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Potentiality of Binucleate *Rhizoctonia* Isolates as Root Rot Causing Pathogens on Faba Bean

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The present study was conducted to identify and determine the pathogenic capabilities of 78 binucleate *Rhizoctonia* isolates isolated from faba bean plants showing root rot and stem canker symptoms. Identification, depending on morphological features, has proved that isolates represented as: two isolates *Rhizoctonia calle*, 26 isolates *R. fumigata*, 48 isolates *R. cerealis* and two species are still unidentified due to edict of identification key. *In vitro* pathogenicity test was achieved for all binucleate isolates comparing with one accurate identified polynucleate *Rhizoctonia solani* (AG4-HGI). All isolates were found to be pathogenic and most of them showed the same degree of pathogenicity as *R. solani*. Potentiality of the most aggressive 4 isolates (belonging to *R. cerealis*) on faba bean as the causal of root rot was assessed comparing with polynucleate *R. solani* on disease incidence and on plant growth characters under greenhouse conditions. Binucleate tested isolates showed significantly greater disease incidence, and showed significant depressed effect on both shoots and roots compared with control. Results indicated the importance of binucleate *Rhizoctonia* spp. as causal agents of faba bean root rot.

Keywords: Binucleat *Rhizoctonia* spp., Morphological characters, Pathogenicity and *Vicia faba* L.

Rhizoctonia diseases cause significant losses of various important economic crops under greenhouse and field conditions (Botha *et al.*, 2003; Sharma-Poudyal *et al.*, 2015; Yang *et al*., 2015). Strains of polynucleate and other binucleate *Rhizoctonia* spp. are capable of attacking a wide range of plant hosts, causing severe symptoms including: seed decays, damping-off of seedlings, root rots, stem cankers, and foliage diseases (Botha *et al.*, 2003; Simonetta *et al.*, 2007; Sharma-Poudyal *et al.*, 2015).

The genus *Rhizoctonia* includes a complex of genetically distinguishing species, with a wide virulence exhibit preference for certain hosts (Anderson, 1982). Both polynucleate and binucleate *Rhizoctonia* spp. have been divided into number of anastomosis groups (AGs) based on hyphal anastomosis (Ogoshi *et al*., 1983, Sneh *et al*., 1991). *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is a polynucleate species that has been divided to 14 AGs. *Rhizoctonia zeae* and *R. oryzae* are polynucleate with the holomorphs *Waitea circinata* var. *zeae* and *W. cercinata* var. *circinata*, respectively (Sneh *et al.*, 1991). Binucleate *Rhizoctonia* spp. (holomorph: *Ceratobasidium* spp. and *Tulasnella* spp.) are divided into 19 AGs (Ogoshi *et al*., 1983; Ogoshi, 1985).

Binucleate *Rhizoctonia* spp. and hypovirulent *R. solani* isolates have been demonstrated as a biocontrol agent against *R. solani* or *Pythium* spp. diseases in a variety of hosts (Cubeta and Echandi, 1991; Herr, 1995; Poromarto *et al.,* 1998; Erper *et al*., 2013). As well, many researchers showed that binucleate *Rhizoctonia* spp. show ability as virulent, less virulent or avirulent on plant hosts (Eken and Demirci, 2003; 2004; Cedeno *et al*., 2006; Tuncer and Eken, 2013; Ünal *et al.,* 2014; Sharma-Poudyal *et al.,* 2015). Binucleate *Rhizoctonia* include several important plant pathogenic fungi on several host plants such as strawberries (Botha *et al*., 2003), potato (Araki *et al.,* 1979), turfgrass (Burpee, 1980), peanut (Oniki and Araki, 1981; 1982), alfalfa (Cedeno *et al*., 2006), and ornamental plants (Hyakumachi *et al*., 2005; Molaei *et al*., 2014).

This study was undertaken to 1) Identify binucleate isolates of *Rhizoctonia* spp. isolated from faba bean plants showed root rot and stem canker. 2) Determine the pathogenic ability of isolated binucleate *Rhizoctonia* under laboratory and greenhouse condition. 3) Evaluate the effect of pathogenic isolates on growth characters of faba bean plants grown in infested soil.

Materials and Methods

1. Isolation and identification of the pathogen:

Faba bean plants (*Vicia faba* L.) with root rot and stem canker symptoms were collected from Qalyubia and Sharqia growing area. Root and basal stem portions with distinct lesions were rinsed with tap water to remove adhesive soil then cut into pieces and 3 parts were put in plates containing sterilized 2% water agar (WA). Plates were incubated at 25˚C and examined after 24 hr. *Rhizoctonia*-Like mycelia were purified by hyphal-tip then sub-cultured on potato sucrose agar (PSA) media. Hyphal characteristics were examined microscopically to confirm that they matched with the description of *Rhizoctonia* spp. (Parmeter and Whitney, 1970).

Morphological features of mycelial growth were observed from upper side and down side of plates and all isolates were divided into two main groups: binucleate and polynucleate according to nuclei stain by trypan blue (0.5%) dissolved in lactophenol (Burpee *et al.,* 1978). Slide culture technique was used to determine number of nuclei in apical compartments. Sterilized glass slide was covered by PSA media and after solidification a small pit of fungal growth was put in the central part of the slide. Slides were put in sterilized petri dishes then incubated at 25±1˚C. The stain was added directly on young hyphae growing on agar media and left till hyphae were stained. Stained hyphae were covered with a microscopic slide cover and examined by light microscopy. Nuclei are stained dark blue. Seventy eight isolates represent as binucleate *Rhizoctonia* were identified depending on color of colonies, diameter of hyphae, measures of moniloid cells, and sclerotia characters according to Sneh *et al.* (1991). All parameters (hyphae and moniloid cells) were determined in 15 cells by using light microscopy at x 400 magnification.

2. Pathogenicity test:

2.1. Under laboratory conditions:

Faba bean seeds (Giza 2 cv.) were rinsed with tap water, and then sterilized superficially with sodium hypochlorite (2%) for 5 min. Seeds were washed with sterile distilled water then dried throw filter papers. For germination, seeds were placed in plastic box contained wetted filter paper in the dark. Apparently healthy germinated seeds have been selected then peeled.

All binucleate isolates were tested for their pathogenicity. *Rhizoctonia solani* AG4-HGI was isolated from faba bean and identified according to sequences of ITS1-5.8S rDNA-ITS4 (Mohamed *et al.,* 2015a) and was used for comparison. Sterile germinated faba bean peeled seeds were put on fungal growth growing on potato sucrose agar medium. Plates were incubated at 25±1°C for three days in the dark, and then disease index was determined according to Mohamed *et al*. (2014) as follow: 1: apparently healthy seeds; 2: very weak infection; 3: moderate infection; 4: very severe infection; 5: seeds were died and completely covered by fungal mats. Three dishes (each contains 5 seeds) were used as replicates.

2.2. Under greenhouse conditions:

Four binucleate *Rhizoctonia* isolates Nos.1, 34, 38 & 70 which represent the aggressive isolates *in vitro* assay were used to determine their pathogenicity and their effects on morphological features of faba bean plants under greenhouse conditions.

2.2.1. Preparation of fungal inoculum:

Fungal inoculum was prepared on sand amended with Czapek Dox broth medium according to the method described by (Mohamed *et al.,* 2014). Sand used in this study was washed with hydrochloric acid (2N) then with distilled water and dried, finally distributed in 9 cm diameter petri dishes (120 g sand/plate). Sand was drenched by Czapek Dox broth (25 ml/plate). Dishes were autoclaved at 121°C for 30 min. After cooling, dishes were infested by pieces of actively binucleate *Rhizoctonia* isolates growth or polynucleate *R. solani* isolate. Plates were incubated for 10 days in the dark at 25±1°C. Sand dishes contained fungal growth were used as inoculum for infestation of sand pots. One dish of inoculum was used for each pot.

2.2.2. Infestation of sand culture and cultivation of seeds:

Washed and autoclaved sand was distributed in plastic pots diameter 10 cm by adding approximately 430 g sand/pot. Each pot was infested with fungal inoculum (one plate/pot). Infested pots were left for a week with a follow-up irrigation. Apparently healthy faba bean germinated seeds were sown in sand culture (3 seeds/pot) infested or not with binucleate isolates (Nos.1, 34, 38 & 70) and by one strain of polynucleate *Rhizoctonia solani* (AG4-HGI) as a comparison. Pots were irrigated when needed by sterilized distilled water and with Hogland's solution every two days. Each treatment consisted of ten replicates.

2.2.3. Determination of disease index and plant growth characters under plastic house conditions:

Number of emerged plants was recorded. Plants were taken then roots were washed to remove adhered sand. Plant height (cm), leaf number/ plant, foliage weight (g) and root weight (g) were determined 30 days after sowing. Aside from, disease index was determined according to Mohamed *et al.* (2015b) by using scale with a score range of 1 to 9 as follow: 1: no visible symptoms; 2: light discoloration with or without necrotic lesions (5% of root tissue covered with lesions); 3: 15% of root tissue covered with lesions; 4: 25% of root tissue covered with lesions, the tissues remain firm with some deterioration of the root system; 5: 50% of root tissue covered with lesions combined with rotting and reduction of root system; 6: 75% of root tissues affected and combined with severe reduction in the root system; 7: root completely rotted after formation of the root system; 8: hypocotyls and root completely rotted after beginning of root formation; 9: rotted germinated seed and no root system was formed

3. Statistical analyses:

Standard deviation (SD) for all obtained means was calculated between data according to Ghahramani (2000).

Results

1. Isolation and identification of Rhizoctonia isolates:

A total of 104 fungal isolates were isolated from faba bean roots with typical symptoms of Rhizoctonia root rot. Microscopic examination of nuclei stained revealed the presence of 26 isolates belong to polynucleate *Rhizoctonia* spp. and 78 isolates to binucleate *Rhizoctonia* spp. (Table, 1). As shown from Table 1, number of *Rhizoctonia* isolates isolated from plants collected from Qalyubia governorate was higher than that isolated from Sharqia governorate.

Binucleate *Rhizoctonia* isolates (78 isolates) have been selected to identify and procedure the pathogenicity test. Identification of 78 binucleate *Rhizoctonia* isolates according to morphology of colony on PDA media (according to Sneh *et al.*, 1991), width of the main runner hyphae, and diameter of moniloid cells, showed that all isolates belong to three species: *Rhizoctonia calle* (2 isolates), *R. cerealis* (48 isolates), *R. fumigatea* (26 isolates) and two isolates are still unidentified (Tables 2 and 3).

Table 1. Nuclei number of *Rhizoctonia* spp. isolated from faba bean showing root rot symptoms

Source of isolates	Number of Isolates	Polynucleate	Binucleate
Qalyubia	87	17	70
Sharqia	17	9	8
Total	104	26	78
%		25	75

Table 2. Identification of 78 isolates of binucleate *Rhizoctonia* according to Sneh *et al.* (1991)

Isolate	Mycelial color	Hyphae diameter (µm)	Sclerotial color	Sclerotial size (mm)	Width of moniloid cell (µm)	Length of moniloid cell (µm)	Proposed species
1	B	5.37 ± 1.54	B	0.5-3.0	7.10 ± 1.54	18.37 ± 5.15	*R. cerealis*1
2	B	4.16 ± 1.64	B	0.5 -3.0	11.26 ± 2.72	23.22 ± 5.15	*R. cerealis*2
3	WY	6.41 ± 1.34	W	0.5-1.0	7.10 ± 2.29	21.84 ± 7.27	*R. cerealis*3
4	WY	5.89 ± 1.54	W	0.5-1.0	9.53 ± 2.12	19.76 ± 4.26	*R. cerealis*4
5	WY	6.41 ± 1.34	W	0.5-1.0	7.32 ± 2.27	23.40 ± 8.05	*R. cerealis*5
6*	WY	4.68 ± 1.07	LB	0.5-1.0	5.54 ± 0.91	23.57 ± 4.22	*R.* species1
7*	WY	3.98 ± 2.38	LB	0.5-1.0	7.10 ± 1.19	22.01 ± 3.38	*R. cerealis*6
8*	WY	5.02 ± 1.54	LB	0.5-1.0	7.97 ± 1.82	23.22 ± 5.85	*R. cerealis*7
9*	WY	6.24 ± 1.31	LB	0.5-1.0	8.49 ± 1.54	22.53 ± 6.56	*R. cerealis*8
10*	WY	6.06 ± 1.26	LB	0.5-1.0	8.84 ± 1.64	18.89 ± 5.33	*R. fumigata*1
11*	WY	6.06 ± 1.26	LB	0.5-1.0	8.84 ± 2.15	19.06 ± 4.67	*R. fumigata*2
12*	WY	5.20 ± 0.98	LB	0.5-1.0	7.28 ± 2.24	19.93 ± 2.53	*R. fumigata*3
13*	WY	4.68 ± 2.63	LB	0.5-1.0	5.02 ± 0.67	26.34 ± 3.91	*R.* species2
14	WY	3.29 ± 1.19	B	0.5-1.0	12.48 ± 2.24	21.66 ± 5.26	*R. calle*1
15	WY	3.98 ± 1.66	B	0.5-1.0	11.10 ± 2.68	20.62 ± 4.65	*R. calle*2
16	WY	3.98 ± 1.66	B	0.5-1.0	10.40 ± 3.25	23.05 ± 5.71	*R. cerealis*9
17	WY	3.81 ± 1.34	B	0.5-1.0	9.70 ± 2.29	22.36 ± 4.89	*R. cerealis*10
18	WY	6.24 ± 1.31	B	0.5-3.0	9.53 ± 2.53	22.70 ± 5.68	*R. cerealis*11
19	WY	6.58 ± 1.66	B	2.0-7.0	7.10 ± 2.07	17.16 ± 4.48	*R. fumigata*4
20	WY	6.41 ± 1.34	B	0.5-1.0	9.18 ± 2.92	19.58 ± 3.91	*R. fumigata*5
21	WY	6.76 ± 1.31	A	----	7.97 ± 2.85	19.93 ± 4.46	*R. fumigata*6
22	WY	6.06 ± 1.60	B	0.5-1.0	8.32 ± 2.24	21.66 ± 5.53	*R. cerealis*12
23	WY	6.24 ± 1.31	B	0.5-3.0	8.14 ± 3.23	18.89 ± 4.55	*R. cerealis*13
24	WY	6.76 ± 1.31	B	0.5-3.0	11.96 ± 2.74	24.44 ± 5.54	*R. cerealis*14
25	WY	3.98 ± 1.34	B	0.5-1.0	8.46 ± 2.08	18.37 ± 5.68	*R. cerealis*15
26	B	6.58 ± 1.34	B	0.5-3.0	8.66 ± 2.89	18.37 ± 6.40	*R. cerealis*16
27	B	3.98 ± 1.66	B	0.5-3.0	10.40 ± 4.50	19.76 ± 5.08	*R. cerealis*17
28	B	6.58 ± 1.34	B	0.5-3.0	7.45 ± 2.57	15.94 ± 5.80	*R. cerealis*18
29	B	7.10 ± 1.19	B	0.5-3.0	8.32 ± 1.75	15.08 ± 4.30	*R. cerealis*19
30	B	6.76 ± 1.31	B	0.5-2.0	7.10 ± 2.68	21.14 ± 8.50	*R. cerealis*20
31	B	6.68 ± 1.33	A	----	9.47 ± 3.16	22.84 ± 7.29	*R. cerealis*21
32	B	6.24 ± 1.64	B	0.5-3.0	7.80 ± 2.19	22.01 ± 5.63	*R. cerealis*22
33	B	6.58 ± 1.34	A	----	8.66 ± 2.12	20.45 ± 5.63	*R. cerealis*23
34	B	6.58 ± 1.34	B	0.5-3.0	9.01 ± 2.38	19.93 ± 4.88	*R. cerealis*24
35	B	6.58 ± 1.66	B	0.5-3.0	9.36 ± 2.56	19.76 ± 5.36	*R. cerealis*25
36	B	6.24 ± 1.31	B	0.5-3.0	8.32 ± 3.83	18.72 ± 8.17	*R. cerealis*26
37	B	6.06 ± 1.88	B	0.5-3.0	7.62 ± 2.49	15.94 ± 3.78	*R. cerealis*27

Table 2…. continued

38	B	6.76 ± 1.64	B	0.5-3.0	9.53 ± 3.20	22.18 ± 5.37	*R. cerealis*28
39	B	5.20 ± 2.19	B	0.5-3.0	9.53 ± 2.12	16.46 ± 5.44	*R. cerealis*29
40	B	5.20 ± 2.19	B	0.5-3.0	9.18 ± 3.23	16.46 ± 4.46	*R. cerealis*30
41	B	6.93 ± 1.26	B	0.5-3.0	9.70 ± 2.85	25.48 ± 5.74	*R. cerealis*31
42	B	6.41 ± 1.34	B	0.5-3.0	9.53 ± 1.60	20.97 ± 4.95	*R. cerealis*32
43	B	5.72 ± 2.24	B	0.5-3.0	7.10 ± 2.07	25.82 ± 7.89	*R. cerealis*33
44	B	6.41 ± 1.34	B	0.5-3.0	10.22 ± 1.54	19.06 ± 4.97	*R. cerealis*34
45	B	6.76 ± 1.31	B	0.5-3.0	10.05 ± 3.65	19.06 ± 5.78	*R. cerealis*35
46	B	3.98 ± 1.66	B	0.5-3.0	10.58 ± 2.96	19.87 ± 6.17	*R. cerealis*36
47	B	6.06 ± 1.26	B	0.5-3.0	10.05 ± 2.38	23.74 ± 6.04	*R. cerealis*37
48	B	6.58 ± 1.34	B	0.5-3.0	11.09 ± 3.32	23.57 ± 5.51	*R. cerealis*38
49	WY	4.68 ± 1.45	B	1.0-2.0	7.45 ± 2.16	19.58 ± 5.09	*R. fumigata*7
50	WY	5.37 ± 1.82	A	----	7.80 ± 1.96	19.41 ± 4.99	*R. fumigata*8
51	WY	6.58 ± 1.34	B	1.0-2.5	8.66 ± 1.26	19.76 ± 6.57	*R. fumigate*9
52	WY	4.33 ± 1.88	B	1.0-3.0	7.10 ± 1.53	24.09 ± 4.44	*R. cerealis*39
53	WY	5.02 ± 2.07	A	----	9.01 ± 2.16	19.06 ± 4.57	*R.fumigata*10
54	WY	5.89 ± 1.19	B	0.5-1.0	10.40 ± 1.96	22.88 ± 3.95	*R. cerealis*40
55	WY	5.89 ± 1.19	B	0.5-0.1	7.10 ± 1.19	18.89 ± 5.68	*R.fumigate*11
56	WY	5.37 ± 1.82	B	0.5-1.0	7.80 ± 1.96	18.37 ± 5.05	*R.fumigata*12
57	WY	4.33 ± 1.88	B	0.5-1.0	8.35 ± 1.50	22.65 ± 3.29	*R. cerealis*41
58	WY	4.50 ± 2.07	B	0.5-1.0	7.02 ± 1.18	18.37 ± 3.32	*R.fumigata*13
59	WY	4.85 ± 1.93	B	0.5-1.0	7.28 ± 1.07	19.76 ± 1.91	*R.fumigata*14
60	WY	4.68 ± 2.01	B	0.5-1.0	7.97 ± 2.68	18.02 ± 6.62	*R.fumigata*15
61	WY	5.02 ± 1.82	B	0.5-1.0	8.32 ± 3.13	17.68 ± 6.15	*R.fumigata*16
62	WY	4.87 ± 1.66	B	0.5-1.0	8.49 ± 3.32	17.16 ± 5.54	*R.fumigata*17
63	WY	4.16 ± 1.64	A	----	8.80 ± 2.98	18.72 ± 3.95	*R.fumigata*18
64	WY	5.20 ± 1.96	A	----	7.80 ± 2.94	17.68 ± 6.45	*R.fumigata*19
65	B	4.68 ± 1.45	B	0.5-3.0	8.49 ± 1.54	22.70 ± 3.98	*R. cerealis*42
66	B	4.85 ± 1.66	B	0.5-3.0	7.28 ± 2.44	17.85 ± 5.27	*R. cerealis*43
67	B	4.85 ± 1.93	B	0.5-3.0	7.80 ± 2.60	17.68 ± 6.15	*R. cerealis*44
68	B	6.76 ± 1.31	B	0.5-3.0	9.18 ± 1.93	18.72 ± 4.41	*R. cerealis*45
69	B	4.68 ± 1.75	B	0.5-3.0	7.10 ± 3.02	18.20 ± 5.89	*R. cerealis*46
70	B	7.62 ± 0.67	B	0.5-3.0	9.36 ± 1.31	24.78 ± 7.53	*R. cerealis*47
71	B	6.06 ± 1.26	B	0.5-3.0	8.49 ± 2.29	18.20 ± 3.37	*R. cerealis*48
72	WY	3.64 ± 1.64	A	----	9.01 ± 2.75	19.93 ± 4.57	*R.fumigate*20
73	WY	6.76 ± 1.31	A	----	7.80 ± 1.96	18.37 ± 5.85	*R.fumigata*21
74	WY	3.98 ± 1.66	A	----	9.53 ± 2.89	16.12 ± 4.73	*R.fumigate*22
75	WY	5.02 ± 1.82	A	----	7.45 ± 3.23	17.50 ± 6.84	*R.fumigata*23
76	WY	5.20 ± 1.70	B	0.3-1.0	7.10 ± 2.85	18.54 ± 7.21	*R.fumigata*24
77	WY	6.41 ± 1.34	B	0.3-1.0	9.01 ± 2.16	19.24 ± 4.69	*R.fumigata*25
78	WY	6.24 ± 1.91	B	0.3-1.0	8.32 ± 2.81	19.41 ± 5.80	*R.fumigata*26

All types were isolated from Qalyubia, except the eighth type (*) was from Sharqia. A: absent, B: brown, LB: light to brown, WY: white to yellow.

Table 3. Identification of 78 isolates of binucleate *Rhizoctonia* using the key proposed by Sneh *et al.* (1991)

Species	No. of isolates	Species	Number of isolates
R. cerealis	48	*R. fumigata*	26
R. calle	2	*R. species*	2

2. Pathogenicity test in vitro:

Pathogenicity test of 78 binucleate *Rhizoctonia* isolates showed that all isolates were found to be pathogenic on faba bean seeds *in vitro* (Table 4). All isolates showed variance degree of pathogenicity, and most of them showed the same degree of pathogenicity as *R. solani*. Four isolates belonged to species *R. cerealis* (Nos. 1, 24, 28 & 47) were more aggressive on faba bean seeds *in vitro* as compared by other isolates (Table 4).

Table 4. Pathogenicity test of binucleate *Rhizoctoni* isolates *in vitro*

Species	Disease index	Species	Disease index	Species	Disease index
*R. cerealis*1	4.00±1.00	*R. cerealis*17	2.73±1.27	*R. fumigata*10	1.93±0.11
*R. cerealis*2	3.00±1.00	*R. cerealis*18	2.60±1.03	*R. cerealis*40	1.86±0.11
*R. cerealis*3	1.86±0.90	*R. cerealis*19	2.66±0.30	*R. fumigate*11	2.13±0.23
*R. cerealis*4	1.06±0.11	*R. cerealis*20	3.20±0.87	*R. fumigata*12	2.66±0.61
*R. cerealis*5	1.86±0.23	*R. cerealis*21	2.40±0.34	*R. cerealis*41	2.13±0.23
R. species1	3.00±0.72	*R. cerealis*22	2.46±0.41	*R. fumigata*13	2.06±0.11
*R. cerealis*6	1.93±0.11	*R. cerealis*23	2.40±0.34	*R. fumigata*14	2.53±0.50
*R. cerealis*7	2.13±0.23	*R. cerealis*24	3.80±1.11	*R. fumigata*15	2.46±0.41
*R. cerealis*8	2.33±0.57	*R. cerealis*25	2.66±0.57	*R. fumigata*16	2.26±0.64
*R. fumigata*1	2.06±0.30	*R. cerealis*26	2.66±0.57	*R. fumigata*17	2.60±0.60
*R. fumigata*2	2.26±0.46	*R. cerealis*27	3.46±1.50	*R. fumigata*18	1.20±0.34
*R. fumigata*3	2.60±0.87	*R. cerealis*28	3.53±1.50	*R. fumigata*19	1.86±0.11
R. species2	1.40±0.69	*R. cerealis*29	2.80±1.56	*R. cerealis*42	2.53±0.11
*R. calle*1	2.33±0.30	*R. cerealis*30	1.60±0.40	*R. cerealis*43	3.06±0.90
*R. calle*2	2.06±0.11	*R. cerealis*31	2.20±0.34	*R. cerealis*44	2.26±0.46
*R. cerealis*9	1.80±0.72	*R. cerealis*32	3.20±1.58	*R. cerealis*45	2.06±0.11
*R. cerealis*10	2.20±0.34	*R. cerealis*33	1.66±0.30	*R. cerealis*46	2.13±0.23
*R. cerealis*11	2.53±0.50	*R. cerealis*34	1.53±0.61	*R. cerealis*47	3.53±0.75
*R. fumigata*4	1.20±0.20	*R. cerealis*35	2.80±1.92	*R. cerealis*48	2.46±0.50
*R. fumigata*5	2.00±0.00	*R. cerealis*36	3.13±1.20	*R. fumigate*20	2.26±0.23
*R. fumigata*6	2.00±0.00	*R. cerealis*37	3.20±1.63	*R. fumigata*21	2.00±0.00
*R. cerealis*12	1.26±0.46	*R. cerealis*38	2.73±0.46	*R. fumigate*22	2.53±0.50
*R. cerealis*13	2.40±0.69	*R. fumigata*7	2.26±0.46	*R. fumigata*23	2.00±1.00
*R. cerealis*14	2.13±0.80	*R. fumigata*8	1.86±0.23	*R. fumigata*24	2.20±0.20
*R. cerealis*15	2.06±0.11	*R. fumigate*9	2.13±0.41	*R. fumigata*25	1.46±0.50
*R. cerealis*16	2.93±0.90	*R. cerealis*39	2.26±0.46	*R. fumigata*26	1.93±0.11
R. solani (cont.)	3.13±0.61				

3. Pathogenicity test under greenhouse condition:

Four more aggressive isolates (Nos. 1, 24, 28, 47; belonging to *R. cerealis*) *in vitro* test were chosen to test under greenhouse conditions for pathogenicity and plant growth characters. All isolates showed significantly greater disease incidence on faba bean seedlings, and showed the same effect with polynucleate isolate *R. solani* (AG4-HGI) (Fig. 1). They reduced the percentages of emerged seedlings compared with control plants but their effect was lower than that found by polynucleate *R. solani* (Fig. 2). All isolates (polynucleate isolate and binucleate isolates) significantly decreased both shoot and root weight compared with healthy plants (Figs. 3&4). On the contrary, no significant effects were recorded to other plant growth characters including stem length and number of leaves/plant (Figs. 5&6).

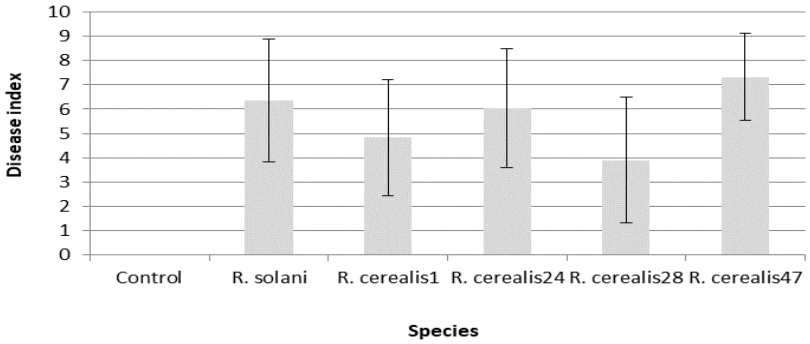

Fig. 1. Pathogenicity test of 4 isolates binucleate *Rhizoctonia cerealis* under greenhouse condition.

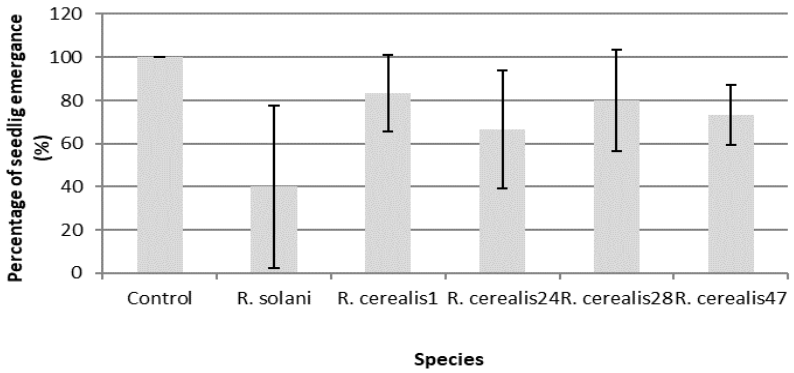

Fig. 2. Effect of 4 isolates binucleate *Rhizoctonia cerealis* on percentage of faba bean seedling emergence, 30 days after planting.

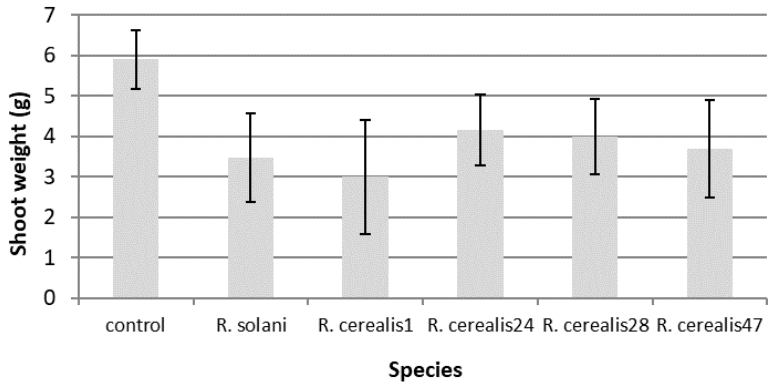

Fig. 3. Effect of 4 isolates binucleate *Rhizoctonia cerealis* on shoot weight of faba bean plants.

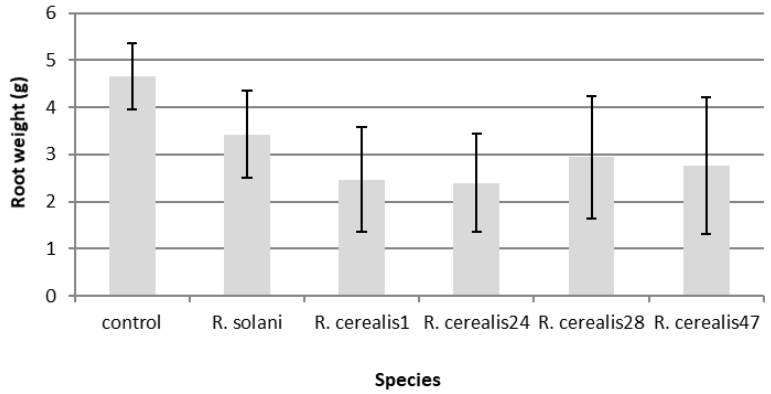

Fig. 4. Effect of 4 isolates binucleate *Rhizoctonia cerealis* on root weight of faba bean plants.

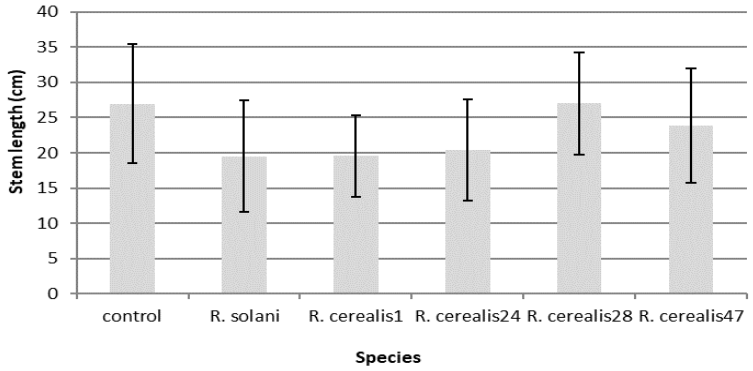

Fig. 5. Effect of 4 isolates binucleate *Rhizoctonia cerealis* on stem length of faba bean plants.

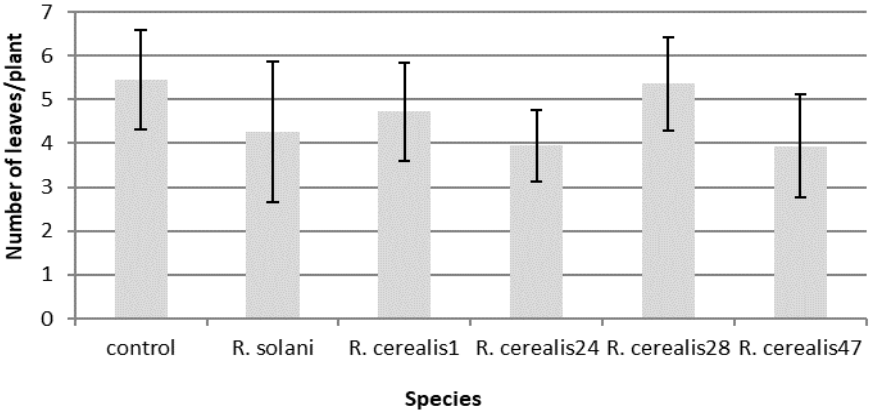

Fig. 6. Effect of 4 isolates binucleate *Rhizoctonia cerealis* on number of leaves of faba bean plants.

D i s c u s s i o n

Rhizoctonia root rot is an important disease of faba bean plants (Rashad *et al*., 2012; Chang *et al*., 2014). Most of researchers focused their studies on the polynucleate *R. solani* as the most common pathogen that has been related with faba bean root rot and with a wide host range (Bolkan and Ribeiro, 1985; Mathew *et al.*, 2012; Chang *et al*., 2014). This study represented the first attempt to focus on binucleate *Rhizoctonia* causing root rot of faba bean in Egypt.

The present study aimed to identify and determine the pathogenicity of binucleate *Rhizoctoni* isolates isolated from faba bean plants showing root rot and stem canker. A total of 104 *Rhizoctonia* isolates were isolated from roots and basal stems of faba bean plants collected from Qalyubia and Sharqia governorate. Number of *Rhizoctonia* isolates isolated from plants collected from Qalyubia region was higher than that isolated from Sharqia region and this may be due to the soil type in both regions, soil type in Qalyubia is heavy clay soil and in Sharqia it is light sandy clay soil. Depending on the number of nuclei in cells close to tips of young hyphae (Sneh *et al*., 1991), *Rhizoctonia* isolates have been divided into two main groups known as polynucleate and binucleate. Identification of binucleate isolates according to key adopted by Sneh *et al*. (1991) appeared that two isolates were identified as *Rhizoctonia calle*, 26 isolates for *R. fumigatea*, 48 isolates to *R. cerealis* and two isolates out of the identification key.

Many researchers showed that binucleate *Rhizoctonia* spp. are pathogenic to some hosts (Botha *et al*., 2003; Hyakumachi *et al*., 2005; Cedeno *et al*., 2006 Molaei *et al*., 2014; Ünal *et al.*, 2014). Among of them, Ünal *et al.* (2014) showed that strains of binucleate *Rhizoctonia cerealis*, belonged to AG-D isolated from soil samples in wheat production areas from Turkey, were found to be pathogenic on susceptible wheat cultivar. All AG-D isolates showed 41-83% disease severity values assessed as pathogens. These results were recorded to be the first in the wheat

field soils in Turkey. Otherwise, there are few studies reporting that binucleate *Rhizoctonia* are pathogenic on legumes crops (Oniki and Araki, 1981; 1982; Yang *et al*., 2005).

The present study revealed that all binucleate *Rhizoctonia* isolates were found to be pathogenic to faba bean germinated seeds *in vitro*. Isolates were differed in their virulence. Among them, four isolates showed more aggressive belonged to species *R. cerealis* have been selected to test their aggressiveness under greenhouse conditions. Tested binucleate isolates under greenhouse conditions showed greater disease incidence on faba bean root, and showed the same effect as compared with polynucleate *R. solani* (AG4-HGI).

The results of this study are in agreement with Yang *et al*. (2005), who showed that twenty binucleate *Rhizoctonia* Chinese isolates belonged to AG-A were pathogenic to pea, soya bean, snap bean and park choy.

According to the key adopted by Sneh *et al.* (1991), nomenclature of binucleate *Rhizoctonia* depends mainly on morphological features of fungal growth. Practically it is very difficult to use these parameters for their nomenclature due to great interference of these characters between species. Therefore, it is very important to find out new features of identification of binucleate *Rhizoctonia* and this will be our aim in the future.

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قدرة العزلات ثنائية الأنوية التابعة لجنس *Rhizoctonia* كمسببات لمرض عفن الجذور في الفول البلدي

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استهدفت الدراسة تعريف ودراسة القدرة المرضية لعزلات من جنس *Rhizoctonia* ثنائية الأنوية معزولة من نباتات الفول البلدي. تم عزل ٧٨ عزلة من جذور نباتات فول تعاني من أعراض مرض عفن الجذور. نتائج التعريف اعتماداً على الصفات المورفولوجية أشارت إلى أن العزلات تنتمي إلى ثلاث أنواع تابعة لجنس *Rhizoctonia* وهي كالتالي: عزلتين من فطـــــر *Rhizoctonia calle*، ٢٦ عزلة تنتمي لفطر *R. fumigatea* ، ٤٨ عزلة تنتمي لفطر *R. cerealis*، بالإضافة لنوعين خارج نطاق مفتاح التعريف. أوضحت نتائج القدرة المرضية في المعمل قدرة جميع العزلات ثنائية الأنوية على إصابة بذور الفول المستنبتة حيث أظهرت درجات مختلفة من الإصابة ، معظم العزلات ثنائية الأنوية أظهرت درجات من الإصابة متقاربة مع العزلة متعددة الأنوية والمستخدمة كمقارنة. تم اختيار أقوى أربعة عزلات في قدرتها المرضية وإختبارها على إصابة جذور الفول البلدي تحت ظروف الصوبة في وجود العزلة متعددة الأنوية كمقارنة. أظهرت الأربع عزلات ثنائية الأنوية درجات إصابة مرتفعة ومتقاربة مع العزلة متعددة الأنوية. أدت الإصابات المرتفعة لجميع العزلات للتأثير المعنوي على صفات كلاً من المجموع الخضري والجذري. أشارت نتائج هذه الدراسة إلى أهمية العزلات ثنائية الأنوية كمسببات لمرض عفن الجذور في الفول البلدي.