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Efficacy of some medicinal plants extract to improve cumin wilt resistance caused by *Fusarium oxysporum* f. sp. Cumini

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Abstract : Cumin (*Cuminum cyminum* L.) is an annual plant known for its pharmaceutical and medicinal importance. Cumin wilt is a serious problem due to the susceptibility of the crop to *Fusarium oxysporum* f. sp. cumini resulting in severe crop losses to growers. The present study was undertaken to evaluate the possibility of using natural plant extracts for combating the negative effect of *Fusarium oxysporum* on growth of cumin. Results clearly indicated that soaking cumin seeds in *Plectranthus amboinicus* aqueous extract at the concentration of 5% prior to sowing in *Fusarium oxysporum* infested soil enhanced growth of cumin plants in terms of plant height, numberof branches and umbels, plant fresh weight and seed yield/plant as compared to the other treatments. The extracts of all tested plants showed higher *invitro*antifungal activity against *Fusarium oxysporum* f. sp. cumini, where 100% inhibition of mycelial growth of *Fusarium oxysporum* f. sp. cumini was recorded with 20% aqueous extracts of all plants under study. Highest antifungal effect was accompanied with high phenols, flavonoids, alkaloids and antioxidants content.
Keywords : *Cuminum cyminum- Fusarium oxysporum- In vitro-* Extracts- Phenols- Flavonoids Alkaloids-Antioxidants.

Introduction

Cumin (*Cuminum cyminum* L.) is an annual plant belongs to Family *Apiaceae*, in addition to its common use as spice in our daily life, recent studies have indicated its pharmaceutical and medicinal importance[1].

The fungal pathogen *Fusarium oxysporum* affects a wide variety of hosts at any age. Tomato, tobacco, legumes, cucurbits, sweet potatoes and banana are a few of the most susceptible plants, but it will also infect other herbaceous plants[2]. Significant loss in cumin yield can be attributed to the adverse effects caused by biotic stresses of which the Fusarium wilt disease is the most serious one[3]. Cumin wilt caused by *Fusarium oxysporum* f. sp. cumini is a destructive disease of this crop and results in yield losses of up to 80%[4].

For many years synthetic pesticides have been used for controlling plant pathogenic caused by fungi or bacteria. However, chemical strategy such as pesticides causes environmental pollution and healthy problems due to their slow biodegradation, phytotoxicity, carcinogenicity and toxic waste in agricultural products[5]. Recently, there is growing interest for possible use of plant secondary metabolites for pest and disease control in agriculture. Biological control has proven to be effective against a variety of pathogenic fungi and bacteria. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action[6]. Some of natural substances showed antifungal, fungistatic or fungicidal activities which

allow protected crops to have an extended shelf life by preventing enzymatic or metabolic processes of microorganisms[7].

Jatropha curcas, Plectranthus amboinicus, Thymus vulgaris and *Withania somnifera* extracts have several biologically active compounds with potent antimicrobial activity and could be used to effectively replace synthetic chemicals with novel mechanisms of action[8,9,10,11,12].

Chemical control using fungicides have been ineffective and the diseases become visible and problematic on cumin especially periods to harvest during seed ripening. Few fungicides would even be permitted for use on an edible seed spice so close to harvest due to residue concern. Accordingly, the aim of this study is improving cumin resistance using natural plants extract.

Materials and Methods

This study was carried out in two successive seasons (2012/2013 and 2013/2014) at Horticulture Research Institute(HRI), Agricultural research Center, Egypt. Cumin seeds cv. 'Baladi' used in this study were obtained from Medicinal and Aromatic plants Research Department, HRI.

Plant Extracts Preparation

100 g fresh aerial parts of all studied plants (*Jatropha curcas, Plectranthus amboinicus, Thymus vulgaris* and *Withania somnifera)* were washed thoroughly under running tap water and sterile distilled water and chopped with 200 ml distilled water (w/v) by using a domestic blender for 1 min at average speed. The mixture were macerated during 24 h at 4ºC. After that, resulting extracts were filtered through double layered Whatman No.4 filter paper for field experiment and sterilized using a 0.45 µm pore size cellulose acetate membrane filter (Cole-Parmer-47 mm) for antimicrobial experiment. Dilutions were prepared (5, 10 and 20 %).

Isolation and identification of the casual pathogen

The pathogen *Fusarium oxysporum* was isolated from wilt symptom showing on cumin plant roots as described by[13] and maintained on PDA medium (Potato Dextrose Agar medium). Fungal were refreshed and maintained on PDA slants. The cultures were streaked on sterile potato dextrose agar and kept in incubator for 7 days at 25°C.

Phytochemical analysis

Phytochemical analysis of four plants extract were performed. Total phenols[14], total flavonoids[15], vitamin C as mg ascorbic acid[16], total alkaloids[17] and antioxidant activities[18] were determined.

Field experiment

Seeds were soaked in the extraction treatments and water (positive control) for 30 min and ten seeds were planted in each clay pot (25 cm) at depth of 2 cm in the first week of November. Plants were fertilized with half strength Hoagland's solution twice a week. The inoculum of the pathogen was prepared by growing the fungus on Barley grain medium for 20 days at 22-25°C. Soil infestation was carried out by mixing the pathogen inoculum thoroughly with sterilized sandy soil at the rate of 5% of soil weight[19]. The infested soil was then placed in sterilized pots. The untreated seeds grown in the sterile soil free of the pathogen (negative control) were also used. The experiment in both seasons contained 12 treatments in addition to the positive and negative control with three replicates containing ten pots each as follow:

1. *Jatropha curcas* aqueous extract concentrations (5, 10 and 20%)
2. *Plectranthus amboinicus* aqueous extract concentrations (5, 10 and 20%)
3. *Thymus vulgaris* aqueous extract concentrations (5, 10 and 20%)
4. *Withania somnifera* aqueous extract concentrations (5, 10 and 20%)

Data of plant growth ; plant height (cm), number of branches, number of umbels, fresh weight/plant (g) and total seed yield/plant (g) were taken in March.

Antifungal activity assay

Autoclaved PDA medium with aqueous extracts of the tested plants were prepared and allowed to solidify. After complete solidification of the medium, five mm disc of seven day old culture of the tested fungi were placed in the centre of the petri plates and incubated at 28± 2°C for six days then observations were recorded by the seventh day. The colony diameter was recorded in terms of cm. PDA medium devoid of extract served as control. For each treatment three replicates were maintained. The fungi toxicity of extracts was calculated in terms of percent inhibition of mycelia growth by using the formula:

% inhibition = dc- dt / dc x 100

Where, dc= Average increase in mycelia growth in control. dt = Average increase in mycelia growth in treatment[20].

Statistical analysis

Each experiment was repeated twice, completely randomized design was used and the represented data were averages. Results were analyzed by analysis of variance (ANOVA) and least significant difference (L.S.D. at 5% level) was also adopted according to[21].

Results

Phytochemical composition of aqueous plant extracts

Results shown in Table (1) demonstrate the analysis of aqueous extracts of *Jatropha curcas, Plectranthus amboinicus, Thymus vulgaris* and *Withania somnifera*. Data clearly revealed that, the aqueous extract of *Jatropha curcas* demonstrated the highest content of phenols (0.060%) followed by *Withania somnifera* (0.049%). Moreover, data showed the highest content of flavonoids in *Withania somnifera* (0.0361 mg/100g) followed by *Jatropha curcas* (0.0257 mg/100g). On the other hand, V.C. content recorded in descending order 20.98, 17.63, 7.693 and 3.881 mg/100g for *Plectranthus amboinicus, Thymus vulgaris, Withania somnifera* and *Jatropha curcas*. *Withania somnifera* contained more alkaloids (0.00695%) as compared to other extracts. Concerning antioxidants, *Thymus vulgaris* recorded the highest value (0.0099 mg/100g).

Table 1. Phytochemical composition of plants aqueous extract

	Phenols (%)	**Flavonoids (mg/100g)**	**V.C. (mg/100g)**	**Alkaloids (%)**	**Antioxidants (mg/100g)**
Jatropha curcas	0.060	0.0257	3.881	0.00084	0.0084
Plectranthus amboinicus	0.020	0.0097	20.980	0.00013	0.0079
Thymus vulgaris	0.029	0.0185	17.630	0.00028	0.0099
Withania somnifera	0.049	0.0361	7.693	0.00695	0.0075
L.S.D. at 5%	0.002	0.0064	0.520	0.00018	0.00065

Field experiment:

Data represented the effect of different concentrations (%) of plants aqueous extract on plant height (cm)of cumin plants during the first and second seasons (Table 2) revealed that, aqueous extract of *Plectranthus amboinicus* gave the tallest plants in average (17.73 and 18.40 cm in the first and second seasons, respectively). In respect to the effect of extract concentration, the lowest concentration (5%)produced the tallest plants in average (18.33 and 18.42 cm during the first and second seasons, respectively), while the highest concentration (20%) produced the shortest plants in average (15.00 and 14.83 cm during the first and second seasons, respectively). Accordingly, soaking cumin seeds in5% *Plectranthus amboinicus* aqueous extract prior to sowing in *Fusarium oxysporum* infested soil was the most effective treatment which gave the maximum values of 22.33 and 23.00 cm for the first and second seasons, respectively.

Table 2. Effect of soaking cumin seeds in aqueous plants extract with different concentrations (%) on plant height (cm) during two seasons

	First season						Second season					
	Cont-	**Cont+**	**5**	**10**	**20**	**Mean**	**Cont-**	**Cont+**	**5**	**10**	**20**	**Mean**
Jatropha curcas	16.67	15.67	16.33	15.67	14.33	15.73	17.67	16.67	17.00	17.00	13.67	16.40
Plectranthus amboinicus	16.67	15.67	22.33	18.00	16.00	17.73	17.67	16.67	23.00	17.67	17.00	18.40
Thymus vulgaris	16.67	15.67	17.67	14.67	14.33	15.80	17.67	16.67	17.33	14.00	15.33	16.20
Withania somnifera	16.67	15.67	17.00	19.33	15.33	16.80	17.67	16.67	16.33	20.67	13.33	16.93
Mean	16.67	15.67	18.33	16.92	15.00		17.67	16.67	18.42	17.33	14.83	
L.S.D. at 5%	Plant=0.539		Conc.=0.603		Plant x conc. =1.316		Plant=0.774		Conc.=0.865		Plant x conc. =1.843	

Results of branches number as affected by soaking treatments of cumin seeds using aqueous extract of the tested plants at different concentrations (%) are illustrated in Table (3). Concerning the effect of plant type, aqueous extract of *Jatropha curcas* had negative effect on branches number where it gave the lowest means (11.13 and 11.53in the first and second seasons, respectively) as compared to other tested plants. On the other hand, using lower concentrations (5 and 10 %) of the extracts enhanced number of branches recording 14.50 and 13.25, respectively for the first season also 14.50 and 14.17, respectively for the second season as compared with all other treatments. As for the interaction, data revealed that, using *Plectranthus amboinicus* aqueous extract at the lowest concentration (5 %) gave the highest number of branches (16.33 and 17.00) during both first and second seasons, respectively, compared with the other treatments.

Table 3. Effect of soaking cumin seeds in aqueous plants extract with different concentrations (%) on branches number during two seasons

	First season						Second season					
	Cont-	**Cont+**	**5**	**10**	**20**	**Mean**	**Cont-**	**Cont+**	**5**	**10**	**20**	**Mean**
Jatropha curcas	12.33	11.33	11.67	10.67	9.67	11.13	12.67	12.33	10.67	11.67	10.33	11.53
Plectranthus amboinicus	12.33	11.33	16.33	13.67	12.67	13.27	12.67	12.33	17.00	14.33	13.00	13.87
Thymus vulgaris	12.33	11.33	15.33	13.67	12.33	13.00	12.67	12.33	14.33	14.67	10.67	12.93
Withania somnifera	12.33	11.33	14.67	15.00	12.00	13.07	12.67	12.33	16.00	16.00	10.33	13.47
Mean	12.33	11.33	14.50	13.25	11.67		12.67	12.33	14.50	14.17	11.08	
L.S.D. at 5%	Plant=0.437		Conc.=0.488		Plant x conc. =0.999		Plant=0.556		Conc.=0.621		Plant x conc.= 1.366	

Results of umbels number as affected by soaking treatments of cumin seeds using aqueous extract of the tested plants at different concentrations (%) are illustrated in Table (4). Concerning the effect of plant type, aqueous extracts of both *Plectranthus amboinicus* or *Withania somnifera* had beneficial effects on number of umbels recording as average 15.87 and 16.00, respectively for the first season also 15.60 and 16.07, respectively for the second season as well. On the other hand, aqueous extract of *Jatropha curcas* had negative effect on umbels number where it gave the lowest means (12.20 and 11.60in the first and second seasons, respectively) as compared to other tested plants. Concerning extract concentration, the lowest concentration (5%) followed by the concentration of 10 % produced more umbels in average during both first season (18.00 and 16.17, respectively)or second season(17.92 and 15.92, respectively).As for the interaction, data revealed that, using *Plectranthus amboinicus* aqueous extract at the lowest concentration (5 %) seems to be more effective regarding number of umbels (20.67 and 22.33forbothfirst and second seasons, respectively).

Table 4. Effect of soaking cumin seeds in aqueous plants extract with different concentrations (%)on umbels number during two seasons.

	First season						Second season					
	Cont-	**Cont+**	**5**	**10**	**20**	**Mean**	**Cont-**	**Cont+**	**5**	**10**	**20**	**Mean**
Jatropha curcas	13.00	12.67	14.00	11.00	10.33	12.20	13.33	11.33	13.00	11.00	9.33	11.60
Plectranthus amboinicus	13.00	12.67	20.67	17.67	15.33	15.87	13.33	11.33	22.33	18.00	13.00	15.60
Thymus vulgaris	13.00	12.67	19.67	16.67	14.33	15.27	13.33	11.33	17.33	15.33	12.67	14.00
Withania somnifera	13.00	12.67	17.67	19.33	17.33	16.00	13.33	11.33	19.00	19.33	17.33	16.07
Mean	13.00	12.67	18.00	16.17	14.33		13.33	11.33	17.92	15.92	13.08	
L.S.D. at 5%	Plant=0.587		Conc.=0.657		Plant x conc. =1.316		Plant=0.617		Conc.=0.690		Plant x conc.= 1.548	

Results of plant fresh weight (g) as affected by soaking treatments of cumin seeds using aqueous extract of the tested plants at different concentrations (%) are illustrated in Table (5). Concerning the effect of plant type, *Jatropha curcas* aqueous extract gave the lowest fresh weight (0.89 and 0.96 g in the first and second seasons, respectively) as compared to other tested plants. On the other hand, using lower concentrations (5 and 10 %) of the extracts enhanced plant fresh weight (1.74 and 1.69 g, respectively for the first season also 1.83 and 1.66 g, respectively for the second season) as compared with all other treatments. As for the interaction, data revealed that, using *Plectranthus amboinicus* aqueous extract at the lowest concentration (5 %) gave the maximum plant fresh weight(2.35 and 2.80g for both first and second seasons, respectively).

Table 5. Effect of soaking cumin seeds in aqueous plants extract with different concentrations (%) on plant fresh weight (g) during two seasons.

	First season						Second season					
	Cont-	**Cont+**	**5**	**10**	**15**	**Mean**	**Cont-**	**Cont+**	**5**	**10**	**15**	**Mean**
Jatropha curcas	1.05	0.99	0.92	0.78	0.73	0.89	1.23	1.00	0.95	0.78	0.83	0.96
Plectranthus amboinicus	1.05	0.99	2.35	1.92	1.19	1.50	1.23	1.00	2.80	2.04	1.05	1.62
Thymus vulgaris	1.05	0.99	1.89	1.79	1.43	1.43	1.23	1.00	1.80	1.64	1.26	1.38
Withania somnifera	1.05	0.99	1.79	2.29	1.12	1.45	1.23	1.00	1.76	2.17	1.11	1.45
Mean	1.05	0.99	1.74	1.69	1.12		1.23	1.00	1.83	1.66	1.06	
L.S.D. at 5%	Plant=0.068		Conc.=0.076		Plant x conc. =0.177		Plant=0.116		Conc.=0.129		Plant x conc. = 0.269	

Results of seed yield/plant (g) as affected by soaking treatments of cumin seeds using aqueous extract of the tested plants at different concentrations (%) are illustrated in Table (6). Regarding the effect of plant type, aqueous extracts of both *Plectranthus amboinicus* or *Withania somnifera* had beneficial effects on seed yield/plant recording as average 1.85 and 1.89 g, respectively for the first season also 1.90 and 1.82 g, respectively for the second season as well. On the other hand, aqueous extract of *Jatropha curcas* had negative effect on seed yield/plant where it gave the lowest means (1.44 and 1.49 gin the first and second seasons, respectively).On the other hand, using lower concentrations (5 and 10 %) of the extracts enhanced plant seed yield/plant recording as average 1.98 and 1.88 g, respectively for the first season also 2.06 and 1.85 g, respectively for the second season as compared with all other treatments. As for the interaction, data revealed that, using *Plectranthus amboinicus* aqueous extract at the lowest concentration (5 %) showed highest seed yield/plant(2.56 and 2.89 g for both first and second seasons, respectively).

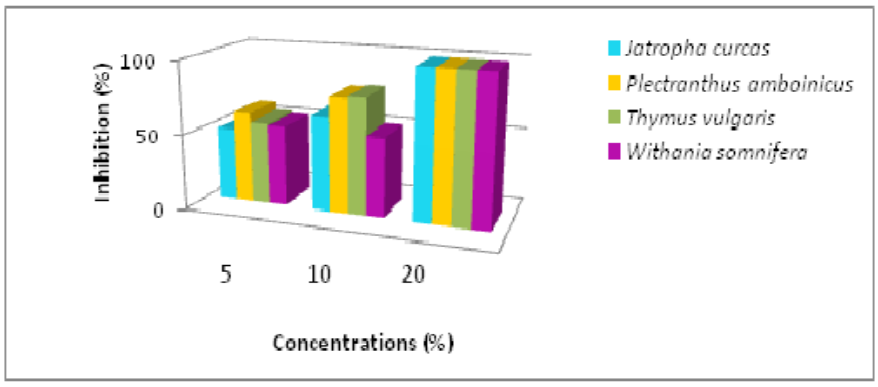
Table 6. Effect of soaking cumin seeds in aqueous plants extract with different concentrations (%)on seed yield /plant (g) during two seasons.

	First season						Second season					
	Cont-	**Cont+**	**5**	**10**	**15**	**Mean**	**Cont-**	**Cont+**	**5**	**10**	**15**	**Mean**
Jatropha curcas	1.64	1.52	1.33	1.25	1.46	1.44	1.62	1.52	1.52	1.25	1.56	1.49
Plectranthus amboinicus	1.64	1.52	2.56	1.98	1.54	1.85	1.62	1.52	2.89	2.08	1.39	1.90
Thymus vulgaris	1.64	1.52	1.97	1.87	1.56	1.71	1.62	1.52	1.88	1.79	1.32	1.63
Withania somnifera	1.64	1.52	2.06	2.44	1.77	1.89	1.62	1.52	1.96	2.30	1.71	1.82
Mean	1.64	1.52	1.98	1.88	1.59		1.62	1.52	2.06	1.85	1.49	
L.S.D. at 5%	Plant=0.095		Conc.=0.106		Plant x conc. = 0.247		Plant=0.114		Conc.=0.128		Plant x conc. = 0.289	

Generally, soaking cumin seeds in *Plectranthus amboinicus* aqueous extract at the concentration of 5% prior to sowing in *Fusarium oxysporum* infested soil gave the tallest plants, highest branches and umbels number, maximum plant fresh weight and produced more seeds/plant as compared to the other treatments.

Antifungal activity of plant extracts

Growth inhibition of *Fusarium oxysporum* f. sp. cumini were recorded in the aqueous plants extract of four different species(*Jatropha curcas*, *Plectranthus amboinicus*, *Thymus vulgaris* and *Withania somnifera)* with three concentrations (5, 10 and 20%) of each (Figures 1 and 2).The extracts of all tested plants showed high antifungal activity against *Fusarium oxysporum* f. sp. cumini. Ignoring the concentration of plant extracts, *Thymus vulgaris* showed highest mycelial growth inhibition (77.85%), on the other hand *Jatropha curcas* gave the lowest value in this respect (67.31%).Concerning plant extract concentrations, 100% inhibition of mycelial growth of *Fusarium oxysporum* f. sp. cumini was recorded with 20% aqueous extracts of all plants under study, inhibition percentage was gradually decreased with decreasing plant extracts concentration.



L.S.D. at 5% for Plant=1.26Conc.= 1.09Plant x conc.= 2.17

Figure 1. Inhibition (%) of mycelial growth of *Fusarium oxysporum* cultured in PDA incorporated with aqueous plants extract of 5, 10 and 20% concentrations.

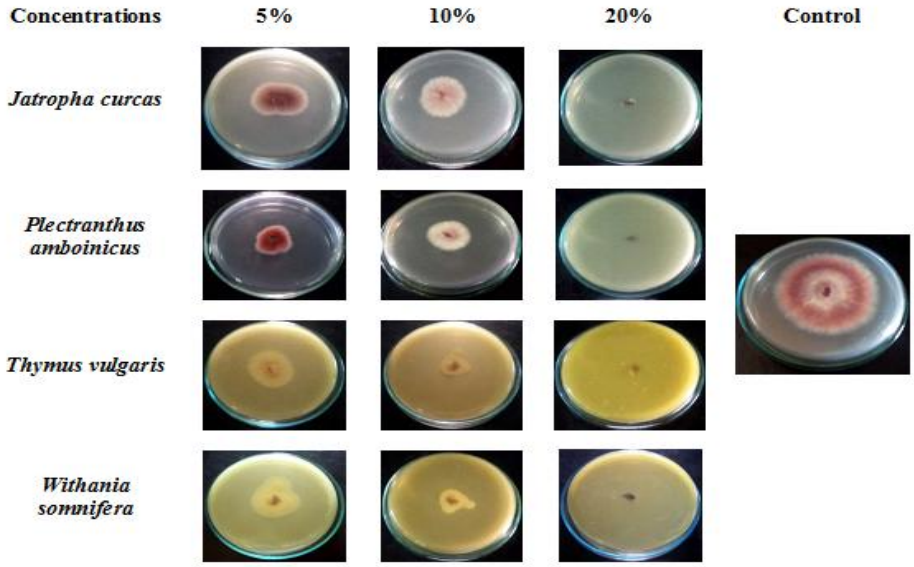


Figure 2. Mycelial growth of *Fusarium oxysporum* cultured in PDA incorporated with aqueous plants extract of 5, 10 and 20% concentrations.

Discussion

Natural plant extracts represent an important role as substitute to chemical fungicides. Our results are quite encouraging because most of screened plants extract showed antifungal activity against *Fusarium oxysporum* f. sp. cumini. This study clearly indicated that soaking cumin seeds in *Plectranthus amboinicus* extract at the concentration of 5% prior to sowing in *Fusarium oxysporum* infested soil enhanced growth of cumin in terms of plant height, No. of branches and umbels, plant weight and seed yield/plantas compared to the other treatments. The obtained results are in agreement with[22] who reported that, fresh and dry leaf aqueous extracts of *V. amygdalina* improve the yield of cowpea plants in terms of pod and grain weight. Also,[23]concluded that, the differences in the inhibitory effect of various plant extracts may be due to qualitative and quantitative differences in the antifungal principles compounds. This was also confirmed by *invivo* pot culture experiment using water extract of *Adhatoda vasica, Jatropha curcas, Sapindus emarginatus* and *Vitex negundo* where there was an increase in the shoot / root length and fresh and dry weight of shoot / root with the consequent reduction in the disease symptoms of the egg plant.

Concerning the growth inhibition of *Fusarium oxysporum* f. sp. cumini our results revealed that, *Thymus vulgaris* showed highest mycelial growth inhibition (77.85%), while *Jatropha curcas* gave the lowest value in this respect (67.31%). Antifungal activity exhibited by these plants may attributes to the presence of secondary metabolites. Plants extract contained phenols, flavonoids, V.C. and alkaloids, these compounds can combat with pathogens by different mode of action. Effect of plant extracts on different enzyme production was reported by[24]who determined the mode of action of extracts on cell wall and enzyme production of fungi, they found that, *Lawsonia inermis* inhibited the production of catalase in *Aspergillus niger* and *Fusarium oxysporum. W. somnifera* were significantly inhibited mycelial growth of fungi. Moreover, [25]stated that, a strong antifungal activity detected in 22 different plant extracts against two soil pathogens, *Fusarium solani* and *Rhizoctonia solani*, and hypothesized that their effect on the fungal mycelium comes both from the inhibition of enzymes within the fungi and the a concomitant induction of endogenous plant proteins associated with defense against biotic stress.

In this respect, [26] found that, lupine seed soaking in antioxidant solutions increases of chlorophyll and carotenoids content in leaves and this reflects the health condition of the plant. Meanwhile, [27]revealed that, antioxidants reduced the area of the root rot/wilt under field conditions and increased the plant vigor and pod yield per plant. The increase in pepper yield may be due to the role of antioxidants in stimulation of physiological processes which reflect the improving vegetative growth that followed by active translocation of the photo assimilation.

The results of this work can as well be further explored for designing integrated pest management strategy for cumin crop. More studies can be done and subsequently recommend in field applications.

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