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Suppression of *Fusarium* Wilt of Chickpea by *Bacillus* spp., *Pseudomonas* sp. and Rhizobacterial Isolate

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This experiment was conducted to evaluate the antagonistic effects of some selected rhizobacteria on *Fusarium oxysporium* f. sp. *ciceris* in a pot experiment. Rhizobacterial isolates (one isolate of *Pseudomonas,* eight isolates of *Bacillus* genera and one bacterium isolate) and two chickpea cultivars (Shendi and Burgeig) were arranged in a factorial pot experiment in CRD with four replicates. The disease incidence and severity were detected weekly. Disease reduction

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percentage was estimated at the end of the study. Generally, the application of rhizobacterial isolates as biological control agent reduced disease incidence compared with the control in both cultivars. The incidence in cultivar Shendi occurred at the third week after inoculation when treated with *Pseudomonas stutzeri* strain W28 (SA3) and *Bacillus subtilis* strain CM14(SA9). For the two cultivars, Shendi and Burgeig, the *Geobacillus* sp. CRRI-HN-1(SA2) and *Bacillus* sp (SA1), respectively had the highest positive effect on disease incidence and severity throughout the experiment compared with the control. These were 45.36 and 44.82% in incidence; 55.36 and 63.89% in severity, respectively.

Keywords: Biological control; disease incidence; severity; in vivo screening.

1. INTRODUCTION

"Chickpea (*Cicer arietinum* L.) is one of the important food legumes it is cultivated in more than 57 countries, it stand third in production following dry beans and peas with a productivity of about 913 kg ha" [1]. "The cultivated chickpea originated in south-eastern Turkey" [2]. "In Sudan, it is a cash crop that generate income for farmers and rural communities and as significant source of protein for poor people. Despite this, production fluctuates widely and farmers face a number of debilitating constrains: the wide spread incidence of diseases, the destructive activities of pests, parasitic weeds, and limited access to quality high-yielding cultivars. The project ICARDA demonstrated high-yield varieties of chickpea to farmers and other stakeholders in the Gezira region, and other areas throughout the River Nile State. In the Gezira, the varieties Salwa and Burgaig performed extremely well, generating and average 4.01 and 3.84 t/ha, respectively, far higher the 1.66 t/ha average achieved by traditional crops" [3]. "More than 50 pathogens was reported so far to infect chickpea in different parts of the world, but only a few of them had the potential to devastate the crop, The important diseases are *ascochyta* blight, dry root rot, stunt (caused by bean leaf roll virus), *botrytis* gray mold, collar rot, black root rot, *phytophthora* root rot, *pythium* root and seed rot and *Fusarium* wilt" [4]. "*Fusarium* wilt caused by *Fusarium oxyspoum* f.sp. *ciceris* is a major constraint to chickpea cultivation through the world" [5]. "Yield losses attribute *Fusarium* wilt varied from 10-15%, but the disease span completely destroy the crop under unfavorable conditions" [6]. "Plant growth promoting rhizobacteria (PGPR) was been proved as biocontrol agents of soil borne plant pathogens, offer an attractive alternative to chemical fertilizers, pesticides and supplements. Thus, the use of PGPR is steadily increasing in agriculture" [7]. "The biological control using rhizosphere inhabitant bacteria is an alternative approach [8,9] and can be a suitable practice for disease control". "Use of biological control agents, such as plant growth promotion rhizobacteria (PGPR), can be suitable approach in control of disease" [10]. "Plant growth promotion rhizobacteria (PGPR), such as *Pseudomonas* and *Bacills* strain, were the major root colonizers [11], and can elicit plant defenses" [12]. "Different mechanisms was reported for their performance such as production of antibiotics, siderphore cyanide hydrogen, competition for nutrition and space,inducing resistance, inactivation of pathogen enzymes and enhancement of root and plant development" [13]. "*Pseudonas* and *Bacillus* strain have great potential in control of *Fusarium* wilt disease of chickpea" [14,15,16, 17]. "Plant growth promoting rhizobacteria (PGPR) have been reported as biocontrol agents of soil borne plant pathogen, offer an attractivelternative to chemical fertilizers, pesticides and supplements. Thus, the use of PGPR is steadily increasing, plant growth. Promoting rhizobacteria are an heterogeneous group of bacteria that can be found in the rhizosphere at the root surfaces and in association with roots which can improve the extent or quality of plant growth directly or indirectly" [11,18]. This study was conducted to assess the antagonistic effect of rhizobacterial isolates against chickpea *Fusarium* wilt. The main objective of this study is the determination of effective rhizobacteria to be used for biological control of *Fusarium oxysporum* f.sp. *ciceris* (FOC).

2. MATERIALS AND METHODS

The study was conducted to assess the antagonistic effect of ten rhizobacterial isolates against *Fusarium* of chickpea wilt using two chickpea cultivars namely, Burgeig and Shendi. Two varieties of chickpeas were used in this study, namely cv. Shendi (susceptible) and cv.

Burgaig (resistant). Soil was prepared by mixing sand and clay soil at 1:1 ratio. The soil was placed into 30×40 inch plastic sacks.

2.1 Rhizobacterial Inocula

Ten isolates of rhizobacteria SA1, SA2, SA3, SA4, SA5, SA6, SA7, SA8, SA9 and SA10 which were identified as *Bacillus* sp., *Geobacillus* sp., *Pseudomonas stutzeri strain* W28, *Bacillus subtilis* strain VT03, *Bacillus subtilis* strain FBRo3. *Bacillus* sp., Bacterium MOBOSA51, *Bacillus tequilensis* strain MML2, *Bacillus subtilis* strain CM14 and *Bacillus subtilis* strain SH23, respectively.

Rhizobacteria isolates were grown in Erlenmeyer flasks (250 ml) containing 100 ml of NA broth and shaken for 24 hrs at a rotary shaker, the growth was diluted with an adequate amount of non-inoculated nutrient broth to obtain a bacterial suspension of 10 ^{8}cfu/ml, using a spectrophotometer (660 nm). Chickpea seeds were surface sterilized with 70% ethanol, then immersed for 2 minutes in 2% sodium hypochloride and washed four times with sterilized distilled water and left for dried. 20 seeds were impressed in Petri-dish filled with bacterial suspension for 24 hrs, then placed onto sterile filter paper moistened with sterilized distilled water in Petri plates (four plates with 20 seeds/plate) and incubated at room temperature for 5 days. Control plates were arranged in similar way, except that they were treated with non-inoculated nutrient broth only.

2.2 Pathogen Inoculant

Ten ml of sterilized water were added to each culture of the pathogen isolates, and the surface of the culture was scraped with a glass spatula to dislodge the chlamydospores. The spore suspensions were transferred to 100 ml sterilized flasks. The concentration of the suspensions were determined with a haemocytometer. High suspension of 9×10^{-2} spore ml $^{-1}$ was prepared from each isolate ready for soil treatment. Half ml of the spores suspension was injected gently beside each one week old seedling using sterilized insulin syringe (Fisher and Toussoun, 1983).

2.3 Pot Experiment

Pot experiment was conducted to evaluate the chickpea Fusarium wilt disease progress (disease incidence and severity). The germinated seeds of the two chickpea cultivars were treated by rhizobacteria isolates SA1, SA2, SA3, SA4, SA5, SA6, SA7, SA8, SA9 and SA10. In addition, the control was represented by germinated seeds treated with non-inoculated nutrient broth. Treated germinated seeds were transferred into 30×40 inch plastic sacks .The plastic sacks were filled with soil enclosed of sand and clay soil at a ratio of 1:1. Treatments and the two cultivars were arranged in a factorial experiment in a Completely Randomized Design (CRD) with four replicates; with one sack per replicate and three plants per sack.

2.4 Assessment of Disease Reaction

Disease reactions were assessed by the incidence and severity of symptoms at 7-day intervals. Severity of symptoms in individual plants of a microplot was assessed on a 0 to 4 rating scale based on the percentage of foliage with yellowing or necrosis in acropetal progression (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%,and 4 = dead plant). Incidence of foliar symptoms, (0-to-1 scale) [16, 19]. The plants displaying the typical symptoms of the *Fusarium* wilt disease were considered infected. The percentage of the disease incidence was calculated using the following equation 1:

$$wilt\ incidence = \frac{No\ of\ plants\ wilted}{Total\ No\ of\ plants} \times 100 \quad (1)$$

Disease reactions were assessed according to the severity of symptoms weekly, the disease severity was assessed by visual estimation adopting the scale shown as following:

0 = No infection* on leaf,
1=1-33% of the leaf were infected
2= 34-66% of the leaf were infected
3= 67-100% of the leaf were infected
4= Dead plant

The disease reduction percentage (DRP) was calculated by the method described by Yun Cao et al. [20] using the following formula:

DRP = 1- DT/DC × 100

(DT = Disease incidence percentages in treatment; DC= Disease incidence percentages in control).

2.5 Statistical Analysis

Statistical analysis for factorial experiments in Completely Randomize Design using STATISTIX 8.0 Analytical Software.

3. RESULTS AND DISCUSSION

3.1 Disease Incidence Progress

Fusarium wilt disease causes yellowing and drying of leaves from the base to upward and finally death of plants. The overall progress of disease incidence in each cultivar presented in Fig. 1 and Table 1. Generally, the application of rhizobacterial isolates as biological control agents reduced disease incidence compared to the control in both cultivars. In Shendi cultivar SA1 and SA2 the incidence occurred in the second week after inoculation. The same trend was observed in Burgeig when treated with SA6. Moreover, the incidence in cultivar Shendi occurred in the third week after inoculation when treated with SA3 and SA9.

For cultivar Shendi, the ten bacterial isolates compared with the control had a positive effect on disease incidence throughout the experiment except for SA4, SA6, SA7 and SA8. For cultivar Burgeig, all bacterial isolates compared with the control had a positively affected disease incidence throughout the experiment except SA4 and SA7. Schroth et al. [21] and Cook et al. [22] stated that there appears to be a non-general correlation between the *in vitro* ability of the antagonists and their ability to suppress diseases in the field. Juhnke et al. [23] noted that biological control depends upon maintaining a threshold population of the antagonist on planting material.

3.2 Main Effect of Cultivars and Bacterial Isolates on Disease Incidence

With the exception of cultivars, non-significant differences were observed among rhizobacterial isolates and for the rhizobacterial isolates × cultivars interaction (Table 2).

The highest disease incidence was recorded in the cultivar Shendi throughout the experiment. In the second week Shendi and Burgeig scored 27 and 11% respectively, whereas in the 8th week 76 and 58% disease incidence were recorded for Shendi and Burgeig, respectively. This result confirms the finding obtained by Ahmed and Adam [24].

3.3 Disease Severity Progress

The overall development of disease severity in each of the two cultivars was presented in Fig. 2. For cultivar Shendi, all bacterial isolates compared to the control had a positive effect on disease severity from the 4th week and onwards except SA4 and SA6. However, for cultivar Burgeig, the ten isolates had a positive effect on severity, compared with the control, throughout the experiment except SA4 and SA6. Anjajah et al. [14] Hervas et al. [25] and Landa et al. [16] reported that *Pseudomonas* and *Bacillus* strain have great potential in control of *Fusarium* wilt disease of chickpea.

3.4 Main Effect of Cultivars and Bacterial Isolates on Disease Severity

A significant difference was observed between cultivars in all weeks except the 4th and 5th ones (Table 1). However non-significant differences were detected among the bacterial isolates in all readings. Additionally, non-significant rhizobacterial isolates × chickpea cultivars were obtained throughout the experiment (Table 3). Concerning the main effect of cultivars the highest disease severity throughout the experiment was observed in cultivar Shendi. In week two disease severity was 0.26 for cultivar Shendi and 0.1 for cultivar Burgeig. In week eight it was 2.84 for cultivar Shendi and 1.91 for Burgeig.

3.5 Effect of Rhizobacterial Antagonist on Disease Index

The disease index is measured in terms of disease incidence and severity. The first symptom of the disease was appeared 12 days after inoculation. Table 1 reveals the simple effects of bacterial antagonists on the disease in each cultivar in week eight as reduction percentages. SA2 and SA1 showed the highest disease incidence reduction (DIR%), this was 45.36 and 44.82% in cultivar Shendi and Burgeig, respectively. The same isolates had the highest disease severity reduction (DSR%), it was 55.36 and 63.89% in cv. Shendi and cv. Burgeig, respectively. On the other hand, SA4 and SA7 had the lowest DRP% in both cultivars, it was similar to the control. Moreover, SA4 scored the lowest DSR%. Karimi et al. [26] reported that *B. subtilis* and *P. aueroginisa* isolates reduced disease severity and more effects in seed treatment (39.47 and 34.21%), respectively. Zaim et al. [27] noted that *Bacillus* isolates reduced disease severity caused by FOC from 60 to 99% in the field trials.

Various substances that promote plant growth are produced by certain rhizospheric microorganisms, including bacteria of the genus

Bacillus and fungi of the genus Trichoderma, and can directly or indirectly influence the metabolism and physiology of the plant [28,29,30], through the synthesis and excretion of phytostimulatory substances, such as phytohormones and volatile organic compounds that reinforce plant immunity [31,32]. For this reason, a study by Oliva-Ortiz et al. [33] pointed out that it is important to study the interaction of the microorganism strain with the chickpea plant and infer the higher grain yield. This fungus is difficult to control because it resists the main fungicides and fumigants, and it can remain dormant in the soil through chlamydospores, structures that represent the initial inoculum for epidemics in the following crop cycles [34,35-37].

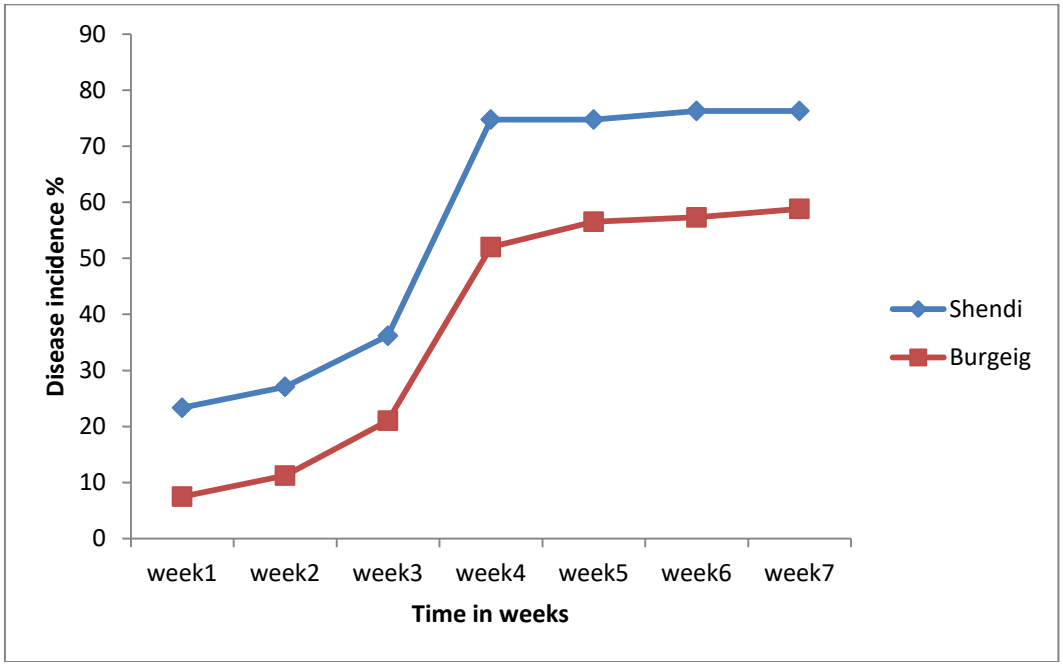

Fig. 1. Main effect of cultivars on disease incidence throughout the experiment

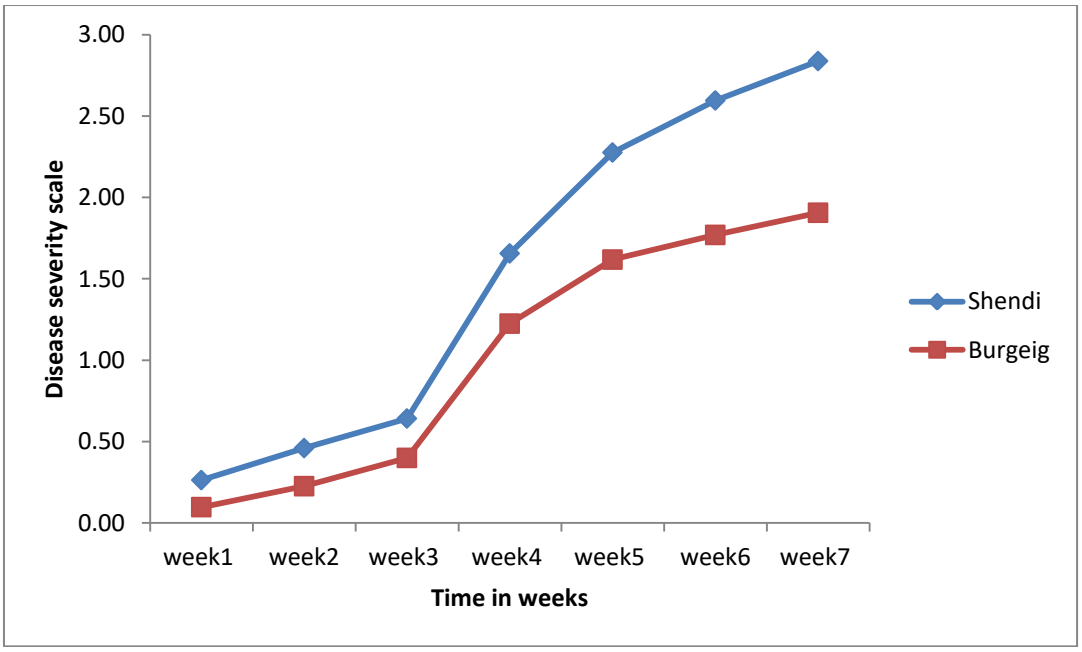

Fig. 2. Main effect of cultivars on disease severity throughout the experiment

Table 1. The effects of bacterial isolates on disease index of *Fusarium* wilt of chickpea in seed treatment in two cultivars after eight weeks from inoculation

	Shendi		Burgeig		Shendi		Burgeig	
	DI	DIR (%)	DI	DIR (%)	DS	DSR (%)	DS	DSR (%)
SA1	66.25	27.60	41.25	44.82	2.50	36.22	1.08	63.88
SA2	50.00	45.36	58.00	22.41	1.75	55.36	1.99	33.44
SA3	66.25	27.60	57.75	22.74	2.42	38.27	1.75	41.47
SA4	91.50	0.00	75.00	-0.33	3.41	13.01	2.58	13.71
SA5	83.00	9.29	49.50	33.78	3.08	21.43	2.08	30.43
SA6	83.00	9.29	66.50	11.04	3.16	19.39	2.25	24.75
SA7	91.50	0.00	75.00	-0.33	3.33	15.05	2.42	19.06
SA8	83.00	9.29	49.75	33.44	3.08	21.43	1.16	61.20
SA9	58.25	36.34	58.00	22.41	2.17	44.64	1.25	58.19
SA10	74.75	18.31	41.50	44.48	2.42	38.27	1.42	52.51
control	91.50	0.00	74.75	0.00	3.92	0.00	2.99	0.00

DI= disease incidence, DS= disease severity,
DIR%= Disease incidence reduction percentage, DSR%= Disease severity reduction percentage

Table 2. Mean square for disease incidence in Chickpea cultivars, rhizobacterial isolates and rhizobacterial isolates x chickpea cultivar throughout the experiment

Source of variation	df	Mean squares						
		Week2	Week3	Week4	Week5	Week6	Week7	Week8
Rhizobacterial isolates	10	702.35	620.77	802.57	1058.16	782.03	914.70	825.66
Chickpea cultivar	1	3319.04**	4006.26**	4279.02*	8027.35**	5474.40*	5792.49*	4959.60*
Rhizobacterial isolates x Chickpea cultivar	10	737.11	603.67	609.06	100.11	167.00	189.99	226.40
Error	66	421.08	534.50	886.52	909.64	926.58	917.05	941.77
CV%		133.58	120.72	107.43	53.65	52.59	51.45	51.60

** and ** denote significant at 5 and 1% level of probability*

Table 3. Mean square for disease severity in Chickpea cultivars, rhizobacterial isolates and rhizobacterial isolates x chickpea cultivar throughout the experiment

Source	df	Mean squares						
		Week2	Week3	Week4	Week5	Week6	Week7	Week8
Rhizobacterial isolates	10	0.13	0.28515	0.51852	1.27429	2.07155	2.42	2.70
Chickpea cultivar	1	0.61**	1.20*	1.29	4.09	9.52**	15.02**	19.10***
Rhizobacterial isolates x Chickpea cultivar	10	0.09	0.17	0.46	0.23	0.32	0.44	0.54
Error	66	0.08	0.26	0.52	1.08	1.34	1.49	1.58
CV %		154.28	149.37	138.74	72.29	59.39	55.90	53.04

** and ** denote significant at 5 and 1% level of probability*

4. CONCLUSION

Based on the study results, the *Geobacillus* sp. CRRI-HN-1 and *Bacillus* sp. had the highest positive effect on disease incidence and severity throughout the experiment compared with the control. Thus the *Fusarium* wilt can conquest in chickpea by used the *Geobacillus* sp. CRRI-HN-1 and *Bacillus* sp. isolate. Further studies could be carried out in order to strengthen the result obtained in this experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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