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Eco-Friendly Control Traits of Common Bean Root Rot Caused by *Macrophomina phaseolina (Tossi)* Goid and *Fusarium equiseti* Using Fungicide Alternatives

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Abstract

Diseased common bean pods, stems, and roots were recognized various fungal pathogens along two growing seasons (2019–2020) in El-Minya governorate, Egypt. Two genera of fungi, *Fusarium* spp. and *Macrophomina phaseolina*, were occurred with pods, stems and roots recording the most predominant that showed the highest frequency, in the case of *Fusarium* 45% and *Macrophomina* 25%.

The most infective *Fusarium* isolate F1 and *Macrophomina phaseolina* isolate M1 were subjected for molecular identification which confirmed that F1 is *Fusarium equiseti* and M1 isolate is *Macrophomina phaseolina*. Soaking seeds in calcium silicate (CaSi) gave significant reduction in disease severity % (DS%) at 0.2 g/l by 40.54%, and 33.3% under soil infestation with *F. equiseti* and *M. phaseolina* respectively. However, CaSi was more effective to protect common bean plants against either *Fusarium equiseti* or *M. phaseolina* infection, than potassium silicate (Psi). Potassium bicarbonate (PB) at 0.2 g/l expressed the highest protection values 47.3% and 54.31% against *F. equiseti* and *M. phaseolina* infection, respectively. Application of (PAA) at 0.2 g/l AA+2.0g/l H_2O_2 as seed soaking resulted in resistant bean plant against *F. equiseti* 20% protection and against *M. phaseolina* 47.0% protection. Salicylic acid (SA) caused significant bean DS reduction. Using 0.2 g/l SA exhibited 35.22% and 32.74% protection against infection by *F. equiseti* and *M. phaseolina*, respectively.

Keywords: *Phaseolus vulgaris*, Potassium silicate, Potassium bicarbonate, Calcium silicate, Peroxyacetic acid and Salicylic acid.

Introduction

The common bean (*Phaseolus vulgaris* L.) is the world's third most important legumes crop after soybeans and peanut. The common bean is an important source of protein, dietary fiber, iron, complex carbohydrates, minerals, and vitamins for millions of people in developing and developed countries. It is primarily consumed as dry seeds (dry beans) but also as green pods (snap beans) and green shelled seeds. Moreover, the total cultivated area in Egypt 67,596 Feddans for green bean production with a yield of about 2,820,897 ton (Mostafa, 2014).

Under Egyptian conditions, common bean plants are infected by different pathogens (Abdou *et al.,* 1999) and physiological disorders. Bean stem rot is a common disease in bean, which causes the most damage to seedlings and older plants which results in significant yield losses. Some soil borne fungi including *Rhizoctonia solani, Macrophomina phaseolina and Sclerotinia sclerotiorum* reported to be the major causal pathogens of stem blight (Saharan and Mehta, 2008 and Sun *et al*, 2015).

Macrophomina phaseolina is primarily soil borne in nature, with heterogeneous host specificity that infects monocots as well as dicots and non-uniform distribution in the soil (Mayek-Perez *et al*., 2001; Su *et al*., 2001). The pathogen is seed-born and seed-to-seedling transmission has been documented in infected seeds (Pun *et al*., 1998). *Macrophomina* infection causes both pre- and post-emergence plant mortality. Characteristic post-emergence symptoms include the development of spindle shaped lesions with dark border and light gray center covered with small pinhead-sized microsclerotia and sometimes pycnidia (Baird *et al*., 2003), Infected seeds are considered the main inoculum source and mean of dispersal of pathogens (Carvalho *et al*., 2011a). Following their introduction into an area, disease may appear in small, isolated foci and, after several seasons, spread throughout the entire area (Abawi and Pastor-Corrales, 1990).

Several approaches have been used to control seed-borne fungi. For instance, fungicides have been used extensively for suppressing seed-borne fungi and increasing the quality and yield of plant (Gupta and Gupta, 2014). However, their excessive use emits toxic pollutants and leaves residues in the environment, harming humans, animals, beneficial insects, and most importantly, inducing pathogen resistance to the chemicals (Van Dyk and Pletschke, 2011). In addition, fungicides may stimulate the growth of non-target pathogens (Gupta and Gupta, 2014), thereby the use of fungicides should be avoided when planning for control strategies for plant diseases (Abolmaaty and Fawaz, 2016).

This study was carried out to 1) isolate the fungi associated with diseased samples of bean, 2) run pathogenicity tests for obtained fungal isolates on common bean plants, 3) identify the most pathogenic fungal isolates through DNA profile analysis and 4) conduct some control trials.

Materials and Methods

Samples collection

Samples of common bean plants (*Phaseolus vulgaris L.* cv Giza 6), naturally infected pods, stems and roots were collected from different locations in El-Minya governorate, samples were placed in a paper bag and stored in a cooler.

Isolation of the causal organisms

The diseased tissues viz pod, roots and stems were washed in running tap water, cut into small pieces about 0.5cm long, surface disinfected with 0.5% $NaOCl_3$ for 3 min, rinsed with sterile distilled water, dried on sterile towel paper, The prepared pieces (5 mm) were placed onto Petri dishes containing potato dextrose agar (PDA) amended with 30 mg/L streptomycin. The plates were incubated at temperature (25° C± 2) and observed for fungal growth. From each isolate obtained by incubation, tips of the hypha from isolated colonies were further sub-cultured, to obtain a purified culture, then sub-cultured in test tubes containing

PDA medium. Frequency of fungi was conducted.

Pathogenicity tests

Koch's postulate was used to test the pathogenicity of the obtained isolates through inoculation of common bean plants cv Giza 6. The inoculum was grown in flasks 500 ml sterilized natural medium containing mixture of (60g barely grains, 40g washed sand and 40 ml water). The flasks were inoculated with uniformed agar disc of desired fungal and incubated at 25°C ± 2 for two weeks to obtain sufficient inocula. Healthy common bean seeds (*Phaseolus vulgaries* L cv. Giza 6 cultivation 95% germination) were used in this study. The test was carried out in pots (15cm) in the green house of Plant Pathology. Department, Faculty of Agriculture, Minia University, El-Minya, Egypt. Pots were sterilized by soaking in 0.5% $NaOCl_3$ solution for 5 min, the disinfested pots were filled with autoclaved clay soil (121ºC for 30 min) mixed with the desired fungal inoculum growing on barely grains at 2.5% (w/w). In check treatment equal amount of the uninoculated substrate was added. After 7 days, bean seeds sterilized by soaking in 0.5% $NaOCl_3$ for 3 min and then washed thoroughly three times with sterilized water then sown in three pots, five seeds/pot. The experiment was designed as a complete randomized with 3 replicates (3 pots/ replicate).

Disease assessment

The arbitrary (0-5) disease scale described by Abd Elrazek et al., (1974) was used to measure the disease severity in which. 0= no infection, 1= 1- 20 % infection; 2 =21-40 % infection; 3= 41-60% infection; 4 = 61- 80 % infection; 5= 91-100% infection. The following equation was used to calculate percentage of disease severity. Disease severity= 0A+1B+2C+3D+4E+5F/5T X 100 Where A, B, C, D, E and F are the numbers of plants corresponding to the numerical grades 0, 1, 2, 3, 4 and 5 respectively, and 5T is the total number of plants (T) multiplied by maximum disease grade 5 (Sharma et al., 2006).

Identification of fungal isolates

The developing fungal colonies were purified by hyphal tip technique. Pure cultures of all isolated fungi were maintained on PDA slants till identification. The isolated fungi were examined microscopically and identified according to the description giving by Booth (1995) and Barnett and Hunter (1998).

Molecular identification of fungal isolates

The most pathogenic fungal isolates F1 and M1were subjected for molecular identification the fungal isolates that were grown in sterile Petri plates containing autoclaved PDA medium and incubated for 7 days at 28°C (Pitt and Hocking, 2009). Cultures were sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. Fungal DNA samples were then sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and rRNA gene sequencing. PCR was performed using internal transcribed spacer (ITS1) (forward) and ITS4 (reverse) primers which were incorporated in the reaction mixture. Primers have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). The purified PCR product (amplicons) was sequenced with the same primers with the incorporation of ddNTPs in the reaction mixture (White *et al*., 1990). The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

Response of bean cultivars to *M. phaseolina* and *F. equesti* infection

Five cultivars of common bean plants viz Bronco, Nebraska, Giza 6, Ouzeria and Polina, were used to study their susceptibility to infection with the most virulent isolates of *Macrophomina phaseolina* and *Fusarium*

equiseti. These cultivars were inoculated and evaluated as described above.

Control traits

Preparation of the peroxyacetic acid (PAA): Stock solutions of two mixtures of acetic acid (AA) and hydrogen peroxide ($H_2 O_2$) were prepared with distilled water then left for at least 10 days before they tested (Galal, 2018). Different concentrations of AA+ $H_2 O_2$ mixtures, i.e. (0.1 g/l AA+ 1.0 g/l $H_2 O_2$, 0.2 g/l AA+ 2.0g/l $H_2 O_2$ and 0.5 g/l AA+5.0 g/l $H_2 O_2$) were prepared for bean pods treatment, while 0.4 g/l AA +0.8 g/l $H_2 O_2$ and 0.4g/l AA +1.2 g/l $H_2 O_2$. Otherwise, safety chemicals e.g., calcium silicate (CaSi), potassium silicate (PSi), potassium bicarbonate (PB) and salicylic acid (SA) were tested at three concentrations, 0.2, 0.1 and 0.05 g/liter were used against *M. phaseolina and F. equiseti* infection. Healthy apparent common bean seeds cv. Giza 6 was surface sterilized and washed thoroughly with sterilized distilled water then air dried. After that seeds were soaked in the test solution for 2h before sowing in pots and disease assessment was evaluated as described above. Disease severity (DS) expressed as total of pre-emergence damping off (Pre-EDO), post-emergence damping off (postedo) plus root rot severity (RRS). Each control experiment was repeated two times along 2020 (Exp. I) and 2021(Exp. II).

Statistical analysis

The protected least significant difference (L.S.D) values at 5 % ($P < 0.05$) were used to test the differences between treatments (Gomez and Gomez, 1984).

Results

1. Frequency of fungi associated with diseased common bean plants

Along two growing seasons, eight genera of fungi viz *Alternaria*, *Aspergillus, Cladosporium, Fusarium, Macrophomina, Penicillium, Pythium and Sclerotinia sclerotiorum* were isolated from different common bean samples (Table 1). Seven genera of fungi, *Alternaria*, *Aspergillus, Cladosporium, Fusarium, Macrophomina, Penicillium,* and *Sclerotinia sclerotiorum* were associated with diseased pods/seeds. While five genera of fungi viz *Alternaria*, *Aspergillus, Fusarium, Macrophomina, and Sclerotinia sclerotiorum* were associated of diseased stems. Meanwhile, four genera of fungi viz., *Fusarium, Macrophomina, Penicillium and Pythium* were isolated from rotted roots. Frequency of fungi was varied by bean parts and growing season. To be noticed, two genera of bean fungi *Fusarium* and *Macrophomina* were common isolates with diseased pods, stems and roods, *Fusarium* spp. were the most frequent and recorded 25% in 2019 and 45% frequency by 2020 growing season, *Macrophomina phaseolina* became after *Fusarium* watched the habited 33.75% frequency in 2019 and 25% by 2020 growing season. Other genera of fungi that associated with diseased common bean showed low frequency viz, *Alternaria* spp. (6.25%) 2019 and (5%) 2020 frequency, *Aspergillus* spp. (5%) 2019 and (6.67%) 2020 frequency, *Cladosporium* spp. (1.25%) 2019 and (1.66%) 2020 frequency, *Penicillium* spp. (6.25%) 2019 and (5%) 2020 frequency, *Pythium* spp. (1.25%) 2019 and (5%) 2020 frequency, *Sclerotinia sclerotiorum* (4%) 2019 and (6.67%) 2020 frequency.

Table 1: Frequency of fungi associated with diseased common bean samples.

Fungi	2019					2020				
	Pods	Stems	Roots	Total	Frequency %	Pods/Seed	Stems	Roots	Total	Frequency%
***Alternaria* spp.**	4	1	-	5	6.25	3	-	-	3	5
***Aspergillus* spp.**	3	1	-	4	5	2	2	-	4	6.67
***Cladosporium* spp.**	1	-	-	1	1.25	1	-	-	1	1.66
***Fusarium* spp.**	10	8	15	33	41.25	5	8	14	27	45
***Macrophomina* spp.**	8	10	4	27	33.75	6	9	4	15	25
***Penicillium* spp.**	3	-	2	5	6.25	2	-	1	3	5
***Pythium* spp.**	-	-	1	1	1.25	-	-	3	3	5
Sclerotinia sclerotiorum	3	1	-	4	5	2	2	-	4	6.67
Total				80	100				60	100

2. Pathogenicity tests

Eleven fungal isolates of the most two frequent genera *Fusarium* spp. (isolates F1-F5) and *Macrophomina phaseolina* (isolates M1-M6) were tested for pathogenicity tests and showed various infectability to common bean plants cv. Giza 6 (Table 2). *Fusarium* isolate F1 resulted the highest disease severity (48%) followed by isolate F2 (44%), F3 (40%), F4 (41%), while isolate F5 was the weakest virulent (38%). As for *Macrophomina phaseolina* isolates, isolate M1 gave the greatest disease severity (61%) followed by M2 (55%). M3 (50%), M4 (34%), M5 (32%) and M6 (26%).

Table 2: Ability of *Fusarium* and *Macrophomina* isolates to infect common bean plants cv. Giza 6. Disease severity (DS%) expressed as total of pre-emergence damping off (Pre-EDO), post-emergence damping off (Postedo) plus root rot severity (RRS).

Fungal isolates	Pre EDO	Post EDO	RRS	DS (%)
F1	10	8	30	48
F2	8	8	28	44
F3	12	6	22	40
F4	10	5	26	41
F5	8	10	20	38
Mean				44.2
M1	15	12	34	61
M2	12	8	35	55
M3	12	8	30	50
M4	8	6	20	34
M5	8	4	20	32
M6	4	4	18	26
Mean				43.6
LSD 0.05				3.7

3. Identification of pathogens

Only two fungal isolates, one of *Fusarium* sp isolate F1 and one of *Macrophomina* isolate M1 were subjected for molecular identification. Data confirmed that isolate F1 could be identified as *Fusarium equiseti* (Fig 1) and isolate M1 as *Macrophomina phaseolina* (Fig 2). Accession number for isolate f1 is OP985038 and accession number for isolate M1 is OP985045.

4. Response of common bean cultivars to infection

Out of five common bean cultivars, Ouzeria cv. exhibited the highest DS 69.6% under *Macrophomina phaseolina* infection and 61.4% disease severity under *Fusarium equiseti* infection (Table 3), followed by Giza 6 cultivar that explored 58.7% and 49.9% DS in case *Macrophomina phaseolina* and *Fusarium equiseti* infection, respectively. The least DS value was pronounced by cultivar Bronco 38.5% and 34.4% DS in case of *Macrophomina phaseolina* and *Fusarium equiseti*, respectively followed by Nebraska 44.4% and 38.6%, and Polina 47.4% and 36.4% under *M. phaseolina* and *Fusarium equiseti* infection, respectively.

Molecular identification of fungal isolates

1- ***Fusarium equiseti*** **AUMC14524 (520 letters)**

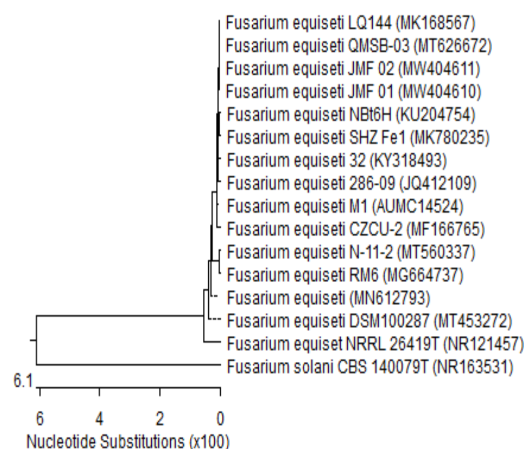

Figure 1: Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (AUMC14524, arrowed) aligned with closely related strains accessed from the GenBank (F. = *Fusarium*). *F. solani*isincluded in the phylogenetic tree as an outgroup strain. Accession number for isolate f1 is OP985038.

2- ***Macrophominaphaseolina*** **AUMC14525 (566 letters)**

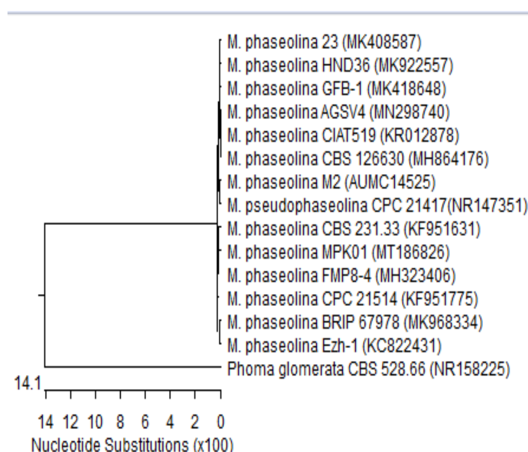

Figure (2): Phylogenetic tree based on ITS sequences of rDNA of the fungal samples isolated in the present study (AUMC14525, arrowed) aligned with closely related strains accessed from the GenBank (M= ***Macrophomina Accession number for isolate M1 is OP985045.***

Table 3: Disease severity (DS%) in common bean cultivars caused by *Macrophomina phaseolina* or *Fusarium equiseti* infection. Disease severity (DS%) expressed as total of pre-emergence damping off (Pre-EDO), post-emergence damping off (Postedo) plus root rot severity (RRS).

Cultivars	*Fusarium equiseti*				*Macrophomina phaseolina*			
	PreEDO	PostEDO	RRS	DS	PreEDO	PostEDO	RRS	**DS**
Bronco	20.2	9	4.5	33.7	29.2	18	9	**56.2**
Nebraska	18	10.5	9	37.5	28.5	18	13.5	**60**
Giza6	26.1	13.5	9	48.6	18.4	13.5	9	**40.9**
ouzeria	32.2	18	13.5	63.7	34.4	22.5	18	**74.9**
Polina	19.5	9	9	37.5	26.1	13.5	9	**48.6**
Mean			9	44.2	29.2	18	9	**56.2**

5. Possible control of common bean root rot

5-1-Using calcium silicate (CaSi)

Data summarized in Table (4) showed significant effect for (CaSi) to reduce DS exhibited by either *Fusarium equiseti* or *Macrophomina phaseolina* infection. Increasing CaSi concentration decreed DS. However, insufficient protection values were explored by the lowest CaSi concentration tested. At 0.2 g/L CaSi concentration, the least DS 33.2% and 45.0 % DS caused by *Fusarium equiseti* and *Macrophomina phaseolina* infection, respectively. A significant reduction in DS was observed by 0.1 g/L CaSi but with lower efficient than 0.2 g/L CaSi concentration. The highest protection *values against Fusarium equiseti* (40.28%) and *Macrophomina phaseolina* (33.33%) infection was pronounced at 0.2 g/l CaSi.

Table 4: Disease severity (DS%) in common bean cv. Giza 6 under *Fusarium equiseti* and *Macrophomina phaseolina* infection as influenced by calcium silicate (CaSi).

CaSi (g/l)	*Fusarium equiseti*				*Macrophomina phaseolina*			
	Exp. I	Ex. II	M	Protection	Exp. I	Ex. II	M	Protection
Untreated	56.4	54.8	55.6	0	66.6	68.4	67.5	0
0.05	54.6	451.	53.0	4.67	64.4	63.4	63.9	5.33
0.1	46.5	42.8	44.6	19.78	60.0	061.	60.5	10.37
0.2	35	31.4	33.2	40.28	45.2	44.6	45.0	33.33

5-2- Using peroxy acetic acid (PAA)

Soaking bean seeds cv. Giza 6 in PAA 2h pre-planting resulted in resistant plants against *Fusarium equiseti or Macrophomina phaseolina* infection (Table 5).The highest protection values were observed by 0.2g L AA + 2.0 g L H_2O_2 while the least protection values were recorded at the lowest concentration 0.05 g/l AA + 0.5 g/l H_2O_2.The highest PAA concentration was reduced DS from 55.6 to 44.1 that gave 20.68% protection against *Fusarium equiseti* infection while the it reduced DS from 67.5 to 35.7% DS that gave 47.11% protection against *Macrophomina phaseolina*.

Table 5: Disease severity (DS) in common bean cv. Giza 6 under *Fusarium equiseti* and *Macrophomina phaseolina* infection as influenced by Peroxy acetic acid (PAA).

PAA (g/l)	*Fusarium equiseti*				*Macrophomina phaseolina*			
	Exp.I	Exp.II	M	Protection	Exp.I	Exp.II	M	**Protection**
Untreated	56.4	54.8	55.6	0	66.6	68.4	67.5	**0**
0.05	53.4	48.8	51.1	8.0	58.3	55.5	56.9	**18.69**
0.1	50.0	46.4	48.2	13.30	53.3	51.7	52.5	**22.22**
0.2	47	41.2	44.1	20.68	36.6	34.8	35.7	**47.11**

5-3-Using Potassium bicarbonate (PB):

Seed soaking in PB expressed (DS%) reduction common bean cv. Giza 6 under *Fusarium equiseti* and *Macrophomina phaseolina* infection (Table 6). Increasing *PB* concentrations descending DS. At 0.2 g/L PB concentration, minimized DS from 55.6 to 29.3% for *Fusarium equiseti* and 31%.DS incase *Macrophomina phaseolina* given 47.3 and 54.07% protection against *Fusarium equiseti* and *Macrophomina phaseolina* infection, respectively.

Table 6: Disease severity (DS%) in common bean cv. Giza 6 under *Fusarium equiseti* **and *Macrophomina phaseolina* infection as influenced by potassium bicarbonate (Pb).**

Pb (g/l)	*Fusarium equiseti*				*Macrophomina phaseolina*			
	Exp.I	Exp.II	M	Protection	Exp.I	Exp.II	M	**Protection**
Untreated	56.4	54.8	55.6	0	66.6	68.4	67.5	**0**
0.05	53.5	52.7	53.1	4.49	46.6	48.4	47.5	**29.62**
0.1	46.6	41.6	44.1	20.68	43.3	42.7	43	**36.29**
0.2	28.3	30.3	29.3	47.30	30.6	31.4	31	**54.07**

5-4-Using Potassium silicate (PS)

Data reported in Table (7) show effective role for Ps to reduce DS caused by pathogens tested. Increasing Ps concentration led to decrease DS, the least DS value was pronounced by 0.2 g/L Ps concentration that was 37.2% DS in case *F. equiseti* and 39.2% in case *M. phaseolina* reflected 33.09 and 41.72% protection against *F. equiseti* and *M. phaseolina* infection, respectively.

Table 7: Disease severity (DS) in common bean cv. Giza 6 under *Fusarium equiseti* and *Macrophomina phaseolina* infection as influenced by Potassium silicate (Ps).

Ps(g/l)	*Fusarium equiseti*				*Macrophomina phaseolina*			
	Exp.I	Exp.II	M	Protection	Exp.I	Exp.II	M	**Protection**
Untreated	56.4	54.8	55.6	0	66.6	68.4	67.5	**0**
0.05	51.7	48.9	50.3	539.	61.6	60.4	61.0	**9.62**
0.1	40.4	38.8	39.6	28.7	58.3	56.5	57.4	**14.96**
0.2	38	35.3	36.6	34.17	39.9	38.5	39.2	**41.92**

5.5. Using salicylic acid (SA)

Application of SA solution 2h pre plant seed soaking resulted in decrease DS that caused by pathogens tested even at the lowest concentration used (Table 8). Enhancing SA concentration lowering DS. The least DS was achieved by 0.2 g/L SA (45.6) reflected 35.26 and 32.74% protection against infection by *F. equiseti* and *M. phaseolina*, respectively**.**

Table 8: Disease severity (DS%) to common bean cv. Giza 6 under *Fusarium equiseti* and *Macrophomina phaseolina* infection as influenced by Salicylic acid (SA).

SA (g/l)	*Fusarium equisetic*				*Macrophomina phaseolina*			
	Exp.I	Exp.II	M	Protection	Exp.I	Exp.II	M	**Protection**
Untreated	56.4	54.8	55.6	0	66.6	68.4	67.5	**0**
0.05	52.0	50.8	51.4	7.55	55.0	54.7	54.8	**18.8**
0.1	50	46.2	48.1	13.48	48.3	46.4	47.4	**29.77**
0.2	38.6	33.4	36.0	35.25	46.6	45.2	45.9	**32.0**

Discussion

The recent work explored that diseased common bean parts viz., pods, stems and roots resulted various fungal pathogens along two growing seasons (2019–2020) in El-Minya governorate, Egypt. Two genera of fungi, *Fusarium* spp. and *Macrophomina phaseolina,* were occurred with pods, stems and roots recording the most predominant that showed the highest frequency in case *Fusarium* 45% and *Macrophomina* 25%. Beside *Fusarium* and *Macrophomina*, five genera *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp*.*, *Penicillium* spp*., and sclerotinia sclerotiorum* were associated with diseased pods. Meanwhile, fungal genera *Alternaria* spp*.*, *Aspergillus* spp*.*, *Penicillium* spp*.*, *Pythium* spp. and *Sclerotinia sclerotiorum* were accompanied with *Fusarium* and *Macrophomina* that associated with diseased stems/roots of common been. Data explored that the two genera *Fusarium* and *Macrophomina* were the most frequent from pods, seeds also stem, roots. Data are in line with those reported elsewhere by Gill *et al.* (2000) and Naseri, (2015).

Based on the forgoing data, the next step of this study was to evaluate the pathogenicity of five *Fusarium* isolates and six *Macrophomina phaseolina* isolates. Data proved that all the tested isolates were infective to common bean cv. Giza 6. Virulence of fungal isolates tested was

varied, *Fusarium* isolate F1 showed the highest virulent among *Fusarium* isolates and *Macrophomina phaseolina* isolate M1 was the most infective than other *Macrophomina phaseolina* isolates. Data are agreed with several researchers (Abdou *et al*., 2001, Hassan and Galal, 2012 and Abdullah *et al*., 2015).

The most infective *Fusarium* isolate F1 and *Macrophomina phaseolina* isolate M1 were put into consideration throughout the recent work and they were subjected for molecular identification which confirmed that F1 is *Fusarium equiseti* and M1 isolate is *Macrophomina phaseolina*. Data indicate a significant variant in particular response to infection by *Fusarium equiseti* or *Macrophomina phaseolina* similar as reported before (Abdou *et al*., 1999; Mohamad *et al*., 2013 and Zaccaron, 2019)

Silicon (Si) could alleviate plant disease resistance through preventing pathogen penetration (1) via structural reinforcement (Rodrigues and Datnoff., 2015), (2) by inhibiting pathogen colonization through stimulating systemic acquired resistance,(3) through antimicrobial compound production (Fauteux *et al*., 2005 and Van *et al*., 2013), as well as 940 through increasing plant resistance by activating multiple signaling pathways and defense–related gene expression (Fauteux *et al*., 2005 and Vivancos *et al*., 2015). The beneficial effects of Si with regard to plant resistance to disease are attributed to Si accumulation in epidermal tissue, the formation of complexes with organic compounds in cell walls, the induction of phenolic compounds, phytoalexin/peroxidase and regulating pathogen invasion and colonization (Brunings *et al*., 2009 and Sakr, 2016). The effect of Si on plant-microbe interaction and related physical biochemical, and molecular resistance mechanisms have been demonstrated (Wang *et al*., 2017).The obtained data proved that CaSi gave significant reduction in DS at 0.2g/l by 40.54% and 33.3% under infection with *Fusarium equiseti* or *Macrophomina phaseolina*, respectively, Data indicated that CaSi was more effective to protect common bean plants against either *Fusarium equiseti* or *Macrophomina phaseolina* infection, than Ps which reduced DS at 0.2g/LPS by 33.0% and 39.3% protection against *Fusarium equiseti* and *Macrophomina phaseolina*, respectively. The present data are in line with this report precisely (Wang *et al*., 2017 and Shehata *et al*., 2019).

As for potassium bicarbonate (PB), using 0.2g/l expressed the highest protection values 47.3% and 54.31% against *Fusarium equiseti* and *Macrophomina phaseolina* infection, respectively. Potassium bicarbonate was suggested to be fungicide alternative to control deferent plant disease. Bicarbonates, such as PB, have antifungal activity and were also suggested to be used in organic production (Mitre *et al*., 2010; Soliman and El-Mohamedy, 2017; Türkkan *et al*., 2018).

Rather than direct antimicrobial molecules, (reactive oxygen species) ROS are more likely cofactors in redox reactions playing various roles in plant defenses (Torres. 2010). For instance, ROS have been characterized as primary signaling molecules, regulating multiple physiological processed during plant growth and development (De Tullio, 2010). Interestingly, evolutionary considerations based on the nicotinamide adenine dinucleotide phosphate (NADPH) gene family suggest that mechanisms to detoxify ROS were acquired before the plants used ROS as signaling molecules (Mittler *et al*., 2011). Application of 0.2g/l AA+2.0g/l H_2O_2 as seed soaking resulted in resistant bean plant against *Fusarium equiseti* 20% protection and against *Macrophomina phaseolina* 47.0% protection. PAA is considered an environment-friendly product with a reported antifungal activity against *Rhizoctonia solani* and *Botrytis cinerea* (Narciso *et al*., 2007; Ayoub *et al*., 2017; Jo *et al*., 2019) and other phytopathogenic fungi (Galal, 2017; Galal, 2018 and Tantawy *et al*., 2020).

To develop systemic acquired resistance (SAR), a plant must generate a

signal in the pathogen-inoculated tissue that travels (presumably through the vasculature) to the uninoculated portions of the plant, in which it signals defense responses. Radio-tracer studies in tobacco and cucumber initially indicated that some of the SA in systemic leaves was synthesized in the inoculated leaf, raising the possibility that SA was the mobile signal (Mölders *et al*. 1996; Shulaev *et al*. 1995). Consistent with this possibility, pathogen-induced SA was shown to move through the apoplast prior to phloem loading in Arabidopsis (Klessing *et al*, 2018). Using 0.2g/l SA exhibited 35.22% and 32.74% protection against infection by *Fusarium equiseti* and *Macrophomina phaseolina*, respectively. However, SA reacted as antioxidant and reacted as inducers for disease resistance in servant plants against various pathogens (Galal and Abdou, 1996; Klessing et al 2018*)*.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

AA	Acetic acid
BLAST	Basic Local Alignment Search Tool
CIAT	Centro International de Agricultura Tropical
DS	Disease severity
L.S. D	Least significant difference
NADPH	Nicotinamide adenine dinucleotide phosphate
NCBI	National Center of Biotechnology Information
PAA	Peroxyacetic acid
PB	Potassium bicarbonate
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RRS	Root rot severity
SA	Salicylic acid

Reference

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