Plant Pathology Journal 7 (1): 54-64, 2008
ISSN 1812-5387

Prospects for Biocontrol of Brown Rot Disease of Potato *in vitro* and under Greenhouse Conditions

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Abstract: This investigation is a trial to biocontrol of brown rot disease of potato by basidiomycetes, wheat straw and spent mushroom straw. Bird's nest *Cyathus stercoreus* is firstly recorded in Egypt. It was found growing on manured soil at New Damietta. It is identified and isolated from its fruit body. Eight basidiomycetes including *C. stercoreus* were tested to antagonize *Ralstonia solanacearum* (causal agent of brawn rot disease of potato) *in vitro*. All of these fungi inhibited the growth of *R. solanacearum* and the largest inhibition zones were recorded with *Cyathus stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain. Extract from the *Cyathus stercoreus* mycelia was studied using Thin Layer Chromatography (TLC), Infrared (IR) spectroscopic analysis and Nuclear Magnetic Resonance (NMR). The study suggested that the polysaccharide of this fungus has the antibacterial activity. *C. stercoreus* was selected to green house study. Water extract filtrate of raw wheat straw (wers) and of spent mushroom straw (wess) of three *Pleurotus* sp. were tested to inhibit *R. solanacearum* growth by applying disc-filter paper method. RSE filtrate and all the other three filtrates inhibited *R. solanacearum* growth. Water extract of *Pleurotus columbinus* spent wheat straw (wess) had the largest inhibition zone, so, it was selected to further study. In greenhouse experiment, the previous selected factors were used to estimate their ability to biocontrol *R. solanacearum*. The reported results revealed that the mycelial suspension of *C. stercoreus* (Css) had the best effect in reduction of the disease, plant health and plant production. Consequently, this is the first report of using *C. stercoreus* in biological control of plant diseases. Using of spent straw and raw straw powders and their water extracts had good results in controlling the disease and plant productivity.

Key words: Bird's nest, *Cyathus stercoreus*, polysaccharides, mushroom spent straw, *Pleurotus columbinus*, potato plant

INTRODUCTION

Brown rot disease of potato is worldwide disease that damages potato plantation. This disease caused by soilborne bacteria called *Ralstonia solanacearum* (Yabuuchi *et al*., 1995). This bacterium has an unusual wide host range; it can infect more than 200 plants belonging to 50 botanicals families (Hayward, 2000; Salanoubat *et al*., 2002).

Basidiomycetes can be used for biological control of plant diseases. These fungi can produce exudates that can inhibit the growth of plant pathogenic fungi (Hwang *et al*., 2000). The bird's nest *Cyathus* is a widely distributed genus all over the world (Alexopolous *et al*., 1996). Studies on the metabolites from *Cyathus* spp. have thrived for many years since then. Three crystalline antibiotics were isolated from the mycelium of the basidiomycete *Cyathus striatus* strain No. 12. They are highly active against fungi imperfecti and a variety of Gram-positive bacteria, as well as against some Gram-negative bacteria (Anke and Oberwinkler, 1976). Ayer and Ttaube (1973) reported cyathin complex, a new class of diterpenoid from *C. helenae*. A number of new compounds were reported from various species, such as xanthone from *C. intermedius* (Ayer and Taylor, 1976), cyafrin from *C. africanus* (Ayer *et al*., 1978), cyathatriol from *C. earlei* (Ayer and Lee, 1979). The striatals and striatins are unusual group of diterpenoids isolated from cultures of the bird's nest fungus *Cyathus striatus* (Anke *et al*., 2002) and tropical *Cyathus* species (Anke and Steglich, 1988). The striatins are artifacts, formed by extraction of the mycelia with methanol. This compound posses antibacterial and antifungal (Anke and Steglich, 1988). Polysaccharides from some basidiomycetes have been confirmed to have biological activity (Zjawiony, 2004; Guo *et al*., 2004).

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Agriculture practices such as incorporating organic amendments and managing the type and quality of crop residue have a direct impact on plant health and crop productivity. The application of organic amendments that are rich in nitrogen may reduce soil born diseases by releasing allelochemicals generated during product storage or by subsequent microbial decomposition (Bailey and Lazarovites, 2003).

Potential benefits of maintaining cover crop residue on soil surface include reduction in costs association with repeated intensive tillage, improvement in weed and insect control, improving in soil fertility and structure prevention of erosion and soil moisture retention. Residue on the soil surface decreases soil temperature and increase soil moisture (Ferguson and Shew, 2001).

The addition of organic matter from rotational or cover crops may directly or indirectly suppress disease by enhancing production of decomposition products by antagonistic microbial populations that inhibit pathogens and enhance growth (Huang and Huang, 1993; Shetty *et al*., 2000; Gamliel *et al*., 2000). Plant residues may represent physical barrier that reduces contact with the pathogen or the impacts of non-target chemicals (Hau and Beutte, 1983; Ferguson and Shew, 2001). Some organic matter can stimulate the production of lytic enzymes involved in the degradation of plant pathogens. Microbial degradation of plant residues can also produce secondary products with antimicrobial activities that inhibit the growth of plant pathogens (Bardin *et al*., 2004).

Little attention has been paid to wild mushroom in Egypt, although there is few literatures have been published (El-Fallal, 2001, 2003). However there is no any record in gasteromycetes have been done in Egypt. *Podaxis pistillaris* has been recorded in Zaranik protected area, North Sinia (El-Fallal and Khedr, unpublished data).

This investigation was aimed to identify and isolate bird's nest (*Cyathus)* from Egypt and study its antagonistic ability with some other basidiomycetes against *R. solanacearum.* The antagonistic ability of both the powder and the water extract of both mushroom spent straw and raw wheat straw against *R. solanacearum* will also be searched. Then, the best factors will be used to control brown rot disease in greenhouse experiment.

MATERIALS AND METHODS

Antagonistic effect of some basidiomycetes against *R. solanacearum*: Bird's nest *Cyathus stercoreus* has been identified and isolated from fruit body using (Watling, 1980) method. Its antagonistic effect compared with another seven basidiomycetes was carried out by the use of disc agar method, according to Mansour *et al.* (1997). The seven basidiomycetes used in this investigation were provided by the first author. The plates were incubated at 28°C for 24 h. The inhibition zone diameter of each fungus was measured (if it occurs) and the mean value of three replicates was calculated for each. The fungal species having the largest diameter of inhibition zone was selected to further study.

Antimicrobial activity of the selected fungal species: The antimicrobial activity of the selected fungal species was preformed by disc agar method, according to Mansour *et al.* (1997).

The plates of bacteria and yeast were incubated at 28°C for 48 h. Those of fungi were incubated at 25°C for 7 days. Finally, the inhibition zones were measured in millimeters.

The microbial species which used in the present study of antimicrobial activities were recorded in Table 1.

Production and extraction of the mycelia of the best fungus inhibiting the tested bacterium: *Cyathus stercoreus* was grown in Martin medium broth. Slant tube cultures were inoculated into four flasks, each 500 mL Erlenmeyer flasks containing 150 mL of the above media. These flasks were cultivated on rotary shaker (180 rpm) for 15 days at 25°C. Mycelia were collected on Büchner funnel, washed with water and extracted with methanol (2×100 mL). The resulted solutions were evaporated under vaccum. Each residue was dissolved in 2 mL of 10% methanol solution and stored at 4°C.

Thin layer chromatography of the fungal suspension: The combined extracts were concentrated in rotary evaporate. Standard chromatography of fungal extracts were prepared by applying 20 μL extract solution to a silica gel TLC plate. The components separated by

Table 1: The origins of the used microorganisms in studying antimicrobial activities

Group	Microorganisms	Origin
Bacteria	*Pseudomonas flourscence* 40	Department of Botany, Faculty of Science, Mansoura University
	Escherichia coli 55	
	Bacillus cereus	
	Erwinia Carotovra Carotonara 46	
	Staphylococcus aureus 102	
	Bacillus subtilus 28	
Fungi	*Rhizoctonia solani*	Department of Plant Disease, Faculty of Agriculture, Mansoura University
	Fusarium oxysporum	
	Trichoderma viridi	
	Aspergillus fumigatus	
Yeast	*Lipomyces starkeyi*	Department of Microbiology, Faculty of Agriculture, Mansoura University
	Candida albicans	
	Candida lipolytica	
	Saccharomyces cervesia El	

preparative TLC (Alugram silica gel TLC plates) Benzene-acetone-acetic acid (7:3:0.5, v/v/v). Chromatograms were detected by UV-light (254 nm).

Identification of the most active band against *R. solanacearum*: The most active band against *R. solanacearum* was determined using Jasco FT Infrared spectroscopic analysis (IR)-460 plus-Japan and analyzed by using Varian-Gemini-200 HZ Nuclear Magnetic Resonance (H^{-1}NMR and C^{13} NMR) at Microanalytical Center at Cairo university.

Effect of mushroom spent straw and wheat straw on the growth of *R. solanacearum*

Collection of mushroom spent straw and raw straw: The mushroom spent straw used in this investigation are mushroom wastes from the mushroom house of Faculty of Science at New Damietta, Mansoura university obtained directly after the cropping of three *Pleurotus* species (*P. columbinus*, *P. pulmonarius* and *P. floridanus*) on wheat straw. The superficial layer of spent straw was removed and the samples were collected from 5-10 cm depth. Five samples were taken for each treatment. These samples were collected aseptically in clean plastic bags. The samples of each treatment were mixed together to form composite sample which divided into separate samples. The samples were stored in cool place. The raw wheat straw was obtained from the same straw before spawning. It was also kept in cool place in clean bags.

Preparation of water extracts of spent and raw straws: Samples were grounded well to be fine powder. Sterilized distilled water was added to the fine powder of each sample in a ratio of 20: 1 (volume: weight) in a clean dry flask. The flasks were shaken at speed 200 rpm for 24 h. The water solutions were filtered through double layer of cheese cloth under a strong hand pressure. The supernatants were centrifuged at 4000 rpm for 30 min. The supernatants were divided into two portions for each treatment. One portion was sterilized by filtrations through fitted glass filter and the other portion was sterilized by autoclaving. The sterilized water extracts were kept at 4°C.

Estimation of the antagonistic activity of water extracts against *R. solanacearum*: The antagonistic activity of water extracts against *R. solanacearum* was estimated by filter paper disc method, according to Mansour *et al.* (1997).

Glasshouse experiment: This experiment was carried out at the Faculty of Agriculture farm, Mansoura University, Egypt, during the period from the 3rd of February to 25th of May 2005 at open air. The plastic sacs of 30 cm diameter were filled with 15 kg non-sterilized soil. This soil was composed of a mixture of clay and sand 1:1 (w: w). Six replicates were prepared for each treatment. This experiment was conducted according to complete randomized design.

Soil infestation: Soils of each plastic sac were soaked with water and left to dry for 72 h. The sacs were infested by *R. solanacearum* (about 10^6 cfu); 100 mL for each sac. The sacs were left to dry for about 48 h before sowing.

Potato used for sowing: Sponta potato cultivar was used in this experiment. The potato tubers were sliced to portions more than 30 g and contain two eyes or more.

Preparation of biological control treatments: The used treatments were located under the following main categories:

- ***Cyathus stercoreus* Egyptian strain (Cs):** The fungus was inoculated on PDA plates of 9 cm diameter and incubated at 28°C for 14 days. Each plate was blended in an electric blender with 1 L sterilized tap water to prepare fungal suspension (Css).
- ***Pleurotus columbinus* spent straw (ss):** Fine powder and its water extract (wess) of 5% concentration were prepared as mentioned before.
- **Raw straw (rs):** Fine powder and 5% of its water extract (wers) were prepared as mentioned earlier.

There were 10 treatments and control (only infested soil, without any biocontrol agent). Five treatments were treated only before sowing and the other 5 treatments were treated both before sowing and after 70 days of sowing. The five treatments treated before sowing were as the following:

- Three treatments in which sliced potato tubers were soaked for 10 min before sowing. These are mycelial suspension of *Cyathus stercoreus* (Css) and water extract of both spent straw (wess) and raw straw (wers).
- Two treatments in which the sliced potato tubers have not been treated but only 1 g of the fine powder of ss and rs were added near them.

The other five treatments which were treated before sowing and after 70 days were as the following:

- Three treatments were treated as mentioned in (i) and drenched after 70 days with 100 mL of Css, wess and wers.

- Two treatments were treated as mentioned in (ii) and 1 g of fine powder of ss and rs were added on the surface of pot near the plant.

Storage of potato tubers after harvest: In order to show the latent infection of brown rot disease, after harvest, the non-infected tubers were stored at room temperature (20-26°C) for 60 days. The tubers were examined for presence of the studied disease at the end of the storage period. The percentage of infected tubers was calculated for each treatment after storage period and also percentage of total infected tubers for each treatment was calculated.

The results were statistically analyzed with the Statistical Analysis System (SAS, 1988). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using Least Significant Differences (LSD) at p = 0.05 and Duncan's multiple range test (Duncan, 1955).

RESULTS

The fungus *Cyathus stercoreus* (Schw.) de Toni has been identified according to Brodie (1975), Phillips (1985) and Smith *et al*. (1981). The fungus can be described as follow : As the name suggests, fruit bodies of Bird's nest fungi looks like small bird's nest full of eggs (Fig. 1a). These eggs are small capsules known as peridioles that contain spores. They belong to the family Nidulariaceae. Peridioles gray to black; wall of fruiting body 3 layered. Fruiting body 5-15 mm high, 4-8 mm wide, at times with a basal pad of brownish mycelium vase-shaped; exterior fibrillose, golden brown to russet brown when young, in age nearly glabrous and blackish; inner surface smooth, pale to dark lead colour. Epiphragm soon evanescent, spores up to 40 μm in diameter, subglobose, often varying in size within a single fruit body. Scattered to cespitose on dung, manured ground and round sawdust in New Damietta garden. The culture characteristics: The mycelium was yellow brownish with clamp connections (Fig. 1b) and the reverse side on PDA was pale yellow.

Table 2: Antagonistic activities of some basidiomycetes against *R. solanacearum*

Fungus	Diameter of inhibition zone (mm)*
Cyathus stercoreus Egyptian strain	17
Agaricus campester Egyptian strain	17
Podaxis pistillaris Egyptian strain	14
Corticium vellereum Egyptian strain	13
Pleurotus columbinus	12
Volvariella volvaceae	13
Pleurotus sajor-caju	13
Lentinus striatus	12

*: The diameter of disc of the fungus is 0.9 mm and the diameters of inhibition zones were measured directly from the plates; the recorded values were the mean values of three replicates for each treatment

Antagonistic effect of some basidiomycetes against *R. solanacearum*: The causal pathogen of brown rot disease was isolated from infected tubers, collected from different locations in Dakahlia and Damietta Governorates. It is identified and the pathogenicity test was preformed (unpublished data). All tested basidiomycetes had antagonistic effect against *R. solanacearum* (Table 2). The largest recorded inhibition zone diameter (17 mm) was observed with *Cyathus stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain. The inhibition zone of *Podaxis pistillaris* Egyptian strain was the second (14 mm), then that of *Corticium vellereum* Egyptian strain and *Volvariella volvaceae* (13 mm). The smallest inhibition zone (12 mm) was recorded with *Pleurotus columbinus* and *Lentinus striatus*.

Cyathus stercoreus was selected for further studies during this investigation because it showed the best inhibition zone and had not form fruiting body in soil in ordinary circumstances.

TLC of the mycelial extract of *Cyathus stercoreus* and identification of the most active band against *R. solanacearum*: Developed TLC plate is presented in Fig. 2. The isolated most active compound was analyzed by IR spectra which showed bands at 3645.9, 3676.5 and 3334.6 cm^{-1} (OH), 2855.6 cm^{-1} (CH_3), 1676.8-1650.5 cm^{-1} (C = C or C = N), 989.9-1560.5 cm^{-1}. H^1-NMR spectrum displayed signals at δ 3.11-3.52, 3.55-3.77 and 4.2 and 5.07-5.25. ^1H NMR spectrum showed a saccharide pattern of signals having the signal of anomeric proton at δ 5.08 ppm and a signal sequence like this of glucose (from δ 3.0-4.0 ppm) indicating a glucan. The ^{13}C-NMR spectrum showed six carbon signals at δ 90.65, 69.94, 69.57, 68.46, 67.12 and 57.96 ppm supporting the glucose unit of this glucan.

Effect of water extracts of mushroom spent wheat straw on *R. solanacearum* growth: Table 3 shows that all of the four tested water extracts inhibited the growth of *R. solanacearum* after their sterilization by filtration. In contrast their autoclaving resulted in three of the tested water extracts inhibited the growth of *R. solanacearum*;

Table 3: Inhibition of *R. solanacearum* growth by water extracts of *Pleurotus* spp. spent wheat straw (ss) and wheat straw (rs)

	Inhibition zone (mm)*	
Treatment	Filtration	Autoclaving
Pleurotus columbinus ss	10	7
Pleurotus pulmonarius ss	9	6
Pleurotus floridanus ss	8	-
Raw wheat straw (rs)	8	7

*: The diameter of filter paper disc is 0.5 mm and the diameters of inhibition zones were measured directly from the plates; the recorded values were the mean values of three replicates for each treatment

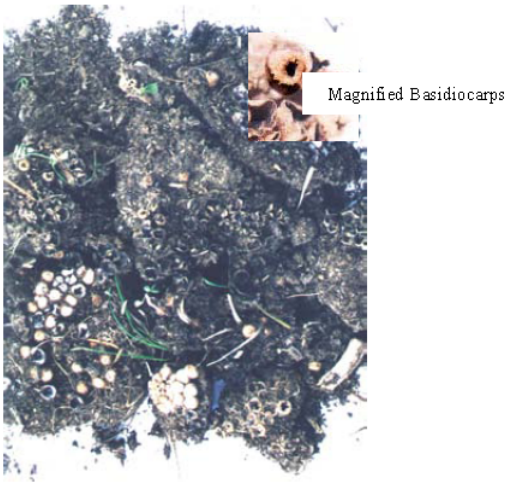

Fig. 1a: Basidiocarps of *Cyathus stercoreus*

Fig. 1b: Mycelial growth of *Cyathus stercoreus*

Fig. 2: TLC separation of mycelial suspension of *Cyathus stercoreus*. Developmental system: Benzene: Acetone:Acetic acid (70:30:5 v/v/v). Detection: 254 nm short UV

Table 4: Antimicrobial activity of water extracts of *Pleurotus columbinus* waste (wess) and water extract of raw wheat straw (wers) and mycelial growth of *C. stercoreus*

	Inhibition zone (mm)		
Indicator	Wess*	Wers*	Cs**
Bacteria			
Staphylococcus aureus 102	-	-	-
Bacillus subtilus 28	-	-	-
Bacillus cereus	-	-	16
Escherichia coli 55	-	-	9
Pseudomonas flourscence 40	-	7	-
Erwinia carotovora carotovora 46	7	-	-
Fungi			
Rhizoctonia solani	15	-	22
Fusarium oxysporum	-	-	-
Trichoderma viridi	-	-	-
Aspergillus fumigatus	-	-	20
Yeast			
Lipomyces starkeyi	-	-	-
Candida albicans	-	-	10
Candida lipolytica	-	-	-
Saccharomyces cervesia El	7	-	-

*: The diameter of filter paper disc is 0.5 cm, -: No inhibition zone was detected, the diameters of inhibition zones were measured directly from the plates; the recorded values were the mean values of three replicates for each treatment. ** : The diameter of the fungal disc is 0.5 cm

Table 5: Plant height and disease incidence after 80 days of sowing

Treatments	Plant height (cm) after 80 days sowing	Disease incidence after 80 days of sowing
1 Control	11.67^{d}	83.33^{a}
2 Css 1	18.50^{b}	16.67^{b}
3 Css 1 + Css 2	19.00^{ab}	16.67^{b}
4 (wess)1	18.50^{b}	33.33^{ab}
5 (wess)1 + (wess)2	19.83^{ab}	33.33^{ab}
6 (ss-1g)1	12.50^{d}	66.67^{ab}
7 (ss-1g)1 + (ss-1g)2	14.83^{c}	50.00^{ab}
8 (wers)1	19.00^{b}	33.33^{ab}
9 (wers)1 + (wers)2	20.00^{a}	33.33^{ab}
10 (rs-1g)1	12.67^{d}	66.67^{ab}
11 (rs-1g)1 + (rs-1g)2	15.33^{c}	50.00^{ab}

Values in the column represent the means of 6 values for potatoes plant. Means followed by different letter(s) within a column are significantly different according to Duncan's multiple range test (p = 0.05). Control: Only infested soil without any biocontrol agent, Css: mycelial suspension of *Cyathus. stercoreus* Egyptian strain; ss: *Pleurotus columbinus* spent wheat straw; rs: wheat straw; we, water extract, 1: treatment before the sowing and 2: treatment after 70 days of sowing. In treatments Css1, wess and wers, potato pieces were soaking in the respective solution or filtrate for 10 min. In treatments Css2, (wess)2 and (wers)2 the treatments were watered by 100 mL of respective suspension or filtrate. In treatments (ss-1g)1 and (rs-1g)1, one gram of the respective fine powder was added in the hole before sowing. (ss-1g)2 and (rs-1g)2 referred to that 1g of corresponding fine powder had been added on the soil surface near each plant of the corresponding treatment(s)

where water extract of *Pleurotus floridanus* spent straw did not inhibit the growth of *R. solanacearum*. In case of sterilization by filtration, the largest recorded inhibition zone diameter was obtained with the water extract of *P. columbinus* spent straw (10 mm), followed by the inhibition zone caused by *P. pulmonarius* (9 mm). While, the inhibition zone diameter of water extract of *P. floridanus* spent straw was equal to that of water extract of raw straw (wers) (8 mm).

In case of sterilization by autoclaving, the antibacterial activities of the extracts decreased. The largest inhibition zone diameter was obtained with water extract of both *P. columbinus* spent straw and wers (7 mm). The diameter of inhibition zone in case of water extract of *P. pulmonarius* spent straw was only 6 mm.

Antimicrobial activity of *Cyathus stercoreus*, water extract of *P. columbinus* spent straw (SS) and raw straw (RS): The largest diameter of inhibition zones resulted from both Cs and wess against *Rhizoctonia solani* (22 and 15 mm, respectively). It is clear from Table 4 that Cs showed higher inhibition ability than wess.

Cyathus stercoreus inhibited the growth of five tested species. However, wess inhibited the growth of three tested microorganisms (Table 4). On the other hand, from the fourteen tested microbes, wers only inhibited the growth of *Pseudomonas flourscence*.

Greenhouse experiment

Observation of plant after 80 days of sowing: The tested treatments generated significant variation in plant height after 80 days (Table 5). All tested treatments had more significant increase in plant height than the control except the two treatments No. 6 and 10. So, the addition of 1 g of fine powder ss and rs aside pieces of potato plants at sowing time had no significant effect.

Using wers once and twice, wess twice and Css once and twice are equally (no significant difference between them) stimulators to the highest plants length. On the other hand there is no significant difference between treatment No. 2 and 3. Therefore the use of mycelial suspension of *C. stercoreus* (Css) one time before sowing is sufficient and it is not significantly different from the use of this suspension twice as mentioned above. The results illustrated in Table 5 also revealed that the use of wess or wers twice (soaking the potato pieces before sowing and watering by 100 mL of wess after 70 days) is not significantly better than using it once.

The two treatments No. 11 and 7 had no significant difference between each other and they had more significant increase than the two treatments 6 and 10. So, the addition of 1g of fine powder of ss and rs near the plants before and after sowing generates greater significant increase in plant height than using each of them only before sowing.

Most of the potato plants of the control treatment (83.33) were infected with the brown rot disease after 80 days of sowing (Table 5). The best treatments in resistant the brown rot after 80 days were recorded with the two treatments of Css (2 and 3) with no significant difference between both. However, the data also indicated that there were no significant differences between all the other treatments and the control.

Table 6: Means weight of tubers for each pot

Treatments	Mean weight of tubers for each pot (g)
1 Control	48.33^{e}
2 Css 1	60.83^{c}
3 Css 1 + Css 2	80.00^{a}
4 (wess)1	72.33^{b}
5 (wess)1 + (wess)2	80.50^{a}
6 (ss-1g)1	66.67^{b}
7 (ss-1g)1 + (ss-1g)2	83.33^{a}
8 (wers)1	67.50^{b}
9 (wers)1 + (wers)2	71.67^{b}
10 (rs-1g)1	55.00^{d}
11 (rs-1g)1 + (rs-1g)2	61.00^{c}

Values in the column represent the means of 6 values for pots. Means followed by different letter(s) within a column are significantly different according to Duncan's multiple range test (p = 0.05). Control: Only infested soil without any biocontrol agent, Css: mycelial suspension of *Cyathus. stercoreus* Egyptian strain; ss: *Pleurotus columbinus* spent wheat straw; rs: wheat straw; we, water extract, 1: treatment before the sowing and 2: treatment after 70 days of sowing. In treatments Css1, wess and wers, potato pieces were soaking in the respective solution or filtrate for 10 min. In treatments Css2, (wess)2 and (wers)2 the treatments were watered by 100 mL of respective suspension or filtrate. In treatments (ss-1g)1 and (rs-1g)1, one gram of the respective fine powder was added in the hole before sowing. (ss-1g)2 and (rs-1g)2 referred to that 1 g of corresponding fine powder dad been added on the soil surface near each plant of the corresponding treatment(s)

Table 7: Percentages of total infected tubers

Treatments	% of total infected tubers
1 Control	100.00^{a}
2 Css 1	27.28^{ef}
3 Css 1 + Css 2	15.00^{f}
4 (wess)1	65.65^{bcd}
5 (wess)1 + (wess)2	48.33^{de}
6 (ss-1g)1	64.39^{bcd}
7 (ss-1g)1 + (ss-1g)2	47.22^{de}
8 (wers)1	86.94^{ab}
9 (wers)1 + (wers)2	77.78^{abc}
10 (rs-1g)1	53.89^{cde}
11 (rs-1g)1 + (rs-1g)2	59.72^{cd}

Values in the column represent the means of 6 values for pots. Means followed by different letter(s) within a column are significantly different according to Duncan's multiple range test (p = 0.05). Control: Only infested soil without any biocontrol agent, Css: mycelial suspension of *Cyathus. stercoreus* Egyptian strain; ss: *Pleurotus columbinus* spent wheat straw; rs: Wheat straw; we, water extract, 1: Treatment before the sowing and 2: Treatment after 70 days of sowing. In treatments Css1, wess and wers, potato pieces were soaking in the respective solution or filtrate for 10 min. In treatments Css2, (wess)2 and (wers)2 the treatments were watered by 100 mL of respective suspension or filtrate. In treatments (ss-1 g)1 and (rs-1 g)1, one gram of the respective fine powder was added in the hole before sowing. (ss-1 g)2 and (rs-1 g)2 referred to that 1g of corresponding fine powder dad been added on the soil surface near each plant of the corresponding treatment(s)

Productivity of potato tubers at harvesting time: The results indicated in Table 6, showed that all tested treatments significantly stimulated more increase in the weight of tubers than the control. The best results were recorded with the treatments (No. 7, 5 and 3); addition of 1 g of fine powder of ss before and after sowing, soaking in wess and drenching with 100 mL wess after 70 days and applying Css before and after sowing. They are equally (not significantly different) efficiently supporting the increase in the weight of tubers.

The results of Table 6 also revealed that using wess twice caused more significant increase in potato tubers weight than wers and the effect of addition of 100 mL of wers after 70 days was significantly different from only soaking plants in this extract for ten minutes before sowing.

All the potato tubers of the control were infected after the storage (Table 7). All treatments had a significant reduction of infected tubers except the treatment No. 8 and 9 in which potato pieces were soaked in wers for 10 min before sowing and addition of 100 mL of wers after 70 days. The lowest significant reduction was recorded with treatments No. 4 and 6 (using ss as powder or water extract before sowing). There was a significant difference between treatments No. 5 and 9 i.e., the use of wess twice (at sowing time and after 70 days) had better result, in this factor, than the use of wers twice.

There were no significant difference between treatments No. 6, 7, 10 and 11, therefore the use of 1 g of ss or rs twice were not better than their uses once. There was no significant difference between using ss or rs in this factor.

The best percentages of reduction of infected tubers were recorded with treatments No. 3 and 2 with no significant difference between them. Therefore, the treatments of potato pieces by soaking in Css or soaking before sowing and watering of plants, after 70 days of sowing, by 100 mL of Css had the best results for this tested factor.

DISCUSSION

All the eight tested fungal species inhibited the growth of *R. solanacearum*. Therefore, all of them may produce active antibacterial metabolites against *R. solanacearum*. The largest zone of inhibition was reported with both *Cyathus stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain, which may be due to the antibacterial metabolites secreted by them. These results were similar to the results of Anke and Oberwinkler (1976), Heim and Anke (1988), Hwang *et al.* (2000) and Lui and Zhang (2004). Smania *et al.* (2001) showed that *Ganoderma applanatum* (Pers.) Pat.) demonstrated antimicrobial activity against Gram-positive *Bacillus cereus, Staphylococcus aureus* and less activity against the Gram-negative *E. coli* and *P. aeruginosa*. The antibacterial activity of *Podaxis pistillaris* has been detected by Lindequist *et al.* (2005).

Rai and Tidke (2005) evaluated 17 mushrooms for antimicrobial activity against human pathogenic bacteria

and fungi. They reported that these mushrooms could be used as antimicrobial agents. In a recent *in vitro* study, extracts of more than 75% of polypore mushroom species surveyed showed antimicrobial activity and 45% of 204 mushroom species (polypores and gilled mushrooms alike) inhibited growth of a wide variety of microorganisms (Suay *et al*., 2000).

In this study the IR spectrum of the purified *Cyathus stercoreus* suspension showed a strong absorption at 3334.6 cm^{-1} due to hydroxyl groups. ^{1}H NMR spectrum showed a saccharide pattern of signals having the signal of anomeric proton at ϭ 5.08 ppm and a signal sequence like this of glucose (from ϭ 3.0-4.0 ppm) indicating a glucan. The ^{13}C-NMR spectrum showed six carbon signals supporting the glucose unit of this glucan. Kacurakova *et al.* (2000) found that the region between 1000-1500 cm^{-1} in IR is really a carbohydrate finger print region. Suay *et al.* (2000) recorded that *Lepista nuda* (Bull.) Cooke, presumably rich with polysaccharides, retarded *Candida albicans. Laetiporus sulphureus* isolated from nature demonstrated antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria during agar and submerged cultivation including methicillin-resistant strain of *Staphylococcus aureus* (MRSA) and glycopeptide-resistant strain of *Leuconostoc mesenteroides* (Ershova *et al*., 2003). Polysaccharides from *Lentinus edodes* and *Schizophyllum commune* exhibited activity against *Bacillus subtilis, S. aureus, Candida albicans* and *Saccharomyces cerevisiae* (Wasser and Weis, 1999). Polysaccharides extracts of some mushrooms stimulated beneficial bacteria (*Lactobacilli*), while reducing harmful bacteria (*E. coli*) (Guo *et al*., 2004).

The water extract of raw wheat straw and spent straw of *Pleurotus* spp. sterilized by autoclaving or filtration inhibited the growth of *R. solanacearum.* These results may be due to that wheat straw contains antibacterial compounds which were soluble in water and so present in the water extracts. The difference in the inhibitory effect of water extract of *Pleurotus* spp. spent straw attributed to the production of some secondary metabolites from the shikimic acid and cinnamic acid pathways during lignocellulosic degradation by *Pleurotus* spp. which may have antibacterial activity (Shimada *et al*., 1989). Okamoto *et al.* (2002) came to the same conclusion, they found that *P. ostreatus* produces p-anisaldehyde which has antibacterial activity and its production is parallel to Mn peroxiodase. Also, Velázquez-Cedeño *et al.* (2002) indicated that the two basidiomycetes *Pleurotus ostreatus* and *P. pulmonarius* had the ability to produce some extracellular lignocellulytic enzymes. It has also been found that *Pleurotus columbinus*, *P. pulmonarius*, *P. sajor-caju* and *P. floridanus* can produce all the ligninolytic enzymes (El-Fallal and El-Diasty, 2006).

Furthermore, the inhibition zones of tested water extracts sterilized by filtration had significantly increased than their corresponding ones sterilized by autoclaving. These results may be due to that these secondary metabolites are degraded by high temperature.

The application of water extract of both ss and rs once and twice equally significantly increased plants height. The addition of both once and twice increased tubers weight but wess is significantly better than wers. The application of wess had significantly reduction in percentage of infected tubers. In contrast wers was not significantly different from the control in reducing the percentage of infected tubers. On the other hand wess and wers did not cause significant decrease from the control to reported disease incidence of potato plants of 80 days age.

Therefore, these water extracts generated significant increases in plant height and potato weight and significant decreases in infected potato tubers. These results may be due to that these water extracts contain water soluble compound act as fertilizers and biocontrol agents. These results had agreement with El-Fallal and Migahed (2003) who reported that water extract of *Pleurotus floridanus* spent straw can be used as biofertilizer and reducing the suppressive effect of *Botrytis fabae* on broad bean plant. The use of water extract of compost for controlling foliar diseases has been reported by Yohalem *et al.* (1994). The relatively better results of wess filtrate than wers filtrate confirmed the results of this study *in vitro* which indicated that wess filtrate had significant higher antibacterial activity, against *R. solanacearum*, than wers filtrate.

There were no significant difference have been recorded in plant height or disease incidence when rs or ss powders were added to the soil. In contrary they increased tuber weights and decreased in percentage of total infected tubers. However, using rs or ss powders twice had no significant difference on disease incidence and on total infected tubers but had significant increase in plant height and tubers weight. These results may be due to the low antimicrobial effect by using 1 g straw which was not enough to reach to the lateral roots of the plants. While, the increase in plant height may be attributed to the enhancement of soil fertility, soil structure and soil moisture retention. Also, these powders play a role in prevention of erosion. Residue on the soil surface decreases soil temperature and increase soil moisture. Moreover, these powders of plant residues may play a role in weed and insect control (Ferguson and Shew, 2001). These results revealed that ss and rs

powders can play three roles in plant health and crop productivity. Firstly, their ability to improve soil properties may be referred to their effect on soil aeration, structure, drainage and moisture holding capacity, (Abo El-Fadl *et al*., 1995; He *et al*., 1995; Davey, 1996). Secondly, their role as fertilizers may be due to increase N mineralization (Gök *et al*., 2002). Also, these powders contain valuable amount of sugars, nitrogen, cations, hormones and some phenolic compounds (Shukery *et al*., 1999; Shen and Shen, 2001; El-Fallal and Migahed, 2003). Thirdly, these organic matters may directly or indirectly suppress disease by enhancing production of decomposed products by antagonistic microbial populations or stimulating the production of lytic enzymes involved in the degradation of plant pathogens (Huang and Huang, 1993; Shetty *et al*., 2000; Gamliel *et al*., 2000). Microbial degradation of plant residues can also produce secondary products with antimicrobial activities that inhibit the growth of plant pathogens (Bailey and Lazarovites, 2003; Bardin *et al*., 2004).

The benefits of applying organic amendments for disease control are incremental, generally slower acting than chemical fumigants or fungicides, but may last longer and their effects can be cumulative (Bailey and Lazarovites, 2003).

Moreover the priority of ss than rs may be due to the ability of *Pleurotus columbinus* to produce extracellular enzymes that enhance the properties of ss as antimicrobial agent and fertilizer than rs. This explanation depends on the results of Velazquez-Cedeňo *et al*. (2002) and El-Fallal and El-Diasty (2006) where they reported the ability of *Pleurotus* spp to produce extracellular enzymes which can degrade lignocellulytic compounds of straw.

In the green house experiment, the best results were obtained by plants that had been treated by soaking of potato pieces in mycelial suspension of *Cyathus stercoreus* (Css) for 10 min directly before sowing which generated significant increase in plant height after 80 days of sowing and significant increase in mean weight of potato tubers for each pot at harvesting time. Also, this treatment had a significant decrease in disease incidence after 80 days of sowing and in percentage of total infected tubers. The watering (drenching) of plants by 100 mL of this fungal suspension had no significant effect on the last four factors mentioned, except the mean weight of tubers where the drenching by this suspension had significant increase of this factor.

The priority of treatments of soaking in Css fungal suspension than all the other treatments could be resulted from the mode of action of glucan extracted from this fungus that has antibacterial activity against the studied pathogen as has been detected in this study. The antimicrobial activity of the polysaccharides from basidiomycetes has been mentioned previously (Wasser and Weis, 1999; Ershova *et al*., 2003; Guo *et al*., 2004). Moreover, Halsall (1993) showed that the nitrogenase activity of *Beijerinckia indica* B15 was stimulated by coinoculation with *Cyathus stercoreus* both in axenic culture and in native soil. He found that *Cyathus stercoreus* can degrade lignocellulose so, showed degree of ability to cross-feed diazotrophs. Its lignocellulolytic ability has been also confirmed by El-Fallal and El-Diasty (2006).

Furthermore, there were no significant differences between the disease incidence values with treatments of soaking potato plants in fungal suspension or water extracts of both spent straw or raw straw while there was a significant priority of soaking potato pieces in the tested fungal suspension than soaking in wess and wers in the percentage of total infected tubers. These results may be due to production of polysaccharides by *Cyathus stercoreus* which have a continuous effect during the growth of potato plants, while the antibacterial agent of wess and wers may be exhausted or broken down during the growth of potato plants and had no effect on studied bacterium in the last stages of potato growth.

This study recommended the use of *Cyathus stercoreus* suspension in the biological control of the disease and as a biofertilizer for potato plant. Also it suggests that raw straw or spent straw can be used as a carrier for Css fungus and also may be used as a carrier for any other biocontrol agent to enlarge the antimicrobial activity and to take advantage of the other mentioned mode of action mechanisms of these organic matters.

REFERENCES

Abo El-Fadl, I.A., M. Abou baker and A.M. El-Gamal, 1995. Effect of different organic manure composts on roselle plants (*Hbiscus sabdariffe* L.) and soil characteristics. Egypt. J. Soil. Sci., 45: 50-61.

Alexopolous, C.J., C.W. Mims and M. Blackwell, 1996. Introductory Mycology. 4th Edn. John Wiley and Sons, New York, pp: 488.

Anke, T. and F. Oberwinkler, 1976. The striatins-new antibiotics from the basidiomycete *Cyathus striatus* (Huds ex Pers.) Willd. J. Antibiotics, 30: 221-225.

Anke, T. and W. Steglich, 1988. Neue wirkstoffe aus basidiomyceten. Forum Mikrobiologie, 11: 21-25.

Anke, T., U. Rabe, P. Schu, T. Eizenhöfer, M. Schrage and W. Steglich, 2002. Studies on the biosynthesis of striatal-type diterpenoids and the biological activity of herical. Z. Naturforsch., 57c: 263-271.

Ayer, W.A. and H. Ttaube, 1973. Metabolites of *Cyathus helenae*: A new class of diterpenoids. Can. J. Chem., 51: 3842-3854.

Ayer, W.A. and D.R. Taylor, 1976. Metabolites of the Bird's nest fungi. Part 5. The isolation of 1-hydroxy-6-methyl-8-hydroxymethylxanthone. A new xanthone from *Cyathus intermedius* synthesis via photoenolisation. Can. J. Chem., 54: 1703-1707.

Ayer, W.A., T. Yoshida and D.M.J. Van Schie, 1978. Metabolites of Bird's nest fungi. 9. Diterpenoid metabolites of *Cyathus africanus*. Can. J. Chem., 56: 2113-2120.

Ayer, W.A. and S.P. Lee, 1979. Metabolites of the Bird's nest fungi. Part 11. Diterpenoid metabolites of *Cyathus earlei* Lloyd. Can J. Chem., 57: 3332-3337.

Bailey, K.L. and G. Lazarovites, 2003. Suppressing soil-born diseases with residue management and organic amendments. Soil Tillage Res., 72: 169-180.

Bardin, S.D., H.C. Huang and J.R. Moyer, 2004. Control of *Pythium* damping-off of sugar beet by seed treatment with crop straw powders and a biocontrol agent. Biol. Control, 29: 453-460.

Brodie, H.J., 1975. The Bird's Nest Fungi. University of Toronto Press, Toronto, CA.

Davey, C.B., 1996. Nursery Soil Management-Organic Amendments. In: (Tech. Coordinators), National Proceedings, Forest and Conservation Nursery Associations. General Technical Report PNW-GTR-389. Landis, T.D. and D.B. Douth (Eds.). USDA Forest Service PNWRS, pp: 6-18.

Duncan, D.B., 1955. Multiple ranges and multiple F-test. Biometrics, 11: 1-42.

Ershova, E. Iu, O.V. Tikhonova, L.M. Lur'e, O.V. Efremenkova, O.V. Kamzolkina and I.V. Dudnik, 2003. Antimicrobial activity of *Laetiporus sulphureus* strains grown in submerged culture. Antibiot. Khimioter, 48: 18-22.

El-Fallal, A.A., 2001. Decolorization of the dye Poly R-478 and oxidation of some phenols by some basidiomycetes. Bull. Fac. Sci., Assiut. Univ., 30: 271-283.

El-Fallal, A.A., 2003. Agaricales from the countryside and grasslands of east Delta region, Egypt. J. Environ. Sci., 26: 339-352.

El-Fallal, A.A. and F.F. Migahed, 2003. Metabolic changes in broad bean infected by *Botrytis fabae* in response to mushroom spent straw. Asian J. Plant Sci., 2: 1059-1068.

El-Fallal, A.A. and G.G. El-Diasty, 2006. Evaluation of Egyptian white-rot fungi for their ability to produce ligninolytic enzymes and decolorization of Poly R. Bull. Fac. Sci., Elminia Univ., 17 (1): 1-44.

Ferguson, L.M. and B.B. Shew, 2001. wheat straw and its impact of three soil borne pathogens of peanut in microplots. Plant Dis., 85: 661-667.

Gamliel, A., M. Austerweil and G. Kritzman, 2000. Non-chemical approach to soil borne pest management-organic amendments. Crop Prot., 19: 847-853.

Gök, A.C.M., I. Onaç, L. Inal and T. Sağlamatimur, 2002. The effect of wheat straw, corn straw and tobacco residues on denitrification losses in field planted with wheat. Turk. J. Agric. For., 26: 349-353.

Guo, F.C., B.A. Williams, R.P. Kwakkel, H.S. Li, X.P. Li, J.Y. Luo, W.K. Li and M.W. Verstegen, 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci., 83: 175-182.

Halsall, D.M., 1993. Inoculation of wheat straw to enhance lignocellulose breakdown and associated nitrogenase activity. Soil Biol. Biochem., 25: 419-429.

Hau, F.C. and M.K. Beutte, 1983. Effects of chlorothalonil on the virulence and physiology of a non targeted pathogen, *Sclerotinia minor*. Phytopathology, 37: 475-479.

Hayward, A.C., 2000. Encyclopedia of Microbiology. 1st Edn. Vol. 4. Lederberg, Academic San Diego, pp: 32-42.

He, X.T., T.J. Logan and S.J. Traina, 1995. Physiological and chemical characteristics of selected as municipal solid waste composts. Quality, 24: 543-552.

Heim, J. and T. Anke, 1988. Antibiotics from basidiomyctes XXIX: Pilatin, a new antibiotically active marasmane derivative from cultures of *Flagelloscypha pilathii agreer*. J. Antibiotics, 41: 1752-1757.

Huang, H.C. and J.W. Huang, 1993. Prospects for control of soilborne plants pathogens by soil amendment. Curr. Top. Bot. Res., 1: 23-235.

Hwang, E., B. Yun and Y. Kim, 2000. Phellinsin A, A novel chitin synthases inhibitor produced by *Phillinus* sp. PL3. J. Antibiotics, 53: 903-911.

Kacurakova, M., P. Capek, V. Sasinkova N. Wellner and A. Ebrngerova, 2000. FT-IR study of plant cell wall model compound: Pectic polysaccharides and hemicellulose. Carbohydrates Polymer, 43: 195.

Lindequist, U., T.H.J. Niedermeyer and W.D. Jülich, 2005. The pharmacological potential of mushroom. Evidence-based Complement. Altern. Med., 2: 285-299.

Lui, Y. and K. Zhang, 2004. Antimicrobial activities of *Cyathus* species. Mycopathologia, 157: 185-189.

Mansour, F.A., S.A. El-Sayed and S.A. El-Sherbiny, 1997. Caeseorhodomycin, A new antibiotic produced by a bacterial strain isolated from Egyptian soil. Egypt. J. Microbiol., 32: 69-81.

Okamoto, K., S. Narayama, A. Katsuo, I. Shigematsu and H. Yanase, 2002. Biosynthesis of p-anisaldehyde by white rot basidiomycete *Pleurotus ostreatus*. J. Basic Bioeng., 93: 207-210.

Phillips, S., 1985. Mushrooms and Other Fungi of Great Britain and Europe. Pan Books Ltd., Cavaye Place, London.

Rai, M.K. and G. Tidke, 2005. Biotechnological potential of mushrooms: Drugs and dyes production. Int. J. Med. Mushrooms, 7: 213.

Salanoubat, M., S. Genin, F. Artiguenave, J. Gouzy, S. Mangenol, M. Artat and A. Billautt *et al*., 2002. Genome sequence of the plant pathogen *Ralstonia solanacearum*. Nature, 415: 497-502.

SAS., 1988. SAS/STAT User's Guide-Release 6.03 Edn. SAS Institute, Cary, NC.

Shen, Q.R. and Z.G. Shen, 2001. Effects of pig manure and wheat straw on growth of mung bean seedlings grown in aluminum toxicity soil. Bioresour. Technol., 76: 235-240.

Shetty, K.G., K.V. Subbarao, O.C. Huisman and J.C. Hubbard, 2000. Mechanism of broccoli-mediated *Verticillium* wilt reduction in cauliflower. Pytopathology, 90: 305-310.

Shimada, M., H. Aohta, T. Kurosaka, T. Hattori, T. Higuchi and M. Takashi, 1989. Roles of Secondary Metabolism of Wood Rotting Fungi in Biodegradation of Lignocellulosic Materials. In: Plant Cell Wall Polymers, Lewis, N.G. and M.G. Paice (Eds.). Am. Chem. Soc., Washington DC, pp: 412-425.

Shukery, W.M., A.A. El-Fallal and H.M.S. El-Bassiouny, 1999. Effect of spent wheat straw growth, growth hormones, metabolism and rhizosphere of *Cuminus sativus*. Egypt J. Physiol. Sci., 23: 39-69.

Smania, A., F.D. Monache, C. Loguericio-Leite, E.F.A. Smania and A.L. Gerber, 2001. Antimicrobial activity of basidiomycetes. Int. J. Med. Mushrooms, 3: 87.

Smith, A.H., H.V. Smith and N.S. Weber, 1981. How to Know Non-Gilled Mushrooms. WMC Brown Company, Dubuque, Iowa.

Suay, I., F. Arenal, F. Asenio, A. Basilio, M. Cabello, M.T. Diez, 2000. Screening of basidiomycetes for antimicrobial activities. Antonie van Leeuwenhoek, 78: 129-139.

Velázquez-Cedeño, M.A., G. Mata and J. Savoie, 2002. Waste-reducing cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* on coffee pulp: Change in the production of some lignocellulytic enzymes. World J. Microbiol. Biotechnol., 18: 201-207.

Wasser, S.P. and A.L. Weis, 1999. Medicinal properties of substances occurring in higher basidiomycete mushrooms: Current perspective. Int. J. Med. Mushrooms, 1: 31-62.

Watling, R., 1980. How to Identify Mushrooms to Genus V: Cultural and Developmental Features. Eureka, Calif. Mad River Press, pp: 168.

Yabuuchi, E., Y. Kosako, I. Yano, H. Hotta and Y. Nishiuchi, 1995. Transfer of two *Burkholderia* and *Alkanigens* species to *Ralstonia* genus. Nov.; Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff, 1973) Comb. Nov. and *Ralstonia eutropha* (Davis, 1969) comb. Microbiol. Immunol., 39: 897-904.

Yohalem, D.S., R.F. Harris and J.H. Andrews, 1994. Aqueous extracts of spent mushroom substrate for foliar disease control. Compost. Sci. Util., 4: 67-74.

Zjawiony, J.K., 2004. Biologically active compounds from aphyllophorales (polypore) fungi. J. Nat. Prod., 67: 300-310.