 was higher than that recorded in 2010/2011 growing season. Foliar application of the biocide, Bio-Zeidfollowed by compost tea showed significant reduction to the severity of cumin blight disease as compared to untreated control. In this respect, [16] grow *T. harzianum* in liquid cultures containing chitin or fungal cell walls as sole carbon source. They mentioned that the fungus produced glucanase and chitinase in the medium. These enzymes, seem to play an important role in the antagonistic action of *T. harzianum* against a wide range of fungal plant pathogens.

Although foliar application of Dithane M-45 performed best in reducing the severity of cumin blight, howeverit did not perform better in increasing the growth and yield parameters of cumin as compared to compost tea. The four possible modes of action of compost tea against pathogens possibly includes, induced resistance against pathogens, inhibition of spore germination, inhibition of lesion expansion and antagonism and competition with pathogens. The foliar application of compost tea offers some micronutrients to plants and may also triggers some signaling pathways in plants. One of the leading explanations is the induction of systemic acquired resistance (SAR). Zhang *et al.* [28] mentioned that compost tea induced systemic acquired resistance in cucumber and *Arabidopsis*. Furthermore, compost tea may enrich the beneficial microbial community in the plant surfaces which in turn compete with other foliar pathogens and reduced their growth and development on the plant surfaces. The antifungal activities of compost tea which may inhibit the growth of other foliar pathogens and increase crop vigor were also reported by Kerkeni *et al.*[15].

Table 5: Disease severity and cumin yield parameters after spraying with the biocide followed by compost teaunder open field conditions at Qureshiyah , Gharbyia governorate in 2010/2011 and 2011/2012 seasons.

Sprayed Materials	D.S., %	Av. Yieldparameters in 2010/2011 season				D.S., %	Av. Yieldparameters in 2011/2012 season			
		No. umbels / Plant	No. seeds/ Umbel	1000 seeds weigh(g)	Seed yield (g/m^2)		No. umbels / Plant	No. seeds / Umbel	1000 seeds weigh(g)	Seed yield (g/m^2)
Biocide	16.7	15.0	13.0	4.8	572.8	19.2	15.8	12.0	3.7	493.5
compost tea	15.5	13.7	12.0	4.2	558.6	18.4	14.5	11.6	4.1	540.3
Biocide+compost	11.4	14.3	11.0	3.7	544.5	13.6	13.2	10.8	3.5	530.2
DithaneM-45	8.7	12.5	10.8	3.3	525.1	9.5	11.2	10.5	3.1	520.6
Unsprayd(Control)	35.3	11.7	10.0	3.0	490.3	37.1	11.0	10.0	2.9	480.8
LSDat 5%	1.9	1.2	n.s.	0.2	13.1	2.4	1.0	0.2	0.3	9.3

Foliar spray of the biocide followed by compost tea may be used as alternative environment friendly means for plant disease control and increase plant growth and yield parameters with maximum profit. Further investigations are required to draw clear conclusion on the use of compost tea in controlling fungal diseases.

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