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Biological Control of Chickpea Wilt Caused by *Fusarium oxysporum* f. sp. *ciceris**

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Abstract: This study was conducted in an attempt to control chickpea *(Cicer arietinum* L.) wilt, caused by *Fusarium oxysporum* f. sp. *ciceris,* using antagonistic properties of soil microorganisms. It also aimed at avoiding problems resulting from the use of chemical fungicides. A *Trichoderma* sp. was isolated from the rhizosphere of a resistant chickpea variety (ICCV-2) and a *Bacillus* sp. from the rhizosphere and rhizoplane of the same variety. Both microorganisms proved to be effective in controlling the disease. In addition, *Trichoderma harzianum*, which was obtained from Giza Research Station in Egypt, was also antagonistic to *Fusarium oxysporum* f. sp. *ciceris* Wilt incidence was significantly reduced when chickpea was grown in pots containing soil mixed with any of the three antagonists or when chickpea seeds were initially treated with the seed-dressing fungicide Vincit at 2 ml/kg seeds. *Trichoderma harzianum* proved to be the best bioagent as it gave the lowest disease incidence. In the field, the two *Trichoderma* spp. were as effective as Vincit in causing reduction in the wilt incidence. At the higher concentration of 140 g/m^2, the two antagonists were effective throughout the growth period, but they were less effective at the lower concentration of 70 g/m^2 particularly at the seedling stage.

Key words: Biocontrol; chickpea wilt; *Fusarium oxysporum*

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INTRODUCTION

Biocontrol of plant diseases has been defined as any condition or practice under which survival or activity of a pathogen is reduced through the agency of another living organism (except man) leading to a reduction in the incidence of the disease caused by the pathogen (Garrett 1965). Biocontrol, using antagonistic microorganisms, proved to be successful in many countries for controlling various plant diseases (Sivan 1987).

Chickpea (*Cicer arietinum* L.) is the world's third most important legume crop, after beans and peas (Saxena 1990). In the Sudan, the relatively long and cool winter season in the north is more suitable for chickpea production than the central and southern parts of the country. Recently, it was found that the crop could be successfully grown in Hawata area in eastern Sudan and Jebal Marra in western Sudan (Sheikh-Mohamed 1996).

Chickpea wilt, caused by *Fusarium oxysporum* (Sehlechtend) f. sp. *ciceris* (Padwick) Matuo and K. Sato, is a major constraint to production in the Sudan (Freigoun 1980) and wherever chickpea is grown , particularly in the Indian Subcontinent and the Mediterranean Basin (Haware 1990).The pathogen is both seed and soil borne, facultative saprophyte and can survive in the soil for up to six years in the absence of a susceptible host (Haware *et al.* 1996), thus it markedly reduces the potential of crop rotation as a disease management strategy. The most effective and practical method for the control of the disease worldwide is the use of resistant cultivars (Kraft *et al.* 1994).The effectiveness of this method is threatened by the existence of pathogenic races in *Fusarium oxysporum* f. sp. *ciceri*s (Nikam *et al*. 2007).

Synthetic chemical fungicides are being used successfully for the control of various fungal diseases, but indiscriminate use of these chemicals has led to the development of fungicide resistance and, more important, environmental pollution, posing potential risk to animal and human health (Lyon *et al*. 1995). In the Sudan, a number of fungicides were used against *Fusarium oxysporum* f. sp. *ciceris* including Tecto-TM, Benlate,

Quinolae, Vincit and Vitavax. The use of Vincit or Vitavax was effective in reducing the wilt incidence, particularly in the seedling stage, but none of them increased seed yield significantly (Suliman 1997).

This investigation was carried out with the objective of evaluating the effect of some bioagents in reducing the incidence of chickpea wilt. The study also aimed at avoiding hazards resulting from using fungicidal chemicals.

MATERIALS AND METHODS

Isolation and identification of the causal pathogen

Five isolates of *Fusarium oxysporum* f. sp. *ciceris* were obtained from samples of infected chickpea plants, showing the typical symptoms of the fusarium wilt disease. Three isolates were collected from Wad Hamid (Khartoum State) and two isolates from Hudeiba Research Farm (River Nile State). Small pieces were taken from roots of the collected samples, washed thoroughly with tap water then sterilized in 2% sodium hypocholorite solution for three minutes, plated on potato dextrose agar medium (PDA) and incubated at room temperature for 7 days. The developed fungi colonies were isolated and purified using the hyphal tip technique. Isolated fungi were identified as *Fusarium oxysporum* f. sp. *ciceri*s by using the morphological characteristics of mycelia and spores as described by Nelson *et al*. (1983).

Pathogenicity test

Each isolate of *F. oxysporum* f. sp. *ciceri*s was used to infest sterilized soil in a separate plastic tray in which ten sterilized seeds of each of the susceptible chickpea variety JG-62 and the resistant variety ICCV-2 were sown, in a row 20 cm long and at depth of 3 cm. Temperature in the screen house was in the range of 20^{o}-30^{o}C. The procedures used were those described by Ali (1995). The test was carried out in a randomized complete block design (RCB) with three replicates for each combination. The pathogenic isolate was recovered and cultured in sand-maize meal media for 10 days at 30^{o}C.

Bioagents

Two species of *Trichoderma* were used. These were *Trichoderma* sp. isolated from rhizosphore of chickpea variety ICCV-2 grown at Hudeiba Research Farm and *Trichoderma harizanum* obtained from Giza Research Station, Egypt. In addition, *Bacillus* sp. isolated from rhizosphere and rhizoplane of ICCV-2, grown at Wad Hamid, was included in the study.

Effect of fungal and bacterial antagonists on chickpea wilt disease

A conidial suspension was prepared by mixing 7-day old cultures of *Fusarium oxysporum* f. sp. *ciceris* on PDA with sterilized distilled water and then filtered through three layers of cheese cloth.The suspension was adjusted at a concentration of 140 x 10^6 conidia/ml, using a series of dilutions and direct slide counting. The two *Trichoderma* spp. were grown on a sand-sorghum medium (1:3 w/w) for two weeks. The bacterium was cultivated on a nutrient broth medium for 48 hours.

The experiment was carried out in pots (30 cm diameter) containing a mixture of sand and clay in the ratio of 1:1 (w/w). The soil was sterilized in an oven at 120^{o}C for 24 hours. The pots were arranged in a RCB design with three replicates for each treatment. Inocula of the fungal antagonists were mixed with the soil at the rate of 10 g/kg soil. The bacterial suspension was added at the rate of 10 ml/kg soil and then mixed thoroughly.

As a control, the spore suspension of *Fusarium oxysporum* was mixed alone with the soil at the rate of 50 ml/kg soil. Other controls were also used in the tests for comparison; these comprised pots inoculated with each of the three bioagents alone, i.e. without the subsequent addition of the pathogen. Chickpea seeds were sown without the addition of any of the biocontrol agents or the pathogen.

The pots were watered and left wet for four days so as to enhance successful establishment of the microorganisms. Then, they were inoculated with the spore suspension of the pathogen at the same rate mentioned above and watered and left wet for four more days. Chickpea seeds (variety Shendi), disinfected by 2% sodium hypochlorite, were sown at 10 seeds per pot. The procedure described by Hassanein *et al.*

(1997) was adopted in this study. Data on germination percentage were taken 7 days after sowing. Incidence of wilt disease was recorded at biweekly intervals up to 45 days. This trial was carried out for two successive seasons (1997/98 and 1998/99).

Field evaluation of the effect of the fungal and bacterial antagonists on chickpea wilt disease

A field trial was conducted at Hudeiba Research Farm in 1998/99 season to test the effect of two *Trichoderma* spp. on the fusarium wilt of chickpea (variety Shendi).The most predominant pathogen in the sick plot was *F. oxysporum* f. sp. *ciceri*s. Inocula of the fungal antagonists were added to the top of the ridge at the rates of 70 and 140 g/m^2 and mixed carefully to a depth of 20 cm. The seeds were also treated with the seed dressing fungicide Vincit 5 FS at the rate of 2 ml/kg seeds and included in the trial for comparison. As a control, untreated seeds were also sown in the sick plot.

The experimental design used was RCB with three replicates. The plot size was 2.7 x 5 m. Each plot consisted of 4 ridges, 5 metres long. Chickpea seeds were sown at the rate of 2 seeds/hole. Inter- and intra-ridge spacings were 60 and 10 cm, respectively. The sowing date was 10 November 1998.

Irrigation was carried out at 10–day intervals. Data on germination percentage were taken after two weeks from planting, and incidence of wilt disease was recorded at biweekly intervals.

RESULTS

Pathogenicity test

The isolates from Hudeiba Research Station infected susceptible chickpea cultivar JG-62 causing wilt disease with mortality percentage of more than 50%. The three isolates from Wad Hamid were not pathogenic to the two chickpea cultivars. They gave mortality percentage in the range of 0% – 20%. The chickpea cultivar ICCV-2 was resistant to all isolates of *F. oxysporum* f. sp. *ciceri*s.

Effect of the antagonists on chickpea wilt incidence

There were no significant differences in germination percentage between the treatments and the control (soil infested only with the pathogen) except for the combination of *Trichoderma harziamum* and *F. oxysporum* f. sp. *ciceris* (Table 1). Wilt symptoms were observed 15 days after planting. Incidence of the disease was greatly reduced when chickpea seeds were treated with Vincit or when the soil was mixed with any of the three antagonists. No disease symptoms were detected on plants grown in pots treated only with the antagonists without the addition of the pathogen, or in pots with sterilized soil which was not treated with either the antagonists or the pathogen. The highest infection percentage was recorded when only the pathogen was used.

The results revealed that both Vincit and *Trichoderma harzianum* were the most effective as they significantly reduced wilt incidence over a period of 45 days (Table 1). Although soil treatment with the other two antagonists caused significant reductions in disease incidence as compared to the untreated control, yet they were not as effective as the first two treatments particularly during the early stage when wilt symptoms were first observed. However, all treatments gave more or less the same results during the subsequent period up to end of the experiment.

The data obtained from the field experiment showed that there was no significant difference in germination percentage among all treatments (Table 2). The fungal antagonists, i.e. *Trichoderma harzianum* and the isolated *Trichoderma* sp. as well as Vincit effectively decreased wilt incidence in chickpea during the seedling, flowering and podding stages. In all treatments, the wilt incidence decreased towards the podding stage, except for the control which showed the highest incidence at maturity.

When the isolated *Trichoderma* sp. was used at the inoculum density of 70 g/m^2, it did not significantly affect the disease severity during the seedling and flowering stages as compared to the control. Although better results were obtained at 140 g/m^2 compared to 70 g/m^2, the difference in disease incidence was not significant. No difference in disease incidence was detected at the seedling stage when either of the two *Trichoderma* spp. were at the higher inoculum density or when the seeds were treated with Vincit.

Table 1. Effect of the bioagents and the seed-dressing fungicide Vincit on the incidence of chickpea wilt (average of two seasons)

Treatment	Germination (%) *	Wilt incidence (% mortality)**at reading interval (weeks)		
		2	4	6
Fusarium oxysporum f. sp. *ciceris*+ Vincit	10.00a	1.08c	4.04b	4.04b
F. oxysporum f. sp. *ciceris* +*Trichoderma harzianum*	9.30b	1.08c	3.34b	4.34b
F. oxysporum f. sp. *ciceris* + *Bacillus* sp.	9.65ab	2.78b	4.50b	4.50b
F. oxysporum f. sp. *ciceris* + *Trichoderma* sp.	10.00b	3.60b	4.81b	4.81b
F. oxysporum f. sp. *ciceris*	10.00a	5.43a	6.57a	6.57a
Trichoderma harzianum	9.49ab	0.71c	0.71c	0.71c
Trichoderma sp.	9.66ab	0.71c	0.71c	0.71c
Bacillus sp.	10.00a	0.71c	0.71c	0.71c
Control	10.00a	0.71c	0.71c	0.71c
S.E. $\pm$	0.16	0.68	0.46	0.46
C.V.(%)	2.9	44.8	18.8	18.8

* Germination percentage values transformed to the arc sine

** Percentage values were transformed according to the formula $\sqrt{x+0.5}$.

Means having the same letter(s) within each column are not significantly different at P=0.05, according to Duncan's Multiple Range Test.

Table 2. Effect of the *Trichoderma* spp. (at different rates) and the fungicide Vincit on wilt incidence of chickpea at different stages of growth in the field

Treatment*	Germination (%)	Wilt incidence** (% mortality) at		
		Seedling	Flowering	Podding
Trichoderma harzianum 1	56.8a	2.50a	2.03b	1.73b
Trichoderma harzianuma 2	57.1a	2.23c	1.80b	1.00b
Trichodrma sp.1	51.1a	2.87ab	2.77a	1.70b
Trichodrma sp.2	52.6a	2.30bc	1.77b	1.33b
Vincit	46.9a	2.03c	1.93b	1.47b
Control	58.9a	3.40a	2.90a	3.63a
S.E. $\pm$	5.6	0.26	0.30	0.73
C.V. (%)	17.9	12.6	16.70	45.7

* Inocula of *Trichoderma harzianum* and *Trichoderma* sp. were used at the rates of 70 (1) and 140 (2) g/m^2. Vincit was used at 2 ml/kg seeds.

** Percentage values were transformed according to the formula $\sqrt{x+0.5}$.

Means having the same letter (s) within each column are not significantly different at P=0.05, according to Duncan's Multiple Range Rest.

DISCUSSION

The result of the pot experiment indicated that the wilt incidence was significantly reduced when any of the three antagonists or the seed-dressing fungicide Vincit was used. Among the antagonists, *Trichoderma harzianum* proved to be the best as it gave comparatively lower disease incidence. This result may be due to the very high growth rate of this fungus (Dennis and Wibster 1971), which might be expected to produce high inoculum density in the soil that leads, subsequently, to a great suppression in the growth of the pathogen. Similar results were obtained in Egypt with *Trichoderma harzianum* which was effective in controlling wilt in four chickpea cultivars (Hassanein *et al*. 1997). The results of this study are also in conformity with the findings of Prasad *et al*. (2002) who reported that soil application of T. *harzianum* and *T. viride*, one week before sowing, effectively reduced wilt and wet root rot of chickpea.

Bacillus sp. was as effective as the other two antagonists against the chickpea wilt disease, particularly after a month from planting. This result is in agreement with that of Hervas *et al*. (1997) who reported that treatment of seeds of a susceptible chickpea variety (ICCV-4) with an isolate of *Bacillus* sp. reduced the disease caused by the chickpea wilt pathogen.

The two *Trichoderma* species were as effective as the seed dressing fungicide Vincit in causing reductions of the wilt incidence on chickpea in the field. At the higher concentration (140 g/m^2), the two antagonists were effective in reducing the disease incidence, but they were less effective at the lower concentration (70 g/m^2). These results are in agreement with those reported by Castejon-Munoz and Oyarzum (1995) who stated that "the dose of the antagonists and the opportunities for competition may influence their effectiveness". The biological equilibrium between a biocide and other soil micro-flora, being in favour of the antagonist, leads to a satisfactory antagonistic effect against the pathogen (Deore and Savant, 2001).

In conclusion, more work is needed to determine the most effective amount of inoculum of each antagonist in reducing the disease incidence and also the proper method and time of its applications.

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المكافحة الحيوية لمرض الذبول الفيوزارمى فى نبات الحمص*

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موجز البحث: إستهدفت هذه الدراسة مكافحة مرض الذبول الفوزارمي *Fusarium oxysporum* فى محصول الحمص باستخدام الكائنات الدقيقة بالتربة ذات الفعالية في مكافحة مسبب المرض. كما هدفت أيضا إلى تفادي الأضرار المترتبة على استخدام المبيدات الكيميائية. اتضح من خلال الدراسة أن الفطر *Trichoderma* sp.، الذي تم عزله من منطقة جذور صنف الحمص المقاوم *ICCV-2*، والبكتيريا *Bacillus* sp. التي عزلت من منطقة الجذور وحواليها لنفس الصنف، لهما مقدرة عالية على مكافحة الفطر المسبب لمرض الذبول. كما أثبت الفطر *Trichoderma harizanum* ، الذي تم الحصول عليه من محطة بحوث الجيزة بجمهورية مصر العربية، فعاليته في مقاومة المسبب للمرض. لوحظ حدوث انخفاض معنوي في معدل إصابة الحمص بمرض الذبول عند زراعته في أصص تحتوي على تربة معاملة بأي من الكائنات الثلاثه أو حين معاملة بذوره بالمبيد الفطري فنست (Vincit) بمعدل 2 مللتر/كجم بذرة. وقد تفوق الفطر *T.harizanum* في مكافحة المرض حيث أدى استخدامه إلى أقل نسبة إصابة. وقد أوضحت النتائج التي تم الحصول عليها من تجربة بحقل محطة أبحاث الحديبة فعالية استخدام نوعي الفطر *Trichoderma* في خفض معدل الإصابة بالمرض حيث كانت النتائج مشابهة لنتائج استخدام المبيد الفطري فنست. وقد كان تأثير المعدل الأكبر للقاح (140 جرام/ متر^2) لنوعي الفطر *Trichoderma* فعالاً أثناء فترة نمو المحصول إلا أنه كان أقل فعالية في حالة المعدل الأقل للقاح (70 جرام/متر^2) خاصة في طور البادرة.

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