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Seed and Soil Treatments as Integrated Control Measure Against Faba Bean Root Rot Pathogens

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ABSTRACT

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In vitro tests, increasing concentrations of acetic acid, propionic acid and furfural were significantly able to gradually reduce the linear growth of tested soilborne fungi. Furfural had a superior effect on fungal growth, causing a complete growth inhibition of *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii* at a concentration of 3 mL/L, while acetic acid and propionic acid reached the same effect at a higher concentration of 5 mL/L. A similar trend was observed when the pathogenic fungi grown in dual culture against seeds imbibed in 1% (10 ml/mL, v:w of 100% *a.i.*) tested chemicals. The presence of acetic acid, propionic acid and furfural in the growth media enhanced the antagonistic ability of *Trichoderma harzianum* against the pathogenic fungi resulted in further decreasing their growth. Germination percent of seeds imbibed in 1% (10 ml/mL, v:w of 100% *a.i.*) of tested chemicals decreased significantly by prolonging the storage period up to the fourth month to reach a range between 48-61%. Under pot and field experiments, evaluation of faba bean seeds soaking or soil drenched with aqueous solutions (v:v) of acetic acid, propionic acid or furfural at the rate of 10, 20 and 30 mL/L, against root rot incidence caused by *R. solani*, *F. solani* and *S. rolfsii* was performed. The applied treatments cause a highly significant effect on root rot incidence at both pre- and post- emergence of plant growth stages of faba bean compared with control. However the applied treatments varied in their effect on disease incidence, the faba bean seeds imbibed in 1% (10 ml/mL, v:w of 100% *a.i.*) of acetic acid, propionic acid or furfural sown in soil artificially infested with bio-agent showed a higher significant reduction on disease incidence than seeds imbibed only in chemical solutions. Furthermore, when soil was drenched with aqueous solution of acetic acid, propionic acid or furfural no significant difference was observed between the two applied treatments, the imbibing seed alone or combined with the bioagent infested soil. In the light of the results obtained in the present study, a promising applicable technique for using such fumigants as seed dressing integrated with biocontrol treatment might be considered as a safe, cheap and easily applied method against soilborne plant pathogens.

Keywords: acetic acid, propionic acid, furfural, faba bean, root rot, seed and soil treatments

INTRODUCTION

Crops diseases caused by soilborne plant pathogens and resulted in the yield losses in many countries. Root rot of faba bean (*Vicia faba*) is considered *Fusarium solani*, *Sclerotium rolfsii* and *Rhizoctonia solani* to be the most deleterious diseases [21, 3, 9]. Root rot disease occurs during the growing season of crops from seedling to flowering stages, and may to cause pre-emergence infection. So far, apart from scientific and practical difficulties, there is no economic way to control the crop diseases. 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. Root rot of faba bean usually occurs at the seedling stage of faba bean but may occur earlier at the pre-emergence stage, and results in the necessity of replanting the missed hills or dead plants. In Egypt, due to the economic importance of faba bean, farmers often grow consecutively on the same land, which leads to a high build up of pathogen inoculums, and causes serious yield losses which can reach up to 12%. The current management strategy relies on the intensive use of fungicides. In addition, chemical control does not give satisfactory control of the root disease. Application of biological control using antagonistic microorganisms has proved to be successful for controlling various plant diseases [28]. However, it is still not easy and costly in application. It can serve as the best control measure under greenhouse conditions. This disease is particularly prevalence in sandy soils in Egypt. The 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. The present research focuses on finding compounds that are safe to humans and the environment, *e.g.* acetic and propionic acids and furfural, and may provide an alternative control of many soil and seed-borne pathogens. Short-chain volatile fatty acids such as acetic, propionic, butyric, valeric, and caproic acids are metabolic products of bacterial anaerobic fermentation in liquid manures [23]. These compounds kill human and animal pathogens [19] and food spoilage organisms [15]. Acetic and propionic acids are universal metabolic intermediaries and occur in plants and animals [4]. Therefore they were commonly used in food manufactures as antimicrobial preservative or acidulates in a variety of industrial food products [10]. Acetic acid inhibits many species of bacteria, yeasts, and molds. It is known to be an effective antifungal agent, particularly on the black molds, *Aspergillus niger* van Tiegh and *Rhizopus nigrificans* Ehr. [11]. The major volatile fatty acids acetic, propionic, and isobutyric acids in the liquid swine manure was able to kill microsclerotia of the wilt fungus *Verticillium dahliae* when added to acidic soils [38]. Acetic acid and propionic acids are frequently added to silage and fruit to prevent rot, and used as food preservatives [18, 34]. On the other hand, it was reported that propionic acid is a registered fungicide and bactericide, to control fungi and bacteria in stored grains, hay, grain storage areas, poultry litter, and drinking water for livestock and poultry [5]. Propionic acid was reported to reduce the risk of mold development in stored rice, sorghum, and groundnut seeds [29]. 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 [6]. Furfural [2-Furancarboxaldehyde] is a naturally occurring compound present in some essential oils and in foods such as bread, baked products, and coffee [27]. It is prepared industrially by treatment with hot sulfuric acid of pentosans contained in agricultural residues, such as cereal straw, brans, and sugarcane bagasse. It was reported that furfural's solubility in water might permit application through drip or other irrigation systems to prolong treatment [25]. They conclude that furfural has a considerable nematicidal activity, and its physical and chemical properties are relative safe to humans [4]. These studies suggest that furfural should be considered for development into a broad-spectrum nematicide-microbiocide. Furfural reduced populations of *Pythium ultimum* and *F. oxysporum* and increased stem height compared with the untreated controls [17]. Meanwhile, metham sodium, furfural + metham sodium, sodium azide, and chloropicrin significantly reduced the incidence of Liatris (*Liatris spicata*) stem rot caused by *Sclerotinia sclerotiorum* [17].

The objective of the present work was to evaluate the inhibitory activity of acetic acid, propionic acid and furfural on the growth of some soilborne pathogenic fungi and the bio-agent *Trichoderma harzianum in vitro*, as well as their efficacy for controlling faba bean root rot when

used as seed treatment under greenhouse and field conditions.

MATERIALS AND METHODS

Seeds pathogens and bioagent

Faba bean seeds (Giza 3 cv.) used in the present work were obtained from the Legumes Crops Research Department, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Virulent pathogenic fungal isolates of *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii* as well as antagonistic isolates of *Trichoderma harzianum* were obtained from Plant Pathology Department of the National Research Centre, Giza, Egypt. These microorganisms were isolated from the rhizosphere of healthy and diseased roots of various leguminous crops, in Delta and Middle Egypt regions, and proved to be of high pathogenic or antagonistic ability during previous work at the same department. Fungal cultures were maintained on PDA [Potato dextrose Agar (Difco Laboratories, Detroit, MI)] slants at 5±1℃ as stock cultures until use. All isolates were refreshed by growing at the optimum growth conditions at the beginning of the present experiments.

Effect on fungal growth

The inhibitory activities of acetic acid, propionic acid and furfural at concentrations of 1, 2, 3, 4 and 5 mL/L on the linear growth of pathogen and antagonistic fungus were evaluated. Moreover, the antagonistic ability of *T. harzianum* against the pathogenic fungi was also evaluated under the same tested concentrations.

Certain volumes of each of acetic acid, propionic acid and furfural were poured into individual flasks containing sterilized PDA before solidifying and rotated gently to ensure even distribution of proposed concentration, and then poured into 90-mm Petri dishes. Amended plates were inoculated with a 5-mm disk of each fungus to be tested at the centre of a plate and incubated at 25℃. The incubation period was 5 and 7 days for antagonistic and pathogenic fungi, respectively.

The inhibitory activity of faba bean seeds treated with (10 ml/mL, v:v) of acetic acid, propionic acid and furfural, as 100% *a.i.* against pathogenic fungi and bioagent was also evaluated *in vitro* performed on PDA medium using the dual culture technique [15]. A 5-mm disk of each fungi was placed onto the PDA plate 10 mm from the edge of the Petri dish. A treated seed was placed on the opposite side of the dish at the same distance. The control treatment was untreated faba bean seeds with a mycelial disk of either a pathogens or antagonist alone. Both experimental and control dishes were assigned to a completely randomized design, with five replicates per treatment. All inoculated Petri dishes were incubated at 28±1℃ and the diameter of fungal growth away from and toward the inhibitor agent was measured when the tested fungal growth in the control treatment had reached the edge of the PDA plate This test was repeated three times and the inhibitor effect was calculated as the reduction percent in colony diameter growth compared to the control.

Effect on antagonistic ability

A 5-mm mycelial disk of *Trichoderma harzianum* was placed onto the PDA, 10 mm from the edge of the Petri dish. Another mycelial disk of pathogen fungal growth culture was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with a mycelial disk of either a pathogen or antagonist alone at the same conditions. Experimental design, incubation period, replicates, fungal growth measurements were carried out as described above.

Effect on seed germination

Seeds of faba bean (500 g/each treatment) were imbibed in 1% (10 ml/mL, v:w) of acetic acid, propionic acid or furfural, as 100% *a.i.* for 6 h [7]. The imbibed seeds were then air-dried on filter paper for 1 h in a laminar flow hood and packed into glass jars sealed with a 45-mm membrane, and stored at 15℃ until required. Another group of surface-sterilised faba bean seeds (using 3% sodium hypochlorite for 5 min, then air- dried) were prepared as control treatments.

Germination of these faba bean seeds was estimated after zero, two, three and four months, storage period. Twenty seeds of each treatment were placed onto wet filter paper inside a sterilised petri dish (180-mm diameter) and incubated at room temperature (20-28℃) for one week. The percentage of germinated seeds was then calculated. All *in vitro* tests were repeated three times, and five replicates were used for each particular treatment.

Greenhouse tests

The evaluation of soaked faba bean seeds or soil drenched with aqueous solutions (v:v) of acetic acid, propionic acid and furfural at the rate of 1, 2 and 3% (10000, 20000 and 30000 μL/L), in addition to biocontrol agent *T. harzianum* was performed in pot experiments under greenhouse conditions of Plant Pathology Dept., NRC, Egypt. Experiments were carried out in a sandy loam soil artificially infested with the bioagent fungus *T. harzianum* and/or the root rot pathogens. Fungal mass production used for soil infestation was obtained by growing the tested isolates on autoclaved sand barley medium [2] [sand:barley (1:1 w:w) + 40% water]. Soils were amended individually at a ratio of 5% (w:w) with antagonistic and/or pathogenic fungal cultures and mixed thoroughly to ensure equal distribution of fungal inocula, then filled in plastic pots (25-cm-diameter) and irrigated every second day for 1 week before sowing.

Another set of soil amended with root rot pathogens (5% w:w) was thoroughly mixed individually with aqueous solutions (v:v) of acetic acid, propionic acid or furfural respectively at the rate of 1, 2 and 3%, with one liter per cubic foot of soil (1), then covered with plastic sheet. The sheet was removed 3 days later, and the treated soil was then filled in plastic pots (20-cm-diameter) and infested individually with inocula of the bio-agent *T. harzianum* relevant to each treatment.

Seeds of faba bean were soaked in aqueous solutions (v:v) of acetic acid; propionic acid or furfural at the rate of 1, 2 or 3% for 2 hr, then picked up and air dried. The surface sterilized faba bean seeds (using 3% sodium hypochlorite for 5 min, then picked up and air-dried) were prepared as control treatments.

Soaked and surface sterilized faba bean seeds were sown, five seeds per pot, six pots per replicates in each treatment. The average percentage of root rot incidence at the pre- and post-emergence of growth stages was recorded up to 45 days of sowing (the experimental period). All of the previous procedures were repeated three times.

Disease assessment

Percentage of root rot incidence at the pre-emergence stage was calculated as the number of absent seedlings relative to the number of seeds sown. Meanwhile, the percentage of root rot incidence at the post-emergence stage was calculated as the number of diseased plants relative to the number of emerging seedlings. At the end of the experiment, faba bean plants were carefully pulled out from pots after being flooded with water in order to leave the root system undamaged. Plant roots showing rot lesions in addition to the visual root rot symptoms on the shoot system were considered diseased plants. Isolation from infected germinated faba bean seeds at the pre-emergence stage and infected bean plants at the post-emergence stage was carried out. Undeveloped, germinated seeds which were picked up from the soil, and the diseased faba bean plants were both washed and sterilized with 3% sodium hypochlorite, then subjected to re-isolation in order to identify the causal pathogens.

Field trials

The activity of integrated treatment of bio-agent *T. harzianum* and soaked faba bean seeds with 1% (v:w) of each acetic acid, propionic acid and furfural, as 100% *a.i.* against root rot incidence was evaluated under field conditions. This study was performed in a naturally heavily infested field with faba bean root rot pathogens, at Al-Aiat territory, Giza Governorate, Egypt. A field experiment was established which consisted of (3.5 ×6.0 m) plots, composed of 12 rows and 25 cm spacing between plants within a row. Inoculum of *T. harzianum* grown on sand-barley medium at the ratio of 120 g/m^2 [2] were incorporated in the top of 20 cm of the soil surface at planting row sites.

Three replicates (plots) per each relevant treatment were used in a completely randomized block design. Three seeds of faba bean per hole were used in all the treatments. Plots received the usual agricultural practices *e.i.* NPK fertilizer and irrigation etc. Percentage of root rot incidence at the pre- and post-emergence of growth stages was investigated and calculated 15 and 45 days after sowing date.

Statistical analysis

All *in vitro* and *in vivo* experiments were set up in a complete randomized design. One way analysis of variance (ANOVA) was used to analyze the obtain results concerning the follows: (i) Differences between tested chemicals as well as microbial antagonistic inhibitor effect

and linear growth of pathogenic fungi *in vitro*, (ii) Differences between tested chemicals on the viability of stored treated faba bean seeds under *in vitro* conditions, (iii) Differences between integrated treatments of seed soaked with chemicals, bio-agent, or soil drenched with tested chemicals against root rot pathogens under greenhouse and field conditions. General Linear Model option of the Analysis System SAS [27] was used to perform the analysis of variance. Duncan's Multiple Range Test at $P \leq 0.05$ level was used for means separation [39].

RESULTS

Laboratory tests

The effect of increased concentrations of acetic acid, propionic acid and furfural on the growth of root rot pathogens were presented in Table 1. Results showed that all tested chemicals were able to reduce gradually the linear growth of tested soilborne fungi by increasing their concentrations. Furfural had superior effect on fungal growth that caused a complete growth inhibition of *R. solani*, *F. solani*, *S. rolfsii*, at concentration of 3 mL/L, while acetic acid and propionic acid reached the same effect at a higher concentration of 5 mL/L. Acetic acid had a higher effect on fungal growth than propionic acid at all tested concentrations. On the other hand, a tolerant reaction to the tested chemicals up to 5 mL/L was observed in the growth of the bioagent *T. harzianum*. Its growth was reduced by 21.0, 12.4 and 16.1% at the highest concentration 5 mL/L of acetic acid, propionic acid and furfural, respectively.

The *in vitro* evaluation of inhibitory effect of imbibed faba bean seeds with 1% (1 mL/100 g seeds) of acetic acid, propionic acid or furfural on root rot pathogens was tested. The data presented in Table 2 show that the growth of pathogenic fungi was reduced by the inhibitory action produced by treated seeds. This reduction in fungal growth was observed in descending order following the seed dressed with acetic acid, propionic acid and furfural. The fungal growth of *F. solani* showed high sensitivity to applied treatments followed by *S. rolfsii* and *R. solani*. Complete reduction in the growth of *F. solani* was observed when exposed to seeds dressed with acetic or propionic acids, while treated seeds with furfural resulted in a high growth reduction recorded as 83.3%. A lesser effect was observed on the growth of the bioagent *T. harzianum* with its growth reduced by 8.7, 7.4 and 2.6% when exposed to the dressed seeds with acetic acid, propionic acid and furfural, respectively.

Table 1. Growth reduction (%) of pathogenic and antagonistic fungi in response to different concentrations of acetic acid, propionic acid and furfural *in vitro*

Tested materials	Concentration (mL/L)	Fungal growth reduction (%) [1]			
		R. solani	*F. solani*	*S. rolfsii*	*T. harzianum*
Acetic acid	1	37.2 ±2.3 g [2]	33.3 ±2.6 g	37.2 ±2.4 g	0.0 ±0.1 j
	2	65.6 ±1.3 de	63.9 ±2.4 de	62.2 ±2.6 de	9.9 ±1.4 ij
	3	83.3 ±2.6 b	75.0 ±2.4 d	83.3 ±2.7 b	14.0 ±2.2 hi
	4	88.9 ±1.8 a	85.6 ±2.8 b	87.8 ±2.3 a	19.4 ±2.6 hi
	5	100.0 ±0.1 a	100.0 ±0.1 a	100.0 ±0.1 a	21.0 ±1.8 h
Propionic acid	1	29.4 ±2.5 gh	19.4 ±1.8 hi	14.4 ±1.8 n	0.0 ±0.1 j
	2	53.3 ±2.7 f	46.7 ±2.2 f	30.6 ±2.5 gh	1.6 ±1.7 j
	3	75.0 ±1.3 d	68.9 ±2.4 d	75.0 ±2.3 d	4.1 ±2.6 j
	4	82.8 ±1.7 b	80.6 ±2.6 b	79.1 ±1.8 bd	9.8 ±2.4 ij
	5	100.0 ± 0.1 a	100.0 ±0.1 a	100.0 ±0.1 a	12.4 ±2.1 i
Furfural	1	23.9 ±2.3 h	31.1 ±2.3 k	21.1 ±1.6 h	0.0 ±0.1 j
	2	58.9 ±1.8 e	60.6 ±2.8 e	64.4 ±2.8 de	0.0 ±0.1 j
	3	100.0 ±0.1 a	100.0 ±0.1 a	100.0 ±0.1 a	3.1 ±2.7 j
	4	100.0 ±0.1 a	100.0 ±0.1 a	100.0 ±0.1 a	12.4 ±1.8 i
	5	100.0 ±0.1 a	100.0 ±0.1 a	100.0 ±0.1 a	16.1 ±1.6 i

[1] Reduction (%) in mycelial growth was calculated in acetic acid; propionic acid and furfural concentrations relatively to the fungal growth (90 mm diameter) in control treatment.

[2] Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 2. Growth reduction (%) of pathogenic and antagonistic fungi in response to imbibed seeds with 1% (1 mL/100 g seeds) of acetic acid, propionic acid and furfural *in vitro*

Seed dressing	Fungal growth reduction (%)[1]			
	R. solani	*F. solani*	*S. rolfsii*	*T. harzianum*
Acetic acid	74.4 ±2.8 cd[2]	100.0 ±0.1 a	83.3 ±2.7 b	8.7 ±1.7 e
Propionic acid	72.2 ±2.7 d	100.0 ±0.1 a	72.2 ±2.6 d	7.4 ±1.8 e
Furfural	77.8 ±2.8 c	83.3 ±2.4 d	77.8 ±2.2 c	2.6 ±1.4 g

[1] Values are the reduction in the growth of pathogenic fungi exposed to treated faba bean seeds calculated relatively to their growth on medium with untreated seeds.

[2] Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Presented data in Table 3 revealed that the presence of acetic acid, propionic acid and furfural in the growth media exhibit an enhancement to antagonistic ability of *T. harzianum* against the pathogenic fungi resulted in the increase of their growth reduction. A significant reduction in the growth of *R. solani*, *F. solani* and *S. rolfsii* was observed at concentrations of 1 and 2 mL/L of acetic acid, propionic acid and furfural comparing with their reduction in the presence of *T. harzianum* only. A complete reduction in the growth of pathogenic fungi was observed in growth media amended with all tested chemicals at concentration of 3 mL/L and more.

The influence of acetic acid, propionic acid and furfural used at concentration of 1% *a.i.* (v:w) as faba bean seeds imbibitions on their germination was determined *in vitro*. Data in Table 4 show that the germination percent of imbibed seeds decreased significantly by prolonging the storage period to reach its minimum at the fourth month. The highest reduction in germination was recorded as 48, 56 and 61% for the stored seeds imbibed with acetic acid, propionic acid and furfural, respectively compared with 98% in untreated seeds. Data obviously showed that the best storage period is two months since compounds treatment remained 89-91%, which blank control was 99% of seed germination.

Table 3. Effect of different concentrations of acetic acid, propionic acid and furfural on the antagonistic ability of *Trichoderma* spp. against root rot pathogens *in vitro*

Tested materials	Concentration (mL/L)	Pathogenic fungal growth reduction (%)[1]		
		R. solani	*F. solani*	*S. rolfsii*
Acetic acid	1	81.1 ±2.6 b[2]	80.0 ±2.1 b	81.1 ±2.6 b
	2	88.9 ±2.4 d	85.6 ±2.4 c	86.7 ±2.2 cd
	3	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
	4	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
	5	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
Propionic acid	1	81.1 ±2.6 b	80.0 ±2.8 b	80.0 ±2.6 b
	2	85.6 ±2.4 cd	83.3 ±2.5 c	83.3 ±2.1 c
	3	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
	4	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
	5	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
Furfural	1	81.1 ±2.6 b	80.0± 2.7 b	81.1 ±2.4 b
	2	85.6 ±2.4 cd	83.3 ±2.6 c	84.4 ±2.6 c
	3	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
	4	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
	5	100.0 ±0.1 d	100.0 ±0.1 d	100.0 ±0.1 d
Untreated medium	0	78.9 ±2.3 a	75.6 ±2.4 a	77.8 ±2.2 a

[1] Values are the reduction in the growth of pathogenic fungi in the presence of bioagent and/or tested chemicals calculated relatively to their growth in growth medium free of these factors.

[2] Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Greenhouse experiments

The efficacy of soaked faba bean seeds or soil drench with water solutions (v:v) of acetic acid, propionic acid and furfural at the rate of 10, 20 and 30 mL/L, in combination with the bioagent *T. harzianum* against root rot incidence caused by *R. solani*, *F. solani* and *S. rolfsii* was evaluated in a pot experiment using soil artificially infested with the pathogen under greenhouse conditions. The incidence of pre-, and post-emergence root rot are presented in Tables 5 and 6. Presented data show that the applied treatments caused a highly significant effect on root rot incidence at both plant growth stages of faba bean comparing with control. A significant effect was observed in the treatment of the seed soaking combined with soil infestation with the bioagent treatments (Table 5). The

Table 4. Germination (%) of imbibed seeds with 1% (1 mL/100 g seeds) of acetic acid, propionic acid and furfural (v:w) at different storage periods

Seed treatment	Seed germination (%)				
	0	1	2	3	4
Acetic acid	98[1] ±0.1a[2]	95 ±0.3 a	89 ±0.4 b	78 ±1.1 c	48 ±1.8 e
Propionic acid	98 ±0.1 a	96 ±0.2 a	90 ±0.8 b	72 ±1.7 c	56 ±1.4 e
Furfural	97 ±0.2 a	97 ±0.2 a	91 ±0.8 b	76 ±1.1 c	61 ±1.8 e
Control	99 ±0.1 a	99 ±0.1 a	99 ±0.1 a	98 ±0.1 a	98 ±0.1 a

[1] Storage period (month).

[2] Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 5. Root rot incidence of faba bean in response to integration between *T. harzianum* and/or soaked seeds in acetic acid, propionic acid, or furfural solution[1] in soil infested with pathogenic fungi[2] under greenhouse conditions

Soil infestation with bioagent	Seed treatment	Con. (mL/L)	Root rot incidence (%)					
			Pre-emergence stage[3]			Post-emergence stage[4]		
			R. solani	*F. solani*	*S. rolfsii*	*R. solani*	*F. solani*	*S. rolfsii*
Non	Acetic acid	10	6.7 ±1.7 h	10.0 ±0.1 g	10.0 ±0.1 g	21.4 ±2.6 b	22.2 ±2.7 b	18.5 ±2.6 c
		20	6.7 ±1.7 h	6.7 ±1.6 h	6.7 ±1.6 h	17.9 ±2.3 cd	14.3 ±2.2 e	17.9 ±2.3 cd
		30	3.3 ±1.4 i	3.3 ±1.3 i	3.3 ±1.4 i	6.9 ±1.8 h	13.8 ±2.6 ef	10.3 ±2.2 g
	Propionic acid	10	6.7 ±1.7 h	10.0 ±2.1 g	6.7 ±1.6 h	14.3 ±2.4 e	18.5 ±2.6 c	10.7 ±2.3 g
		20	6.7 ±1.7 h	6.7 ±1.6 h	6.7 ±1.7 h	10.7 ±2.2 g	17.6 ±2.3 cd	10.7 ±2.1 g
		30	3.3 ±1.4 i	3.3 ±1.3 i	3.3 ±1.4 i	10.3 ±2.2 g	13.8 ±2.1 ef	6.9 ±1.6 h
	Furfural	10	6.7 ±1.7 h	10.0 ±2.1 g	10.0 ±2.2 g	14.3 ±2.4 e	14.8 ±2.2 e	11.1 ±2.3 g
		20	6.7 ±1.7 h	6.7 ±1.6 h	6.7 ±1.7 h	10.7 ±2.1 g	14.3 ±2.1 e	10.7 ±2.4 g
		30	3.3 ±1.4 i	3.3 ±1.3 i	3.3 ±1.4 i	10.3 ±2.1 g	13.8 ±2.1 ef	10.3 ±2.3 g
T. harzianum	Acetic acid	10	6.7 ±1.8 h	6.7 ±1.6 h	6.7 ±1.6 h	10.7 ±2.3 g	14.3 ±2.2 e	7.1 ±1.8 h
		20	3.3 ±1.4 i	3.3 ±1.3 i	3.3 ±1.4 i	10.3 ±2.1 g	13.8 ±2.1 ef	6.9 ±1.6 h
		30	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j
	Propionic acid	10	6.7 ±1.7 h	6.7 ±1.6 h	6.7 ±1.6 h	10.7 ±2.2 g	14.3 ±2.1 e	7.1 ±2.1 h
		20	3.3 ±1.4 i	3.3 ±1.3 i	3.3 ±1.4 i	6.9 ±1.7 h	13.8 ±2.1 ef	6.9 ±1.6 h
		30	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j
	Furfural	10	3.3 ±1.4 i	3.3 ±1.3 i	3.3 ±1.4 i	6.9 ±1.8 h	10.3 ±2.3 g	6.9 ±1.8 h
		20	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	3.3 ±1.4 I	6.7 ±1.8 h	3.3 ±1.4 i
		30	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j	0.0 ±0.1 j
Control			33.3 ±2.8 a	30.0 ±2.8 a	36.7 ±2.7 a	40.0 ±2.6 a	38.1 ±2.8 a	47.4 ±2.7 a

[1] Seeds were imbedded for 2 hr in acetic acid, propionic acid, or furfural water solution at 1, 2 or 3% (v:v).

[2] Soil artificially infested individually with the root rot pathogens at the rate of 5% w:w.

[3] Values are the incidence of infection rated as percentage of emerged plants relative to the number of seeds sown in soil artificially infested with pathogenic fungi. Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

[4] Values are the incidence of infection rated as percentage of infected plants relative to the number of emerged plants in soil artificially infested with pathogenic fungi. Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 6. Root rot incidence of faba bean in response to integration between *T. harzianum* and/or soil drench with acetic acid, propionic acid or furfural solution[1] in soil infested with pathogenic fungi[2] under greenhouse conditions

Soil infestation with bioagent	Soil drench	Con. (mL/L)	Root rot incidence (%)					
			Pre-emergence stage[3]			Post-emergence stage[4]		
			R. solani	*F. solani*	*S. rolfsii*	*R. solani*	*F. solani*	*S. rolfsii*
Non	Acetic acid	10	6.7 ± 1.6 d	6.7 ± 1.4 d	6.7 ± 1.6 d	14.3 ± 2.2 c	14.3 ± 2.3 c	21.4 ± 2.6 b
		20	6.7 ± 1.6 d	6.7 ± 1.4 d	6.7 ± 1.6 d	10.7 ± 2.1 d	14.3 ± 2.1 c	14.3 ± 2.2 c
		30	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
	Propionic acid	10	10.0 ± 2.2 cd	10.0 ± 2.4 cd	10.0 ± 2.6 cd	25.9 ± 2.6 b	22.2 ± 2.6 b	25.9 ± 2.8 b
		20	6.7 ± 1.8 d	6.7 ± 1.7 d	6.7 ± 1.6 d	14.3 ± 2.3 c	21.4 ± 2.6 b	21.4 ± 2.4 b
		30	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
	Furfural	10	10.0 ± 2.3 cd	6.7 ± 1.6 d	10.0 ± 2.6 cd	17.9 ± 2.6 bc	14.3 ± 2.6 c	18.5 ± 2.1 bc
		20	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
		30	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
T. harzianum	Acetic acid	10	6.7 ± 1.8 d	10.0 ± 2.3 cd	10.0 ± 2.8 cd	17.9 ± 2.4 bc	22.2 ± 2.6 b	18.5 ± 2.1 bc
		20	6.7 ± 1.8 d	6.7 ± 1.6 d	6.7 ± 1.8 d	10.71±2.1 d	14.3 ± 2.1 c	14.3 ± 2.3 c
		30	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
	Propionic acid	10	6.7 ± 1.7 d	6.7 ± 1.8 d	10.0 ± 2.7 cd	21.4 ± 2.6 b	14.3 ± 2.1 c	25.9 ± 2.8 b
		20	6.7 ± 1.6 d	3.3 ± 1.4 de	10.0 ± 2.6 cd	14.3 ± 2.1 c	13.8 ± 2.2 c	22.2 ± 2.1 b
		30	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
	Furfural	10	3.3 ± 1.4 de	3.3 ± 1.4 de	6.7 ± 1.7 d	13.8 ± 2.1 c	13.8 ± 2.2 c	14.3 ± 2.2 c
		20	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
		30	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e	0.0 ± 0.1 e
Control			33.3 ± 2.8 a	30.0 ± 2.7 a	36.7 ± 2.6 a	40.0 ± 2.8 a	38.1 ± 2.6 a	47.4 ± 2.8 a

[1] Soil were drenched with acetic acid, propionic acid or furfural aqueous solutions at 10, 20 or 30 mL/L (v/v).

[2] Soil artificially infested individually with the root rot pathogens at the rate of 5% w:w.

[3] Values are the incidence of infection rated as percentage of emerged plants relative to the number of seeds sown in soil artificially infested with pathogenic fungi. Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

[4] Values are the incidence of infection rated as percentage of infected plants relative to the number of emerged plants in soil artificially infested with pathogenic fungi. Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

treated seeds showed a protective effect against disease incidence. Furthermore, increasing concentration gradually of acetic acid, propionic acid and furfural used for seeds imbibitions showed a parallel decline in the percentage of disease recorded up to the concentration of 30 mL/L when a lowest reduction of root rot incidence was observed at both growth stages of pre- and post- emergence, while a complete reduction of disease incidence was recorded at the same concentration when combined with biocontrol treatment. Treated seeds sown in infested soil with either *R. solani*, *F. solani* or *S. rolfsii* showed a significant reduction of pre-emergence root rot incidence, started from no infection up to 10.0% for acetic acid; propionic acid and furfural either alone or combined with bioagent treatments, compared with the untreated control treatments, which recorded as 33.3, 30.0 and 36.7%, in respective order to fungal soil infestation. It is also interesting to note that a greater reduction in disease incidence was observed at the pre-emergence stage of plant growth than that at the post-emergence stage.

The data presented in Table 6 show a significant reduction in root rot incidence at both pre- and post-emergence stages in soil drenched with water solution of acetic acid, propionic acid or furfural compared with untreated control treatment. The disease incidence was reduced by increasing acetic acid, propionic acid and furfural concentrations. No significant difference was observed between the two applied treatments, the soil drench alone or combined with soil infestation with the bioagent *T. harzianum*. Percentages of root rot incidence at pre-emergence stage presented in a significant lower records ranged between 3.3 and 10.0% than that recorded at post emergence stage as a range of 10.7 to 25.9 for acetic acid, propionic acid and furfural either alone or combined with bioagent treatments, compared with the untreated control treatment which recorded as 33.3, 30.0,

36.7 and 40.0, 38.1, 47.4% at pre-, and post-emergence stages, respectively. Furfural applied as soil drench showed a highly superior effect on root rot incidence followed by acetic and propionic acids treatments. Soil drench with furfural solution at concentration of 2% caused a complete reduction in disease incidence, while the same effect was observed at concentration of 3 mL/L for acetic and propionic acids treatments.

Field experiment

Imbibed faba bean seeds with 1% (v:w) of acetic acid, propionic acid or furfural, as 100% *a.i.* alone or in combination with *T. harzianum* as artificial soil infestation was evaluated against the incidence of root rot in a field experiment with soil naturally infested with the root rot pathogens. The data presented in Table 7 show a significant reduction in root rot incidence at both pre- and post-emergence stages for applied treatments compared with the untreated control. Although the applied treatments varied in their effect on disease incidence, the imbibed faba bean seeds sown in soil artificially infested with bio-agent showed a higher significant reduction on disease incidence than imbibed seed only. At pre-emergence stage, the percentage of root rot incidence at treatment of only dressed seeds with acetic acid, propionic acid and furfural recorded as 9.1, 9.9 and 6.5% which reduced significantly when combined with *T. harzianum* soil infestation treatment to reach 4.4, 3.9 and 2.4%, respectively compared with control treatment as 11.9%. Recorded percentage of root rot incidence at post-emergence stage followed the same trend. Furfural proved to be the superior treatment for reducing root rot disease incidence significantly at both pre- and post-emergence stages followed by acetic and propionic acids in respective order either when applied as seed imbibitions alone or combined with soil infestation with *T. harzianum*.

DISCUSSION

Application of fungicides and fumigants have provided a good control of soil pathogens and a broad spectrum of activity and have been popular with growers all over the world [20] although they are cheap, but hazardous, and sometimes difficult to apply [36]. Evaluation of acetic acid, propionic acid and furfural as seed dressing or soil drench alone or in combination with soil infestation with the biocontrol agent *T. harzianum* against faba bean root rot incidence were conducted. Acetic and propionic acids have been thoroughly proved to be the antimicrobial food preservatives and acidulants [11]. Fumigation with acetic, formic, and propionic acids are reported to prevent decay of cherries, stone fruit, pome, and citrus fruit by destroying spores of four different pathogens on the fruit surface [34]. It was shown that vapor of acetic and propionic acids would kill spores of pathogenic fungi on the surface of fruits [31, 32]. The use of propionic acid and propionates has been directed primarily against molds, although some yeasts and bacteria, particularly gram-negative strains,

Table 7. Root rot incidence of faba bean in response to integration between soil infestation with *T. harzianum* [1] and/or seed imbibitions with 1% acetic acid, propionic acid or furfural [2] under natural field conditions

Soil infestation with bioagent	Seed treatment	Root rot incidence (%)	
		Pre-emergence stage [3]	Post-emergence stage [4]
None	Acetic acid	9.1 ±2.8 b	10.5 ±2.6 b
	Propionic acid	9.9 ±2.8 b	10.6 ±2.4 b
	Furfural	6.7 ±2.6 d	8.5 ±2.6 c
T. harzianum	Acetic acid	4.4 ±2.4 f	5.8 ±1.8 e
	Propionic acid	3.9 ±1.8 e	4.9 ±1.7 f
	Furfural	2.4 ±1.6 h	3.7 ±1.6 g
Control		11.9 ±2.4 a	12.6 ±2.3 a

[1] Soil artificially infested with *T. harzianum* inoculum grown on sand-barley medium at the ratio of 120 g/m^2 through incorporation in the top of 20 cm of the soil surface at planting row sites considering relevant treatments.

[2] Seeds were imbibed with 1% (v:w) of each acetic acid, propionic acid and furfural as 100% *a.i.*

[3] Values are the incidence of infection rated as percentage of emerged plants relative to the number of sown seeds. Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

[4] Values are the incidence of infection rated as percentage of infected plants relative to the number of emerged plants. Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

may also be inhibited [10]. Similar results were obtained in this study, whereas acetic and propionic acids caused *in vitro* complete inhibition in the growth of the root rot pathogenic fungi *R. solani*, *F. solani* and *S. rolfsii* at concentration of 5 mL/L when added to their growth media (Table 1) as well as reduced the fungal growth by about 72.2 to 100% when applied as seed dressing (Table 2), meanwhile the antagonistic fungus *T. harzianum* showed less response to the same concentration resulted in enhancement of its antagonistic ability against pathogenic fungi at the same concentration or below. This observation was also true in furfural treatment. In this regards, El-Mougy *et al*. (2008) reported that 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. Furfural treatments in this study had superior effect on either pathogenic fungal growth or disease incidence than that recorded by acetic and propionic acids treatments. This result could be explained through what reported previously by Sholberg *et al*. (1998). They stated that because acetic and propionic acids are chemically closely related, they probably have the same mode of action on fungal spores. Interestingly, when calculations are made to determine the molar amount of acetic and propionic acids that was effective in killing fungal spores, the acids were effective at the same molar concentration of 33 μM. This indicates that the same number of molecules of each short-chain organic acid is required to kill fungal spores.

Regarding the use of acetic, propionic acids and furfural as seed treatment, a preliminary *in vitro* tests were carried out for the purpose of define their affective concentration on faba bean seed germination, and the result revealed that concentration above 1% (v:w) as 100% *a.i*. or 40 ml/L as aqueous solution caused phytotoxicity to treated seeds which indicated by blackened and pitting of the seeds surface and mainly direct complete failure of their germination. Similar observations were also recorded by Sholberg *et al*. (1998) on cherries, citrus pome and stone fruits treated by vapors of acetic, propionic and formic acids. Under greenhouse and field trails the applied treatments varied in their effect on disease incidence, although they significantly reduced root rot incidence compared with untreated control treatment. The dressed faba bean seeds sown in soil, either artificially or naturally infested with bioagent *T. harzianum*, showed a higher reduction on disease incidence than dressed seed only. On the other hand, soil drench with acetic, propionic acids or furfural solution showed no difference when the biocontrol agent was applied as integrated treatment (Table 6). This result could be attributed to the inhibitory effect of the used compounds at 30 mL/L which affected both pathogenic and antagonistic fungi. Similarly, it was reported that soil fumigation destroys the natural equilibrium between pathogens and antagonistic microorganisms [22]. It was reported that application of acetic acid vapors prevented further infection of some seeds inoculated with *Aspergillus flavus* [32]. It was also reported that fumigation of legume seeds with acetic acid vapor caused complete inhibition of natural infection by *A. flavus*, *Penicillium* sp., *F. moniliforme* and *A. niger* [12]. Furthermore, fumigated legume seeds showed protective effect against fungal invasion by these fungi. However, few literature reported the efficacy of furfural against fungi and bacteria, but the metabolism and effects of furfural 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 furfural alcohol in anaerobic fermentation [37]. The fungicidal properties of furfural was first reported during the study of its control of *R. solani* in potato [14, 24]. Soil treated with furfural also controlled the southern blight (*Sclerotium rolfsii*) in lentil, while stimulating development of *Trichoderma* spp. and bacteria antagonistic to *S. rolfsii* [8]. A similar effect was also reported for tomato wilt (*F. oxysporum*) [35] and liatris (*Liatris punctata*) stem rot (*S. sclerotiorum*) [17]. Pot and field experiments indicated that furfural (6 mL/L) combined with bioagent treatments proved to have superior suppressive effect against tomato root rot (*F. solani* and *R. solani*) incidence comparing with each individual treatment [13].

The obtained results in this study showed that acetic, propionic acids and furfural can have a considerable fungicidal activity against faba bean root rot pathogens especially when combined with biocontrol agent. Their physical and chemical properties suggest potentials for

commercial formulation and application as a broad spectrum use against soilborne pathogens.

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摘 要

El-Mougy, N. S.[1, 2], and Abdel-Kader, M. M.[1] 2009. 種子與土壤處理綜合防治蠶豆根腐病菌. 植病會刊 18: 75-87. ([1] 埃及國家研究中心植物病理系；[2] 聯絡作者，電子郵件：nehal_nrc@yahoo.com)

體外抑菌試驗顯示隨著醋酸、丙酸 (propionic acid) 及糠醛 (furfural) 溶液濃度的增加，可顯著降低土傳病原眞菌的生長。糠醛濃度在 3 mL/L 時，可完全抑制 *Rhizoctonia solani*、*Fusarium solani* 和 *Sclerotium rolfsii* 的生長，然而醋酸與丙酸溶液濃度則須高於 5 mL/L 時，才可達到完全抑制上述三種土傳病原眞菌的生長。利用共培養 (dual culture) 試驗得知蠶豆種子浸泡 1% 醋酸、丙酸或糠醛溶液後，亦可降低上述三種土傳病原眞菌的生長。評估醋酸、丙酸或糠醛溶液對 *Trichoderma harzianum* 拮抗能力的影響，結果顯示此三種化學藥品可提高 *T. harzianum* 拮抗上述三種土傳病原眞菌生長的能力。蠶豆種子經浸泡 1% 醋酸、丙酸或糠醛溶液後，其種子儲藏至第四個月時，發芽率明顯降低至 48-61% 左右。在網室與田間試驗中，以蠶豆種子浸泡 1% 醋酸、丙酸或糠醛溶液後，再種植至含有 *T. harzianum* 的土壤中，結果顯示其防治效果較單獨使用化學藥品佳；若以土壤澆灌方式進行試驗，其防治效果則與種子單獨浸泡化學藥品或種子浸泡化學藥品再配合 *T. harzianum* 相同。綜合上述研究結果，本研究顯示蠶豆種子先浸泡燻蒸化學藥劑再配合生物防治劑，應是一種安全、便宜且方便施用以防治土傳病原眞菌的方法。

關鍵詞：醋酸、丙酸、糠醛、蠶豆、根腐病、種子與土壤處理