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Fungicide Alternatives for Controlling Cantaloupe Root rot Incidence under Plastic Houses Conditions

Nehal S. El-Mougy, M. M. Abdel-Kader, S.M. Lashin

Abstract* - Evaluating the efficacy of different plant resistance inducers and/or bio-agents treatments against root diseases incidence revealed that soil drench with treatments, mixture of [Humic & Folic acids + Furfural + Trichoderma harzianum] and the bio-agent [Furfural + Trichoderma harzianum] resulted in highly reduction in the incidence of root rot disease of cantaloupe grown under plastic house conditions comparing with fungicides treatment. The obtained results revealed that cantaloupe seedlings grown in plastic house showed 4.6 and 5.8% root rot incidence at treatments of mixtures of [Humic & Folic acids + Furfural + Thricoderma harziamum] and [Furfural + Trichoderma harzianum], respectively. Meanwhile root rot disease infection of cantaloupe seedlings was recorded as 12.4% in control (fungicide treatment). It could be conclude that, antagonistic fungal and bacterial bio-agents and/or furfural, Humic & Folic acid are the effective agents capable of protecting cantaloupe plants against root rot pathogens under plastic house conditions.

***Index Terms*—Biocontrol, cantaloupe, fungicides alternatives, plastic houses, root rot.**

I. INTRODUCTION

The words cantaloupe and muskmelon are used rather loosely and are interchangeable. All varieties and types of muskmelon belong to the same genus and species; however, cantaloupe belongs to a different botanical variety (subgroup) than winter type muskmelons. Cantaloupes are the most important type of muskmelon grown in Egypt. Cantaloupes, and more specifically the winter type muskmelons, are better adapted to the drier southwestern areas of the state where foliage diseases are less prominent. Although hot, dry weather is favorable for cantaloupes, they can be grown successfully under plastic houses conditions if diseases can be managed.

Cantaloupe is susceptible to several diseases that attack the roots, foliage, and fruit [1,2,3] . The most common diseases have been damping off, root rot/vine decline, and root-knot nematode [4,5,6,7].

Disease control is essential in the production of high-quality cantaloupes. A preventive program that combines the use of cultural practices, genetic resistance, and chemical control as needed usually provides the best results. Cultural practices are useful for limiting the establishment, spread, and survival of pathogens that cause cantaloupe diseases. Many of the fungal, bacterial, and nematode pathogens survive in old crop debris and in soil. Diseases such as gummy stem blight and Fusarium wilt are known to be carried on seed. This can lead to rapid disease development and spread in greenhouse. The management of soil-borne plant pathogens is particularly complex because these organisms live in or near the dynamic environment of the rhizosphere, and can frequently survive a long period in soil through the formation of resistant survival structures. The current management strategy relies on the intensive use of fungicides. In addition, chemical control does not give satisfactory control of the root disease.

Present research focuses on finding compounds that are safe to human and environment. An alternative to pesticide application is that, it may be possible to utilize a scheme of inducible plant defenses which may provide protection against a broad spectrum of disease-causing pathogenic microorganisms. Furfural [2-Furancarboxaldehyde] is a naturally occurring compound, present in some essential oils and in foods such as bread, baked products, and coffee. It is prepared industrially by treatment with hot sulphuric acid of pentosans contained in agricultural residues, such as cereal straw, brains, and sugarcane bagasse. Furfural is a new pesticide active ingredient intended for the use as a fumigant to control root infesting plant parasitic nematodes and fungal plant diseases. The technical formulation (Furfural Technical) contains 99.7% furfural and is for the use in formulating end-use products and is applied to growing media and/or soils in greenhouses and field. Also, [8] reported that most of drip irrigation treatments reduced populations of *Pythium ultimum* and *F. oxysporum* and increased stem height compared with the non treated controls. Metham sodium, furfural + metham sodium, sodium azide, and chloropicrin significantly reduced the incidence of Lateris stem rot caused by *Sclerotinia sclerotiorum*. On the other hand, Humic acid can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests [9,10], stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water and stimulated the soil microorganisms [8,9]. Several reports indicated the efficiency of Humic acid in reducing some plant diseases. In this respect, [10] reported that the most effective treatments for suppression gray mould disease caused by *Botrytis cinerea* in Geranium plants was compost tea plus kelp extract and HA.

On the other hand, biological control agents (BCAs) inhibit plant pathogens through one or more of the following mechanisms: mycoparasitism, competition for key nutrients and colonization sites, production of antibiotics, or stimulation of plant de-fence mechanisms [11]. It is well known that, *Trichoderma* are present in all soil and they are the most cultural fungi. *Trichoderma* species are strongly antagonistic to other phytopathogenic fungi. They produce hydrolytic enzymes which are believed to play an important role in the parasitism of phytopathogenic fungi. Moreover, [12] reported that fluorescent *Pseudomonas* and *Trichoderma harzianum* T-22 applied in combination and alone, for controlling *Fusarium oxysporum* f. sp. *lycopersici* of tomato was studied in the greenhouse. They found that all biocontrol agents applied individually reduced disease incidence, while treatments as combination showed more protective effect.

Therefore the present research focuses on finding compounds that are safe to humans and the environment, *e.g.* Humic & Folic acids and Furfural as well as biocontrol agents which may provide an alternative control of many soil and seed-borne pathogens. The objective of the present work was to evaluate fungicide alternatives and /or bio-agents against root rot incidence when used as soil drench under greenhouse.

II. MATERIALS AND METHODS

Plant Materials

Seeds of Cantaloupe (cv. Yatherb 7), were used in the present study.

Fungicides Alternatives

Furfural and Humic & Folic acid (mixture) were obtained from El-Nasr Company for chemical industry, Egypt.

Bio-agents

The tested antagonistic fungus were *Trichoderma ha-zianum*, was isolated from cucumber, cantaloupe, tomato and pepper grown in plastic houses under protected cultivation systems and showing root rot disease symptoms [13]. The present bio-agent proved its antagonistic ability against the cantaloupe root rot pathogens under *in vitro* conditions [14].

Evaluating the efficacy of different plant resistance inducers and/or bio-agents treatments against root diseases incidence was carried out under naturally infested plastic house conditions at Nubaria location, Beheira Governorate, Egypt. The following treatments were applied as soil drench:

1. A mixture of: [Humic & Folic acids (5ml/L) + Furfural (10ml/L) + *Thricoderma harziamum* $10x10^{10}$ cfu/mL (10ml/L)].
2. A mixture of: [Furfural (10ml/L) + *Thricoderma harziamum* $10x10^{10}$ cfu/mL (10ml/L)].
3. Control (received only the fungicides approaches following the protective program recommended by Agriculture Research Centre, Protected Cultivation, Ministry of Agriculture and Reclaimed soil) stated in Table (1).

The experimental plastic house consists of 5 rows, each divided into 3 parts 20m long, and every part considered as one replicate. Five replicates were used for each particular treatment in complete randomized design. The proposed treatments were prepared in laboratory of Plant Pathology Dept., NRC and sent to certain locations for application. The prepared solution mixture was incorporated into the cultivated row site at the rate of 20L/row in 60m long 5 days before vegetables transplants. Cantaloupe transplants were planted and received recommended agriculture practices, *i.e.* irrigation and fertilization. Monitoring and scouting of root rot incidence were recorded up to 45 days from transplanting. Percentage of root rot disease incidence was recorded as the number of diseased plants relative to the number of planted seedlings, then the average of disease incidence in each treatment was calculated.

Table (1) Protective program against root rot fungal diseases for growing cucumber and cantaloupe in plastic houses and its financial cost *

Time of Treatment	Fungicide	Concentration (ml & gm/100 liter)	Dose / plastic house (gm & ml)
After 3 days from cultivation	Chagrin	100 ml	70 ml
After 7 days from previous spray	Maxim-L	100 ml	100 ml
After 7 days from previous spray	Rizolex–T	200 gm	200 gm

* The followed protective program is recommended by Agriculture Research Centre, Protected Cultivation, Ministry of Agriculture and Reclaimed soil.

Statistical analysis

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between applied treatments and disease incidence. A general linear model option of the analysis system SAS [15] was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was used for means separation [16].

III. RESULTS AND DISCUSSION

Application of soil treatment with either mixtures of [Humic & Folic acids (5ml/L) + Furfural (10ml/L) + Thricoderma harziamum 10x1010 cfu/mL (10ml/L)] and [Furfural + Trichoderma harzianum] resulted in announcement effect against root rot disease incidence of grown vegetable seedlings in commercial plastic houses at different locations. Presented data in Table (2) reveal that cantaloupe seedlings grown in plastic house at Nubaria location showed 4.6 and 5.8% root rot incidence at treatments of mixtures of [Humic & Folic acids (5ml/L) + Furfural (10ml/L) + Thricoderma harziamum 10x1010 cfu/mL (10ml/L)] and [Furfural +Trichoderma harzianum], respectively. Meanwhile root rot disease infection of cantaloupe seedlings was recorded as 12.4% in control (fungicide treatment). These applied soil treatment in respective order could reduce disease incidence calculated as 62.9 and 53.2% over fungicide treatment (Fig. 1).

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Table (2) Percentage of root rot incidence in response to application of plant inducers formula against root diseases of Cantaloupe grown in plastic houses under protected cultivation system (Nubaria location)

Treatment	Root rot incidence (%)
Humic & Folic acids + Furfural + *T. harzianum**	4.6 c
Furfural +*T. harziamum* *	5.8 b
Control (Fungicide application)	12.4 a

Mean values within column followed by the same letter are not significantly different ($P \leq 0.05$).

* The prepared solution mixture was incorporated into the cultivated row site at the rate of 20L/row in 60m long.

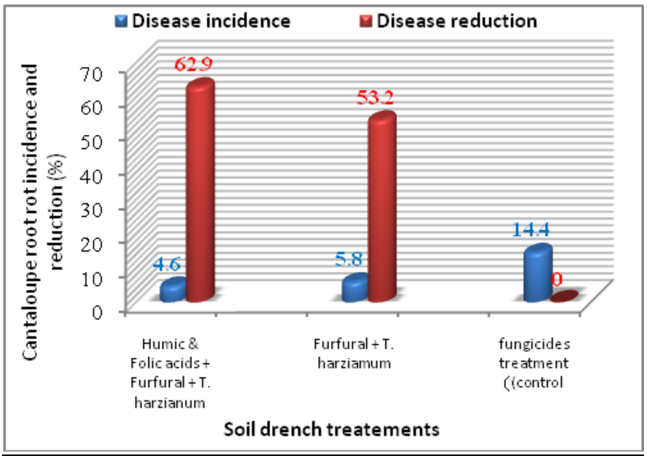

Fig. (1). Root rot incidence and reduction in response to application of plant inducers formula against root diseases of Cantaloupe grown in plastic houses under protected cultivation system (Nubaria location)

In the present study combination of bio-agents and fungicides alternatives was investigated. Similar report[17] reported that several tests revealed that soil drenches or seed treatments of a number of compounds, especially inducers of resistance, combined with strain BS8651 enhanced effectiveness and consistency of the biological control agents against Pythium-damping off in cucumber. Many investigators explain the role of humic acid in plant diseases reduction. The role of humic acid in plant diseases may be due to the correlation between these acids and plant health. Humic acid are used both for the production of new cell biomass and to produce energy. Followed by de-animation into the keto acid which inter into the Tri Carboxylic acid (TCA) cycle, which play important role in plant resistance [18]. The role of Humic acid in overcoming the harmful effects of chocolate spot and rust diseases in faba bean plant may be due to the increase in chitinase activity [19,20] and stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water [21,22] also, regulate hormone level, improve plant growth and enhance stress tolerance [23]. Humic acid is a suspension, based on potassium humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests [24] which consequently increase yield of plant. Foliar application of HA (25% active HA) consistently enhanced antioxidants such as á-tocopherol, â-carotene, superoxide dismutases, and ascorbic acid concentrations in turf grass species [25]. These antioxidant may play a role in the regulation of plant development, flowering and chilling of disease resistance [26,27]. Amino acids have a chelating effect on micronutrient when applied, that make the absorption and transportation of micronutrients inside the plant is easier due to its effect on cell membrane permeability. Some of these micronutrients play roles in plant resistance by regulating the levels of auxin in plant tissues by activating the auxin oxidize system [28] and by it appears to be required in synthesis of intermediates in the metabolic pathway, through tryptophan to auxin [29]. Consequently auxin lead to increase in total phenol, calcium content and activity of catechol oxidize, these materials protect plants against pathogen stress [30]. However, not much can be found in the literature regarding the efficacy of furfural against fungi and bacteria, the metabolism and effects of furfural in eukaryotic cells have been investigated for yeast cells. In this case, the conversion of furfural depends on the rate of oxidizing in yeasts. Furfural is oxidized to furoic acid under aerobic conditions, and it is reduced to furfuryl alcohol in anaerobic fermentation [31]. The authors indicated that when furfural was added to the culture medium, both cellulose and *β*-glucosidase activities decreased with increasing furfural concentration. Furthermore, [32] first studied the fungicidal properties of furfural, reporting control of *R. solani* in potato. More recently, [33] demonstrated that soil treatments with furfural control southern blight caused by *S. rolfsii* in lentil, while stimulating development of *Trichoderma* spp. and bacteria antagonistic to *S. rolfsii*. These reports confirm the present findings. Furfural caused the reduction in root-rot incidence, being 75.43% comparing with untreated control when applied alone. Combined treatments with furfural and either bacterial or fungal bio-agents showed a lower effect, although they reduced the disease incidence by more than 41% [34]. A similar effect was also reported for tomato wilt caused by *F. oxysporum* [35] and stem rot of liatris (*Liatris punctata*) caused by *S. sclerotiorum* [8]. Moreover, botanical aromatics, furfural, citral and benzaldehyde showed potential for control of both fungal pathogens and phytoparasitic nematodes [36] and they did not reduce colonization of cotton roots by plant growth promoting rhizobacteria (PGPR). Furthermore, Pamphlet sheet of Protect [37,38] has demonstrated efficacy in the control of plant parasitic nematodes and fungal pathogens, *i.e. Pythium, Fusarium, Phytophthora* and *Rhizoctonia*. Protect is a contact soil treatment that kills nematodes by irreversibly damaging the cuticle and kills fungi by reacting with the cellular wall and disrupting cellular functions. Also, it is obvious from Multigaurd fate sheet that it controls root infesting plant parasitic nematodes and fungal plant pathogens such as *Pythium, Phytophthora, Fusarium* and *Rhizoctonia*.

For the effective biological control of soilborne plant pathogens, a major consideration has been given to proliferation of the antagonist after introduction into the soil. Among the desirable attributes of a successful antagonist is its ability to produce inoculums in excess and to survive, grow, and proliferate in soil and the rhizosphere [39]. The antagonistic organisms have been known to be capable of colonizing in the rhizosphere compatibly responding to the crops [40,41]. In many cases, biological control of soil borne plant pathogens was successfully conducted in greenhouse or fields [42,43,44]. One interesting aspect of biocontrol agent-induced suppression of disease is the reported affect of *T. harzianum* on development of gray mould disease caused by *B. cinerea* through a reduction in its pathogen city [45,46]. Production of cysteine protease enzymes by Trichoderma was reported to inhibit the activities of hydrolytic enzymes – especially polygalacturonases – in the pathogen, which are important pathogen city factors in Botrytis and many other fungi [45,46]. The proteases inactivated the pathogen enzyme by cleaving the molecule. Retaining proteases from Trichoderma culture filtrates and from infected bean leaves, and was reversed by adding protease inhibitors [45]. For such an interaction to be evoked on the plant leaf surface infected with *B. cinerea*, the interacting organisms must spatially and temporally occupy the same niche and be in close proximity to one another, providing localized protection.

In conclusion, antagonistic fungal and bacterial bio-agents and/or furfural, Humic & Folic acid are the effective agents capable of protecting cantaloupe plants against root rot pathogens under greenhouse conditions. However, more detailed studies are required to elucidate formulations of antagonists and alternative fungicides for more successful protection against such soil-borne

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AUTHOR'S BIOGRAPHY

First Author: Department of Plant Pathology, National Research Centre – Egypt, Email: Nehal_nrc@yahoo.com

Second Author: Department of Plant Pathology, National Research Centre – Egypt, Email: mokh_nrc@yahoo.com

Third Author: Department of Plant Pathology, National Research Centre – Egypt, Email: lashin2004@hotmail.com