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Biological Control of Wilt and Stem-Canker of Potato by Antagonism

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ABSTRACT

Fishmeal used as a soil amendment increased the microbial activity, addition of fishmeal to the soil infested with the pathogens (*Fusarium oxysporum* and *Rhizoctonia solani*) led to a remarkable reduction in the percentage of disease compared to the soil non-amended with fishmeal. Eleven fungal isolates, five yeast isolates were isolated from the rhizosphere and stem associated soil of potato plant. Soil samples were collected from a field cultivated with potato from Riyadh region, Saudi Arabia. Four fungal isolates, *i.e. Fusarium sambucinum, Penicillium oxlacium, F. solani, F. oxysporum* were characterized by their potent and remarkable antagonistic activities against *F. oxysporum*, but the antagonistic fungi against *R. solani* were *P. oxlacium, Trichoderma* sp., *Gliocladium* sp., and *Fusarium sambucinum.* The use of rhizosphere yeast species led to increase in inhibition of the pathogens, an increase in inducting of resistance of potato plants and an increase in growth measurements of potato plants. The non-pathogenic *F. sambucinum* was the best antagonist of *F. oxysporum. Gliocladium* sp. was a good antagonist of *R. solani,* together with *Trichoderma* sp. and *Gliocladium virens* reduced *R. solani* severity. The ability of yeast isolates to produce inhibitory metabolites active against these pathogens, was tested *in-vitro* and *in-vivo*. The *in-vivo* assay of these fungal isolates demonstrated their ability to promote the growth of potato plants in the greenhouse.

Key words: *Fusarium oxysporum, Rhizoctonia solani*, Rhizosphere microorganisms, potato growth parameters, potato diseases, antagonists.

INTRODUCTION

Potato (*Solanum tuberosum L.*) is one of the most important crops. Potato plants are liable to attack with vulnerable viral, bacterial, and fungal diseases. Wilt and stem-canker of potato caused by *Fusarium oxysporum* and *Rizoctonia solani,* respectively are the most important fungal diseases causing great reduction in both quality and quantity of tuber yield (Alabouvette *et al.* 2006).

Microbial antagonism implies direct interactions between two microorganisms sharing the same ecological niche, by parasitism, antibiosis and competition of nutrients. Olivain and Alabouvette (1997) observed intense colonization of the surface of tomato roots by a strain of non-pathogenic *F. oxysporum* that competed with a strain of *F. oxysporum* f. sp. *lycopersici*. It was reported that two species of rhizosphere yeast fungi, *i.e. Saccharomyces unispora* and *Candida stealolytica* having antagonistic and inhibitory effects on the growth of *F. oxysporum* (El –Mehalawy, 2004).

Biological control of plant pathogenic fungi and bacteria may be accomplished through the destruction of existing inoculum, exclusion from the host, or the suppression or displacement of the pathogen after infection (Alabouvette *et al.* 2006).

Yeast fungi, as biofungicides, were reported to inhibit many plant pathogens. There are a variety of fungal species and isolates that have been examined as biocontrol agents but *Trichoderma* species clearly dominate, perhaps reflecting their ease of growth and wide host range. Hanson (2000) reported that the three fungal antagonists, *Trichoderma viride, T. harzianum* and *Gliocladium virens* have significantly inhibited the mycelial growth and sclerotial production of *R. solani.*

Amendment of soil with organic matter increases disease suppressiveness, presumably due to enhanced microbial activity. This occurs without a reduction in inoculum's density of pathogens. *F. oxysporum,* the fungal causal agent of potato wilt disease, a well represented species among the communities of soil borne fungi, in every type of soil all over the world. This species is also considered as a normal constituent of the fungal communities in the rhizosphere of plants (Gordon and Martyn, 1997). Many types of organic matter have been used for soil amendments; including fishmeal, chitinous materials as soil cakes.

A strain of non-pathogenic *F. oxysporum,* Fo47 was isolated from a suppressive soil that can control *Fusarium* wilt of several plants such as carnation, cyclamen, flax, and tomato (Albouvette and Couteaudier, 1992). *R. solani* is a soil borne pathogen responsible for severe damage on many crop species; on potato causes delayed emergence, lesions on stem (stem canker) and stolon, and sclerotial formation on tubers (Black scurf) (Campion *et al.* 2003). *R. solani* attack occurs wherever potato is grown and may be responsible for potato yield reduction; also affect the quality of tubers through size disturbance of alternation of skin aspect "sclerotia decrease tuber marketability". Attack on stolon may induce the development of mis-shapen tubers (Jeger *et al.* 1996). To control *R. solani* on potatoes, the inoculum source must be considered. Frank and Leach (1980) showed that

both soil-borne and tuber-borne inocula are etiologically important and also indicated that the pathogen may be present on the tuber surface even though sclerotia are not visible. Several microbial antagonists have shown potentiality for control of *R. solani* on potato or other host crops. *Trichoderma harzianum* and *Gliocladium virens* have successfully suppressed *R. solani* on several pathosystems (Lewis and Larkin, 1997), Asaka and Shoda, (1996) reported that fermentation biomass (FB) of *Trichoderma* and *Gliocladium* species have significantly reduced soil-born levels of *R. solani* in laboratory tests. Isolates that reduced tuber-borne sclerotium viability in the field might be expected to reduce disease incidence and severity as well. The use of bio-agents for controlling soil-borne diseases including stem canker and black scurf of potato was investigated. (Abada and Abdel Aziz, 2002). They found that using bioagents have caused significant reduction in infection with stem canker and black scurf of potato in pot and in field experiments, with significant increase in the produced tuber yield. *Trichoderma* and *Gliocladium* spp. secrete diverse secondary metabolites with antibiotic properties, including polyketides, terpenoids, polypeptides and metabolites derived from α-amino acids and *Trichoderma harzianum* produces harzianic acid (Sawa *et al.* 1994).

The biocontrol product can be mass-produced on a readily available substrate in a process that the inoculum is produced in a simple culture vessel, incubated in the dark at 20°C, and can be applied as a conidial suspension or as a granular material (Babu *et al.* 2004). They also showed that solid substrate with relatively low protein content such as seeds of sorghum, millets, maize, rice and maize stalks encouraged sporulation, thus rice, wheat, sorghum and maize were used for producing most conidia.

The objective of this study was to evaluate the potential of selected bio-agents for the biological control of potato pathogens, *F. oxysporum* and *R. solani* causing wilt and stem canker and to evaluate the improvement in the growth parameters of potato.

MATERIALS AND METHODS

1- Soil samples: (isolation and estimation of filamentous fungi from the rhizosphere of potato plant)

Many types of organic matter have been used for soil amendments. These include seaweed extracts, fishmeal, chitinous materials as soil cakes, barley straw, sorghum stubble, and fowl manure. The fishmeal used as soil amendment increases the microbial activity and led to a remarkable reduction in disease incidence of *R. solani* (Sneh *et al.* 1971).

Soil samples were collected from a field cultivated with potato in Riyadh region, Saudi Arabia. The soil was used for cultivating potato plants in the greenhouse, then for the isolation of antagonists from the rhizosphere of these plants. Two sets of pots (3 kg of soil) were used. The first set (10 pots) contained fishmeal amended soil. Twenty g of sterilized fishmeal at 121°C for 30 min. were mixed with the soil in each pot and watered every 2-3 days for one week. Soil amendment was carried out twice for increasing the microbial activity of antagonists. The second set (10 pots) contained non-amended soil. Two potato seed pieces were sown in each pot at a depth of 7-8 cm of soil. Pots were placed in the greenhouse, watered every 2-3 days with equal amounts of distilled water. After 4-5 weeks from sowing, rhizosphere soil was collected by removing intact plant roots with adhering soil. Ten g of rhizosphere soil were dispensed into volume sterile 0.1% (w/v) agar in distilled water; suspensions were shaken for 30 min. then left to settle down. Isolation of rhizospheric fungi (antagonists) was carried out using the soil dilution plate method according to Johnson and Curl (1972). Isolation was made on Weikerham agar, and Sabouraud agar media. After incubation at 28°C for 6 days, colonies were counted and expressed as CFU/g dry rhizosphere soil. Fungal isolates were identified to the genus and species levels using the following Donsch *et al.* (1993).

2- Isolation and identification of rhizosphere yeast fungi

Rhizosphere yeast fungi were isolated using the soil dilution plate method of Johnson and Curl (1972), on peptone yeast malt-extract agar (PYMA) and Nutrient agar (NA) media. Yeasts isolates were stored at 4°C and sub-cultured on PYMA slants. Yeast fungi isolates were identified to the genus and species levels according to Kreger Van-Rij, (1984) and Barnett *et al.* (2000).

3- Isolation and identification of rhizosphere filamentous fungi

Healthy potato plants with intact roots and adhering soil were collected from potato fields. Ten g. of rhizosphere soil were dispersed into sterile 0.1% (w/v) agar in dist. water, shaken for 30 min. and left to settle down. All fungal isolates were identified according to Nelson *et al.* (1983).

Isolation and identification of *Fusarium oxysporum*, the causal agent of potato wilt disease

Healthy potato plants or having symptoms were collected from potato field, isolation of *F. oxysporum* was carried out within 24 h by washing the whole plants under running water. Isolation was made, roots and stems bases of

infected plants were surface sterilize for 3 min. in 0.5% NaOcl. They were then rinsed several times in sterile distilled water before being placed on sterile filter paper in laminar flow cabinet to dry for 30 min. (Deman *et al.* 1993). Once dried, the roots and stem bases were excised aseptically and placed on (1) PDA amended with yeast extract and the antibiotic streptomycin (2) Sabouraud agar amended with yeast extract and the antibiotic streptomycin. The plates were incubated in the dark at 25±2°C for 4 days and observed daily for fungal growth. Colony morphology of *F. oxysporum* was assessed on potato dextrose yeast extract Agar (PDYA), and SYA (Sabouraud yeast-extract agar) at 28°C in the dark for 7 days. Identification of *F. oxysporum* was based on morphological characteristics of the cultures growing on the two media.

4- Pathogenicity test of *F. oxysporum* in the greenhouse

Pathogenicity tests for isolated *F. oxysportum* and *R. solani* were done in the greenhouse to ensure virulence of the isolates and to determine if they were the causal agent of potato wilt and stem-canker diseases.

(A) Inoculum's preparation of *F. oxysporum*:

F. oxysporum isolate was grown on sterilized crushed maize seeds at 28°C for 7 days in the dark. Crushed maize seeds were prepared by adding 40 grams of crushed seeds with 10 ml of dist. water into 250 ml conical flasks and kept overnight. Flasks were then autoclaved at 121°C for 30 min on three successive days. Flasks were then inoculated under aseptic conditions with three plugs (5mm diameter) from the actively growing margin of *F. oxysporum* colonies. Flasks were incubated at 28°C in the dark for 2 weeks, and were occasionally shaken to ensure uniformity of colonization. Non inoculated flasks were used as control.

(B) Soil amendment with fish meal

Twenty grams of fish meal were sterilized at 121°C for 30min. then mixed within a set of pots (10 pots) containing about 3-4 kg steam pasteurized soil and then left for 7 days, watered every 2-3 days. Amendment with fish meal was repeated twice, whereas non-amended soil served as control.

(C) Soil infestation

F. oxysporum inoculum's was dispersed through the steamed soil in 4 sets of pots (each set consisted of 10 pots), filled with 3-4 kg soil in each. The first set, contained the pathogen inoculums in fishmeal amended soil, the second set which contained amended soil received the same quantities of autoclaved maize seeds to serve as control. The third set, received the pathogen inoculums in non-amended soil, the fourth set contained non-amended soil, and received the same quantities of autoclaved maize seeds to serve as control. The pathogen was used in three inoculum's levels (10^4, 10^5 and 10^6 CFU/g soil). Two potato seed pieces were sown in each pot. Pots were placed in the greenhouse, watered every 2-3 days with equal amounts of water. The growing potato plants were observed for wilt symptoms after 4-6 weeks. Re-isolation of *F. oxysporum* was carried out from the soil, and from the roots of plants showing wilt symptoms.

5- *In-Vitro* screening of the antifungal activity of microorganisms

Yeasts and fungal isolates were examined for their ability to antagonize *F. oxysporum* and *R. solani* using the dual culture method readings (Henis and Inbar, 1968).

Dual culture technique

Fungal antagonists were examined for *in-vitro* antagonism against *F. oxysporum* and *R. solani* by dual culture technique. Potato dextrose agar medium was inoculated with two disks (5mm diameter) each of fungal antagonist; disks were positioned on both sides of (5mm diameter) disk of each pathogen. Distance between disks was approximately 5 cm, cultures were grown at 25°C±2°C in the dark. Inhibition of pathogen growth in direction of antagonist was observed within 2-6 days of incubation.

Detection of inhibitory activities of fungal antagonists' culture filtrates

Fungal antagonists were incubated on potato dextrose broth for 6 weeks. At the end of each week, the broth cultures were filtered; filtrates were sterilized using Millipore filter. The inhibitory activities of these filtrates were tested against *F. oxysporum* and *R. solani.* PDA plates were performed at their edges to form holes using sterile cork borer. 50µl of each filtrate was placed in the holes; sterile water was placed instead of the filtrate in control plates. *F. oxysporum* or *R. solani* mycelia were inoculated at the center of these plates using sterile pointed needle. Plates were incubated at 28°C, inhibition of pathogen growth around these holes was checked after 2-4 days.

Detection of antagonistic activities of yeasts and filamentous fungi, against *F. oxysporum* and *R. solani* in the greenhouse

Preparation of pathogenic inocula

The pathogenic *F. oxysporum* and *R. solani* were grown on PDA medium at 25°C for 7 days in the dark. Crushed maize seed-based inocula were prepared by adding 100g of crushed seeds with 30 ml of dist. water into 250ml conical flask and kept over night. The flasks were steamed without pressure for 10 min. then autoclaved at 121°C for 30 min. on 3 consecutive days. Under aseptic conditions, the maize seeds were then inoculated with eight agar plugs (2mm. diameter) cut from the actively growing margin of *F. oxysporum* and

R. solani colonies. The flasks were incubated at 25°C in the dark for one week and were occasionally shaken to ensure uniformity of colonization. Non-colonized maize seeds which had been autoclaved twice served as control. Prior to use, small amounts of the colonized and control maize seeds were plated onto PDA medium to confirm the presence or absence of target pathogens.

Inoculum's preparation of the tested yeast fungi

The inocula of the tested yeast fungi, was prepared by placing 50g moist wheat bran with 30 ml distilled water into 500 ml conical flask, autoclaved at 121°C for 30 min. on three successive days as described by Roiger and Jeffers, (1991). The mixture was inoculated aseptically with a suspension (25ml) of spores and cells of the desired fungus, in 10% Tween 80 and incubated at 28±2°C in the dark for two weeks. The flasks were shaken to ensure uniformity of growth of each microorganism. Non-colonized wheat bran which had been autoclaved served as the control.

Preparation of antagonistic filamentous fungi inocula

The fungal antagonists tested against pathogenic *F. oxysporum* were non-pathogenic *F. sambucinum*, non-pathogenic *F. solani*, non-pathogenic *Penicillium oxalicum* and *Tricloderma* species. Antagonists tested against pathogenic *R. solani* were *Gliocladium* sp. *Trichoderma* sp. and *P. oxalicum*. Inocula from all antagonists were prepared on crushed maize seeds in the same way described before. Inoculum's concentrations of the fungal antagonists were *Penicillium oxalicum* ($12.8x10^5$), *Fusarium sambucinum* ($0.35x10^5$), *Fusarium solani* ($0.03x10^5$), *Trichoderma* sp. ($5.0x10^5$) and *Gliocladium* sp. ($5.14x10^5$).

Preparation of pathogens inocula

Inocula of *F. oxysporum* and *R. solani* were prepared by the same way stated previously for the greenhouse assay. The concentration of pathogenic *F. oxysporum* was $55x10^6$ CFU/g soil. As well, the selected antagonistic fungal isolates were also prepared by the same way stated before for the greenhouse. Concentrations of antagonists used were *F. solana* ($70x10^6$ CFU/g soil), *F. sambucinum* ($101x10^6$ CFU/g soil), *P. oxalicum* ($5x10^7$ CFU/g soil) and *Gliocladium* sp. ($6x10^6$ CFU/g soil).

Isolation of *Rhizoctonia solani*, the fungal causal agent of potato stem-canker disease

Diseased potato stems showing typical symptoms of stem-canker were collected from potato fields. Small pieces of underground stem were excised, surface-disinfested in 1% NaCl for 30 sec., rinsed with distilled water then placed in Petri-dishes of 2% antibiotic water agar (AWA), consists of: water agar (2%), streptomycin sulphate (100µg/ml), and chlorotetracycline Hcl (50µg/m), and PDA media, consists of: potato (200 g), glucose (20g), agar (20g), and distilled water (IL). After incubation for 72h at 25°C, hyphae were transferred onto PDA slants and kept at 4°C.

Identification of *Rhizoctonia solani* isolates

The colony morphology of isolates was assessed on 2% water agar and PDA media, after 48-72h of incubation at 25°C. Identification was based on morphological and microscopical characteristics (Parmeter *et al.*, 1970).

Greenhouse pathogenicity test of *Rhizoctonia solani*

Certified potatoes of cultivar whose dormancy had been broken and free from sclerotia were surface sterilized by dipping in 95% ethanol followed with soaking in 1% NaCl for 5 min. Tubers were rinsed twice in sterile dist. water, air dried then cut into single-eye seed pieces (40-60 gram each) immediately before planting. 150 cm of steam pasteurized soil was added in each pot (10 pots), then two seed pieces were placed on the soil surface and covered with 50 cm of soil. Disks (5mm diameter) taken from the margin of 2-3 days-old cultures of *R. solani* on PDA plates were placed on the soil about 1 cm above the seed pieces and covered with 250 cm of soil. Control included tubers treated with disks of PDA and were prepared by the same way stated previously in *F. oxysporum*.

***In-vitro* detection of antifungal activity of rhizosphere yeasts and fungi**

The ability of antagonists to inhibit (antagonize) *F. oxysporum* and *R. solani* growth was detected *in-vitro* on Hussein's fish meal extract agar HFMEA and PDA media. All antagonists were examined against pathogenic *F. oxysporum* and *R. solani*. Antagonists were streaked at one side of (HFMEA) plates, incubated for 7 days to allow the production and diffusion of metabolites into the agar. Two agar disks (5mm diameter) one contained *F. oxysporum* mycelium and the other containing *R. solani* were placed onto the opposite sides of the antagonist inoculated plates. Other *F. oxysporum* and *R. solani* mycelia disks were also placed on un-inoculated HFMEA separately as control. Hussein's fishmeal extract agar (HFMEA) consists of: fishmeal (20g), glucose (20g), peptone (5g), Nacl (0.5g), CaCo3 (anhydrous) (3g), agar (20g), and dist. water (IL) pH=7. Cultures were incubated in the dark at 25°C for 7 days, and plates were examined for inhibition of pathogens growth after 7 days. The level of inhibition was determined as described by Yuen and Crawford (1995). The level of inhibition (Δr) was defined as the subtraction of pathogen growth radius (r_o in cm) of a control culture from the distance of growth of pathogen in the direction of antagonist

colony (r in cm), where:

$$\Delta r = r_o - r$$

$$\text{Inhibition percentage} = \frac{\Delta r \times 100}{r_o}$$

Effect of rhizosphere microorganisms on potato plant growth

Some growth parameters of potato plants, plant height, fresh weight and average number of leaflets plant were determined after the removing any soil particles from the shoot and the time of harvest. After 4-5 weeks, several parameters were used to assess the effect of antagonistics on potato plants such as height of the shoots, fresh weight of shoots and number of the leaflets. The pathogens and antagonists were re-isolated from the lower and upper soil layers respectively.

Statistical analysis

The results of biological control studies are presented as average (mean ± SD) of six readings. The statistical analyses were carried out using ANOVA by SPSS V17 to determine the degree of significance between treatments. P-value was used as a critical value to accept the significance of the tested effect. P-value <0.001 then accept the significance of the tested factor.

RESULTS AND DISCUSSION

1- Microbial population of filamentous fungi and yeasts of rhizosphere of potato plants

Colony forming units of filamentous fungi were more in case of isolation on Sabouraud medium ($60x10^2$), than isolation on Weikerham medium (36×10^2). Eleven different isolates of filamentous fungi were isolated and identified as; *Absidia* sp. (Rai and Pathak, 1981), *Alternaria* sp. (Dixit and Gupta, 1982), *Aspergillus clavatus* (Olutiola, 1977), *Aspergillus flavus* (Paster *et al.* 1993), *A. niger* (Paul and Banerjee, 1984), *F. oxysporum* (Fuch *et al.* 1999), *F. sambucinum* (Schisler *et al.* 1995), *F. solani* (Larkin and Fravel, 1998), *Gliocladium* sp. (Howell, 1987), *Mucor* sp. (Rai and Pathak, 1981) and *Penicillium oxalicum* (DeCal *et al.* (2000). Five different isolates of yeast fungi were isolated from rhizosphere of potato plants as shown in tables (3) and (4).

As shown in table (1), microbial activity of soil amended with fishmeal was higher than that of non-amended soil. Data also showed the total counts of yeast colonies in non-amended soil compared to the counts of the amended one. The count was higher on NA medium than on PYMA (five different isolates of yeasts). These results are in agreement with those of Lumsden *et al.* (1983).

Table (1): Colony forming unit of the rhizosphere yeasts isolated on different media

CFU of yeasts/g dry soil			
PYMA medium		NA medium	
Control soil	Amended soil by fishmeal	Control soil	Amended soil by fishmeal
$10x10^2$	$11x10^2$	$85x10^2$	$88x10^2$

Readings are the average of 5 plates

Isolation of *Fusarium oxysporum*, the causal agent of potato wilt

The fungus was isolated from naturally wilted potato plants on PDYA and SDYA media in plates incubated at 28±2°C. The isolated fungus was purified and identified according to its morphological characters as *F. oxysporum.*

2- Pathogenicity test of *F. oxysporum*

Germination of potato seed pieces began after 18 days from sowing. Wilt symptoms started to appear on plants after 4 weeks as severe stunting of shoots followed by wilting of most leaves, started from the lower to the upper newly formed ones. Leaves first became dull green, eventually lost color and dried. In advanced stages of the disease, lower parts of the stalk became dry, shrunken, and shallow, finally, leaves dropped and the whole plant collapsed and died. Symptoms were severe when the highest inoculum's of *F. oxysporum* (10^6 spores CFU/g soil) was used. No wilt symptoms were observed on control plants. In both fishmeal amended and non-amended soil, symptoms were slightly lower on plants grown in the amended soil. Wilt symptoms were decreased due to the pathogen inoculum's levels (10^5 and 10^4 CFU/g soil) (Table 2).

Table (2): Pathogenicity assay of different inoculums densities of *F.oxysporum* against potato plants in the greenhouse.

Soil treatments	% wilt at inoculum density of CFU/g soil		
	10^4	10^5	10^6
A	70	81	87
B	0	0	0
C	74	81	90
D	0	0	0

A: Soil amended with fishmeal, infested with *F. oxysporum*
B: Soil amended with fishmeal, none infested with *F. oxysporum*
C: Soil non-amended with fishmeal, infested with *F. oxysporum*
D: Soil non-amended with fishmeal, none infested with *F. oxysporum*
Readings are means of 5 pots (10 plants) for each treatment.

3- Isolation and identification of *Rhizoctonia solani*, the causal agent of potato stem disease

Isolates of *R. solani* were recovered on 2% antibiotic water agar and PDA media, from stem lesions of 10 diseased potato plants showing typical symptoms of (stem canker). The isolates were identified due to their morphological characteristics

according to Ogoshi (1985) by the presence of multinucleate cells, branching near the distal septum of cells, constriction of the branch near the point of origin.

Pathogenicity test of *R. solani*

Infested potato plants showed symptoms of disease, like delayed emergence, pruned shoots, dark brown lesions on plant stem bases, dwarfism, yellowing and curling of leaves. In addition, small green tubers were noticed on the base of stems at the soil surface. Control plants did not show any symptoms of stem-canker. *Rhizoctonia solani* was re-isolated from the soil, and underground stolons of infested plants.

4- *In-vitro* detection of antifungal activity of antagonistic yeasts

A) *In-vitro* detection of antifungal activity of rhizosphere yeasts against *F. oxysporum:*

Data recorded in table (3) showed that the antagonistic activities of yeast isolates obtained from the rhizosphere of potato plants.

As shown in table (3), yeast isolates No. 2 and 4 were found to have the remarkable antagonistic activities against *F. oxysporum* than the other isolates.

B) *In-vitro* detection of antifungal activity of some yeast isolates against *Rhizoctonia solani*

Inhibitory activity of the tested yeasts species (5-11%); against each of *R. solani* and *F. oxysporum* was very weak in most cases (6-18.75%) (Tables 3 and 4).

Isolation of yeast fungi

Screening the yeast fungi isolates for antagonistic activities against the pathogenic fungi causing wilt and stem canker potato (*F. oxysporum and R. solani)* indicated that out of the five yeast fungi isolates three showed weak degrees of antagonistic activities against the pathogenic fungi (*F. oxysporum* and *R. solani*) as shown in (Tables 3 & 4).

As the antagonistic activities of yeasts were expressed in terms of the percentage of inhibition of growth of *F. oxysporum and R. solani.* Isolates No. (3 & 4) inhibited the growth of *F. oxysporum* and *R. solani* by a percentage of inhibition (5-18.75%) *Saccharomyces cerevisiae* and *Candida stealdytica.*

5- *In-vitro* detection of antifungal activities of rhizosphere fungi against *F. oxysporum*

Activity of the tested fungi against *F. oxysporum* is recorded in table (5). Inhibitory activity of fungal species ranged between (25-43%). However, *Aspergillus clavatus, A. flavus, A. niger, Mucor* sp., *Absidia* sp. and *Alternaria* sp. showed a very weak inhibitory activities to linear growth of *F. oxysporum* (6-12.5%) (Table 5).

Table (3): Antagonistic activities of yeast isolates against *F. oxysporum*

Tested Organism	Pathogenic fungus *(F. oxysporum)*			
	r_o	r	Δr	% inhibition
1-Hansenula arabitolegenes	1.6	1.4	0.2	12.5
2-Candida incommunis	1.6	1.3	0.3	18.75
3-Saccharomyces cerevisiae	1.6	1.5	0.1	6.25
4-Candida stealolytica	1.6	1.3	0.3	18.75
5-Candida glabrata	1.6	1.5	0.1	6.25

r_o: Fungal growth radius of a control culture (in cm)
r: Distance of fungal growth in direction of the fungal colony (in cm) (antagonist).

Table (4): Antagonistic activities of some yeast isolates against *R. solani.*

Tested Organism	Pathogenic fungus *(R. solani)*			
	r_o	r	Δr	% inhibition
1- Hansenula arabitolegenes	1.8	1.7	0.1	5.5
2- Candida incommunis	1.8	1.8	0	0
3- Saccharomyces cerevisiae	1.8	1.6	0.2	11
4- Candida stealolytica	1.8	1.6	0.2	11
5- Candida glabrata	1.8	1.7	0.1	5.5

For explanation refer to footnote of table (5)

Table (5): Antagonistic activities of fungal isolates against *F. oxysporum*

Isolated Fungi	Tested pathogenic fungus			
	r_o	r	Δr	% inhibition
1-Fusarium sambucinum	1.6	1.2	0.4	25
2-Fusarium oxysporum	1.6	1.2	0.4	25
3-Fusarium solani	1.6	1.0	0.6	37.5
4-Penicillium oxalicum	1.6	0.9	0.7	43
5-Aspergillus clavatus	1.6	1.5	0.1	6
6-Aspergillus flavus	1.6	1.4	0.2	12.5
7-Aspergillus niger	1.6	1.5	0.1	6
8-Mucor sp.	1.6	1.5	0.1	6
9-Absidia sp.	1.6	1.4	0.2	12.5
10-Alternaria sp.	1.6	1.5	0.1	6

For explanation refer to footnote of table (3)

Table (6): Antagonistic activities of fungal isolates against *R. solan*

Isolated Fungi	Tested pathogenic Fungus *(R. solani)*			
	r_o	r	Δr	% inhibition
1-Fusarium sambucinum	1.9	1.6	0.3	15.7
2-Fusarium solani	1.9	1.7	0.2	10.5
3-Fusarium oxysporum	1.9	1.4	0.5	26
4-Aspergillus clavatus	1.9	1.8	0.1	5.2
5-Aspergillus flavus	1.9	1.8	0.1	5.2
6-Aspergillus niger	1.9	1.8	0.1	5.2
7-Mucor sp.	1.9	1.9	0	0
8-Absidia sp.	1.9	1.9	0	0
9-Alternaria sp.	1.9	1.9	0	0
10-Gliocladium sp.	1.9	1.5	0.4	21
11-Trichoderma sp.	1.9	1.5	0.4	21

For explanation refer to footnote of table (3)

Isolation and screening of the rhizosphere microorganisms for antagonistic activities against potato wilt and stem canker fungi

A) Isolation of mold fungi:

Eleven fungal isolates were recovered from the rhizosphere associated soils of potato. Screening the fungal isolates for antagonistic activities against fungi causing wilt of potato, indicated that out of the eleven fungal isolates recovered, some isolates showed variable degrees of antagonistic activities against the pathogenic fungi, *i.e. Fusarium F. oxysporum* and *Rhizoctonia R. solani* (Tables 5 and 6). Comparing all the antagonizing fungal isolates, it was found that 4 of them were characterized by their remarkable and potent antagonistic activities than the other isolates. Isolate No. 4 *(Penicillium oxalicum)* inhibited *F. oxysporum* by 43%.

El-Mehalawy (2004) found that *Saccharomayces unispora* and *C. steatolytica* as a rhizosphere yeast fungi had antagonistic and inhibitory effects on the growth of *F. oxysporum.*

Isolation of yeast fungi

Screening the yeast fungi isolates for antagonistic activities against the pathogenic fungi causing wilt and stem canker potato (*F. oxysporum* and *R. solani)* indicated that out of the five yeast fungi isolates recovered, 5 isolates showed weak degrees of antagonistic activities against the pathogenic fungi (*F. oxysporum* and *R. solani*) as shown in (Tables 5 and 6).

As the antagonistic activities of yeasts were expressed in terms of the percentage of inhibition of growth of *F. oxysporum and R. solani.* As shown in tables (5&6), isolates No. (3) & (4) inhibited the growth of (*F. oxysporum and R. solani* by a percentage of inhibition between 5-18.75%.

B) *In-vitro* detection of antagonistic activities of fungal isolates against *Rhizoctonia solani*

The retardation in growth of the tested pathogens might be due to the production of antifungal antibiotics, lytic enzymes or due to competition for nutrients and space (Lorito *et al.* 1996). Howell and Stipanovic (1995) stated that the antifungal antibiotics gliotoxin and gliovirin produced by *Gliocladium virens* were associated with its efficacy as biocontrol agent of seedling diseases caused by *R. solani.* These results are supported by Howell (2003) who found that some isolated fungi are known to be very good competitors for nutrients, space and infection sites at the rhizoplane in spite of they are unable to produce inhibitory antibiotics.

Metabolites extracted from solid and liquid cultures of *F. sambucinum* showed a very weak inhibitory activity against the pathogenic *F. oxysporum.* This means that it may produce weak antifungal antibiotics. Metabolites extracted from liquid and solid cultures of *Gliocladium* sp. caused weak lysis of *R. solani* colony. Metabolites extracted from solid culture of *P. oxalicum* had the most inhibitory activities against the pathogenic *F. oxysporum.* These results are in agreement with those obtained by Howell (2003). Metabolites extracted from liquid cultures of *F. solani* showed a weak inhibitory activity against the pathogenic *F. oxysporum.*

In conclusion, the non-pathogenic *F. sambucinum* was the best antagonist for *F. oxysporum. Gliocladium* sp. was a good antagonist for *R. solani. Trichoderma* sp. and *Gliocladium virens* reduced *R. solani* activities (Corley *et al.* 1994). Dennis and Webster (1971) were the first to describe the antagonistic properties of *Trichoderma* in terms of antibiotic production.

6- *In-vivo* detection of antagonistic activities of yeasts against *F. oxysporm* and *R. solani* under greenhouse conditions

Data of *in-vivo* antagonism of yeasts against *F. oxysporum* table (7) and those recorded for *R. solani* are shown in table (8).

Tested isolates gave parameters of plant growth lower than those of the control soil. This means that, these isolates can show very weak antagonistic activities against the pathogens. Such results are in accordance with those recorded previously using in-vitro assays. These results are in agreement with those of Carling *et al.* (1986).

7- *In-vivo* detection of antagonistic activities of the isolated fungi against *F. oxysporum* in the greenhouse

In-vivo test results of the isolated fungi against the pathogenic *F. oxysporum* are recorded in table (9). In positive control, potato plant growth values were greatly retarded compared to the negative control plants. This may be due to the effect of the pathogenic *F. oxysporum,* as a result of the wilt disease.

Results obtained from the greenhouse experiment were very promising, since potato plant growth values were promoted by all the tested fungal antagonists with different degrees, compared with the positive control infested with the pathogen. A possible mechanism for protection of the potato plants against the pathogens was the colonization of roots and hypocotyls which works as a protective masking barrier at recognition and infection sites, thus prevented host penetration by the pathogens, and improved plant health and yield through protecting the root from pathogens (Sneh *et al.* 1989). Hide *et al.* (1985), stated that most stem canker disease develops before shoots emerged,

Table (7): Effect of antagonistics yeast isolates on plant parameters in soil infested with *F. oxysporium* under greenhouse conditions

Soil treatments		Parameters of potato plant		
Pathogen	Antagonist	Plant height (cm)	No. of leaflets/plant	Fresh wt. (g)/plant shoot
		Mean ± SD	Mean ± SD	Mean ± SD
Fusarium oxysporum	Hansenula arabitolegenes	60.000 ± 0.163	79.000 ± 0.163	18.000 ± 0.163
	Candida incommunis	52.000 ± 9.851	88.000 ± 16.672	24.000 ± 4.547
	Saccharomyces cerevisiae	50.000 ± 0.163	80.000 ± 0.163	20.000 ± 0.163
	Candida stealolytica	62.000 ± 11.746	90.000 ± 17.051	20.000 ± 3.789
	Candida glabrata	56.000 ± 0.163	88.000 ± 0.163	26.000 ± 0.163
Control soil (-ve control)		63.000 ± 11.935	89.000 ± 16.861	28.000 ± 5.305
Positive control		50.000 ± 9.473	75.000 ± 14.209	18.000 ± 3.410
ANOVA	F	7.546	15.560	54.122
	P-value	0.011*	<0.001*	<0.001*

Positive control: pathogen + potato plant Control soil: soil without pathogen + potato plant
Results are the average of 5 pots for each treatment
-ve control: uninfected plants (Soil without pathogen or antagonist)
Highly significant at P<0.001 using ANOVA

Table (8): Effect of antagonistics of yeast isolates on plant parameters in soil infested with *Rhizoctonia solani* in the greenhouse conditions

Soil treatments		Parameters of potato plant		
Pathogen	Antagonist	Plant height (cm)	No. of leaflets/plant	Fresh wt. (g)/plant shoot
		Mean ± SD	Mean ± SD	Mean ± SD
Rhizoctonia solani	Hansenula arabitolegenes	53.000 ± 7.709	81.000 ± 11.782	20.000 ± 2.909
	Candida incommunis	57.000 ± 8.291	85.000 ± 12.364	25.000 ± 3.637
	Saccharomyces cerevisiae	58.000 ± 8.437	83.000 ± 12.073	28.000 ± 4.073
	Candida stealolytica	63.000 ± 9.164	86.000 ± 12.510	29.000 ± 4.218
	Candida glabrata	61.000 ± 8.873	79.000 ± 11.491	24.000 ± 3.491
Control soil (-ve control)		61.000 ± 8.873	94.000 ± 13.673	31.000 ± 4.509
Positive control		50.000 ± 7.273	78.000 ± 11.346	19.000 ± 2.764
ANOVA	F	7.546	5.6546	16.881
	P-value	0.001*	0.022*	<0.001*

N= 5 pots Highly significant at P<0.001 using ANOVA

Table (9): *In-vivo* detection of the antagonistic activities of the isolated fungi against *F. oxysporum* under greenhouse conditions

Soil treatments		Parameters of potato plant		
Pathogen	Antagonist	Plant height (cm)	No. of leaflets/plant	Fresh wt. (g)/plant shoot
		Mean ± SD	Mean ± SD	Mean ± SD
F. oxysporum	*F. solani*	80.920 ± 15.330	155.000 ± 0.163	16.850 ± 0.163
	F. sambucinum	82.600 ± 15.649	132.000 ± 0.163	31.400 ± 5.949
	P. oxalicum	73.040 ± 13.837	131.000 ± 0.163	29.700 ± 0.163
	Trichoderma sp.	49.400 ± 9.359	80.000 ± 0.163	15.820 ± 2.997
Control soil (-ve control)		56.100 ± 10.628	79.000 ± 0.163	23.700 ± 0.163
Positive control		34.800 ± 6.593	57.000 ± 0.163	11.100 ± 2.103
ANOVA	F	60.525	85.550	22.136
	P-value	<0.001*	<0.001*	<0.001*

N = 5 pots Highly significant at P<0.001 using ANOVA

although it was gradually increased later when new shoots arising both from seed tubers or as branches on shoots with damaged apices (pruned shoots), became infected before emergence. It was shown that early infection and pruning of shoots have been considered the most important phase of stem-canker disease causing delayed emergence, stunted plants and a decrease in the number of stems.

Soil treatment with the non-pathogenic *F. sambucinum* and *F. solani,* gave the highest plant height, compared to plants in both of negative control and positive control (Table 9). In respect to the number of leaflets, treatment with *F. solani* showed the highest number of leaflets compared with both positive and negative control. Soil treated with *P. oxalicum* gave a moderate increase in plant growth compared to the control, whereas soil treated with *Trichoderma* sp. showed better growth than that of the positive control. Obtained results are in agreement with those obtained by Carling *et al.* (1986). The retardation in growth of the tested pathogens might be due to the production of antifungal antibiotics, lytic enzymes or due to competition for nutrients and space (Lorito *et al.* 1996).

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