PSM Microbiology ISSN: 2518-3834 (Online) – An International Peer-reviewed Journal

Research Article 2018 | Volume 3 | Issue 2 | 43-54

Article Info

Open Access
Citation: Hassanein, N.M., Shoala, T., Gouda, S.A., 2018. *In vitro* Studies on Biological Control of *Drechslera* species Causing Brown Spot Disease in Rice Plants. PSM Microbiol. 3(2): 43-54.

Received: February 2, 2018

Accepted: May 8, 2018

Online first: June 7, 2018

Published: July 13, 2018

***Corresponding author:**
Tahsin Shoala;
Email:
tahsinshoala2000@gmail.com

Scan QR code to see this publication on your mobile device.

In vitro Studies on Biological Control of *Drechslera* species Causing Brown Spot Disease in Rice Plants

Naziha M. Hassanein[1], Tahsin Shoala[2]*, Shaymaa A. Gouda[1]

[1]Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
[2]College of Biotechnology, Misr University for Science and Technology, Cairo, Egypt.

Abstract
Drechslera species are amongst common fungal pathogens of rice, causing leaf spot diseases. Infection with *Drechslera* species causes quantitative and qualitative damage to small grains and rice plants. In the present study, rice rhizosphere mold and yeast fungi were isolated from El-Dakahlia and El-Qaliubiya governorates. Results indicated that in case of mold fungi, *Fusarium* and *Aspergillus* were the most dominant isolates (520 and 410 CFU per gram, respectively) while *Monodictys* and *Phaeodactylium* represented the lowest ones (10 CFU per gram for each). Concerning yeast fungi, *Stephomoascus* and *Candida* gave the highest CFU count per gram (90 and 60 CFU per gram respectively). Isolation of pathogens from infected rice leaves and grains from El-Dakahlia and El-Qaliubiya governorates indicated that *Drechslera specifier* gave the highest frequency percentages (56 % and 53.33 % respectively), while *Drechslera rostrate* gave 33 % and 26.66 % for both governorates respectively. *Penicillium decumbens thom* gave the highest antagonistic activity against *Drechslera specifier* (83.9 %). Biological control of *Drechslera* species could be successfully applied by using *Penicillium decumbens thom* as a bio-agent.
Keywords: Antagonism, biological control, *Drechslera*, leaf spot and rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide. It is staple food for more than half of the world's population. It's known to be a healthy, nutritious and multipurpose food because of its complex carbohydrates structure which is converted by the body's digestive processes into glycogen, then stored in muscle tissues and released as energy when activity demands. Being the food staple of most Egyptians, rice is locally consumed at a rate of 35-40 kg/capita/annum (Ahmed, 1998; Jatoi *et al.,* 2015).

Rice crop is subjected to be infected by many factors that may be biotic (pathogens) or abiotic (environmental factors). However, major biotic diseases are rice blast, brown spot, bacterial leaf blight and leaf streak, sheath blight, sheath rot, Fusarium wilt, stem rot, Tungro virus and false smut. These diseases either attack rice plants at any growth stages or infect rice grains post-harvest, which adversely affect its yield in both quality and quantity per unit area (Hajano *et al.,* 2011; Arain, 2013).

Brown spot is one of the most commonly occurring and dangerous diseases worldwide. In Egypt, the disease comes in the second rank after blast disease; because it causes both quantity and quality losses in rice crop that may range from 5-45% loss in the crop yield (Jatoi *et al.,* 2015).

Rice brown spot disease caused by *Drechslera* species, the pathogen attacks the crop from seedling to milk stage. It attacks coleoptile, leaf blade, leaf sheath and glume, being most prominent on leaf blades and glumes. Typical symptoms on rice leaves include, brownish spots with grey or whitish center, cylindrical or oval in shape resembling sesame seeds usually with yellow halo. On glumes; black or dark brown spots are produced resulting in discolored and shriveled grains. Under favorable conditions, the fungus may penetrate the glumes and leave blackish spots on the endosperm. The pathogen has also been reported to cause brown to dark brown lesions on panicle stalk at the joint of flag leaf to stalk (Singh, 2005).

Chemical control normally available to reduce effectively and extensively the effects of brown spot disease on young plants, but field application of these chemical fungicides may not always be desirable. Extreme and inappropriate application of these fungicides affects human health, animal and environment. Many of these chemicals are also too expensive for the resource of poor farmers (Shabana *et al.,* 2008). Our current research may offer alternative and safe strategies which are economically reasonable and eco-friendly as biocontrol approach like botanical pesticides or biological agents for managing rice plant pathogens (Jitendiya and Chhetry, 2013).

Many microorganisms from the rhizosphere can positively influence plant growth and plant health and are referred to as PGPR (Plant growth promoting rhizo-microorganisms). These microbes can act as biocontrol agents in several ways, including niche exclusion, bio antagonism and induction of induced systemic resistance (ISR) against infection by fungal, bacterial and viral pathogens in different plant speciesince biocontrol is a key component of integrated disease management (Shyamala and Sivakumaar, 2012). Biological control practices require an integrative approach, and more knowledge than chemical control (Alsohiby *et al.,* 2016).

The aim of this work was to study the *in vitro* capacity of fungi to control *Drechslera* spp. which causing brown spot disease of rice plant.

MATERIALS AND METHODS

Samples collection

Rice rhizosphere soil

Rhizosphere soils obtained from fields cultivated with rice plants from El-Dakahlia and El-Qaliubiya governorates were used in this study during year from 2014-2015.

Infected rice plant parts

Infected rice plants with typical symptoms (grains and leaves showing brown spot symptoms) were collected randomly from El-Dakahlia and El-Qaliubiya fields during 2014 season. Samples were collected and labeled in plastic bags, stored inside ice box, and brought to the laboratory for further studies.

Isolation and identification of rice rhizosphere fungi

Ten grams of rhizosphere soil transferred to 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water, and then the samples were shaken and left to settle down. 10 ml of the supernatant solution was transferred to 250 ml Erlenmeyer flask containing 90 ml of sterile distilled water. This dilution was prepared from the concentrations of 10^{-1} to 10^{-4}. Aliquots of 0.1ml was spread with a sterile glass rod over the surface of three isolation media (i) Sabourad's yeast extract agar (SYA) (ii) Potato dextrose agar (PDA) and (iii) Czapek's Dox agar (CDA). The petri-dishes were incubated at 28 ± 2^0C for 5-7 days. 10 replicates were used to confirm the results. The colonies were purified and sub-cultured on their selective media then stored at 4^oC in slants (Chandrashekar *et al.,* 2014).

Fungal isolates were identified to the genus and species levels according to the macroscopic features (texture and color) and microscopic features (slide culture technique) by staining with lacto phenol cotton blue and examined under compound microscope for conidiophores, conidia and arrangement of spores (Chandrashekar *et al.,* 2014) according to the references of: a) Carmichael *et al.* (1980) for hyphomycetes; b) Ellis (1971) and (1976) for dematiaceous hyphomycetes; c) Hesseltine (1955) and Gilman (1957) for general Mucorales; d) Gilman (1957), Toussoun and Nelson (1968) and Booth (1971) for the

genus *Fusarium*; e) Pitt (1979) for the genus *Penicillium* and f) Raper and Fennelli (1977) for the genus *Aspergillus*.

Isolation and identification of rice pathogens

Diseased leaves pieces collected randomly from EL-Dakahlia and EL-Qaliubiya fields were immersed in absolute alcohol, surface sterilized in one percent sodium hypochlorite solution for 30 seconds, rinsed three times in sterilized water, dried with sterilized filter paper then placed in sterilized Petri dishes containing freshly prepared PDA medium. Five grains and pieces of diseased plant parts were placed in each Petri dish and incubated at 25^0C for five days to induce sporulation of the fungi. Growing fungal colonies were purified and multiplied on PDA slants then stored at 4^oC. The isolated fungal species were identified on the basis of their morphological and microscopical characteristics (Hajano *et al.,* 2011; Venkateswarlu *et al.,* 2015).

Calculation of the isolation frequency of fungal pathogens was carried out using a total of 20 and 25 leaves and grains samples from EL-Dakahlia governorate respectively and a total of 30 samples from both leaves and grains of EL-Qaliubiya governorate. Isolation frequency was calculated as follows:

$$Fr\ (\%) = (ns / N) \times 100$$

Where: Fr = Frequency; ns = the number of isolates and N = the total number of collected plant samples.

Identification of *Drechslera* species

Identification of *Drechslera* species was based on morphological and microscopic characteristics of the culture growing on PDA media at 28 ± 2^0C for 7 days using the references of Ellis (1971 and 1976).

***In vitro* antagonistic activity of rhizosphere fungi against *Drechslera* species**

Rhizosphere fungi were examined for their antagonistic activity against brown leaf spots and grain pathogens by using dual culture technique. The isolates were streaked-inoculated to one side of PDA medium (Hassanein, 2010; Hassanein *et al.,* 2010) then incubated for 3 days to allow the production and diffusion of metabolites into the agar. An agar disk containing leaf and grain spot pathogens mycelium was placed into the opposite side of the inoculated plates. Pathogens mycelia discs were also placed on un-inoculated potato dextrose agar separately as controls. Cultures were incubated at 28 ± 2^0C for 6 days and the plates were examined for inhibition of pathogens growth. The level of inhibition was determined as described by Yuan and Crawford (1995). Briefly, the level of inhibition was defined as the subtraction of the pathogen's growth radius in the direction of antagonistic fungus colony {γ (in cm)}, where $\Delta\ \gamma = \gamma_0 - \gamma$. Inhibition was indicated when mycelial growth of pathogens in the direction of fungi colony was retarded.

RESULTS

Isolation and identification of rice rhizosphere fungi

Thirty filamentous fungal species belonging to 12 genera and 5 yeast species belonging to 4 genera were isolated from rice rhizosphere soil of El- Dakahlia and El-Qaliubiya governorates (Tables 1 and 2).

A total number of 1370 CFU per gram were isolated from EL-Dakahlia governorate represented the highest CFU per gram count (720 CFU per gram) while EL-Qaliubiya governorate represented by 650 CFU per gram (Table 1). Also, *Stephanoascus* sp. and *Candida* spp. represented the most dominant yeast species and represented by 90 and 60 CFU per gram respectively.

Fusarium and *Aspergillus* were the most dominant genera and represented by 520 and 410 CFU per gram respectively while *Monodictys* and *Phaeodactylium* represented the lowest ones (10 CFU per gram for both).

Concerning yeast fungi, Table 2 showed that genera *Candida* (*C. guil.* And *C. utilis*) and Cryptococcus were isolated only from El- Dakahlia governorate while *Rhodotorula* was recorded only in El-Qaliubiya. On the other hand, the genus *Stephanoascus* was recorded in both governorates and represented by 70 and 20 CFU per gram respectively. In general, the large numbers of yeast were isolated from El- Dakahlia governorate rather than El-Qaliubiya governorates.

Isolation and identification of rice leaves and grains pathogens

In this study, 8 fungal species were isolated from the pre-harvest rice leaves and grains samples showing brown spot symptoms. Table (3) showed that, the highest numbers of isolates were recorded from leaves (29 isolates) while the lowest one was obtained from grains (6 isolates) from El-Qaliubiya governorate. *Drechslera specifier* showed the highest percentage frequency 43.33 % and 40 % isolated from leaves of El-Qaliubiya and El-Dakahlia, respectively. *Drechslera rostrata* followed *Drechslera specifier* in frequency percentage value (25 % and 23.33 % isolated from leaves of El-Dakahlia and El-Qaliubiya, respectively). Photomicrograph of *Drechslera rostrate* and *Drechslera speciefer* isolated from naturally infected rice plants are indicated in Figures (1) and (2) respectively.

Table 1. Count and frequency of mould fungi isolated from rhizosphere of rice plants cultivated in El- Dakahlia and El- Qaliubiya

Genus number	Genus	Species number	Species	CFU per gram		Total CFU
				El -Dakahlia	El-Qaliubiya	
1	*Alternaria*	1	*Alt. citri*	-	10	**60**
		2	*Alt. dianthi*	-	50	
Total genus count				**-**	**60**	
2	*Aspergillus*	3	*A. aculaetus*	10	150	**410**
		4	*A. candidus*	20	-	
		5	*A. famigatus feresenius*	-	10	
		6	*A. flavipes*	40	-	
		7	*A. nidulans*	-	160	
		8	*A. parasiticus*	10	-	
		9	*A. terrus*	-	10	
Total genus count				**80**	**330**	
3	*Cladosporium*	10	*Cl. cladosporiodes*	30	60	**100**
		11	*Cl. state of venturia*	-	10	
Total genus count				**30**	**70**	
4	*Fusarium*	12	*F. fusarioides*	30	-	**520**
		13	*F. lateritium*	200	-	
		14	*F. moniliforme*	70	-	
		15	*F. moniliforme var anthophilium*	20	50	
		16	*F. moniliforme var subglutinans*	-	30	
		17	*F. poae*	60	30	
		18	*F. solani*	-	30	
Total genus count				**380**	**140**	
5	*Gliomastix*	19	*Gli.. cerealis*	**60**	**10**	**70**
6	*Monodictys*	20	*Mono. castaneae*	-	**10**	**10**
7	*Nigrospora*	21	*Ni.* state of *khuskia oryzae*	**60**	-	**60**
8	*Penicillium*	22	*P. decumbens*	-	20	**70**
		23	*P. dimorphosporum*	20	-	
		24	*P. digitatum*	10	-	
		25	*P. miniolutеum*	-	10	
		26	*P. verrucuola*	10	-	
Total genus count				**40**	**30**	
10	*Phaeodactylium*	27	*Pha. alpiniae*	**10**	-	**10**
11	*Thielaviopsis*	28	*Thi.* state of *ceratocystis moniliforme*	**40**	-	**40**
12	*Trichoderma*	29	*Tri. lignorum*	10	-	**20**
		30	*Tri. glaucum*	10	-	
Total genus count				**20**	**-**	
Total count				**720**	**650**	**1370**

Table 2. Count and frequency of yeasts isolated from rhizosphere of rice plants cultivated in El- Dakahlia and El-Qaliubiya

Genus number	Genus	Species number	Species	CFU per gram		Total CFU
				El -Dakahlia	El- Qaliubiya	
1	*Candida*	1	*C. gulieurimondii*	10	-	**60**
		2	*C. utilis*	50	-	
Total genus count				**60**	**-**	
2	*Cryptococcus*	3	*Cr. laurentii*	**30**	-	**30**
3	*Rhodotorula*	4	*Rh. mucilaginosa*	-	**10**	**10**
4	*Stephanoascus*	5	*St.* sp.	**70**	**20**	**90**
Total count				**160**	**30**	**190**

Table 3. Isolation frequency of fungal pathogens recovered from rice plants from El- Dakahlia and El- Qaliubiya

Genus	Species	El- Dakahlia				El-Qaliubiya			
		Leaves		Grains		Leaves		Grains	
		No. of isolates	Fr[a] (%)	No. of isolates	Fr[a] (%)	No. of isolates	Fr[a] (%)	No. of isolates	Fr[a] (%)
Alternaria	*Alt. betroselini*	1	5	-	-	2	6.66	1	3.33
	Alt. cinerariae	-	-	-	-	1	3.33	-	-
Drechslera	*D. rostrata*	5	25	2	8	7	23.33	1	3.33
	D. specifiera	8	40	4	16	13	43.33	3	10
Fusarium	*F. graminearum*	1	5	2	8	2	6.66	-	-
	F. merismoidescorda	-	-	2	8	-	-	-	-
	F. sporotrichoides	2	10	-	-	1	3.33	-	-
Pyricularia	*Pyricularia* sp.	-	-	-	-	3	10	1	3.33
Total isolates		17		10		29		6	
Total samples		20		25		30		30	

a: Frequency (Fr %) = (ns/N) x 100

Where,

ns: the number of isolates where a genus species occurred

N: the total number of collected plant samples

Fig. 1. Photomicrographs of *Drechselara rostrata* isolated from naturally infected rice plants.

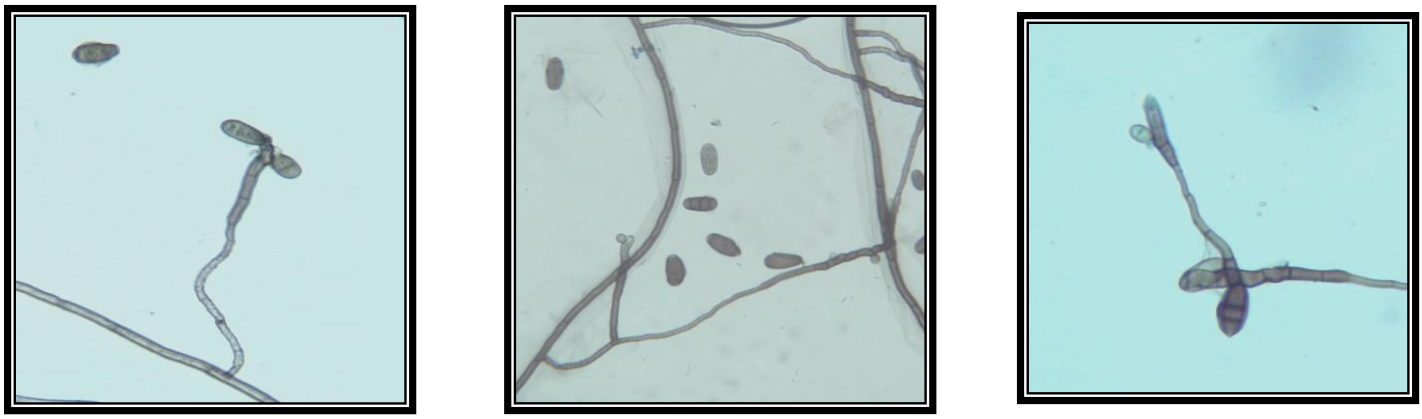

Fig. 2. Photomicrographs of *Drechselara specifiera* isolated from naturally infected rice plants.

***In vitro* antagonistic activity of rhizosphere fungal isolates against brown leaf spot pathogens of rice plant**

Result showed positive antagonistic activity of 15 isolates from rhizosphere fungi against leaf spot pathogens *Drechslera specifiera* and *Drechselara rostrate* shown in Table (4) and (5) and Figures (3) and (4).

Table (4) and Figure (3) showed that *Penicillium decumbens*, *P. dimorphosporum, Cladosporium* state of *venturia* and *Cl. cladosporiodes* have positive antagonistic activity against *Drechslera* state of *cochlibolus specifier* and gave inhibition percentage of 83.9%, 72.8%, 67.9% and 63.1% respectively. The highest antagonistic activity was recorded by *Penicillium decumbens.*

On the other hand, antagonistic activity of the rhizosphere fungi against *Drechslera rostrate* is indicated in Table (5) and Figure (4). Results showed that *Aspergillus terreus* gave the highest antagonistic percentage against *D. rostrate* (82.3 %) followed by *Aspergillus parasiticus*, *A. aculetus*, *Penicillium dimorphosporum* and *P. digitatum* with equal inhibition percentage (77.5 %).

Table 4. *In vitro* antagonistic activity of the selected rhizosphere fungi against *Drechselara specifiera*

Antagonistic fungus	*Drechselara specifiera*			
	γ_0	γ	$\Delta\gamma$	% inh.
Penicillium decumbens	2.06	0.33	1.73	83.9
Penicillium dimorphosporum	2.06	0.56	1.5	72.8
Cladosporium state of *venturia*	2.06	0.66	1.4	67.9
Cladosporium cladosporiodes	2.06	0.76	1.3	63.1

Where:

γ_0 : Pathogen growth radius of a control culture (cm).

γ : Pathogen growth radius in direction of the antagonistic fungus colony (cm).

$\Delta\gamma = \gamma_0 - \gamma$.

% inh. : Percentage of inhibition ($\Delta\gamma / \gamma_0$).

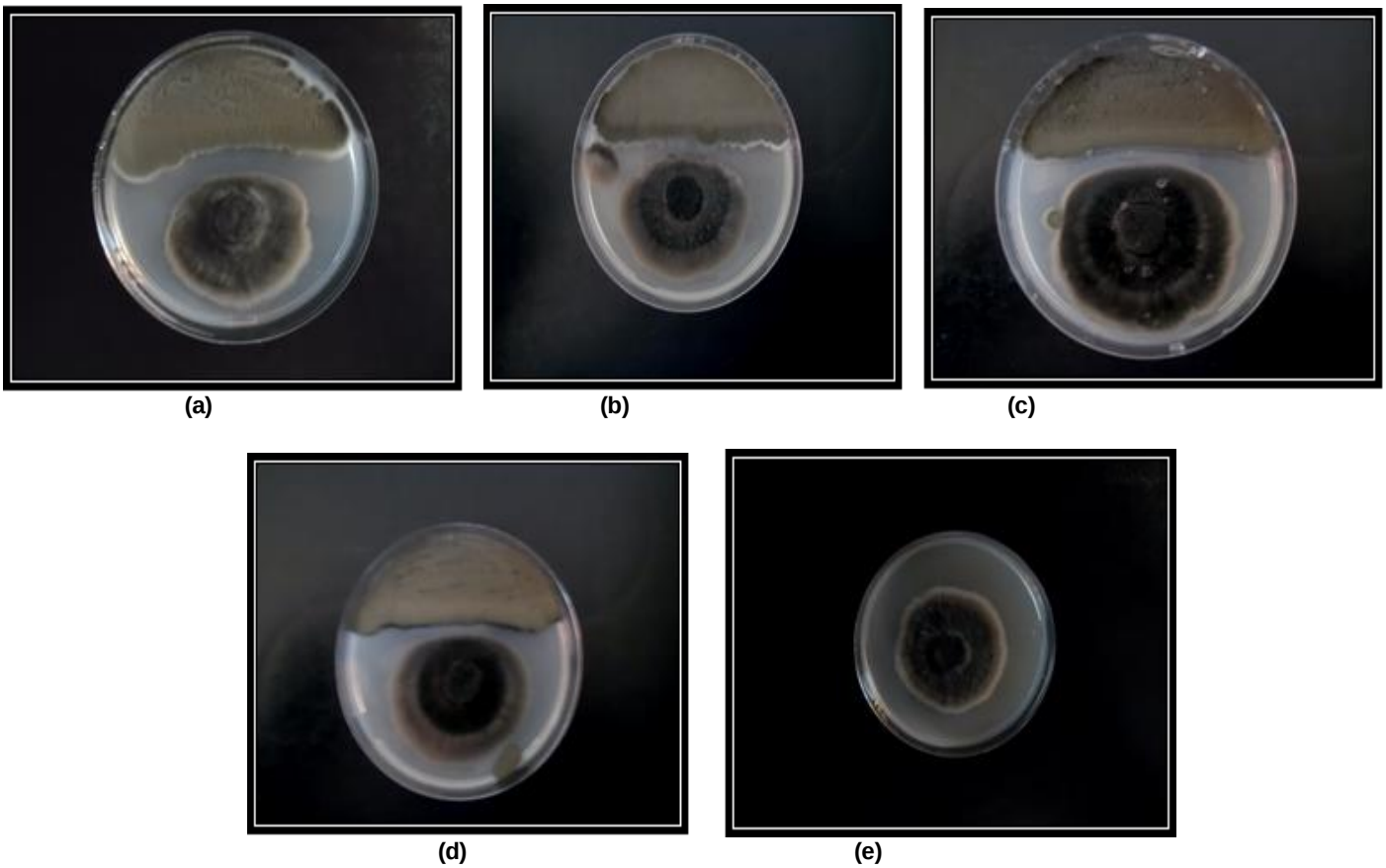

Fig. 3. *In vitro* antagonistic activity of the selected rhizosphere fungi against *Drechselara* state of *Cohlibolus specifier*, where: (a) *Penicillium decumbens*; (b) *Penicillium dimorphosporum*; (c) *Cladosporium* state of *venturia*; (d) *Cladosporium cladosporiodes*; (e) Control.

Table 5. ***In vitro*** **antagonistic activity of the selected rhizosphere fungi against** ***Drechslera rostrata.***

Antagonistic fungus	*Drechslera rostrata*			
	γ_0	γ	$\Delta\gamma$	% inh.
Aspergillus terrus	1.16	0.2	0.96	82.3
Aspergillus parasiticus	1.16	0.26	0.9	77.5
Aspergillus aculaetus	1.16	0.26	0.9	77.5
Penicillium dimorphosporum	1.16	0.26	0.9	77.5
Penicillium digitatum	1.16	0.26	0.9	77.5
Cladosporium cladosporiodes	1.16	0.3	0.86	74.1
Aspergillus flavipes	1.16	0.33	0.83	71.5
Monodictys castaneae	1.16	0.43	0.73	62.9
Gliomastix cerealis	1.16	0.43	0.73	62.9
Rhodotorula mucilaginosa	1.16	0.46	0.7	60.3
Aspergillus candidus	1.16	0.53	0.63	54.3

Where: γ_0 : Pathogen growth radius of a control culture (cm); γ: Pathogen growth radius in direction of the antagonistic fungus colony (cm); $\Delta\gamma = \gamma_0 - \gamma$; % inh. : Percentage of inhibition ($\Delta\gamma / \gamma_0$).

DISCUSSION

The rhizosphere microbial communities effect plant growth and resistance to disease or even plant death depending on the degree of parasitism and pathogenicity. So that, existed different microorganisms in the rhizosphere could be used as biocontrol agents against plant diseases (Abou-Zeid, 2008). Antibiosis, myco-parasitism and food competition are the main mechanisms of these microorganisms in biological control (Ranasingh *et al.,* 2006; Ghildyal and Pandey, 2008; Umamaheswari *et al.,* 2009). It is required to develop efficient selection approaches to choose biocontrol organisms that can be produced in a large scale at low cost and that retain their viability and efficiency for long periods (Iqbal and Ashraf, 2017).

In the present study, 30 filamentous fungi species belonging to 9 genera and 5 yeast species belonging to 4 genera were isolated from El- Dakahlia and El- Qaliubiya governorates. These results were almost in accordance with those obtained by Venkateswarlu *et al.,* 2015, who indicated that rhizosphere myco-flora represent in genera *Fusarium* sp., *Aspergillus* sp., *Penicillium*sp., *Rhizopus* sp., *Penicillium* sp., *Trichoderma* sp. and *Alternaria* sp.

The current research also indicated that *Aspergillus* and *Fusarium* were the most dominant genera and represented the highest genera in their CFU per gram soil count isolated from El- Dakahlia and El- Qaliubiya governorates soils. These results were in agreement with the previous investigations. *Fusarium* is one of the basic constituents of fungi in the rhizosphere and rhizoplane of many Egyptian plants. Fungi other than *Fusarium* were also reported such as *Aspergillus* and *Penicillium* were commonly isolated from the rhizosphere and rhizoplane of both plants (Abdel-Hafez *et al.,* 2009; Ismail *et al.,* 2009).

Our research work also indicated that the large number of isolated yeast in their CFU count was isolated from El-Dakahlia governorate rather than El-Qaliubiya governorates. These results agree with those reported by Frey (2007) who found that the distribution of microbial biomass, including yeast cells, most likely reflects environmental heterogeneity, which is typical for underground biota. The environmental condition and the type of soil play a vital role in the distribution of yeast and the microbial biomass (Whitfield, 2005).

This study revealed that two *Drechslera* species (*Drechslera specifiera* and *Drechslera rostrata*) were isolated from naturally infected rice plants grown in fields of El- Dakahlia and El- Qaliubiya governorates. At the same time, other fungal agents were commonly seen during the isolation of *Drechslera* sp. such as *Alternaria* spp. and *Fusarium* spp. This might be due to association of different fungal flora such as *Bipolaris oryzae, Alternaria* spp and *Fusarium* spp. may be due to varietal genetic characterization to germinate which was close agreement with the findings of Naeem et al. (2001) and Hafiz et al. (2013).

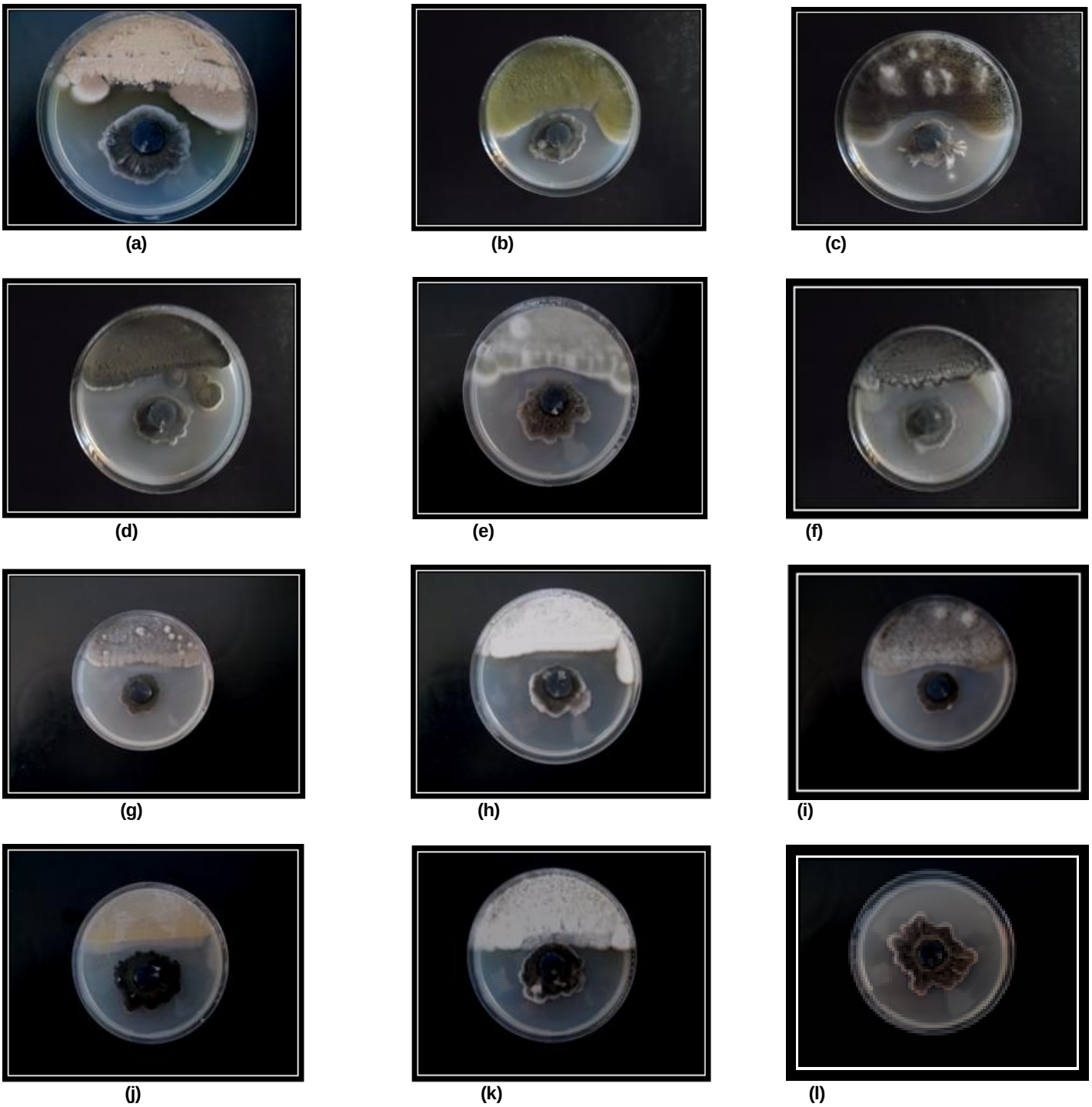

Fig. 4. ***In vitro*** **antagonistic activity of the selected rhizosphere fungi against** ***Drechselara rostrata*****, where:**
(a) ***Aspergillus terrus*****, (b)** ***Aspergillus parasiticus*****, (c)** ***Aspergillus aculaetus*****, (d)** ***Penicillium dimorphosporum*****, (e)** ***Penicillium digitatum*****, (f)** ***Cladosporium cladosporiodes*****, (g)** ***Aspergillus flavipes*****, (h)** ***Monodictys castaneae*****, (i)** ***Gliomastix cerealis*****, (j)** ***Rhodotorula mucilaginosa*****, (k)** ***Aspergillus candids*** **and (l) Control.**

Drechslera avenae is considered to be the cause of *Drechslera* leaf spot on oats and some grass species in Europe (Prończuk, 2000), in both parts of America (Clear et al., 2000 and Mehta, 2001) and in Africa (Scott, 1995). Various *Drechslera* species were previously isolated from various crops in Egypt. Ghany (2012) isolated *Drechslera dactylidis* which reported that cause leaf spot disease or southern leaf blight of Maize in Egypt. Also, EL-Shahir (2014) isolated *Drechslera neergaardii* from broad bean in Upper Egypt.

In vitro inhibition of fungi has been attributed to some factors such as antibiotic production and pH changes in the medium. Jeffries and Young (1994) revealed production of extracellular metabolites (such as antibiotics and lytic enzymes) was one of the mechanisms of antagonism between two fungal isolates (Hassanein *et al.,* 2016). *In vitro* experiments showed that thirty five fungal isolates were obtained from the rhizosphere soil of rice plants and examined for their ability to produce inhibitory compounds against two *Drechslera* sp. in PDA plates. Out of 35 rhizosphere isolates, fourteen isolates showed antagonistic activity against the pathogenic fungi and *P. decumbens*. From the all above, two isolates only showed strong and remarkable antagonistic activity namely (*Penicillium decumbens thom*) against *Drechslera specifier* and *Aspergillus terrus* against *Drechslera rostrata*).

The strong antagonistic activity of *Penicillium decumbens* and *Aspergillus terrus* are with agreement with Hossain et al. (2007) who demonstrated that some species of *Penicillium* are well known for their antagonistic activity against pathogens by producing antibiotics and induce resistance in plants by activating multiple defense signals. Getha et al. (2005) and Gachomo and Kotchoni (2008) also stated *Aspergillus* spp. had also been reported inhibitory to several plant pathogens. Finally, Waing (2015) showed stated that *P. decumbens* showed antagonistic interaction when paired with *F. semitectum.*

ACKNOWLEDGEMENT

We are thankful to Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt, for supporting this study.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

REFERENCES

Abdel-Hafez, S.I., Ismail, M.A., Hussein, N.A., Nafady, N.A., 2009. The diversity of *Fusarium* species in Egyptian soils, with three new record species. The first International Conference of Biological Sciences, March 4-5th 2009, Faculty of Science, Assiut University, Assiut, Egypt. Assiut Uni. J. Bot., (Special Publication No. 1): 129–147.

Abou-Zeid, A.M., Altalhi, A.D., Abd El-Fattah, R.I., 2008. Fungal control of pathogenic fungi isolated from some wild plants in Taif governorate, Saudi Arabia. Mal. J. Microbiol., 4(1): 30-39.

Ahmed, T.A., 1998. Egypt worth of rice cultivation in the Nile delta 24th WEDC Conference sanitation and water for all.

Alsohiby, F.A.A., Yahya, S., Humaid, A.A., 2016. Screening of Soil Isolates of Bacteria for Antagonistic Activity against Plant Pathogenic Fungi. PSM Microbiol., 01(1): 05-09.

Arain, G.N., 2013. Crop manager–agronomy center pivot irrigation system valley irrigation Pakistan (private), limited. P 1-17.

Booth, C., 1971. *Fusarium*, Laboratory guide to the identification of the major species. Common wealth Mycological Institute. Kew, Surrey, England.

Carmichael, J.W., Kendrick, W.B., Conners, I.L., Sigler, L., 1980. Genera of hyphomycetes. Manitoba University of Alberta Press, pp. 386.

Chandrashekar, M.A.; Pai, K.S., Raju, N.S., 2014. Fungal diversity of rhizosphere soils in different agricultural fields of Nanjangud Taluk of Mysore District, Karnataka, India Int. J. Curr. Microbiol. App. Sci., 3(5): 559-566.

Clear, R.M., Patrick, S.K., Gaba, B., 2000. Prevalence of fungi and fusariotoxins on oat seed from western Canada, 1995–1997. Can. J. Plant Pathol., 22: 310–314.

Ellis, M.B., 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute. Kew, England.

Ellis, M.B., 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute. Kew, England.

EL-Shahir, A. A., 2014. Seasonal variation of air, soil and leaf surface fungi of broad bean and cellulolytic ability in Upper Egypt. Afr. J. Plant Sci., 8(2): 118-132.

Frey, S.D., 2007. Spatial distribution of soil organisms. In: Paul EA (ed), Soil Microbiology, Ecology, and Biochemistry, 3rd edn. Academic Press, London, pp. 283-300.

Gachomo, E.W., Kotchoni, S.O., 2008. The use of *Trichoderma harzianum* and *T. viride* as potential biocontrol agents against peanut microflora and their effectiveness in reducing aflatoxin contamination of infected kernels. Biotechnol., 7: 439–447.

Getha, K., Vikineswary, S., Wong, W.H., Seki, T., Ward, A., Goodfellow, M., 2005. Evaluation of *Streptomyces* sp. for suppression of *Fusarium* wilt and rhizosphere colonization in pot grown banana plantlets. J. Microbiol. Biotechnol., 32(1): 24–32.

Ghany, T. M. A., 2012. Fungal leaf spot of maize: pathogen isolation, identification and host biochemical characterization. Mycopath., 10(2): 41-49.

Ghildyal, A., Pandey, A., 2008. Isolation of cold tolerant antifungal strains of Trichoderma sp. from glacial sites of Indian Nimalayan Region. Res. J. Microbiol., 3(8): 559-64.

Gilman, J.C., 1957. A Manual of Soil Fungi. The Iowa State College Press. Ames, Iowa, U.S.A.

Hafiz, M.I., Arshad, N.H., Safdar, A., Khan, J.A., Saleem, K., Babar, M.M., 2013. Behavior of *bipolaris oryzae* at different temperatures, culture media, fungicides and rice germplasm for resistance. Pak. J. Phytopathol., 25 (01): 84-90

Hajano, J., Pathan, M.A., Rajput, Q.A., Lodhi, M.A., 2011. Rice blast-mycoflora, symptomatology and pathogenicity. IJAVMS, 1(5): 53-63.

Hassanein, N.M., 2010. The role of biotic and abiotic agents in the control of damping off and wilt of bean plants. Egypt. J. Exp. Biol. (Bot.), 6: 21 - 31.

Hassanein, N.M., Abou Zeid, M.A., Youssef, K.A., Mahmoud, D.A., 2010. Control of tomato early blight and wilt using aqueous extract of neem leaves. Phytopathol. Mediterr., 49: 143 - 151.

Hassanein, N.M., Abd El- Aziz, L.M., Peter, F.F., 2016. Production of cell wall degrading enzymes and mycotoxins by *Fusarium proliferatum* and *Fusarium verticillioides* isolated from maize ears. Austr. J. Basic App. Sci., 52: 22-42.

Hesseltine, C.W., 1955. Genera of Mucorales, with notes on their synonymy. Mycologia., 47: 344 - 363.

Hossain, M.M., Sultana, F., Kubota, M., Koyama, H., Hyakumachi, M., 2007. The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in Arabidopsis thaliana by activation of multiple defense signals. Plant Cell Physiol., 48: 1724–1736.

Iqbal, M.N., Ashraf, A., 2017. Antagonism in Rhizobacteria: Application for Biocontrol of Soil-borne Plant Pathogens. PSM Microbiol., 2(3): 78-79.

Ismail, M.A., Abdel-Hafez, S.I.I., Hussein, N.A., Nafady, N.A., 2009. Monthly fluctuations of *Fusarium* species in cultivated soil, with a new record species. The first International Conference of Biological Sciences, March 4-5th 2009, Faculty of Science, Assiut University, Assiut, Egypt. Assiut Uni. J. Bot. (Special Publication No. 1): 117–128.

Jatoi, G.H., Abro, M.A., Shabana, M., Hussain, S., Mangi, N., Maari S.A., 2015. Efficacy of different Botanical extracts on the linear colony growth of the *Helminthosporium oryzae*. Eur. Academic Res., Vol. III, Issue 7.

Jeffries, P., Young, T.W.K., 1994. Interfungal parasitic relationship. CAB International, Wallingford.

Jitendiya, O.D., Chhetry, G.K.N., 2013. Evaluation of Antifungal Properties of Certain Plants against *Drechslera Oryzae* Causing Brown Leaf Spot of Rice in Manipur Valley. Int. J. Sci. Res. Publications, 3(5): 1-3.

Mehta, Y.R., 2001. Molecular and pathogenetic variability of *Drechslera* isolates form oats. Fitopatol. Bras. 26: 590–596.

Naeem, K., Anwar, S.A., Haque, M.I., Riaz, A., Khan, M.A., 2001. Seed-borne fungi and bacteria of rice and their impact on seed germination. Pak. J. Phytopath., 13(1): 75-81.

Pitt, J.I., 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and Talaromyces. Academic press, New York.

Prończuk, M., 2000. Grass diseases – occurrence and harmfulness in seed production and turf maintenance. Radzików.

Ranasingh, N., Saturabh, A., Nedunchezhiyan, M., 2006. Use of *Trichoderma* in disease management .Orissa Review, September-October, pp.68-70.

Raper, K.B., Fennelli, D.I., 1977. The Genus *Aspergillus*. Williams and Wilikins. Baltimore, USA.

Scott, D.B., 1995. *Helminthosporia* that cause leaf spots on small-grain cereals in South Africa. [In:] Chełkowski J. *Helminthosporia* metabolites, biology, plant diseases *Bipolaris,Drechslera, Exserohilum.* Poznań, *Poland*, 107–137.

Shabana, Y.M., Abdel-Fattah, G.M., Ismail, A.E., Rashad, Y.M., 2008. Control of brown spot pathogen of rice (*bipolaris oryzae*) using somephenolic antioxidants. Braz. J. Microbiol., 39: 438-444.

Shyamala, L., Sivakumaar, P.K., 2012. Antifungal activity of rhizobacteria isolated from rice rhizosphere soil against rice blast fungus *Pyricularia oryzae*. Int. J. Pharm. Biol. Arch., 3(3): 692-696.

Singh, R.S., 2005. Plant Disease (8th edition). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 439-444.

Sunder, S., Singh, R., Agarwal, R., 2014. Brown spot of rice: an overview. Indian Phytopath., 67(3): 201-215.

Toussoun, T.A. and Nelson, P.E., 1968. A pictorial guide to the identification of *Fusarium* species. The Pennsylvania State University press. University Park and London.

Umamaheswari, B., Thakore, B., More, T., 2009. Post-harvest management of ber (*Ziziphus mauritiana* Lamk) fruit rot (*Alternaria alternata* (Fr.) Keissler) using Trichoderma species, fungicides and their combinations. Crop Protec., 28(6): pp.525-32.

Venkateswarlu, N., Sireesha, O., Aishwayra, S., Vijaya, T., Sriramulu, A., 2015. Isolation, screening of rhizosphere fungi antagonistic to rice stem rot disease pathogen *Sclerotium Oryzae*. Asian J. Pharm. Clin. Res., 8(5): 54-57.

Waing, K.G.D., Abella, E.A., Kalaw, S.P., Waing, F.P., Galvez, C.T., 2015. Antagonistic interactions among

different species of leaf litter fungi of Central Luzon State University. Plant Pathol. Quarantine, 5(2): 122–130.

Whitfield, J., 2005. Biogeography: is everything everywhere? Science 310: 960-961.

Yuan, W.M., Crawford, D.L., 1995. Characterization of *Streptomyces lydicus* WYEC-180 as a potential biocontrol agent against fungal root and seed rots. Appl. Environ. Microbial., 61(8): 3119 - 3128.