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Efficacy of furfural and basamid soil treatment for controlling black scurf disease of potato plants under field conditions

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Effect of furfural on black scurf disease of potato plants was evaluated under laboratory and field conditions. The inhibitory effect of different concentrations of furfural on the linear mycelial growth of *Rhizoctonia solani* was evaluated *in vitro*. Results indicate that complete reduction in linear growth was obtained at furfural concentrations of 5000 and 6000 ppm. Meanwhile, the highest reduction was obtained at 4000 ppm which reduced the linear growth by 77.8 %. Under field conditions, four concentrations of furfural, *i.e.* 4000, 5000 and 6000 ppm, in addition to fungicide Basamid (at rate 50 g/m^2 of cultivation soil) were applied to study their effect on black scurf disease incidence of potato plants. Results indicate that the highest significant reduction was obtained with furfural treatments at concentrations of 5000, 6000 ppm and fungicide Basamid which reduced the disease incidence by 86.0, 88.9 and 87.5 %, respectively. Meanwhile, furfural at 4000 ppm showed moderate effect. As for tuber yield the highest increase was obtained with furfural at concentrations of 5000, 6000 ppm and fungicide Basamid which increased the potato yield by 50.0, 55.0 and 50.0 % respectively.

Key words: Black scurf disease - Furfural- Potato - *Rhizoctonia solani*

Introduction

Rhizoctonia diseases of potato are caused by the fungus *Rhizoctonia solani* Kühn can be found on all underground parts of the plant at different times during the growing season. *R. solani* causes black scurf on tubers, stem and stolen canker on underground stems and stolen and occurs wherever potatoes are grown. The symptoms of the disease are found on both above and below ground portions of the plant. Black scurf is the most conspicuous sign of Rhizoctonia disease (Errampalli and Johnston, 2001; Almeida *et al.*, 2007). In this phase of the disease the fungus forms dark brown to black hard masses on

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the surface of the tuber. These are called sclerotia and are resting bodies of the fungus. Although black scurf is the most noticeable sign of Rhizoctonia, stem canker, is the most damaging of the disease as it occurs underground and often goes unnoticed (Grosch *et al*., 2005; Wharton *et al*., 2006). Early in the growing season, the fungus attacks germinating sprouts underground before they emerge from the soil. The sprout may be killed outright if lesions form near the growing tip. Damage at this stage results in delayed emergence and is expressed as poor and uneven stands with weakened plants (Wharton *et al*., 2006).

Controlling such disease mainly depend on fungicides treatments (Rauf, 2000). However, fungicidal applications cause hazards to human health and increase environmental pollution. Therefore, alternative treatments for control of plant diseases are needed.

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 sulfuric acid of pentosans contained in agricultural residues, such as cereal straw, bran, 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 (Al-Hamdany *et al.*, 1999). 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.

On the other hand Gerik (2005) reported that most of drip irrigation treatments supplemented with furfural reduced populations of *Pythium ultimum* and *F. oxysporum* and increased stem height compared with the non-treated controls. Also, He added that Metham sodium, furfural + metham sodium, sodium azide, and chloropicrin significantly reduced the incidence of Liatris stem rot caused by *Sclerotinia sclerotiorum*. Moreover, El-Mougy (2008) reported 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* .

The main objectives of the present research were designed to study the efficacy of Furfural on the mycelia growth of *Rhizoctonia solani* as well as its application as soil treatment against black scurf incidence of potato plants under field conditions and its influence on tuber yield.

Materials and methods

Pathogen and plant material

Different isolates of *Rhizoctonia solani* were isolated from diseased potato tubers samples collected from different fields located at Nubaria region, Behera Governorate, Egypt. This work was done by the auther of the present work.The isolated fungi were tested for their ability to induce Black scurf disease of potato and proved their pathogenicity in this regard. The highly aggressiveness isolate was used in the present study. Potato seeds cv. Diamond were used for cultivation under field conditions.

Laboratory tests

Effect of furfural on linear growth of Rhizoctonia solani

The inhibitor effect of furfural at different concentrations against the linear growth of *Rhizoctonia solani* was evaluated *in vitro*. The furfural concentrations of 1000, 2000, 3000, 4000, 5000 and 6000 ppm were tested. A different certain volumes of furfural were added to flasks containing sterilized PDA medium before its solidifying to obtained the proposed concentrations, then poured in Petri dishes. Another set Petri dishes containing PDA furfural-free medium were kept as control treatment. Disks (6- mm-diameter) taken from the edge of fungus culture were placed in the centre of each Petri plates. Five Petri plates were used as replicates. All Petri plates were incubated at 25 ± 1°C for 7 days, then the average diameter of linear growth was calculated and the percentage of reduction in mycelial growth was calculated for furfural concentrations relative to the control treatment. This test was repeated three times.

Field experiment

Experiments were carried out at the Researches and experimental station (NRC) in Nubaryia region, Behera Governorate, Egypt. The promising concentrations in laboratory experiments were applied under field conditions for two successive growing seasons. The influence of furfural application against black scurf disease incidence and potato tuber yield was determined during the two successive growing seasons.

Field experiments consisted of plots (4x8 m) each comprised of 8 rows and 32 holes/row, were conducted in a Complete Randomized Block design with three replicates (plots) for each particular treatment.

Treatments: Three concentrations of furfural, *i.e*. 4000, 5000 and 6000 ppm in addition to fungicide, Basamid at recommended dose of 50 g/m^2 of soil were applied before potato cultivation.

Application: All plots were irrigated to full water holding capacity. Three days later, the irrigated plots were sprayed with furfural emulsion as well as the fungicide at proposed concentrations at the ratio of 10 L/m^2, then covered with polyethylene sheets for another three days. All plots were cultivated with potato seeds cv. Diamond, three days after polyethylene sheets removal.

Disease assessment: Percent of diseased plant was recorded up to 90 days of planting. Tuber yield of potato (kg $/m^2$) for each treatment was also determined.

All the previous procedures were repeated twice at two successive growing seasons at the same area.

Rhizosphere studies

The influence of furfural and basamid soil treatment at applied concentrations on the total fungal and bacterial counts was studied. The method developed by Louw and Weblely (1959) for studying the microflora of the root region was used. The plate count technique according to Allen (1961) was followed for both total fungal and bacterial counts. Three samples were examined for calculation of total microflora counts, just before potato cultivation and at start of flowering stage and harvest time.

Statistical analysis

Tukey test for multiple comparisons among means was utilized after Neler *et al*. (1985).

Results and discussions

Laboratory tests

The effect of increased concentrations of furfural on the growth of the fungus *R. solani* is presented in Table (1) and Fig. (1). Obtained results showed that furfural was significantly able to reduce gradually the linear fungal growth increasing its concentrations. Complete growth inhibition of *R. solan,* was observed at concentration of 5000 ppm. Meanwhile, the highest reduction was obtained with furfural at 4000 ppm which reduced the linear growth by 77.8 %. Meanwhile, furfural at 2000 and 3000 ppm showed moderate effect (Fig. 1).

Table 1. Average linear growth of *Rhizoctonia solani* in response to different concentrations of furfural

Furfural concentration (ppm)	Average linear growth (mm)
1000	90.0* a
2000	72.3 b
3000	54.4 c
4000	30.6 d
5000	0.0 e
6000	0.0 e
Control	90.0 a

Means followed by the same letter are not significantly different at $P \leq 0.05$.

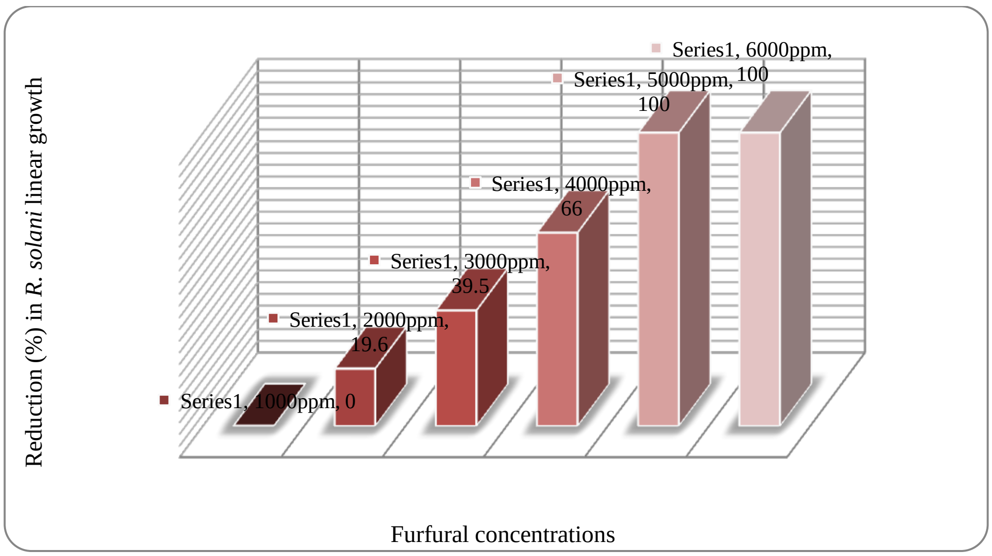

Fig. 1. Reduction in the linear growth of *Rhizoctonia solani* in response to different concentrations of furfural

Similar results concerning the effect of furfural on various soilborne fungi were reported. 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 (Taherza *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. They added that activity of both enzymes decreased by 50% when concentration of furfural increased from 0 to 1.2 g/l (1200 ppm). More recently, El-Mougy *et al.*,

(2008) reported that the linear growth of tested soil-borne pathogenic fungi (*Fusarium solani* and *Rhizoctonia solani*) was dramatically reduced with the increasing of furfural concentrations added to the growth medium up to 4000 ppm where no growth was observed.

Field experiments

In field trials for two successive growing seasons, the efficacy of controlling *R. solani* (Black scurf) on potato using furfural and fungicide measures was determined. The results presented in Table (2) and Fig. (2) showed superior significant effect of furfural and fungicide treatments against the incidence of Black scurf disease comparing with control. The average recorded percentage of potato black scurf disease incidence during the two successive growing seasons was 25.4% in untreated soil naturally infested with the pathogen *R. solani*. This percentage sharply decreased down to 13.0, 5.2 and 4.0% in the presence of applied furfural at 4000, 5000 and 6000ppm, respectively. Meanwhile 4.4% disease incidene was recorded in applied soil with Basamid (50g/m^2).

The highest reduction was obtained with furfural at concentrations of 5000, 6000 ppm and fungicide Basamid which reduced the disease incidence more for about 79.5, 84.2 and 82.6%, respectively during two growing seasons. Meanwhile, furfural at 4000 ppm showed lower effect although it significantly differed than untreated control treatment (Fig. 2).

Table 2. Effect of furfural and Basamid soil treatment on black scurf disease of potato plants under field conditions

Treatment	**Black scurf incidence (%)**		
	First growing season	Second growing season	Average
Furfural 4000ppm	13.7 b	12.4 b	13.0
Furfural 5000ppm	5.8 c	4.5 c	5.2
Furfural 6000ppm	4.3 c	3.7 c	4.0
Basamid 50g/m^2	4.5 c	4.2 c	4.4
Untreated control	26.3 a	24.4 a	25.4

Means followed by the same letter are not significantly different at $P \leq 0.05$.

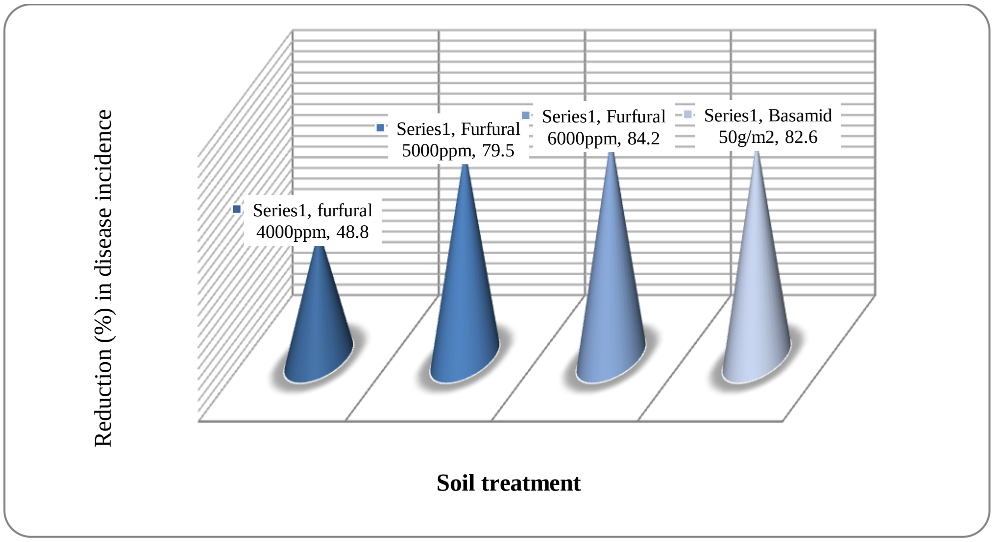

Fig. 2. Reduction (%) in Black scurf disease incidence in response soil application with furfural and basamid

In the present study an interesting observation was noticed, that reduction in disease incidence reflected positively on the produced yield. In this regards, results in Table (3) indicate that the average highest tuber yield was obtained with furfural at concentrations of 5000, 6000 ppm and fungicide Basamid which recorded as 3.1, 3.2 and 3.0 Kg/m^2, respectively. Meanwhile, furfural treatment at 4000 ppm was lesser effective for increasing yield that it cause only an increase of 20% over control treatment (Fig. 3).

Table 3. Effect of soil treatment with furfural and basamid on potato yield under field conditions

Treatment	**Potato tuber yield (Kg/m^2)**		
	First growing season	Second growing season	Average
Furfural 4000ppm	2.3 b	2.5 b	2.4
Furfural 5000ppm	3.0 a	3.2 a	3.1
Furfural 6000ppm	3.1 a	3.3 a	3.2
Basamid 50g/m^2	3.0 a	3.1 a	3.0
Untreated control	2.0 c	2.1. c	2.0

Means followed by the same letter are not significantly different at $P \leq 0.05$.

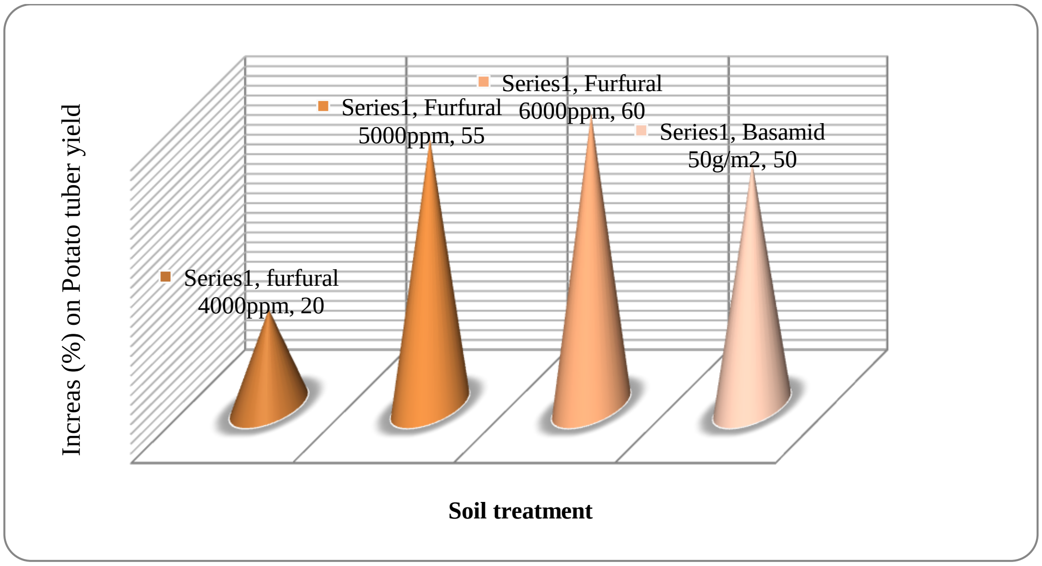

Fig. 3. Increas (%) in Potato tuber yield in response soil application with furfural and basamid

There are a few cited reports explaining the furfural mode of action against soil microflora. Flor (1926) first studied the fungicidal properties of furfural, reporting control of *R. solani* in potato. 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 fungi by reacting with the cellular wall and disrupting cellular functions. Also, it is obvious from Multigaurd fact sheet that it controls root infesting plant parasitic nematodes and fungal plant pathogens such as *Pythium*, *Phytophthora*, *Fusarium* and *Rhizoctonia*. Moreover, drip irrigation treatments reduced populations of *Pythium ultimum* and *F. oxysporum* and increased stem height compared with the non-treated controls (Gerik, 2005). In this respect, 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*. Moreover, 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 (El-Mougy-Nehal, 2008). Using furfural for controlling soil-borne diseases was also reported by Al-Hamdany *et al.* (1999), Stephan *et al.* (2001) and Anonymous (2006).

Rhizosphere studies

Data in Table (4) show a drastic, sharp reduction in fungal and bacterial counts after furfural and Basamid application. The decline in fungal propagules reduced from 178.66 down to (11.74-14.57) and $9.83x10^3$ cfu/g soil and from 187.34 down to (14.31-16.24) and 18.40 x 10^6 cfu/g soil for bacterial cells. During the growing season it was noticed that the total counts of fungi and bacteria in the rhizosphere of potato plants increased as plants grew up reaching their maximum at harvest time where no significant differences were observed between treatments and control. Data also show that the average total fungal counts were higher in furfural treatments than those of fungicide throughout the growing season, while the opposite feature was observed regarding bacterial counts. Another conclusion may be drawn from the fact that the microorganism counts curve showing fungal population of the rhizosphere in furfural treatments were almost a mirror image of those representing bacterial counts in fungicide treatments. These differences could be attributed to the effect of fungicide on fungal population the soil. The high population density of fungi or bacteria introduced through soil treatment technique enables these microorganisms to adapt themselves against environmental conditions (Papavizas, 1982) resulting in the dominance of high population observed.

Application of furfural and benzaldehyde to soil causes both quantitative and qualitative shifts in the composition of the soil bacterial community (Bauske *et al.*, 1997). After decreasing in the first 24 h after application, bacterial populations increased 1 week after application and remained higher than in non-treated control soils for 7 weeks (Kloepper *et al.*, 1999). In the present study, increasing both fungal and bacterial population in treated rhizospheric soil to reach the nearest counts in untreated soil is an expected phenomenon for microbial equilibrium in nature. Initial population of soil microflora increasing throughout the growing season enhanced with favorable conditions lead to rapid propagation in the plant root region, *e.g*. root exudates, plant debris and other organic materials especially in tomato plants which received the traditional fertilizers needed (El-Said, 1997).

Table 4. Frequency of occurrence of fungal and bacterial total counts in rhizosphere region of potato plants in response to furfural application

Treatment	Concentration	Total fungal counts 1 x 10^3/gram of dry soil			Total bacterial counts 1 x 10^6/gram of dry soil		
		before cultivation	45 day after cultivation	at harvest time	before cultivation	45 day after cultivation	at harvest time
	400ppm	14.57 b	44.61 b	263.33 b	16.24 b	68.64 b	414.50 b
Furfural	500ppm	13.37 b	34.76 c	259.24 b	15.34 b	44.27 c	392.33 b
	600ppm	11.74 b	30.84 c	247.68 c	14.31 b	42.36 c	376.43 c
Basamid	50g/m^2	9.83 b	28.46 c	218.35 c	18.40 b	30.64 c	344.37 c
Untreated control		178.66 a	237.78 a	289.48 a	187.34 a	261.68 a	487.58 a

Means followed by the same letter are not significantly different at $P \leq 0.05$.

In conclusion, the obtained results in the present study showed that furfural can have a considerable fungicidal activity in the soil. Its physical and chemical properties suggest potential for commercial formulation and application. These factors combine with its relative safety to humans, low price and its ready degradation by soil microorganisms (Canullo *et al.*, 1992). Furthermore, 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 and Steyn, 2006). It is suggested that furfural could be considered as a broad spectrum micro-biocide.

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