Response of Released Chickpea Cultivars to some *Fusarium oxysporium* f.sp *ciceris* Isolates in Sudan

Abstract

Chickpea seeds in Sudan is an economically important, as a cash crop that generates income for farmers and rural communities, and as a significant source of protein for poor people. It is used increasingly as a substitute for animal protein This study was conducted to screen eight chickpea cultivars viz Salawa, Burgeig, Wadhamid, Jebelmarra, Hawatta, Shendi, Atmour and Mattama using eighteen (18) isolates of *Fusarium oxysporum* f.sp *ciceris* (*FOC*) isolated from infected plants of chickpea displaying the characteristic symptoms of *Fusarium* wilt disease in winter season from different locations in The Sudan. Pot experiment was carried out to assess disease intensity in term of disease incidence (DI) and disease severity (DS). After seven weeks from inoculation 19 out of 144 isolated-cultivar combinations do not showed disease symptoms. The cultivar Burgeig found to be immune to all *Fusarium* wilt isolates in second and third week after inoculation. After seven weeks from inoculation, the least DI and DS registered in Burgeig, whereas the highest ones observed in cultivar Shendi. The remaining cultivars showed different response to FOC isolates. Regarding disease development, the high jump of incidence and severity occurred between third and fourth week after inoculation. The FOC isolate S9 seems to be more virulent and aggressive compared to the others.

Key words: Fusaruim wilt, *Cicer arietinum*, Screening, Sudan

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important food legumes in the most countries of the world with the productivity of about 913 kg ha^{-1} (FAO, 2012). The cultivated chickpea originated in south-eastern Turkey (van der Maesen, 1984). In Sudan, it is a cash crop that generate income for farmers in rural communities-and as significant source of protein for people. The production fluctuates widely and farmers face a debilitating constrains such as the wide spread incidence of diseases, the destructive activities of pests, parasitic weeds, and limited access to quality high-yielding cultivars. The ICARDA has demonstrated high-yield varieties of chickpea to farmers

and other stakeholders in the Gezira region of Sudan and other areas throughout the River Nile State. In the Gezira, the varieties Salwa and Burgaig have performed extremely well, generating (ICARDA, 2014). More than 60 pathogens have been reported so far to infect chickpea in different parts of the world, but only a few of them have the potential to devastate the crop, The important diseases are *ascochyta* blight, dry root rot, black root rot, *phytophthora* root rot, *pythium* root and seed rot and *Fusarium* wilt (Nene et al., 1991). *Fusarium* wilt (*Fusarium oxyspoum* f.sp. *ciceris*) is a major constraint to chickpea cultivation through the world (Nikam *et al*., 2011). The yield losses attribute vary about (10-15%), but the disease span completely destroy the crop under unfavorable environment (Cherif *et al*., 2007).

The use of resistant cultivars is the most effective and practical mean to control *Fusarium* wilt (Mahmood *et al*., 2011). However, the efficiency of resistant cultivar in managing a disease can be seriously limited by pathogenic variability occurring in pathogen populations, including the existence of pathogenic races and pathotypes (Jimenez-Gasco *et al*., 2004). There are eight races of *F*. *oxysporum* f.sp. *ciceris* which are identified by reaction on a set of differential chickpea cultivars (Jimenes-Gasco and Jimenes-Diaz, 2003). This study aim to screen the released Sudanese chickpea cultivars using some *Fusarium oxysporum* f.sp.*ciceris* isolates.

2. Materials and Methods

2.1 Isolation of the pathogen:

Eighteen isolate of *Fusarium oxysoprum* f.sp *ciceris* were isolated from infected plants of chickpea displaying the characteristic symptoms of *Fusarium* wilt disease in winter (2013) from different locations in centeral Sudan (El- Madina Arab, Ganeb, Abugota, El-Moaileg and Agricultural Research Corporation-Madani) and in Northeren Sudan from Hudeiba Research Station, (three isolates from each location).

The roots and stem of infected plants were washed in running tap water to remove soil before isolation to avoid contamination. The roots were cut into small bits of the size (5-10 mm), These bits were then surface sterilized with 0.1 percent mercuric chloride for 2 minutes and washed with three changes of sterilized water to remove traces of mercuric chloride. Each bit was blot dried and four bits each placed on the solidified potato dextrose agar (PDA) plates. These plates

were then incubated at 27 C^0 for seven days. The fungal growth was transferred to the plates of PDA.

Fusarium species were maintained on PDA slants and were stored at 4°C till use (Hend *et al*., 2012).

2.2 Chickpea genotypes:

In order to evaluate the varietal response of different chickpea cultivars to *F. oxysporum* f. sp. *ciceris* (Foc), a pot experiment was conducted at Department of Crop Sciences nursery, Kordofan University El-obied-Sudan, in the month of November 2013. Eight chickpea cultivars viz., Wad-Hamed, Mattama, Burgaig, Hawata, Shandi, Gebel Marra and Atmour obtained from Agricultural Resaerch Corporation, Plant Breeding-Hudeiba Research Station, El-Damer, Sudan. Screened for the source of resistance against eighteen isolates of *Fusarium oxysporum* f.sp *ciceris* the causal agent of chickpea wilt disease, isolated from the most important chickpea regions in Centeral and Northeren Sudan El-Madina Arab,Ganeb, Abugota, El-Moaileg, Agricultural Research Corporation-Madani and Hodeiba Research Station (three isolates from each location).

Treatments were arranged in factorial experiments in a complete block design. The treatment consisted of 3 replicates with one pot per replication and three plants per sack.

2.3 Preparation of the host plant

Soil prepared from sand and clay soil at the ratio of 1:1 the soil was placed into 30x40 inch plastic sacks. Seeds of each variety were surface sterilized and four seeds were sown in each sack.

2.4 Preparation of the pathogen inocula:

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. Concentratin of the suspentions were determined with a haemocytometer. A 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).

Inoculated plants were kept in nursery with three replicates adopting factorial design.

2.5 Disease assessment

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 pro-gression (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%,and 4 = dead plant). Incidence of foliar symptoms, I(0-to-1 scale) (Landa .,2001).(Navas-Cortes *et al* ., 1998).

Calculation of disease incidence:

The plants displaying the typical symptoms of the *Fusarium* wilt disease were considered infected. Percentage of the disease incidence was calculated using the following formula:

$$wilt\ incidence = \frac{No\ of\ plants\ wilted}{Total\ No\ of\ plants} \times 100$$

Calculation of disease severity:

The disease severity assessed by visual estimation adopting the scale presented in Table 1.

Table 1: The Adopted Disease Severity Scale for *Fusarium* wilt Disease.

Scale	Designation of Disease Severity
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

*infection: Displayed the typical *Fusarium* wilt disease symptoms.

2.6 Statistical analysis

Statistical analysis for factorial experiments in completely randomize design using MSTATC program.

3. Results and Discussion

Fusarium wilt disease cause yellowing and drying of leaves from the base to upward and finally death of plants (plate 1).

The study was conducted to screen eight (8) chickpea cultivars viz Salawa, Burgeig, Wadhamid, Jebelmarra, Hawatta, Shendi, Atmour and Mattama using eighteen (18) isolates of *Fusarium oxysporum* f.sp *ciceris* (*FOC*).

The overall development of disease incidence in the eight cultivars presented in Figure 1

The second week after inoculation: highly significant differences were obtained among cultivars and isolates. Burgeig found to be immune to all *Fusarium* wilt isolates in this week, while the other 7 cultivars were susceptible. The highest infection (11.6) was recorded in cultivar Shendi which infected by fifteen (15) *FOC* isolates. Regarding isolates the highest infection (13.88) recorded in Isolate S7, whereas the lowest one (1.38) registered in Isolate S11 and S17.

The third week after inoculation: shows highly significant differences among cultivars and isolates in this week. The cultivars Burgieg still immune to all Foc isolates, and cultivars Shendi scored 17.49. In addition, Shendi infected by all isolates except S10 and S13. Other chickpea cultivars scored less than 10% disease incidence. The most virulent isolate was S9 which scored 19.33 whereas the lowest one was S8 with 2.75 disease incidence.

The fourth week after inoculation: all chickpea cultivars were affected by the causal fungus isolates in the fourth week after the inoculation. Analysis of variance revealed highly significant differences among cultivars and isolates. The highest disease incidence (46.79) scored by the cultivar Shendi and the lowest one (6.42) scored by the cultivar Burgeig. Its worthily notice that Burgeig immunity to some isolates break after three weeks from inoculation. The largest disease incidence (30.54) recorded in isolate S9 and the smallest one (9.67) obtained in isolate S16.

The fifth week after inoculation: highly significant differences were observed among cultivars and isolates. The lowest disease incidence 16.40 % attained by the cultivar Burgieg and the highest one 73.72% attained by the cultivar Shendi. The Isolates S9 and S16 cause the highest (58.38) and the lowest (26.38) disease incidence, respectively.

The sixth week after inoculation: analysis of variance showed highly significant differences between cultivars and isolates. In this week, the cultivar Burgieg scored the lowest disease incidence (16.98 %) while Shendi scored the highest disease incidence (76.07 %). Concerning the main effect of isolates, the highest disease incidence (58.4) registered in S9 and the lowest one (32) registered in S16.

The seventh week after inoculation: the lowest disease incidence (16.98%) registered in cultivar Burgieg whereas the highest one (76.07) still registered in cultivar Shendi. The Isolate S9 cause the highest disease incidence (59.7) and the Isolate S16 cause the lowest one (34.7).

The eighth week after inoculation: highly significant differences were obtained among cultivars and isolates. Burgeig seem to be more resistant to most *FOC* isolates. Interestingly, the lowest disease incidence (17.58) was registered in this cultivar. Whereas the cultivar Shendi infected by all *FOC* isolates. Moreover, the highest infection (77.82 %) in this week recorded in its canopy. The most virulent Isolate was S9, it gave 59.75 disease incidence, while the lessen virulent *FOC* isolates was S2. It gave 36.7 disease incidence.

The overall development of disease severity in the eight cultivars presented in Figure 2.

The second week after inoculation: highly significant differences were obtained among cultivars and a significant differences were obtained between the isolates. Burgeig found to be immune to all *Fusarium* wilt isolates in this week, while the other 7 cultivars were susceptible. The highest disease sevirity (0.2) was recorded in cultivar Atmour. Regarding isolates the highest infection (0.25) recorded in Isolate S4, whereas the lowest one (0.02) registered in Isolates S1,S11 and S17. The third week after inoculation: Analysis of variance showed non-significant differences among cultivars and isolates. The fourth week after inoculation: all chickpea cultivars were affected by the causal fungus isolates in the fourth week after the inoculation. Analysis of variance revealed highly significant differences only among cultivars. The highest disease severity (0.79) scored by the cultivar Shendi and the lowest one (0.11) scored by the cultivar Burgeig. The fifth week after inoculation: highly significant differences were observed among cultivars and isolates. The lowest disease severity .036% attained by the cultivar Burgieg and the highest one 1.81% attained by the cultivar Shendi. The Isolates S9 cause the highest (1.39) and the lowest disease severity (0.67) attained by S17. The sixth week after inoculation: in this week, the cultivar Burgieg scored the lowest disease severity (0.6) while Shendi scored the highest disease severity (2.74). Concerning the main effect of isolates, the highest disease severity (2.15) registered in S18 and the lowest one (1.04) registered in S16.

The eighth week after inoculation: highly significant differences were obtained among cultivars and isolates. Burgeig seem to be more resistant to most *FOC* isolates. Interestingly, the lowest

disease severity (0.68) was registered in this cultivar. Whereas the cultivar Shendi infected by all *FOC* isolates. Moreover, the highest infection (3.06 %) in this week recorded in its canopy. The most virulent Isolate was S9, it gave 2.39 disease severity, while the lessen virulent *FOC* isolates was S16. It gave 1.35 disease severity.

Effect of cultivars x FOC isolates on disease severity: non significant cultivar x FOC isolates interaction was detected in all weeks except week six.

Figure 3 shows that all cultivars exhibit immunity (severity = 0.00) against a few FOC isolates except Jebelmarra and Shendi. The highest severity (4.00) reported in cultivar Jebelmarra with S9 and S18.

In this study and after seven weeks from inoculation 19 out of 144 isolated-cultivar combinations do not showed disease symptoms. Navas-Cortes et al. (2000), Sibtain et al.(2001) and Chaudhry et al. (2006) observed conciderable variation in response of chickpea genotypes when inoculated by FOC races. This might be due to the fact that the races of FOC differ in pathogencity and virulence, depending on the susceptibility of the cultivar. Other factors favoring the development of FOC are high temperature, amount of inoculums and excess soil water (Navas-Cortes et al ., 2000). Moreover, Shinde et al. (2010) concluded that both the resistance and wilt is polygenic and that may have genes with secondary effects which modify the response to the disease. According to disease incidence, based on the main effect at the end of the experiment (the seventh week after inoculation), cultivars could be divided in three groups viz, < 30% incidence which include only Burgeig (17.58%), 30%< and > 60%, include (Wadhamid (33.35%), Mattamma (42.70%), Hawatta (43.32%) Salawa (46.27%) and Atmour (49.74%), >60% incidence which include Shendi (77.82%). The results of Burgeig and Shendi is in accordance with Ahmed and Adam (2014). Concerning disease incidence progress (Figure 1) for the different cultivars, it is appear that the great change in incidence occurred between the third and fourth week after inoculation. Then incidence progress slightly in all cultivars. The slow and fast development of disease incidence observed in Burgeig and Shendi, respectively. Kumar *et al*. (2013) reported that development of disease is slow in resistant lines and fast in susceptible lines. Furthermore, he suggested field screening at reproductive stage for genotypes exhibit resistant at early growth stage and became susceptible at reproductive stage.

Similarly, based on the main effect at the end of the experiment (the seventh week after inoculation) the chickpea cultivars could be divided as follows:

(i) 1 ≥ severity, represented by Burgeig (0.68),(ii) 1< severity ≤ 3, this include Wadhamid (1.33), Mattama (1.63), Hawatta (1.72), Salawa (1.87), Jebemarra (1.94) and Atmour (1.98). (iii) more than three severity, which include Shendi (3.06).

Regarding FOC isolates, no significance differences were observed among the eighteen them after seven weeks from inoculation for disease incidence and severity, but the isolate S9 seems to be the more virulent and aggressive compared to other FOC isolates.

4. **Conclusion**:

In this study it could be concluded that the cultivars Burgeig and Shendi were the best and worst one respectively. The high jump of incidence and severity occurred between third and fourth week after inoculation. The FOC isolate S9 seems to be more virulent and aggressive compared to the other FOC isolates. Generally in this study the release chickpea cultivar, Burgaig was found to be the most resistant cultivated variety to *Fusarium oxysporium* f.sp *ciceris*. Further studies should be carried out in future to confirm this results.

Healthy plant

A

B

C

Plate 1. Disease development symptoms from A to C

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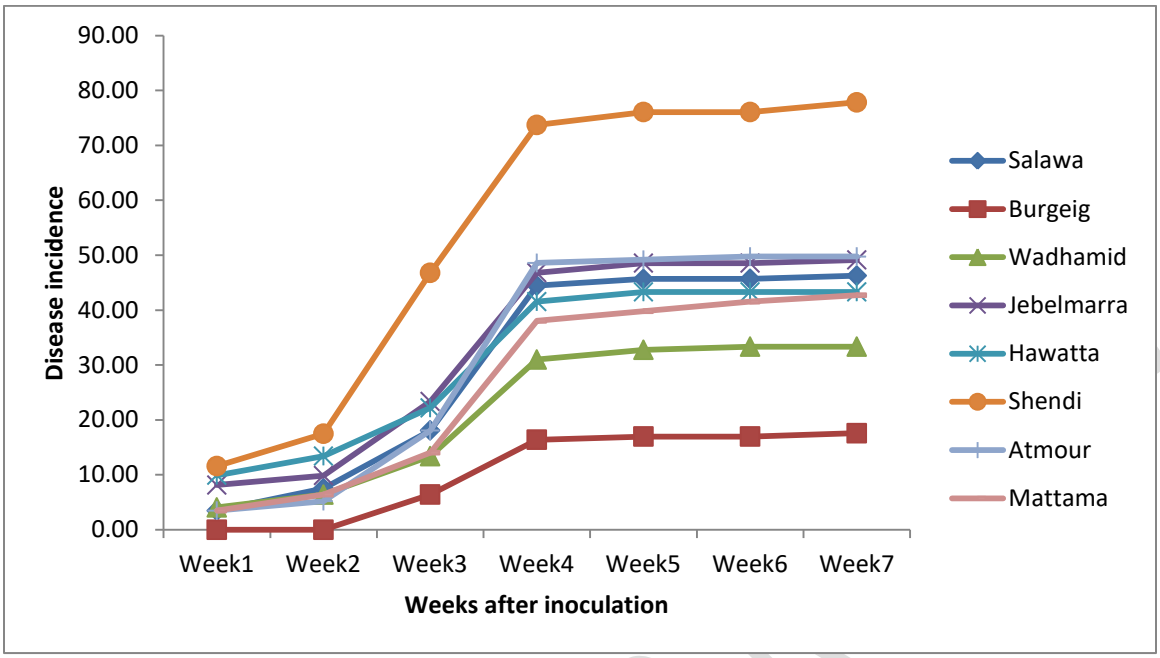

Figure 1. Disease incidence progress in chickpea cultivars

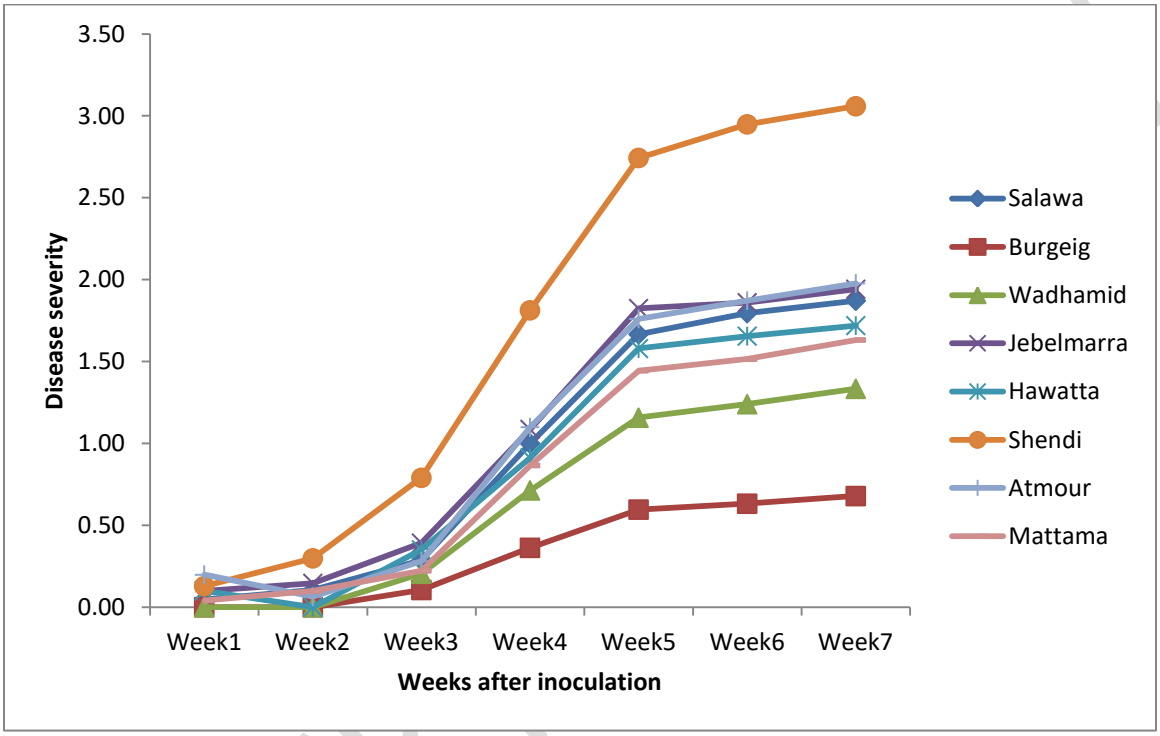

Figure 2. Disease severity progress in chickpea cultivars

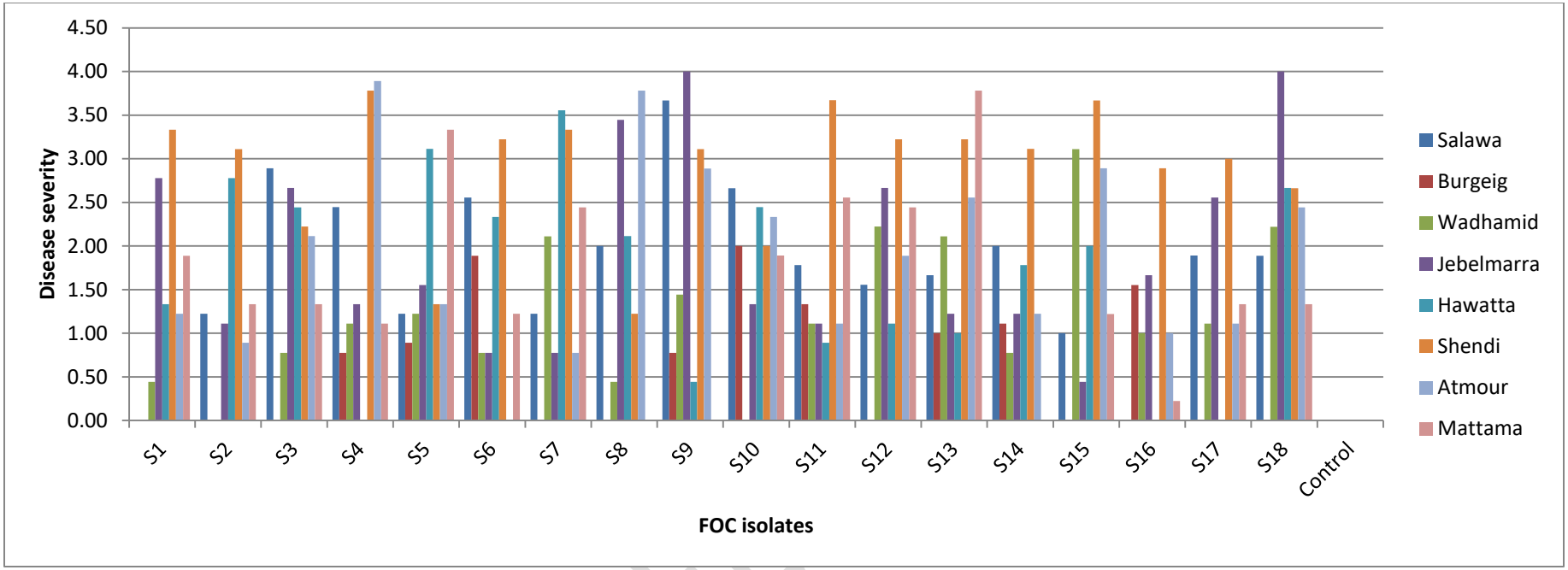

Fig. 3. Effect of chickpea cultivar x FOC isolates interaction on disease severity.

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