Australian Journal of Basic and Applied Sciences, 6(5): 241-248, 2012
ISSN 1991-8178

Evaluating the Efficacy of Different Plant Resistance Inducers and/or Bio-Agents Treatments Against Root Diseases Incidence of Some Vegetables Under Protected Cultivation System

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Abstract: The efficacy of different plant resistance inducers and/or bio-agents treatments against root diseases incidence of some vegetables were evaluated under plastic houses conditions. The evaluated treatments were applied as soil drench before transplanting at commercial plastic houses located at Giza and Qualubia governorates. These treatments were evaluated against root rot incidence of cucumber, tomato and pepper plants. The obtained results revealed that soil drench with treatments, mixture of [Humic & Folic acids (5ml/L) + Furfural (10ml/L) + *Trichoderma harzianum* $10x10^{10}$ (10ml/L)] and the bio-agent [*Trichoderma harzianum* $10x10^{10}$ (10ml/L)] resulted in highly reduction in the incidence of root rot disease of tested Cucumber, Tomato and pepper vegetables comparing with other applied treatments as well as untreated control. The addition of a biological control agent in combination with plant resistance inducers resulted in increased symptomless plant stand over the biological agent. These methods characterized as environmentally safe, bioactive natural products which able successfully to control phytopathogenic fungi in crop production systems.

Key words: bio-agents, chemical resistance inducers, cucumber, pepper, plastic house, root rot, tomato.

INTRODUCTION

Cultivated vegetables are subjected to attack by several plant pathogenic fungi during different stages of plant growth starts from seed sowing up to seedling to flowering stages, and may to cause pre-emergence infection, thus forcing the farmer to replant the missed hills or dead plants. Soilborne plant pathogens considered the major problems in agricultural production throughout the world, reducing yield and quality of crops. Damping-off, Root rot and Wilt of vegetables caused by *Fusarium solani*, *Fusariumm oxysporum*, *Sclerotium rolfsii, Rhizoctonia solani, Alternaria solani, Macrophomina phaseolina* and *Pythium* spp. to be the most deleterious diseases (Abdel-Rehim *et al*., 1987; Celar, 2000; Ramamoorthy *et al*., 2002; Hibar *et al*., 2006 and Steinkellner *et al*., 2008). These harmful parasites cause considerable losses in plant stand as well as quality and quantity of produced yield throughout root diseases incidence of various cultivated crops. The current management strategy relies on the intensive use of fungicides. In addition, chemical control does not give satisfactory control of the root disease. Therefore, concern of pesticides use with respect to human health and environment has brought increasing interest in alternatives use by avoiding negative effect on the environment. Biological control is an alternative to the use of chemical pesticides. Biological fungicides may act to suppress the population of the pathogenic organisms through competition with pathogenic organisms. Stimulated plant growth, which may allow plants to quickly outgrow any pathogen effects, or damage the pathogen by means of toxins produced (Cook, 2000). Bio-control agents are derived from natural materials such as animals, plants, bacteria, fungi and certain minerals. The application of biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases in many countries (Sivan, 1987). It is still not easy and costly in application, however it can serve as the best control measure under greenhouse conditions. In recent years, several attempts have been made to overcome this obstacle by applying fungal and bacterial antagonistic microorganisms (Sivan and Chet, 1986; Parke *et al.,*1991; King and Parke, 1993; Kim *et al.,*1997; Sunick *et al.,* 1997; Abdel-Kader, 1997; Whipps and Lumsden, 2001and McLean *et al*., 2004). With the knowledge of the adverse effects of synthetic fungicides worldwide, attention is rapidly, being shifted to non-synthetic, safer alternatives. The present research focuses on finding compounds that are safe to humans and the environment, *e.g.* chemical resistance inducers and/ or bioagents.

In this regards, plant products are characterized as having a wide range of volatile compounds could be used as alternative anti-bacterial and antifungal treatments (Jenny 2000). It is evident from reviews by several investigators that Humic and Fulvic acids have been early recorded to have appositive effect against plant pathogens and their cells biological activities (Vaughan *et al*., 1985; Hoitank and Fahy 1986; Zhang *et al*., 1996). On the other hand, furfural is a naturally occurring compound, and recently used as a new pesticide active ingredient intended for the use as a fumigant to control root infesting plant parasitic nematodes and fungal plant diseases. Moreover, Gerik (2005) reported that most of drip irrigation treatments reduced populations of *Pythium*

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ultimum and *F. oxysporum* and increased stem height compared with the nontreated controls. Metham sodium, furfural + metham sodium, sodium azide, and chloropicrin significantly reduced the incidence of Liatris stem rot caused by *Sclerotinia sclerotiorum*.

The objective of the present work was to evaluate the suppression activity of some fungicides alternatives applied as soil drench before transplanting against root rot incidence of some vegetables under commercial plastic houses conditions.

MATERIALS AND METHODS

The evaluation of plant resistance inducers and /or bio-agents under natural infestation with vegetable diseases causal organisms against root diseases infection was performed under protected cultivation system conditions in commercial plastic houses of Ministry of Agriculture and Soil Reclamation, A.R.E. at Dokki, Haram and Tookh locations. The cultivated vegetables were Cucumber (at Dokki and Tookh locations); Tomato and Pepper (at Haram location).

Evaluating the efficacy of different plant resistance inducers and/or bio-agents treatments against root infection were applied as soil drench treatment as follows:

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. A mixture of: [Humic & Folic acids (10ml/L) +*Bacillus subtilis* 10x10^{10} cfu/mL (10ml/L)]
4. A mixture of: [Humic & Folic acids (10ml/L) +*Pseudomonas fluorescens* 10x10^{10} cfu/mL (10ml/L)]
5. *Thricoderma harziamum* 10x10^{10} cfu/mL (10ml/L)
6. *Bacillus subtilis* 10x10^{10} cfu/mL (10ml/L)
7. *Pseudomonas fluorescens* 10x10^{10} cfu/mL (10ml/L)
8. Control (received only the recommended fungicide Rizolex-T 50% as transplanting dipping at the rate of 2g/L).

The experimental plastic house consists of 5 rows, each (0.9 x 60m, width x long) contains two cultivated row sites. Each cultivated row site divided into 3 parts 20m long each, and every part considered as one replicate. Three replicates were used for each particular treatment in complete randomized block 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 (distributed for the three replicates) 5 days before vegetables transplants, then mulched with black mulching polyethylene sheets.

Certain vegetables (Cucumber, Tomato, Pepper) 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.

Statistical Analysis:

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between applied treatments. A general linear model option of the analysis system SAS (SAS Institute Inc. 1996) was used to perform the ANOVA. Duncan's multiple range test at $P < 0.05$ level was used for means separation (Winer 1971).

RESULTS AND DISCUSSION

The obtained results in Table (1) and Fig (1) showed the root rot incidence of Cucumber seedlings grown in plastic house at Dokki and Tookh locations. Presented data revealed that all applied treatments have significant drastic effect on root rot incidence comparing with untreated control. Announced highly significant effect of both treatments, mixture of [Humic & Folic acids (5ml/L) + Furfural (10ml/L) + *Trichoderma harzianum* 10x10^{10} (10ml/L)] and [Furfural + *Trichoderma harzianum* 10x10^{10} (10ml/L)] that they could completely inhibit the incidence of root rot disease of cucumber seedling grown at Dokki location, while they record the lowest disease incidence as 1.3 and 1.6% for seedlings grown at Tookh location. A moderate effect was observed with treatments, [*Trichoderma harzianum* 10x10^{10} (10ml/L)], mixtures of [Humic & Folic acids (10ml/L) +*Pseudomonas fluorescens* 10x10^{10} (10ml/L)] and [Humic & Folic acids (10ml/L) + *Bacillus subtilis* 10x10^{10} (10ml/L)] that root rot incidence were recorded as 1.8, 2.2 and 2.7% at Dokki location and 2.7, 3.6, 3.8% at Tookh location, in respective order. Also, the bacterial bio-agents treatments, *Bacillus subtilis* 10x10^{10} (10ml/L) and *Pseudomonas fluorescens* 10x10^{10} (10ml/L) could decrease significantly disease incidence down to 3.8, 4.3

and 4.7, 5.6% for growing seedling at Dokki and Tookh locations, respectively comparing with 15.6 and 16.8% in control treatment.

Table 1: Percentage of root rot incidence in response to application of different formula against root diseases of Cucumber grown in plastic houses under protected cultivation system at Dokki location.

Treatment*	Root rot incidence (%)	
	Dokki location	Tookh location
A mixture of: [Humic & Folic acids +Furfural + *Trichoderma harzianum*]	0.0 g	1.3 f
A mixture of: [Furfural +*Trichoderma harzianum*]	0.0 g	1.6 f
A mixture of: [Humic & Folic acids +*Bacillus subtilis*]	2.7 e	3.8 d
A mixture of: [Humic & Folic acids +*Pseudomonas fluorescens*]	2.2 e	3.6 d
Trichoderma harzianum	1.8 de	2.7 e
Bacillus subtilis	3.8 d	4.7 bc
Pseudomonas fluorescens	4.3 bc	5.6 b
Control	15.6 a	16.8 a

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).
*Each row drenched with 20L

Similar results were obtained with root rot incidence of Tomato and Pepper seedlings grown in plastic house at Haram location. Presented data in Table (2) and Fig (2) showed that all applied treatments have announced significant effect on root rot incidence comparing with control. The applied treatments, mixture of [Humic & Folic acids (5ml/L) + Furfural (10ml/L) + *Trichoderma harzianum* $10x10^{10}$ (10ml/L)]; mixture of: [Furfural (10ml/L) +*Trichoderma harzianum* $10x10^{10}$ (10ml/L)] and the bio-agent [*Trichoderma harzianum* $10x10^{10}$ (10ml/L)] reduced significantly the incidence of root rot disease of Tomato which recorded as 3.2, 3.1 and 3.8% followed by a mixture of: [Humic & Folic acids (10ml/L) +*Pseudomonas fluorescens* $10x10^{10}$ (10ml/L)] and *Pseudomonas fluorescens* $10x10^{10}$ (10ml/L) treatments which recorded root rot incidence as 5.2 and 6.4%, respectively. A moderate effect on disease incidence was observed with treatments, mixture of: [Humic & Folic acids (10ml/L) +*Bacillus subtilis* $10x10^{10}$ (10ml/L)] and *Bacillus subtilis* $10x10^{10}$ (10ml/L) as 7.6 and 8.2%, respectively comparing with 17.6% in untreated check control treatment.

Data also showed similar trend of root rot incidence of pepper plants grown at Haram location. The lowest root rot incidence 3.1, 4.2, 4.8 and 5.1% were recorded at the applied treatments a mixture of: [Humic & Folic acids + Furfural + *Trichoderma harzianum* $10x10^{10}$ (10ml/L)]; a mixture of: [Furfural +*Trichoderma harzianum*]; a mixture of: [Humic & Folic acids +*Bacillus subtilis*] and the bio-agent [*Trichoderma harzianum*], respectively. Furthermore, 6.0, 6.2 and 7.3% root rot incidence of Pepper transplants were recorded at treatments, mixture of: [Humic & Folic acids (10ml/L) + *Pseudomonas fluorescens* $10x10^{10}$ (10ml/L)]; *Bacillus subtilis* $10x10^{10}$ (10ml/L) and *Pseudomonas fluorescens* $10x10^{10}$ (10ml/L), respectively comparing significantly with 18.7% in untreated check treatment.

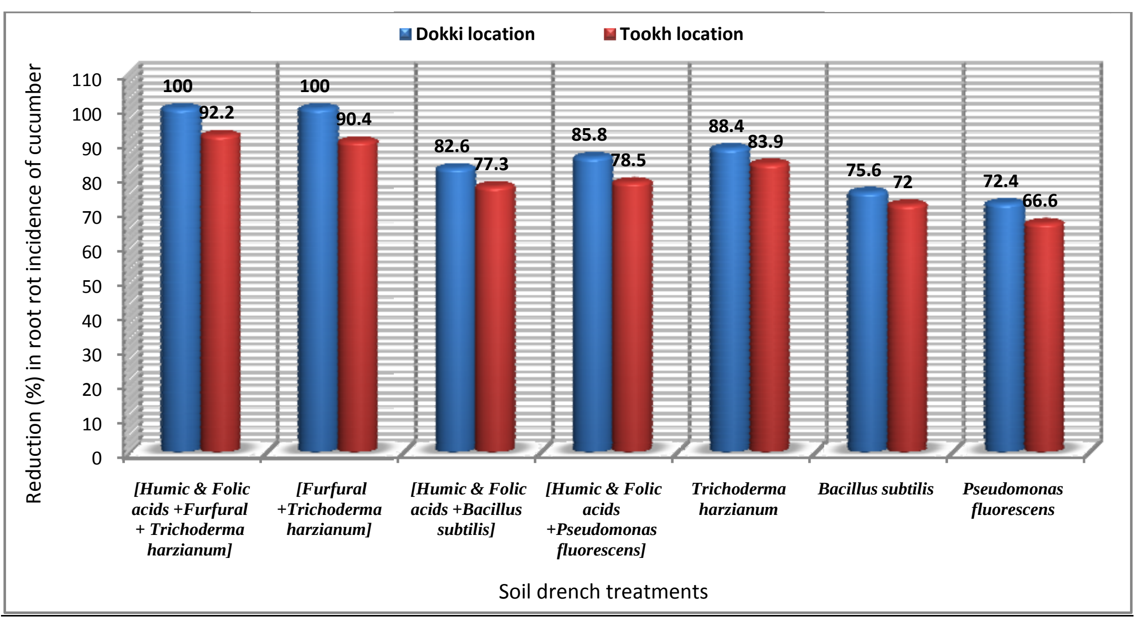

Fig. 1: Reduction in root rot incidence of cucumber in response to applied different formula in plastic houses under protected cultivation system (Dokki and Tookh locations)

Table 2: Percentage of root rot incidence in response to application of different formula against root diseases of Tomato and Pepper grown in plastic houses under protected cultivation system (Haram location).

Treatment*	Root rot incidence (%)	
	Tomato	Pepper
A mixture of: [Humic & Folic acids +Furfural + *Trichoderma harzianum*]	3.2 f	3.1 e
A mixture of: [Furfural +*Trichoderma harzianum*]	3.1 f	4.2 d
A mixture of: [Humic & Folic acids +*Bacillus subtilis*]	7.6 bc	4.8 d
A mixture of: [Humic & Folic acids +*Pseudomonas fluorescens*]	5.2 e	6.0 c
Trichoderma harzianum	3.8 f	5.1 cd
Bacillus subtilis	8.2 b	6.2 c
Pseudomonas fluorescens	6.4 d	7.3 b
Control	17.6 a	18.7 a

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).
*Each row drenched with 20L

On the other hand, illustrated data by Fig (1) showed that the highest reduction in root rot incidence of cucumber plant either grown at Dokki or Tookh locations was observed at the applied treatments of a mixture of: [Humic & Folic acids +Furfural + *Trichoderma harzianum*]; a mixture of: [Furfural +*Trichoderma harzianum*] and the bio-agent [*Trichoderma harzianum*]. They recorded reduction in root rot incidence calculated as (100, 92.2%); (100, 90.4%) and (88.4, 83.9%) for cucumber plant grown at Dokki and Tookh locations, respectively. Meanwhile, the lowest reduction in root rot incidence was recorded as 72.4 and 66.6% at the bio-agent *Pseudomonas fluorescens* treatment for cucumber plant grown at Dokki and Tookh locations, respectively. The rest of the applied treatments caused reduction in disease incidence ranged between (85.8 – 75.6%) and (78.5 – 72.0%), in respective order to relevant cultivated area.

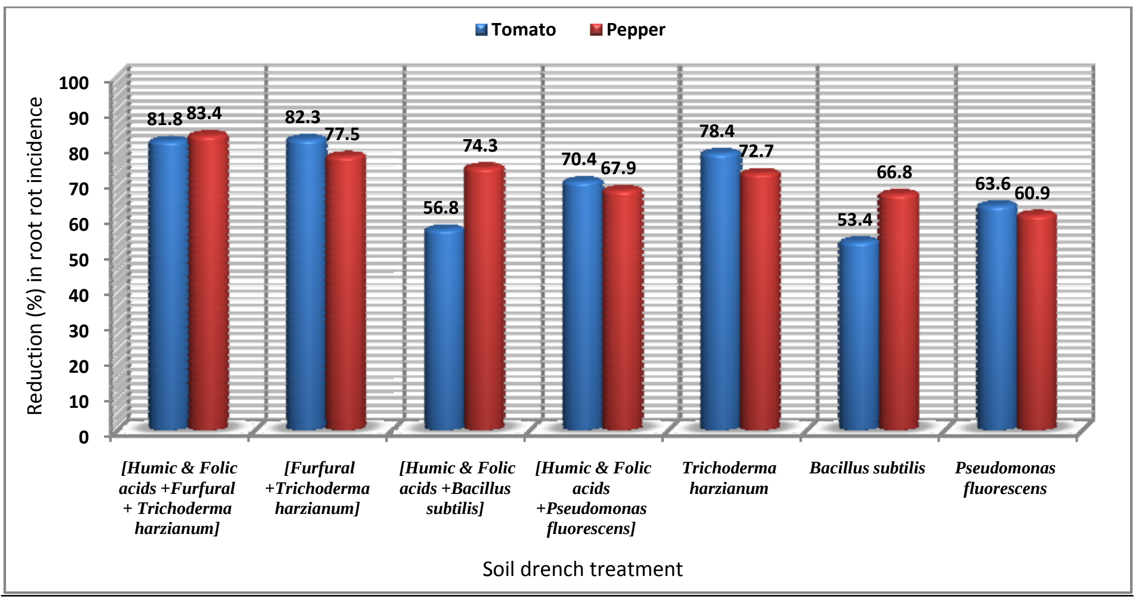

Fig. 2: Reduction in root rot incidence of Tomato and Pepper in response to applied different formula in plastic houses under protected cultivation system (Haram location).

Similarly, illustrated data by Fig (2) revealed that the applied treatments, a mixture of: [Furfural + *Trichoderma harzianum*] followed by a mixture of: [Humic & Folic acids + Furfural + *Trichoderma harzianum*] and [Furfural +*Trichoderma harzianum*], caused the highest reduction in root rot incidence of tomato plants grown at Haram location. The calculated reduction in root rot incidence was 81.8, 82.3 and 78.4%, respectively. As for Pepper plants, the highest reduction in root rot incidence was recorded as 83.4, 77.5 and 74.3% at the applied treatments, a mixture of: [Humic & Folic acids + Furfural + *Trichoderma harzianum*]; a mixture of: [Furfural +*Trichoderma harzianum*] and mixture of: [Humic & Folic acids + *Bacillus subtilis*], respectively. The lowest reduction in root rot incidence was recorded as 63.3 and 60.9% at the bio-agent *Pseudomonas fluorescens* treatment for Tomato and pepper plants, respectively. Meanwhile, the rest of the applied treatments caused reduction in disease incidence ranged between (70.4 – 53.4%) and (67.9 – 66.8%) for cultivated vegetables in respective order.

The obtained results in the present study showed high efficacy of the bio-agents application alone or combined with the fungicides alternatives Humic & Folic acid or Furfural as soil drench treatment before transplanting. A mixture of: [Humic & Folic acids + Furfural + *Trichoderma harzianum*] and [Furfural +*Trichoderma harzianum*] treatments could completely inhibit the root rot disease incidence of cucumber plants grown at Dokki location and also reduced the disease incidence by 90.4 and 92.2% at Tookh location (Table 1 and Fig. 1). Also, the highest reduction in root rot incidence of both tomato and pepper was also recorded at treatment of the bio-agent [*Trichoderma harzianum*] alone or combination treatments of [Furfural +*Trichoderma harzianum*] and [Humic & Folic acids + Furfural + *Trichoderma harzianum*], (Table 2 and Fig. 2). These results are confirmed with those obtained by several investigators. The obtained reduction in invaded vegetable plants with root rot pathogens may be attributed to the high accumulative inoculum potential of the introduced bio-agents into the root region, before sowing and throughout the growing season as well, where they are predicted to have a direct impact on already established *pathogens* population. Similar explanation was reported (Abdel-Kader, 1997), who stated that, soil treatment with biocide *T. harzianum* showed better reduction in root rot incidence of bean followed by seed coating with the biocide. He added that, these differences could be due to the initial inoculum of *T. harzianum* introduced into the soil. Moreover, the high fungal population density introduced through soil treatment technique enables the fungus to adapt itself against environmental conditions (Papavizas, 1982) resulting in dominance of high population of the introduced fungi in the plant rhizosphere. The use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists. Moreover, the combination of such biological control agents (BCAs) with reduced levels of fungicide (integrated control) promotes a degree of disease suppression similar to that achieved with full fungicide treatment (Monte, 2001). Moreover, the application of biological controls using antagonistic microorganisms, has proved to be successful for controlling various plant diseases in many countries (Chao *et al*, 1986; Sivan, 1987; El-Mougy and Abdel-Kader, 2008). Furthermore, 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 inoculum in excess and to survive, grow, and proliferate in soil and the rhizosphere (Baker and Cook, 1974). Various actinomycetes, bacteria, and fungi, which show antagonism to *P. capsici*, exist in soils where peppers are grown (Ahn and Hwang, 1992; Kim and Hwang, 1992). In particular, some antagonistic rhizobacteria such as *Burkholderia cepacia* (Jee *et al*., 1988) and *Pseudomonas aeruginosa* (Kim and Hwang, 1992) very effective against Phytophthora blight in pepper plants under laboratory and greenhouse conditions. Similarly, application of *B. cepacia* granules into soil provided better suppression of Phytophthora blight on red-pepper seedlings, as compared to direct drenching with *Burkholderia cepacia* suspensions (Park *et al*., 1989). Several workers explained the mode of action of antagonistic *Pseudomonas fluorescens* and *Bacillus subtilis* isolates (Peighami-Ashnaei *et al*., 2009; El-Mougy *et al*., 2011). In this regards, the potential of *Bacillus* sp. to synthesis a wide variety of metabolites with antifungal activity is known and in recent years it has been a subject of experiments (Ahimou *et al*., 2000). Most of these substances belong to lipopeptides, especially from surfactin, iturin and fengicin classes. Not so much is known about the mechanism of antifungal activity of these substances produced by *Bacillus* sp. Some of them (iturin and surfactin) are able to modify bacterial surface hydrophobicity and, consequently, microbial adhesion to mycelium surfaces (Ahimou *et al*., 2000). Antibiotics of the iturin group were found to act upon the sterol present in the cytoplasmic membrane of the fungi (Worthington, 1988). Biological control of *Aspergillus niger* by *Bacillus subtilis* was also investigated (Podile and Parkash, 1996). They demonstrated that the bacterial cells initially adhered to the fungus, multiplied and extensively colonized the surface. Rapid growth of bacterial cells on the surface resulted in damage of fungal cell walls. These aspects appear essential in association with the antifungal properties of *Bacillus* sp. used in the biological control of plant diseases.

On the other hand, a more balanced, cost effective and eco-friendly approach must be implemented and adopted farmers. In order to overcome such hazardous control strategies, scientists, researchers from all over the world paid more attention towards the development of alternative methods which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly biodegradable. Such strategy is use of fungicides alternatives, *i.e*. plant resistance inducers (Punja and Grogan, 1982; Smilanick *et al*., 1999; El-Gamal *et al*., 2006; Ragab *et al*., 2009). Humic acid (HA) is a heterogeneous mixture of many compounds with generally similar chemical properties it performs various functions in the soil and on plant growth. Humic substances have been early recorded to have appositive effect against plant pathogens (Hoitank and Fahy 1986; Zhang *et al*., 1996). Also, many studies (Vaughan *et al*., 1985; Visser, 1985) showed that Fulvic acid (FA) have a greater effect on cells biological activities than humic acids (HA) compounds**.** Also, many studies (Vaughan *et al*., 1985; Visser 1985) showed that Fulvic acid (FA) have a greater effect on cells biological activities than humic acids (HA) compounds. The addition of 500 mgl^{-1} of humic acids on the growth medium completely eliminated the inhibition of *P. ultimum* by *R. radiobacter* (Charest *et al*., 2005). Furthermore, *In vitro*, humic acid at 15.0% (v/v) reduced significantly the radical growth and spore germination of *Fusarium solani* the causal agent of dry root rot (El-Mohamedy and Ahmed 2009). Moreover, recently, plant products with antimicrobial properties have notably obtained attention as possible applicants in order to prevent bacterial and fungal growth (Lanciotti *et al.* 2004).

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 (Taherzadeh *et al*. 1999). The authors indicated that when furfural was added to the culture medium, both cellulose and β-glucosidase activities decreased with increasing furfural concentration. The activity of both enzymes decreased by 50% when concentration of furfural increased from 0 to 1.2 g/l (1200 ppm). Furthermore, Flor (1926) first studied the fungicidal properties of furfural, reporting control of *R. solani* in potato. More recently, Canullo *et al.* (1992) 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*. There are a few cited reports explaining the furfural mode of action against soil microflora. In this regard, the end-use product containing 90% furfural in a liquid formulation is registered as commercial products, e.g. Crop guard, Multigaurd protect and Protect etc. (Anonymous 2005, 2006). Pamphlet sheet of Protect (2005–2006) 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 fungal plant pathogens such as *Pythium, Phytophthora, Fusarium* and *Rhizoctonia*. Also, El-Mougy *et al*., (2008) reported that under *in vitro* conditions, the linear growth of tested soilborne pathogenic fungi was dramatically reduced with the increasing of furfural concentrations added to the growth medium up to 4000 ppm where no growth was observed, while the bacterial and fungal bioagents showed more tolerance to these concentrations and failed to grow at 6000 and 7000 ppm, respectively. The added that pot and field experiments indicated that furfural at 6000 ppm combined with bioagent treatments proved to have superior suppressive effect against tomato root rot incidence, caused by *Fusarium solani* and *Rhizoctonia solani*, comparing with each individual treatment. All these reports confirm the present findings. Moreover it is important to indicate that the residue analyses showed no levels of furfural above natural background levels found within the plant or fruit even after multiple applications during the growing season (Rodriguez-Kabana 2006; Steyn 2006).

The obtained results in the present study appears that an urgent investigation of favorable possible applicable method for controlling root diseases, where the addition of a biological control agent in combination with plant resistance inducers could be resulted in increased symptomless plant stand over the biological agent. These methods characterized as environmentally safe, bioactive natural products which able successfully to control phytopathogenic fungi in crop production systems.

ACKNOWLEDGMENT

This work was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No. 1059.

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