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Management of Cumin Blight Disease Caused By *Alternaria Burnsii* By Using Green Chemicals and Biofungicides

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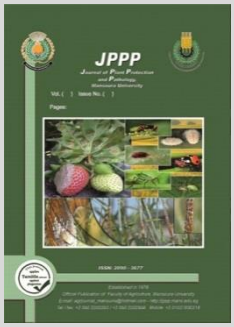

ABSTRACT

The cumin blight disease caused by the fungus *Alternaria burnsii* is the most destructive disease on cumin in its cultivated areas in the world. The fungus *A. burnsii* was isolated from cumin plants infected with blight and its pathogenicity was confirmed on cumin plants. Selected green chemicals (potassium silicate; PS and nano silicon; NSi) were evaluated for their antagonistic effect against *A. burnsii* in the laboratory at different concentrations (50, 100, 200, 300 and 400 ppm for PS and 1.5, 2, 2.5, 3 and 3.5 mM for NSi). Also, the inhibitory effect of *Trichoderma harzianum* and *T. hamatum* was studied in the laboratory using dual culture technique. The biocontrol fungus *T. harzianum* was formulated in four biofungicidal forms and tested against cumin blight in greenhouse and field trials. These forms were: suspension of fresh spores and mycelium in water, emulsion of fresh spores and mycelium with its filtrate in corn oil, emulsion of fresh spores and mycelium without its filtrate in corn oil, and emulsion of dried spores in corn oil, which were all sprayed on cumin plants showing blight symptoms. The emulsion of dried spores of *T. harzianum* at 1×10^6 spores/ml was the most effective in suppressing blight disease, and NSi was in the second place in this regard, thus increasing the yield of seeds and the percentage of volatile oil in the seed compared to the untreated control.

Keywords: cumin, Alternaria blight, *Trichoderma*, formulation, green chemicals

INTRODUCTION

Cumin (*Cuminum cyminum* L.) is an annual herbaceous flowering plant belonging to family Apiaceae, also known as the Umbelliferae family. Cumin is native to the Mediterranean region and eastward into India. Cumin is one of the most extensively used seed spices and is used by people all over the world. It is the second most popular spice in the world after black pepper. The seed content has an essential oil between 2.5 to 4.5%. The seed contains oil whose major component is cuminol due to which a typical pleasant aroma is present in the seed. Owing to the presence of aromatic substances in the herbs, many researchers are attracted to validate experimentally the therapeutic uses of the herb or its seed. It is already exploited in various ayurvedic medicines for various diseases such as stomachache, obesity, and dyspepsia (Agarwal *et al*., 2017). Many phytochemicals are present in cumin seed that are antioxidant, anti-flatulent and contain huge amount of dietary fibers which help in preventing constipation (Rana *et al*., 2018). Cumin seeds are very nutritious and consist of 17.7% protein, 7.7% minerals, 35.5% carbohydrates and 23.8% fat (Pandey *et al*., 2019). It has been observed that spices behave as a bio-nutrient enhancement that improves the flavor, essence and fragrance of food and also controls several diseases. The major cumin diseases observed were wilt (0-60%), blight (0-80%), and powdery mildew (0-54%) (Sharma *et al*. 2013).

Among the major diseases of cumin, Alternaria blight caused by *Alternaria burnsii* is the most devastating disease in major cumin growing areas in the world. . This disease is quite prevalent and destructive as it affects all above ground plant parts including seed, thus causing direct yield loss up to 70% as reported in India (Uppal *et al*., 1938).

Today, biological management of plant diseases is needed as alternative to chemical pesticides that have adverse effects on environment, animal, and humans. Bio-prospecting is searching for new sources of compounds, genes, microorganisms, plants and other natural sources. The biocontrol proficiency of antagonistic microorganisms like *Bacillus*, *Trichoderma*, *Pseudmonas* and some entophytic bacteria has been exploited for the management of the diseases of horticultural crops. Characterization of the metabolites which play a role in the suppression of plant pathogens is also very much needed. The biocontrol efficacy of antagonistic microorganisms depends on a combination of factors such as the characteristics of the antagonistic microorganism, the epidemiology of the target pathogen and the environmental conditions in which the relationship between the pathogen and the antagonist(s) is taking place. Hence, efforts are needed to develop systems by integrating several strategies taking into consideration pathogen biology, cultivar resistance, and epidemiology (Devappa *et al.* 2020). The success of the fungal biological control agent *Trichoderma* in managing plant diseases depends not only on its highly effective propagules and biocontrol mechanisms but also its formulation and delivery systems in relation to the crop, disease and to the

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environment. Two major types of formulations are commonly used: solid and liquid formulations. To prolong shelf life and enhance application efficiency of *Trichoderma*, microencapsulation has been developed. The modes of delivery and application of *Trichoderma* focus on seed treatment employing techniques in seed technology such as solid matrix priming, liquid coating and double coating. The development of biological control systems in formulation of *Trichoderma* is essential to promote sustainable plant disease management, should be concentrated to increase the shelf life of the formulation by developing superior strains that support the increased shelf life, or the organic formulations that support the maximum shelf life with low level of contaminants must be standardized for making biocontrol as a commercial venture. Whatever the limitations these *Trichoderma* products may have, it can be addressed by enhancing biocontrol through manipulation of the environment, accurate strain identification by molecular approach, using mixtures of beneficial organisms, physiological and genetic enhancement of biocontrol mechanisms, and manipulation of formulations. Of late, many small and large entrepreneurs have entered into the commercial production of biocontrol agents resulting into the entry of various bio-control products into the world market (Kumar *et al.*, 2014 and Cumagun, 2014).

Thus, the main objective of this study was to control cumin blight disease by using green chemicals and biological control agents for suppressing the blight pathogen *A. burnsii* in cumin via promoting the plant systemic acquired resistance and / or killing the pathogen.

MATERIALS AND METHODS

1. Isolation and identification of the causal pathogens:

Naturally diseased cumin plants of the cumin cultivar "Baladi", showing typical blight symptoms, were collected from different localities of El-Gharbia governorate (Basion, Qutoor, and Tanta); El-Minia governorate (Maghagha, Beni Mazar, Samallout, and Matai) and Assiut governorate (Diroot, Qossia, and Abnob). Small pieces were taken from diseased plant parts, washed thoroughly with tap water, surface sterilized by immersing for two minutes in 2% sodium hypo chloride solution, rinsed several times in sterilized distilled water, plated on potato dextrose agar (PDA) medium (200g potato, 20g dextrose, 20g agar and 1000 ml water), and incubated at 28±1°C for four days.. After that, the plates were checked out for fungal growth. Purification of the obtained fungal isolates was carried out by using the hyphal tip technique.

Identification of the isolated fungi was carried out by using the morphological characteristics of mycelia and spores as described by (Clements and Chear 1957; Barnett and Hunter, 1972; Rossman *et al*., 1994; Rotem, 1994; Singh *et al*, 2015; and Singh *et al*., 2016).

2. Pathogenicity tests:

Preparation of fungal inoculum: Fungal inocula were prepared by growing the tested isolates on PDA medium in Petri plates at 28ºC for 15 days. At the end of the incubation period, ten ml of sterile distilled water were added to each plate and the growth of fungal colonies was carefully scarped with sterile brush to release fungal spores. Fungal suspensions were collected and filtered through cheesecloth to get rid of agar crumbs. Concentrations of inocula were adjusted by sterile distilled water to get $2.4X10^4$ CFU/ml water (Shekhawat *et al*., 2013 and Singh *et al*., 2015).

Alternaria burnsii isolates that obtained from the survey were examined under greenhouse conditions for their pathogenicity as follows: Black plastic bags (30cm diameter x 30cm height) filled up with sterilized soil mix (50% sand and 50% loam; autoclaved twice in two consequent days at 121˚C for 1 h) were seeded with surface-sterilized cumin seeds (by immersing seeds in 2% sodium hypochlorite solution for 2 minutes followed by several washing with sterilized distilled water) at rate of 5 seeds/plastic bag. Six replicates were used for each isolate.

Inoculation was carried out by spraying using a hand atomizer, 45 days after sowing with the inoculum suspensions of the tested isolates (Deepak *et al*., 2008 and Singh *et al*., 2015). After inoculation, plants were covered with plastic bags for 48 hours to maintain high moisture. After this period, plastic bags were removed and plants were kept under greenhouse conditions until development of the symptoms. Check plants were sprayed with sterilized water and treated in the same manner as untreated control. After 10 days from inoculation, disease incidence and disease severity were recorded. The severity of infection was estimated using a disease scale/grade of 0 to 4 (Shekhawat *et al*., 2013), where 0 = no infection, 1 = 1-25% infection, 2= 26-50% infection, 3= 51-75% infection, and 4= 76-100% infection. The disease index was then calculated according to the following equation:

$$\text{Disease index} = \frac{0A + 1B + 2C + 3D + 4E}{4T}$$

Where 0–4 are the disease scale and A, B, C. D, and E are the numbers of plants in each disease grade and 4T is the total number of plants (T; T= A+B+C+D+E) multiplied by maximum disease grade (4).

3. In vitro experiments

Green chemicals and biological materials were tested in vitro for their inhibitory effect against *A. burnsii* growth in Petri plates as follows:

Potassium silicate solution (PS): Five concentrations of PS in PDA medium (100, 200, 300, 400 and 500 ppm) were used. Control plates were made with PDA only without PS. Five replicates were used for each concentration and the control. All plates received 20 ml of the medium and left for 30 minutes to be solidified, and then inoculated with 0.5 cm disc from the edge of a 7-day-old culture of the pathogen, which placed in the center of plates. The growth inhibition was calculated after 7 days as the percentage inhibition of redial growth relative to the control (Menzies *et al*., 1991).

Nano Silica (NS): Five concentrations of NS in PDA medium (1.5, 2, 2.5, 3 and 3.5 mM (v/v)) were used. Control plates were made with PDA only without NS. Five replicates were used for each concentration and the control. All plates received 20 ml of the medium and left for 30 minutes to be solidified, and then inoculated with 0.5 cm disc from the edge of a 7-day-old culture of the pathogen, which placed in the center of plates. The growth inhibition was calculated after 7 days as the percentage inhibition of

redial growth relative to the control (Khadiga, Hasan *et al*., 2020)

Dual culture assay: Antagonistic fungi were tested for their antifungal activity against *A. burnsii*. The inhibitory effect of antagonistic fungi (*Trichoderma harzianum* and *T. hamatum*) against the pathogen growth was assayed by using the dual culture method described by Sharma (2011). Petri plates (9-cm diameter), each containing PDA (20 ml) were inoculated with a 0.5cm disc of *A. burnsii* at 1 cm from the edge of the Petri dish and then a 0.5 cm disc of the bio-agent (*T. harzianum* and *T. hamatum*) were placed at 1 cm from the opposite edge of the Petri dish. The test was performed in 5 replicates. The paired cultures were incubated at 25±2˚C for 5 days. Plates inoculated with the pathogen only at 1 cm from edge of plate served as a control. The radial growth of individual and dual cultures was recorded then the inhibition percentage of the pathogen was calculated relative to the control by using the following equation:

$$\text{Growth inhibition (\%)} = \langle (R_1 - R_2) / (R_1) \rangle \times 100$$

Whereas, R_1 = inward linear growth in the control plate and R_2 = inward linear growth in the dual culture plate.

4. Preparing *T. harzianum*-based oil emulsion formulation:

The oil phase was prepared by adding lecithin to plant oil in a ratio (1:3 v/v) respectively, and then mixed in a blender to a homogenization. The aqueous phase was made by mixing (1% w/v) sodium alginate, 0.5% maize honey, and tween-80. Oil phase was added to aqueous phase (3:2 v/v) and mixed well into a mixer and then lime (calcium hydroxide) was added to the final mixture at 0.5% (w/v) and the fungal suspension of *T. harzianum* (10%w/v) was added and mixed until homogenization.

5. In vivo experiments

Greenhouse experiment

The most effective treatments resulted from in vitro experiments against *A. burnsii* were used as spray application for management of cumin blight in pots. Black polyethylene bags (30-cm diameter x 30-cm height) were filled with a mixture of sand and soil (1:2) and sown with surface sterilized (0.1% $HgCl_2$ for 2 minutes) cumin seeds (Balady; local cultivar) and irrigated. After germination, thinning was done to maintain five plants per pot. Pots were arranged in a complete randomized design with six replications. After 45 days from sowing plants were sprayed with potassium silicate (200, 300 and 400 ppm), nano silica (2.5, 3, and 3.5 mM), and four biofungicidal formulations of *T. harzianum* namely, suspension of fresh spores and mycelium in water, emulsion of fresh spores and mycelium with its filtrate in corn oil, emulsion of fresh spores and mycelium without its filtrate in corn oil, and emulsion of dried spores in corn oil. Each formulation was tested at four concentrations (0, $1x10^5$, $1x10^6$, and $1x10^7$ propagules/ml). Ridomil gold plus (1.5 g/L) was used for comparison as a chemical fungicide treatment (Kumar *et al*., 2014). After 36 h, all plants were inoculated with the most pathogenic isolate (Ab02) of the pathogen *A. burnsii* ($1x10^6$ propagules/ml). For comparison, six pots inoculated with *A. burnsii* only served as a positive control while another six pots were sprayed with just sterilized distilled water and maintained without treatment served as a negative control. Disease severity was recorded after 10 days of inoculation using a standard disease rating scale (0-4 score). The percent disease index (PDI) and percent efficacy of disease control (PEDC) were calculated by using following formulas given by (Chester, 1959; Wheeler, 1969; Shekhawat *et al.*, 2013; and Singh *et al*., 2015):

$$\text{PDI} = \frac{\text{Sum of all individual disease rating}}{(\text{Total no .of plants assessed}) \times (\text{maximum rating})} \times 100$$

$$\text{PEDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Field experiment

Field experiments were carried out during two successive seasons (2018/2019 & 2019/2020) in a private farm at Damsheet village, Tanta, El-Gharbia governorate. Seeds were sown in plots (0.5x1.5 m), each with 3 rows and 5 holes/row, 2 seeds/hole. After ten weeks of planting, cumin plants were sprayed separately by PS (300 ppm), N Si (2.5 ppm), *T. harzianum*-based emulsion biofungicide ($1x10^6$ dry spores/ml), as well as the chemical fungicide Ridomil gold plus (2g/L water). Disease severity were determined 30 days after spraying as described before (Chester, 1959; Wheeler, 1969; Shekhawat *et al.*, 2013; and Singh *et al*., 2015).

Determination of seed yield:

This study was carried out during 2019/2020 growing season at the experimental farm at Damsheet village. Seed samples were collected at random from each treatment at the harvesting stage for assessment of seed yield. Weight of 100 seeds/plant in grams was estimated for each sample. Four replicates were used for each treatment.

Determination of volatile oil content in seeds:

This study was carried out during 2019/2020 growing season at the experimental farm at Damsheet village. Seed samples were collected at random from each treatment at the harvesting stage for assessment of volatile oil content in seeds. The fully ripened fruits of cumin plants were air dried for a week, covered at night with polyethylene sheet. Random samples from air dried fruits were selected and crushed immediately before analysis. The crushed fruits were distilled as described by Egyptian pharmacopoeia (1961)

Distillation was continued for 3 hours as reported by Guenther (1961). When distillation was completed, oil was left to stand undisturbed to assure complete separation. Volatile oil yield percentage was then calculated as ml of oil per 100 grams of fruits. Also, volatile oil yield per plant was calculated by multiplying plant fruit yield in grams by their oil percentage as ml/plant. The volatile oil was received in a glass tube and stored in a refrigerator at 4-7°C.

5.2.2.1. GLC analysis: On completion of distillation, the collected essential oil was dried over anhydrous Na_2SO_4. Then immediately, oil analysis (1 u1 samples) was performed by capillary GC (FID at 230c, 100: injection split at 220c) on a 25 m Carbowax 20 MWOT columns operated at 4ml/min. H2 and programmed from 45 c (5 min. hold) to 180 c at 10 c/min. FID out-put was electronically integrated and calculated based on the internal standards. Yield and relative percent of major oil constituents were also determined. Identification of oil components based on RT were confirmed by GC-MS comparison of retention times and

mass spectra to authentic standards. This method is adopted by El-Keltawi and Croteau (1986).

6. Statistical analysis:

All data were statistically analyzed using CoStat 6.311 software (2005) for the analysis of variance (ANOVA) (Gomez and Gomez 1984). All comparisons were first subjected to ANOVA and significant differences among treatments means were determined using Duncan's Multiple Range test at $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Results

Disease survey:

Data presented in Table (1) and Fig (1) showed that percentages of naturally infected cumin plants with Alternaria blight varied according to the cultivated area. The natural infection ranged from 15 to 33.2% the 1st season of survey and 20 to 35% in the 2nd season. Percentages of infection were higher in the 2nd season than those of the 1st one in all governorates. It was noticed that the disease decreased from the north to the south. El-Gharbia governorate came in the front position as infected plant percentages recorded 30.2 and 35% in the 1st and 2nd seasons, respectively. However, Assuit recorded the least percentages of naturally infection (15 and 25% in both seasons, respectively) in comparison with the other governorates.

Table 1. Average percentages of cumin plants naturally infected with Alternaria blight disease in three governorates in Egypt.

Governorate	County	GPS		% Infection	
		Latitude	Longitude	2016/2017 Season	2017/2018 Season
El-Gharbia	Basion	30.945635	30.824903	30.2 c*	32.6 b
	Qutoor	30.544614	30.533259	31.4 b	30.4 c
	Tanta	30.887880	31.039588	33.2 a	35.0 a
El-Minia	Maghagha	28.652938	30.842324	25.8 d	23 h
	Beni Mazar	28.095464	30.754033	24 e	27 e
	Samalout	27.702826	31.039034	22 f	28 d
	Matai	27.566319	30.814468	20 g	25 f
Assuit	El-Qussia	27.192767	31.172034	18 h	20 i
	Dairout	27.184411	31.182561	15 i	20 i
	Abnob	27.268714	31.258243	20 g	24 g
Mean				23.96	26.47

***Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

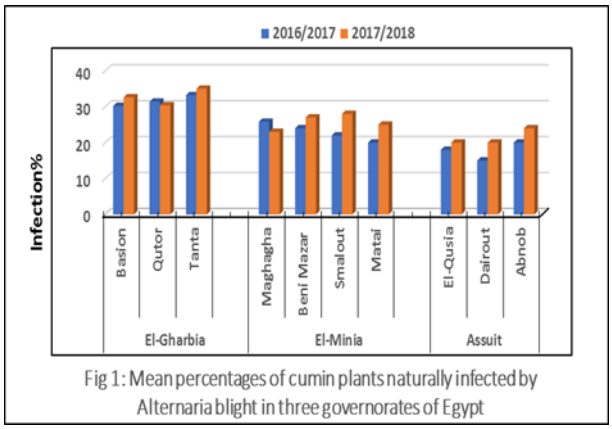

Fig 1: Mean percentages of cumin plants naturally infected by Alternaria blight in three governorates of Egypt

Isolation, purification and identification of the associated fungi to blight disease symptoms:

Isolation trails from infected stems, leaves and umbels of cumin, collected from Assuit, El-Gharbia and El-Minia governorates on PDA medium yielded 14 fungi (Table 2). Isolated fungi were purified and identified as: *Alternaria burnsii*, *Aspregillus* sp., *Aspregillus niger*, *Aspregillus flavaus*, *Bipolaris* sp., *Cephalospourium* sp., *Cladosporium* sp., *Chatomium* sp., *Curvularia* sp., *Drechslera* sp., *Nigrospora* sp., *Penicillium* spp., *Stemphylium* sp., and *Trichoderma* spp. *A. burnsii* had the highest frequency percentage (81.9%). Whereas, frequency (%) of the other isolated fungi ranged between 0.2 to 2.9%

Table 2. Mean repeats (%) of fungi isolated from ten areas observed cumin blight disease symptoms.

Fungi	Frequency (%) at locations										Mean frequency
	1	2	3	4	5	6	7	8	9	10	(%)
Alternaria burnsii	39a*	42a	40a	44a	43a	40a	47a	38a	46a	37a a	81.9a
Aspregillus flavus	0e	2b	0d	8b	0d	0d	0c	0c	1c	0d	2.1c
Aspregillus niger	0e	0c	0d	1c	0d	0d	0c	0c	8b	1cd	2.0c
Aspergillius sp.	0e	0c	0d	0c	3c	0d	0c	0c	0d	1cd	0.8g
Bipolaris sp.	0e	0c	0d	0c	0d	5b	0c	3b	0d	0d	1.6d
Cephalosporium sp.	0e	0c	0d	0c	0d	0d	0c	4b	0d	0d	0.8g
Chatomium sp.	0e	0c	0d	0c	0d	0d	0c	4b	0d	1cd	0.9fg
Cladosporium sp.	1de	0c	0d	0c	0d	0d	0c	0c	0d	0d	0.2h
Curvularia sp. .	3bc	1bc	5b	0c	0d	1d	0c	3b	0d	2c	2.9b
Drechslera sp.	2cd	2b	0d	0c	0d	0d	0c	0c	0d	0d	0.8g
Nigrospora sp.	4b	0c	2c	0c	0d	0d	0c	0c	0d	0d	1.2ef
Penicillium spp.	0e	0c	0d	0c	0d	3c	2b	0c	0d	6b	2.2c
Stemphylium sp.	1de	1bc	0d	0c	3c	0d	2b	0c	0d	0d	1.4de
Trichoderma spp.	1de	0c	0d	0c	5b	0d	0c	0c	0d	0d	1.2ef

***Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

Pathogenicity tests:

Pathogenicity tests of ten isolates of *A. burnsii*, isolated from different localities at three governorates confirmed that they were all pathogenic to cumin plants exhibiting typical symptoms of Alternaria blight disease. The disease symptoms started to show as minute lesions (0.1-0.4cm) on umbels, leaves and stems after 4, 5, and 7 days, respectively. Dark brown to black lesions (0.8-1.2 cm) developed10 days after inoculation on all infected plant tissues. The adjacent lesions coalesced very rapidly especially on umbels and leaves. Disease severity ranged between 30-95% 10 days after inoculation. Isolate "Ab02"of *A. burnsii* was the most virulent isolate, since it yielded the highest disease severity percentage followed by isolate "Ab03" (Table 3).

In vivo experiment

Effect of potassium silicate and nano silicon on the linear growth of *A. burnsii* (isolate # Ab02):

The mycelial growth of *A. burnsii* (isolate # Ab02) varied in its sensitivity against PS and NSi. In general, suppression of the fungal growth was increased when the concentration of each silicon source was increased (Table 4). Suppression, however, was observed at 50 ppm for the PS Complete growth inhibition was obtained at 2.5 mM or above for NSi and 200 ppm or above for PS (Table 4). Both species of *Trichoderma* had moderate level of growth inhibition against *A. burnsii* (Table 4).

Table 3. Pathogenicity tests of 10 isolates of *A. burnsii* to cumin plants (45-day-old), grown under greenhouse conditions.

Isolate code	Disease severity (%)(15 days after inoculation)
Ab01	70c*
Ab02	95 a
Ab03	85b
Ab04	65d
Ab05	60e
Ab06	65d
Ab07	35h
Ab08	40g
Ab09	50f
Ab010	30i
Untreated control	0.0j

***Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

Table 4. influence of green chemicals, biological control agents, and a chemical fungicide on the superficial growth of *A. burnsii* on PDA.

Treatment	Concentration	Radial growth (cm)	Growth inhibition (%)
Nano silicon (NSi)	1 mM	7.6± 0.400 b*	15.6cd
	1.5 mM	4.5± 0.300c	50.0bc
	2 mM	2.3±0.300 e	74.4ab
	2.5 mM	0.0±0.000 h	100a
	3 mM	0.0±0.000 h	100a
Potassium silicate (PS)	50 ppm	1.50±0.400 f	83.0ab
	100 ppm	0.80±0.200 g	91.1ab
	200 ppm	0.0±0.000 h	100a
	300 ppm	0.0±0.000 h	100a
	400 ppm	0.0±0.000 h	100a
Untreated Control	PDA	9.00±0.300 a	0d
T. harzianum	Dual culture on PDA	2.80±0.200 d	68.9ab
T. hamatum	Dual culture on PDA	2.90±0.300 d	67.8ab
Untreated Control for biocontrol agents	PDA	9.00±0.200 a	0d

***Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

Greenhouse experiment

Effect of green chemicals and antagonistic bioagents on disease severity of Alternaria blight in greenhouse

The effect of spraying four *T. harzianum* formulations, PS, NSi and Ridomil gold plus on cumin plants artificially inoculated with *A. burnsii* was investigated (Table 5). All treatments used significantly decreased the severity of disease, 10 days after spraying. In all cases, disease severity was always decreased by increasing the concentration of each treatment. It was 95.5% in the untreated control (sprayed with water only). *T. harzianum* dry spores (at 1×10^7 spores/ml) in suspension emulsion was the most effective treatment in suppressing disease, followed by NSi. The chemical fungicide Ridomil gold plus gave 92.01% disease control.

Field experiment

Effect of spraying four *T. harzianum* formulation, nano silicon, potassium silicate and the chemical fungicide Ridomil gold plus on severity of Alternaria blight in the field in two growing seasons (2018/2019 and 2019/2020):

Spraying cumin plants with four *T. harzianum* formulations (1×10^6 propagules/ml), NSi (2.5mM), PS (300 ppm) and the fungicide Ridomil gold plus significantly decreased percentages of disease severity under natural infection 30 days after spraying comparing with the untreated control (Table 6). Among the *T. harzianum* formulations tested, emulsion of dried spores was the most effective in suppressing the disease in both seasons. This formulation was the second best (43.9% disease control) after the chemical herbicide (52.44%) and NSi came in the third position (37.81%).The *T. harzianum* suspension in water was the least effective treatment (24.39% disease control) in comparison with the untreated control.

Table 5. Influence of three *T. harzianum* formulations, Nano silicon, potassium silicate and the chemical fungicide Ridomil gold plus, on disease severity under artificial vaccination with *A. burnsii*, 10 days after spraying in the greenhouse.

Treatment	Concentration	(%) Disease severity (after 10 days)	(%) Reduction in disease
Nano silicon (NSi)	0.0	95.5a*	----------
	2 mM	60.0 e	37.17o
	2.5 mM	26.25p	72.51e
	3 mM	25.0q	73.82d
Potassium silicate (PS)	0.0	95.5a	----------
	200 ppm	64.25c	32.72q
	300 ppm	37.75l	60.47i
	400 ppm	30.0o	68.59f
Suspension of fresh *T. harzianum* spores and mycelium in water	0.0	95.5a	----------
	1×10^5 propagules/ml	72.5b	24.08r
	1×10^6 propagules/ml	50.5f	47.12n
	1×10^7 propagules/ml	46.5h	51.31m
Emulsion of fresh *T. harzianum* spores and mycelium with its filtrate in corn oil	0.0 propagules/ml	95.5a	----------
	1×10^5 propagules/ml	62.5d	34.55p
	1×10^6 propagules/ml	44.0i	53.93l
	1×10^7 propagules/ml	39.5k	58.64j
Emulsion of fresh *T. harzianum* spores and mycelium without its filtrate in corn oil	0.0 propagules/ml	95.5a	----------
	1×10^5 propagules/ml	50.0g	47.64n
	1×10^6 propagules/ml	36.25m	62.04h
	1×10^7 propagules/ml	31.5n	67.01g
Emulsion of *T. harzianum* dried spores in corn oil	0.0 spores/ml	95.5a	----------
	1×10^5 spores/ml	41.25j	56.81k
	1×10^6 spores/ml	22.25r	76.71c
	1×10^7 spores/ml	18.75s	80.46b
Ridomil gold plus	1.5 g/L	7.63t	92.01a
Untreated control I	----------	0.00	----------

***Values followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

Table 6. Effect of *T. harzianum* formulation, Nano silicon, potassium silicate and the fungicide Ridomil gold plus as spray treatment on Alternaria blight disease severity in cumin plants grown under felid conditions, during 2018/2019 and 2019/2020 seasons.

Treatments	2018/2019 Season		2019/2020 Season	
	% Disease severity (after 10-30 days)	% Disease Control	% Disease severity (after 10-30 days)	% Disease Control
Nano silicon (NSi)	10.5 de*	43.24c	12.75e	37.81c
Potassium silicate (PS)	12.50 cd	32.43d	13.25 d	35.37d
Suspension of fresh *T. harzianum* spores and mycelium in water	16.00 ab	13.51g	17.50 b	24.39g
Emulsion of fresh *T. harzianum* spores and mycelium with its filtrate in corn oil	13.50 bc	27.03f	14.25 c	30.48f
Emulsion of fresh *T. harzianum* spores and mycelium without its filtrate in corn oil	12.75 cd	31.08e	13.50 d	34.15e
Emulsion of *T. harzianum* dried spores in corn oil	9.00 e	51.35b	11.50 f	43.90b
Ridomil gold plus	8.00 e	56.76a	9.75 g	52.44a
Untreated control	18.50 a	----------	20.50 a	----------

***Values followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

- **Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate and the chemical fungicide Ridomil gold plus as spray treatments on weight of 100 seeds and their essential oil content (ml) in field trials in two growing seasons (2018/2019 and 2019/2020):**

All the tested treatments except the Suspension of fresh *T. harzianum* spores and mycelium in water significantly increased the yield of dry seeds per plant and oil yield (ml/100g dry seeds) in comparison with the untreated control in both seasons (Table 7). With regard to the weight of 100 seeds, the chemical fungicide and the emulsion of *T. harzianum* dried spores in corn oil gave the greatest yield with no significant differences between them in both seasons (Table 7, Fig. 2). With regard to the yield of essential oil, the chemical fungicide, the emulsion of *T. harzianum* dried spores in corn oil, and NSi gave the greatest yield with no significant differences between them in both seasons (Table 7, Fig. 3).

Table 7. Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate and the chemical fungicide Ridomil gold plus as spray treatments on weight of 100 seeds and their essential oil content (ml) in field trials in two growing seasons (2018/2019 and 2019/2020).

Treatments	2018/2019 season				2019/2020 season			
	Weight / 100 seeds (g)	Increase (%)	Essential oil (ml/100 g seed)	Increase (%)	Weight / 100 seeds (g)	Increase (%)	Essential oil (ml/100 g seed)	Increase (%)
Nano silicon (NSi)	0.388b*	6.89	6.7ab	15.52	0.450b	17.80	7.6ab	22.58
Potassium silicate (PS)	0.390ab	7.44	6.2c	6.98	0.430bc	12.57	7.4ab	19.35
Suspension of fresh *T. harzianum* spores and mycelium in water	0.365cd	0.55	6.1cd	5.17	0.390d	2.09	6.5c	4.83
Emulsion of fresh *T. harzianum* spores and mycelium with its filtrate in corn oil	0.385bc	6.06	6..3c	8.62	0.420c	7.540	7.1b	14.52
Emulsion of fresh *T. harzianum* spores and mycelium without its filtrate in corn oil	0.388b	6.89	6.4bc	10.34	0.450b	17.80	7.3ab	17.74
Emulsion of *T. harzianum* dried spores in corn oil	0.405ab	11.57	6.8a	17.24	0..478ab	25.13	7.5ab	20.96
Ridomil gold plus	0.410a	12.95	6.9a	18.97	0.490a	28.27	7.8a	25.81
Untreated control	0.363d	----------	5.8d	----------	0.382de	---------	6.2c	--------

***Values followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

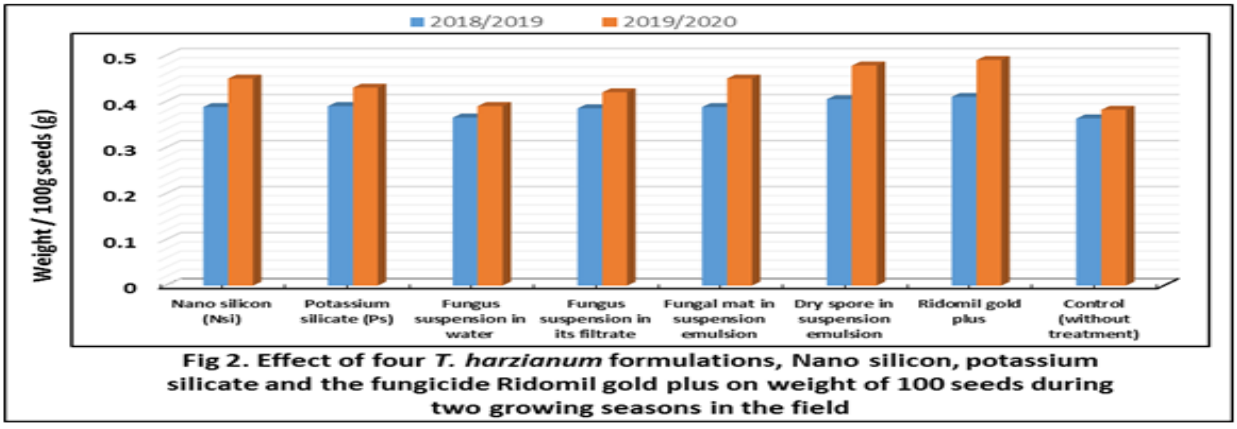

Fig 2. Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate and the fungicide Ridomil gold plus on weight of 100 seeds during two growing seasons in the field

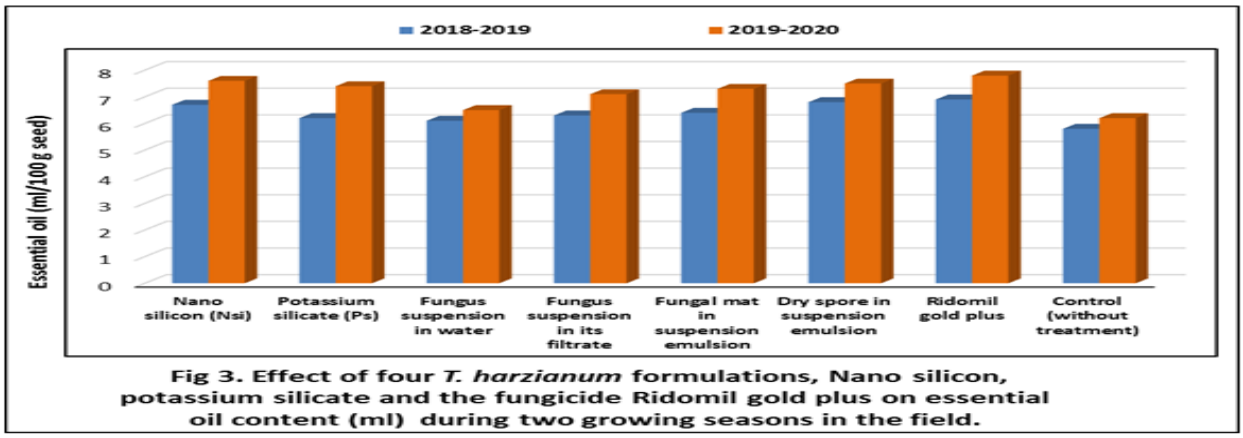

Fig 3. Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate and the fungicide Ridomil gold plus on essential oil content (ml) during two growing seasons in the field.

- **Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate, and the fungicide Ridomil gold plus on major compounds in cumin essential oil in cumin seeds of plants grown under field conditions during 2019/2020 seasons.**

The active substance cumin aldehyde represents the most important constituent of oil with regard to its quality. Cumin aldehyde increased by 60.49 and 54.32% when plants sprayed with Ridomil gold plus and emulsion of *T. harzianum* dried spores in corn oil, respectively with no significant difference between them comparing with the untreated control (Table 8, Fig. 4).

Table 8. Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate, and the fungicide Ridomil gold plus on major compounds in cumin essential oil in cumin seeds of plants grown under field conditions during 2019/2020 seasons.

Treatment	Constituents (%)			
	Myrcene & Phellandrene	y-terpinene	Cumin aldehyde	1ncrease
Nano silicon (NSi)	22.3ab*	38.9c	11.0b	35.80
Potassium silicate (PS)	21.1deF	38.6cd	10.2c	25.93
Suspension of fresh *T. harzianum* spores and mycelium in water	18.1cd	42.6a	9.1d	12.35
Emulsion of fresh *T. harzianum* spores and mycelium with its filtrate in corn oil	21.6cd	38.8c	9.7d	19.75
Emulsion of fresh *T. harzianum* spores and mycelium without its filtrate in corn oil	21.8bc	38.2d	10.1d	24.79
Emulsion of *T. harzianum* dried spores in corn oil	22.7a	35.5e	12.5a	54.32
Ridomil gold plus	22.8a	34.2f	13.0a	60.49
Untreated control	20.6e	41.4b	8.1e	----------

***Values followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$).**

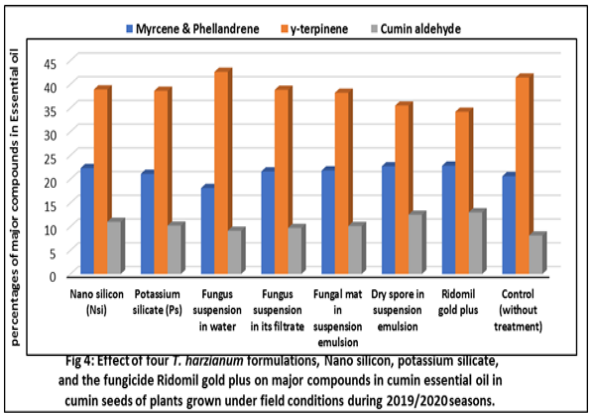

Fig 4: Effect of four *T. harzianum* formulations, Nano silicon, potassium silicate, and the fungicide Ridomil gold plus on major compounds in cumin essential oil in cumin seeds of plants grown under field conditions during 2019/2020 seasons.

Discussion

Cumin is one of the ancient aromatic crops grown in Egypt. A few years ago, a severe occurrence of cumin blight has been observed on cumin cv. Baladi cultivated in Egypt causing a drastic reduction in seed yield and quality,

During the course of this study, *A. burnsii* was the most common fungus isolated from cumin plants. Pathogenicity test revealed that this fungus attacks cumin plant causing blight disease, which leads to disease ultimately causing yield loss of seeds and reduction in volatile oils content and the major constituents of oil. The quality of seeds is also affected by the disease, whereas seeds produced by affected plants became shriveled and discolored. The severe Alternaria blight may lead to plant kill. Such results are in line with those reported by Uppal *et al.* (1938); Pandey (2010); Sharma *et al.* (2013), and Singh *et al.* (2016) who studied the effect of Alternaria blight on seed yield and volatile oil of other crops.

In parallel to these results, Marschner (2012) reported that K is a major plant element which plays a major part in photosynthesis, a variety of physiological processes, maintenance of water status and protein synthesis in plant tissues. . Our results showed that the application of NSi caused a significant reduction in DS and DI of cumin Alternaria blight.. Such findings agree with those of Sapre and Vakharia (2016) and Das *et al.* (2017). They may be due to that NSi increases cell wall thickness. Khadiga Hasan *et al.* (2020) in their ultrastructure and physiological study on faba bean reported that NSi has increased the cell wall thickness while the other cell organelles were kept in a good shape. In addition, NSi treatment increased the total phenols. In addition, it improved the activities of protective enzymes peroxidase (POD) and polyphenol oxidase (PPO). Besides, it enhanced the accumulation of protein in infected plants in comparison with the pathogen-alone treatment.

This research was carried out using a modern method for reducing the detrimental effect on cumin plants caused by *A. burnsii*. Aqueous solutions of the PS and NSi, and the spore suspensions of antagonistic biological control agents *T. harzianum* and *T. hamatum* besides Ridomil fungicide increased the survival of cumin plants even the plants were grown under the stress of *A. burnsii*. The in-vitro studies showed a significant inhibition effect of these materials against the growth of the pathogen *A. burnsii*.

Many researchers found that *Trichoderma* spp. are very strong biocontrol agents against several plant diseases. Vey *et al.* (2001); Raaijmakers *et al.* (2009) stated that some toxic metabolites against phytopathogens were produced by *Trichoderma*, which include alamethicins, harzianic acid, peptaibols, tricholin, 6-penthyl-α-pyrone, antibiotics, massoilactone, gliovirin, viridian, heptelidic acid and glisoprenins.

The results presented here show that the most effective plant treatments for best seed yield of cumin plant were Ridomil fungicide, *T. harzianum* dry spore in suspension emulsion and NSi. Similar findings were reported by Sunder (2005) and Shilkha and Pandey (2013) who reported that the resistance to blight disease was significantly increased by spraying plants with biocontrol agents. Plant disease control with *Trichoderma* builds on its metabolic versatility, ability to degrade organic substrates, tolerance to microbial inhibitors on the plant.

The in-*vivo* studies insured a good potential of spraying cumin plants with Ridomil fungicide, *T. harzianum* dry spore in suspension emulsion, and NSi for combating Alternaria blight disease and increasing the content of the photosynthetic pigments and total phenols. Phenolic compounds are known as stimulant to the immune system of the plants as described by Taiz and Zeiger (2002) and Ibrahim *et al.* (2015). Elwakil *et al.* (2019).

Our results of field trials insured that spraying cumin plants with *T. harzianum* dry spore in suspension emulsion before showing disease symptoms significantly reduced the incidence and severity of Alternaria blight disease. They significantly increased the content of the photosynthetic pigments and the total phenols in plants,

The qualitative analysis of major constituents of essential oil of the tested cumin seeds were determined. They were Myrecene, α-phellandrene, y-terpene and cumin aldehyde. Data indicated also that the cumin plants sprayed with *T. harzianum* dry spore in suspension emulsion were superior in their contents of oil over the untreated control plants .Such results demonstrate that the essential oil contents of cumin plants might play a role in cumin resistance to the disease. Similar finding was reported by Pandey (2010), Kamkar *et al*. (2011) and Singh *et al*. (2015).

REFERENCES

Agarwal, U.; Pathak, D. P.; Kapoor, G.; Bhutani, R.; Roper, R.; Gupta, V., and Kant, R. (2017). Review on *cuminum cyminum* – Nature's Magical seeds. J. Chemical and Pharmaceutical Res., 9(9): 180-187.

Barnett, H. L. and Hunter, B. B. (1972). Illustrated Genera Imperfect Fungi. 2nd Ed. Byrgess Publishing Co., Minnesota, U.S.A. 241 PP.

Chester, K. S. (1959). How sick is the plant? In: Plant Pathology an Advanced Treatise (Eds. J.G. Horshfall and A.E. Diamond). Vol. 1, Academic Press, New York, 199-242 pp.

Clements, F. E. and Chear, G. L. (1957). The Genera of Fungi. Hofiner Publishing Co., New York, 496 pp.

Cumagun, C. J. R. (2014). Advances in formulation of Trichoderma for biocontrol In: Biotechnology and Biology of Trichoderma, Gupta,V.K.;Schmoll,M. and Tuohy,M.G.(2014) PP :527-531.

Das, K. K.; Swamy, G. S.; Biswas, D. and Chnaniya, K. K. (2017). Response of soil application of diatomaceous earth as a source of silicon on leaf nutrient status of guava. Int. J. Curr. Microbiol. App. Sci., 6(4): 1394-1399.

Deepak; Arora, D. K.; Saran, P. L.and Lai, G. (2008). Evaluation of cumin varieties against blight and wilt disease with sowing. Annals of Plant Protection Sciences 16(2): 441-443.

Devappa, V.; Sangeetha, C. G. and Jhansirani, N. (2020). Bio prospecting of diseases of horticultural crops in India In: Emerging Trends in Plant Pathology, Singh K.P.; Jahagirdar, S. and Sarma; B.K.(2021). PP:425-455

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11: 1-42. Cross Ref Direct Link.

EL-Keltawi, N. E. and R. Croteau (1986). Influence of ethephon and daminozide on growth and essential oil content of content of peppermint and sage. Phytochemistry. 25: 128-1288.

Elwakil, M. A.; El-Metwally, M. A.; Khalil, A. E. and Eisa, K. N. (2019). Scaling up the phytochemical contents in the leaves of beetroot using a formulated antioxidant Gawda®. J. Plant Prot. and Path., Mansoura Univ., Egypt. 10 (7): 377-379.

Gomez, k. A. and A. A. Gomez (1984). Statistical Procedures for Agricultural Research. 2nd.ed., John Willey and Sone Inc. New York, pp.: 95-109.

Guenther, E. (1961). The Essential oil. D. Van Nor stand Inc. Com. New York, vol. 1,2., 569 pp.

Ibrahim, M. F. M.; Abd Eljawad, H. G. and Bondok, A. M. (2015). Physiological impacts of potassium citrate and folic acid on growth, yield and some viral diseases of potato plants. Middle East Journal of Agriculture Researches, 04(03): 577-589

Kamkar, B.; Koocheki, A., Mahallati, M. N.; Teixeira da Silva, J.A. Moghaddam, P. R. and Kafi, M. (2011). Fungal diseases and inappropriate sowing dates, the most important reducing factors in cumin fields of Iran, a case study in Khorasan provinces. Crop Protection 30(2): 208-215.

Khadiga A. Hasan, Hoda Soliman, Zakaria, B. and Shabana, Y. M. (2020). Efficacy of nano-silicon in the control of chocolate spot disease of *Vicia faba L.* caused by *Botrytis fabae*. Egyptian J. Basic and App. Sci., 7: 53-67.

Kumar, D. J. (2015). Integrated management of cumin (*Cuminum cyminum* L.) blight caused by *Alternaria burnsii*. J. Trends in Biosciences 17(8): 4621-4624.

Kumar, S.; Thakur, M. and Rani, A. (2014). *Trichoderma*: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. African J. Agri. Res., 9 (53): 3838-3852.

Marschner, H. (2012). Mineral nutrition of higher plants, 3rd edn. Academic Press, New York. USA.

Menzies, J. G.; Ehret, D. L.; Glass, A. D. M.; Helmer, Koch, C. and Seywerd, F. (1991). Effects of soluble silicon on the parasitic fitness of *Sphaerotheca fuliginea* on *Cucumis sativus*. Phytopathology 81:84-88.

Pandy, R., A.K. Misra, H.B. Singh, A. Kalra, and Singh. D. (2019). Diseases of Medicinal and Aromatic Plants and Their Management. Pages 335-339. Today and Tomorrow Printers and Publisher, New Delhi, India.

Ramanujam,B.; Prasad, R. D. ; Sriram S. and Rangeswaran, R. (2010). Mass production, formulation, quality control and delivery of *Trichoderma* for plant disease management. The Journal of Plant Protection Sciences 2(2): 1-8.

Rana, A.; Kumar, V.; Mirza, A. and Panghal, A. (2018). Efficacy of cumin (*Cuminum cyminum* L.) as a bionutrient and its management. Annals of Biology 34(2): 218-222.

Rossman, A. Y.; Palm M. E. and Spielman, L.J. (1994). Literature Guide for the Identification of Plant Pathogenic Fungi. APS Press, St. Paul, Minnesota, U. S.A., 252pp.

Rotem, J. (1994). The Genus Alternaria. APS Press, St. Paul, Minnesota, U.S.A., 326pp.

Sapre, S. S. and Vakharia, D. N. (2016). Role of silicon under water deficit stress in wheat: (Biochemical perspective): A review. Agricultural Reviews., 37(2): 109-116.

Sharma, S. (2010). Studies on cumin blight incited by *Alternaria burnsii* (UPPAL, PATEL AND KAMAT) and its management. M.Sc. Thesis, Anand Agricultural University, Anand.

Sharma, S. and Pandey, R. N. (2011). Evaluation of bacterial and fungicidal bioagent against *Alternaria burnsii* causing blight of blight. J. Plant Disease Sciences 2(6): 191-192.

Sharma, Y. K.; Choudappa, P. C. and Anwer, M. M. (2013a). Efficacy of fungicides for the management of Alternaria blight of cumin. Intel J. Seed Spices 3,1(1): 48-49.

Sharma, Y. K.; Kant, K.; Solanki, R. k. and Saxena, R. P. (2013b). Prevalence of cumin diseases on farmer's field: a survey of Rajasthan and Gujarat states. J. Seed Spices 3(2):46-49.

Shekhawat, N. ; Trived, A. Kumar, A. and Sharma, S. K. (2013). Management of *Alternaria burnsii* causing blight of cumin. International Journal of Plant Protection 6(10): 280-284.

Shikha, S. and Pandey, R. N. (2013). Survival, epidemiology and management of Alternaria blight of cumin. Gujarat. J. Indian 5(10): 639-642.

Singh, N. K.; Patel, D. B.; Chaudhari, S. R.; Morad, B. G.; Rabari, S. M.; Chattopadhyay, A.; Tetarwal, M. L. and Prajapati, M. R. (2016). Characterization of the plant pathogenic isolates of *Alternaria burnsii.* National Academy of Agricultural Science 34(6): 1461-1465.

Singh, S.; Trivedi, A.; Mathur, K. and Padamini, R. (2015). Assessment of yield loss of cumin *(Cuminum cyminum*) caused by Alternaria leaf blight and pathogen recovery from infected seed. Indian Phytopathology 68(3): 350-352.

Sunder, S. (2005). Studies on Cumin Blight Caused by *Alternaria burnsii* (ppal, Patel and Kamat. M. Sc. Thesis, Chaudhary Charan Singh Haryana Agricultural University.

Taiz, L. and Zeiger, E. (2002). Plant Physiology, 3rd Edition. Sinaur Associates Inc., Sunderland, MA, USA., Pp. 224.

Uppal, B. N.; Patel, M. K. and Kamat M. N. (1938). Alternaria blight of cumin. J. Agricultural Science 8(1): 49-62.

Vey, A.; Hoagland, R. E. and Butt, T. M. (2001). Toxic metabolites of fungal biocontrol agents. Pages 311-346 In: Alain Vey, Robert Hoagland and Tarik Butt, Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI, Bristol, UK.

Wheeler, B. E. J. (1969). An Introduction of Plant Diseases. John Willy & Sons Ltd., London, 301 pp.

مكافحة مرض لفحة الكمون المتسبب عن فطر ألترناريا بارنسياى باستخدام الكيماويات الخضراء والمبيدات الحيوية

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يعتبر مرض لفحة الألترناريا في الكمون المتسبب عن الفطر ألترناريا بارنسياي أخطر الأمراض التي تصيب الكمون في مناطق زراعته بالعالم. وقد تم عزل فطر ألترناريا بارنسياي من نباتات الكمون المصابة باللفحة وإثبات قدرته الإمراضية على نباتات الكمون. كما تم تقييم بعض الكيماويات الخضراء (سلكيات البوتاسيوم و نانو سليكون) من حيث قدرتها التثبيطية لنمو الفطر المسبب للمرض في المعمل بتركيزات مختلفة (50 و100 و200 و300 و400 جزء في المليون لسلكيات البوتاسيوم و 1.5 و2 و2.5 و3 و3.5 مللي مولر للنانو سليكون) من أجل تحديد التركيز الأمثل لدراسة تأثيره في مقاومة المرض في ظروف الصوبة والحقل. كما تم دراسة التأثير التثبيطى للفطرين ترايكودرما هارزيانم وترايكودرما هاماتم ضد المسبب المرضى (ألترناريا بارنسياي) فى المعمل. ثم صياغة اكثرهما كفاءة في تثبيط المسبب المرضى في صورة مبيد حيوي تم تقييمه في مكافحة مرض اللفحة في الكمون في ظروف الصوبة والحقل. حيث تم صياغة الفطر ترايكودرما هارزيانم واستخدامه بأربعة صور وهى: معلق جراثيم وميسيليوم الفطر الطازجة في الماء، ومستحلب جراثيم وميسيليوم الفطر الطازجة في راشح المزرعة وزيت الذرة، ومستحلب جراثيم وميسيليوم الفطر الطازجة بدون راشح المزرعة في زيت الذرة، ومستحلب جراثيم الفطر المجففة في زيت الذرة وذلك رشا على النباتات. وقد تبين من هذه الدراسة أن مستحلب الجراثيم المجففة لفطر الترايكودرما بتركيز 1×10^6 كانت أفضل المعاملات على الإطلاق، وكان نانو سليكون في المرتبة الثانية من حيث كفاءتها في مقاومة المرض وبالتالي زيادة المحصول من البذور وزيادة نسبة الزيت الطيار في البذرة مقارنة بالمبيد الفطري.