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Integrated Control against Root Rot and Wilt Diseases of Cantaloupe under Plastic Houses Conditions

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ABSTRACT

Plant samples of cantaloupe grown in plastic houses showing typical root rot and wilt diseases symptoms were collected and subjected to isolation trials. The isolated purified fungal cultures were identified as *Rhizoctonia solani* and *Fusarium oxysporum*. These two fungi had the ability to induce root rot and wilt diseases in high percent to cantaloupe plants under artificial infestation conditions in greenhouse. The efficacy of humic acid and furfural individually or combined with the bioagent *Trichoderma harzianum* was evaluated against root rot and wilt incidence of cantaloupe plants grown in plastic house, under natural infestation, at Nubaria location. *Trichoderma harzianum* as soil drench combined with either humic acid or furfural had superior significant effect for reducing root rot and wilt diseases incidence of cantaloupe comparing with each of them alone. It could be suggested that such soil drench treatments could suppress root rot and wilt pathogens attacked cantaloupe under protective cultivation system.

Keywords: Cantaloupe, root rot and wilt, humic acid, furfural, *Trichoderma harzianum*,

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INTRODUCTION

Cantaloupe (*Cucumis melo* var. *cantalupo*) affected by several diseases which often cause serious crop losses. Root rot and wilt are the most common diseases of Cantaloupe which often cause serious crop losses [1-5].

Soil borne fungi, *Rhizoctonia solani* and *Fusarium oxysporum* f. *melonis* were reported to cause root rot and wilt diseases to cantaloupe in fields [6,7]. For gaining a high-quality of cantaloupes, control of serious diseases is essentially required. Followed a protective program includes physical control (cultural practices and varietal resistance), and chemical pesticides are needed to achieve acceptable results. Utilization of plant inducible resistance compounds may be enhancing the plant defense system against pathogenic microorganisms causing plant diseases. Furfural [2-Furancarboxaldehyde] is a natural compound present in some essential oils, bread, baked products and coffee. Furfural added to drip irrigation treatments system was found to reduce biomass of *F. oxysporum* and *Pythium ultimum* compared with the non treated controls [8]. Furthermore, humic acid have a role as plant growth stimulant in addition it might likewise improve plant resistance defense system against plant diseases and pests [9]. Using compost tea plus kelp extract and Humic acid could provide effective control of gray mould disease (*Botrytis cinerea*) in Geranium [10]. Furthermore, biological control agents (BCAs) were reported to inhibit plant pathogens [11]. The bioagents *Trichoderma* spp. are present in all soil and they are proved their strong antagonistic effect against other phytopathogenic fungi. The aim of the present study focuses on utilizing safe compounds to humans and environment, *e.g*. Humic acid and Furfural individually or combined with *Trichoderma harzianum* which may characterized as fungicide alternatives against root rot and wilt pathogens when used as soil drench under plastic house conditions.

MATERIAL AND METHODS

Plant Materials: Cantaloupe (*cv*. Galia) seedlings were used in the present study.

Fungicides Alternatives: Humic acid and Furfural were obtained from El-Nasr Company for chemical industry, Egypt.

Bio-agents: An isolate of antagonistic fungus *Trichoderma harzianum*, was kindly obtained from Culture Collection Unit, Plant Pathology Department, National Research Centre (NRC), Egypt. This isolate have high antagonistic ability against several plant pathogens and used successfully in various works at the same Department.

Isolations and identification of the causal organisms: Monitoring and scouting of diseases incidence of plants showing root rot and wilt symptoms were uprooted, collected and subjected to isolate the causal organisms. The plant roots were washed, excised into small pieces and disinfested in 0.5% sodium hypochlorite then rinsed several times in sterilized distilled water and dried between two sterilized filter papers. Sterilized root samples were transferred onto Petri dishes containing PDA medium and incubated at 25˚C until the appearance of the fungal colonies. For purification of the isolated fungi, the used techniques of hyphal tip and/or single spore were followed [12]. The purified fungal colonies were microscopically examined and identified according to fungal identification manuals for *Rhizoctonia* sp. [13,14] and [15,16] for *Fusarium* spp. The pure fungal isolates were kept on PDA slants at 5ºC for further studies.

Pathogenicity Test: The pathogenic ability of the isolated fungi was conducted at greenhouse belong to plant pathology Dept., NRC under controlled conditions. A 5-mm disc of mycelal growth from 7 days old culture of each of *R. solani* or *F. oxysporum* was transferred individually to glass bottles containing autoclaved corn-sand medium, .then incubated at 25ºC, until abundant fungal growth. Autoclaved sand and clay soils (1:1, v/v) were mixed and prepared for cultivation. One week before sowing, prepared soil was mixed with fresh fungal inocula of the individual pathogen at the rate of 3% of soil weight. Infested soil according to prevalent infestation was poured into Black plastic bags (25cm - diameter each). Another set of plastic bags full of un-infested soil was served as control treatment. All bags were watered under greenhouse condition for one week before transplanting. Three seedlings of the Cantaloupe seedlings *cv* Galia, were transplanted in each bag at the rate of three seedlings per bag, and five bags were used as replicates for each fungal isolate as well

as control. All bags were irrigated and fertilized as recommended. Cantaloupe plants showing symptoms or wilt diseases incidence were recorded and percentages of diseases incidence were calculated.

Plastic Houses Experiment: The efficacy of Humic acid, Furfural and/or *T. harziamum* treatments for controlling root rot and wilt diseases incidence was evaluated. This experiment was conducted in naturally infested soil with root rot and wilt pathogens in plastic house located at Nubaria location, Beheira Governorate, Egypt during two successive growing seasons 2014/2015.

The following treatments as soil drench were applied:
1. Humic acid (5ml/L).
2. Humic acid + *T. harziamum* $10x10^{10}$ cfu/mL (10ml/L)].
3. Furfural (10ml/L).
4. Furfural (10ml/L) + *T. harziamum* $10x10^{10}$ cfu/mL (10ml/L)].
5. Untreated control.

The experimental plastic house was 8X60m in dimensions comprised into 5 rows and each of 20m long considered as one replicate. Three replicates were used in complete randomized design for each particular treatment. The prepared tested materials were previously prepared in laboratory and sent to the experimental location. Five days before cantaloupe transplanting, the tested treatment was applied into the cultivated row site at the rate of 20L/row. Cantaloupe plants *cv.* Galia received traditional agriculture practices, *i.e*. irrigation and fertilization. All the above mentioned procedures were repeated for the second growing season using another plastic house at the same location

Observations for root rot and wilt diseases incidences were detected at each growing season. Percentages diseases incidence were calculated following the equation of:

The number of diseased plants / the number of planted seedlings X 100

Disease incidence in each treatment throughout the two growing seasons was calculated in average.

Statistical analysis: One-way ANOVA was used to analyze differences between applied treatments and diseases incidence for each year separately. A general linear model option of the analysis system SAS [17] was used to perform the ANOVA. Duncan's multiple range tests at $P \leq 0.05$ level was used for means separation [18].

RESULTS AND DISCUSSION

The two isolated fungi from cantaloupe plants showing root rot and wilt diseases symptoms were identified in respective order as *Rhizoctonia solani* and *Fusarium oxysporum.* The pathogenic ability of the two isolated fungi to induce diseases incidence was evaluated under greenhouse conditions. Presented data in Table (1) revealed that *R. solani* was able to induce only root rot disease in percent calculated as 86.6%. Meanwhile, cantaloupe plants grown in infested soil with *F. oxysporum* showed only wilt disease symptoms. Koch's postulates were fulfilled and confirmed that the isolates from cantaloupe were pathogenic, producing root rot lesions and wilt symptoms similar to those observed on plants in the field. Recorded results by several investigators were in agreement with the present findings. However, a binucleate *Rhizoctonia* sp. was shown to cause root rot on watermelon in Italy where it was isolated among other root rot pathogens from collapsed watermelon plants [6]. Moreover, [19] recorded a new root rot disease of watermelon has been observed sporadically in commercial fields in central Arizona, USA caused by the fungus *Rhizoctonia* sp. Also, *Fusarium oxysporum* f. *melonis* was reported to induce wilt disease to cantaloupe under field conditions [7,20].

Table 1: Pathogenic ability of isolated fungi to induce root rot and wilt diseases of cantaloupe under greenhouse conditions

Soil infestation	Disease incidence %	
	Root rot	wilt
Rhizoctonia solani	86.6 a	0.0 c

Fusarium oxysporum	0.0 c	73.3 b
Control	0.0 c	0.0 c

Mean values within each column followed by the same letter are not significantly different (P ≤ 0.05).

Announced effect against cantaloupe plants root rot and wilt diseases incidence was observed as a result of applied Humic acid (5ml/L) and Furfural (10ml/L) individually or combined with the bioagent *T. harziamum* $10x10^{10}$ cfu/mL (10ml/L) as soil drench treatment. Data presented in Table (2) show that throughout the two growing seasons 2014-2015 records of wilt incidence was higher than that of root rot. No significant differences between the two seasons at any of the same soil drench treatment were observed. Data also, showed that the mean disease incidence of cantaloupe root rot recorded as 12.3 and 16.3% at treatment of furfural and humic acid, compared with 20.9% in control treatment, respectively. These figures reduced significantly when the bioagent *T. harzianum* combined with those treatments. At treatments of [Humic acid + *T. harziamum*] and [Furfural +*T. harzianum*], root rot incidence was recorded as 8.3 and 8.6%, respectively. Also, the efficacy of soil drench with Humic acid and furfural individually or combined with the bioagent against wilt disease incidence showed similar trend. The mean values of wilt incidence were recorded as 20.3 and 24.3% at soil drench with furfural and humic acid, respectively. The combination of bioagent *T. harzianum* enhanced the activity of furfural and humic acid against disease incidence which recorded as 16.6% at both treatments comparing with 36.6% at control treatment.

Data illustrated in Fig. (1) reveal that the mean root rot and wilt diseases of cantaloupe throughout the two growing seasons reduced in respective order by 41.1; 44.5% and 22.0; 33.6% at furfural and humic acid soil drench treatments. More significant root rot and wilt disease reduction was recorded as 58.8; 54.6% and 60.2; 54.6% at both treatments of Furfural + *T. harzianum* and Humic acid + *T. harzianum*, respectively.

Integration of biocontrol and safe compounds agents was investigated, in the present study, as control measures against root rot and wilt diseases incidence. Similarly a number of inducers of resistance enhanced the effectiveness of *Bacillus subtilis* strain BS8651 as biological control agents when used as soil treatment or seed dressing against Pythium damping-off in cucumber [21]. Correlation between humic acid and plant vigor could be considered the role in plant diseases. In this regards, Humic acid is utilized for the purpose of creating new cells and to prepare their vitality. That followed by forming keto acid which enter into the Tri Carboxylic acid (TCA) cycle, which assume to be critical part for inducing resistance in plant [22].

Table 2: Effect of soil drench with fungicides alternatives individually or combined with *T. harzianum* against root rot and wilt diseases of Cantaloupe under plastic house conditions during two successive growing seasons

Soil drench treatment	Disease incidence %					
	Root rot			Wilt		
	First season (2014)	Second season (2015)	Mean	First season (2014)	Second season (2015)	Mean
Furfural	11.4 c	13.2 c	12.3 c	19.7 c	20.9 c	20.3 c
Furfural + *T. harzianum*	8.5 d	8.7 d	8.6 d	16.9 d	16.3 d	16.6 d
Humic acid	15.9 b	16.7 b	16.3 b	23.3 b	25.3 b	24.3 b
Humic acid + *T. harzianum*	8.1 d	8.5 d	8.3 d	16.6 d	16.6 d	16.6 d
Control	20.6 a	21.3 a	20.9 a	35.6 a	37.6 a	36.6 a

Mean values within each column followed by the same letter are not significantly different (P ≤ 0.05).

The part of humic acid for overcoming those hurtful impacts of chocolate spot and rust diseases of faba bean plant might be expected of the expansion in chitinase movement [23,24] What's more incitement plant development through expanded cell division, and additionally optimized uptake for nutrients and water [25,26] also, control hormone level, move forward plant development and improve stress tolerance [27].

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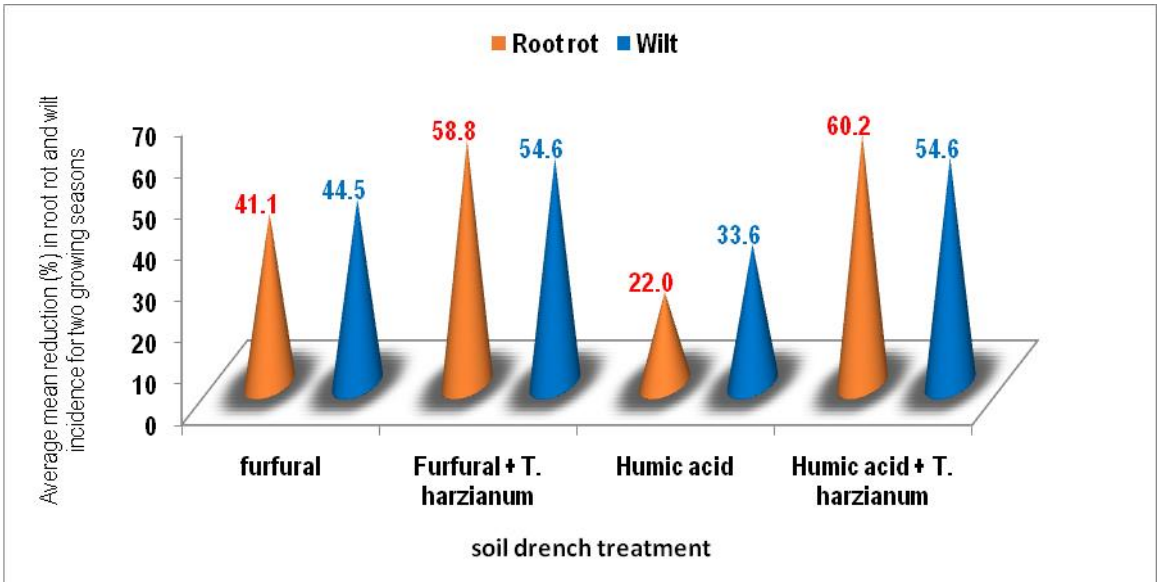

Fig 1: Average mean reduction for two growing seasons in root rot and wilt diseases of Cantaloupe in response to soil drench with some fungicides alternatives individually or combined with bioagent under plastic house conditions

A lack reports could be found in the literature concerning the suppressive effect of furfural against pathogenic fungi and bacteria. In eukaryotic yeast cells the metabolism and effects of furfural have been investigated. In this regards, [28] stated that furfural converted to furoic acid through the oxidization process under aerobic conditions. Meanwhile, in anaerobic fermentation it is reduced to furfuryl alcohol. They added that when furfural was added to the culture medium, both cellulose and β-glucosidase activities decreased with increasing furfural concentration. Control of *R. solani* in potato was the first study concerning the fungicidal properties of furfural [29]. Also, soil treatments with furfural could control southern blight caused by *Sclerotium rolfsii* in lentil could achieved by soil treatment with furfural, in addition, development of antagonistic *agents Trichoderma* spp. and bacteria to *S. rolfsii* was stimulated [30]. Furthermore, a pamphlet sheet of a pesticide Multigaurd Protect [31,32] has demonstrated the efficacy of furfural for controlling plant parasitic nematodes as well as pathogenic fungi, *i.e*. *Phytophthora, Fusarium, Rhizoctonia* and *Pythium.* The mode of action of Multigaurd Protect when used as soil treatment it could kills both nematodes and fungi, which it could damage through irreversibly the cuticle of nematodes. Moreover, it could react with the cellular wall and disrupting cellular functions of fungal cells.

To gain successful biological control against soilborne plant pathogens, establishment and propagation of the introduced antagonists into the soil has a major attention. The fruitful capacity achieved with Antagonistic inoculums which could survive, grow, and proliferate in soil and the rhizosphere against environmental factors [33]. The antagonistic organisms able to colonize rhizosphere in compatible reaction to the crops [34,35]. Several successful attempts in greenhouse or fields were conducted with biological control against soil borne plant pathogens [26,27].

In conclusion, protection of cantaloupe plants against of root rot and wilt pathogens invasion was successfully achieved by utilizing formula contains antagonistic bio-agent and safe compounds, *i.e*. furfural or Humic acid. However, more studies are needed to provide formulations of antagonists and alternative fungicides could be more successful against such soil-borne diseases.

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