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BIOCONTROL OF *Fusurium oxysporum* F. SP*. CICERIS*, THE CAUSAL PATHOGEN OF WILT DISEASE IN CHICKPEA PLANTS BY RHIZOBACTERIA SEED TREATMENT.

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ABSTRACT

Three strains of rhizobacteria, namely *Azospirillium brasilense, Azotobacter chroococcum* and *Klebsilense pneumoneae* and commercial product HALEX were tested for growth promotion of chickpea plants and for controlling *Fusarium oxysporum* f. sp*. ciceris* the causal agent of fusarium wilt disease. In vitro, the rhizobacteria reduced the dry weight of mycelium of fusarium by 47.3-70.7%, and highest suppression was achieved by *K. pneumoneae* and the three mixture isolates. In-vivo significant decrease in disease severity was observed when chickpea seeds were coated with HALEX.This treatment also, increased fresh and dry weight /10 plants. Dry weight increased from 61.18, 26.19 to 79.43, 56.13 g in soil nontreated with the pathogen and from 38.49, 21.06 to 45.61, 27.13g in soil treated with pathogen on cv. PV 60 and cv. JG 62 respectively. Also, significant increases were observed in the number of seeds /100 pod and weight of 100 seeds. Weight of 100 seeds increased from 24.008, 9.59 to 28.73, 10.29 g in soil nontreated with the pathogen and from 17.91, 7.54 to 23.30, 10.007 g in soil treated with the pathogen on cv. PV 60 and cv. JG 62 respectively.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a major source of human and animal food and the world's third most important pluse crop after beans *(Phaseolus vulgris* L.) and peas (*Pisum staivum* L.) Navas *et al*., (2000). Fusarium wilt, caused by *Fusarium oxysporum* Schlectend; Fr. f sp. *cicris* (padwich) Matuo & K. Sato, is one of the most important soilborne diseases of chickpea throughout the world. Yield losses up to 10% have been arbitrated to Fusarium wilt in India, Spain, and up to 40% in Tunisia, Hervas *et al*., (1995). Wilt symptoms including flaccidity, yellowing, and vascular discoloration are induced by this pathogen. This disease was first found in India and it has since been reported in Burma, California, Ethiopia, Malawi, Mexico, Pakistan, Peru, Tunisia, Turkey and USSR, Trapero and Jimenez (1985). Numerous investigations have been conducted to develop system for effective utilization of microbial amendments for biocontrol of soilborne plant pathogens. Induced systemic resistance in plants by growth promotion or by antagonism to pathogens has bean demonstrated in chickpea and many other crops. Anjaiah *et al*., (1998) used *Pseudomonas aeruginosa* to suppressed wilt of chickpea. Landy *et al*., (1997) used *Pseudomonas chlororaphis* to inhibit mycelial growth of *F. oxysporum* f.sp. *ciceris*. Plant growth promoting rhizobacteria were defined by Klopper *et al*., (1980). A mixture of plant growth promoting rhizobacteria consisting of N_2-fixing *Azotobacter* spp. *Azospirillium* spp. and *Klebsiella* spp. has been developed by Hassouna (1973), and registered as HALEX. Hassouna and Aboul -Nasr, (1992) used HALEX

against soilborne plant pathogen in soybean plants. Aboul-Nasr *et al*., (1993) used plant growth promoting rhizobacteria against Fusrium root rot. Farfour (1995) used plant growth promoting rhizobacteria to control *Rhizoctonia* spp. and *Sclerotinia* spp. The objective of this study was focused on testing N_2 fixers *Azotobacter, Azospirillium, Klebsiella* isolates, and the commercial product HALEX, for their potential use in protecting chickpea against Fusarium wilt caused by *F.oxysporum* f. sp. *cicreris.*

MATERIAL AND METHODS

Source of rhizobacteria and HALEX:

Three growth promoting nitrogen fixing rhizobacteria, namely *Azospirillium barasilense, Azotobacter chroococcum,* and *Klebsiell*a pneumoneae, engineered for increased N_2 fixation, Aboul-Nasr *et al*., (1993) were chosen for this study, in addition to HALEX, a mixture of the above rhizobaceria and chemically inert talc powder, which served as a carrier. All the bacterial inocula were kindly provied by M. G. Hassouna. Plant Pathology Department. Faculty of Agriculture, Alexandria University.

Isolation from affected plants.

Fungus was isolated from roots and seeds of an infected chickpea plants. A single spore isolate of the fungus was maintained on potato dextrose agar (PDA). Isolates were identified according to Booth (1971), as *Fusarium oxysporum* f. sp. *ciceris*

Pathogenicity tests.

F. oxysporum f. sp. *ciceris* isolate was grown on sterilized barley grain in 250 ml flasks for 10 days. Inoculum was transferred from the flasks to autoclaved aerated potted soil at the rate of 4.5g/15cm pot, the inoculum was mixed with the soil, watered every day for 10 days, then sown with chickpea seeds at the rate of 10 seeds/pot. Two chickpea cultivar were used, JG 62 (desi type) characterized bye small, angular, colored seeds, and PV 60 (Kabuli type) characterized by large, white seeds. The seeds of these cultivars were obtained from International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. Ten replicates (pots) were used for each cultivar. In check treatment the seeds were sown in autoclaved soil in which no fungi were added. Number of wilted and dead plants were recorders after 28 days from sown.

In vitro assays:

A- Inhibition Zones:

PDA medium was poured into 9-cm diameter sterilized perti dishes. Two straight lines, 5 cm long and 3 cm apart, were streaked with a loopfull of the investigated bacterial suspension (Reddy & Patrick, 1991). The dishes were incubated at 30°C for 48 hours before the fungus (*F. oxysporum* f. sp. *ciceris*) was introduced as a disc, 0. 7 cm in diameter, in a central position between the two lines. The fungal growth was followed daily for 5 days. The

suppressive affects were visually assessed by comparing 10 dishes with their controls (i.e., no bacterial inoculation). The reactions were scored as (-) no suppression (= no inhibition zone), (+) minimal, (++) moderate, and (+++) high suppression (i. e., clear inhibition zone), Farfour (1995).

B- Fungal dry weight:

Fifty milliliters of PD medium were poured into each of five 100 ml conical flasks. The medium was sterilized subsequently, inoculated with cell suspensions of the rhizobacteria individually. Inoculum density was approximately 1.6 x 10^8 cells/flask. A set of flasks was left uninoculated to serve as a control. All flasks were inocubated at 30 °C for 48 hours and then were inoculated with 7 day old fungal culture as 0.7 cm diameter disk to each flask. Each of the three rhizobacteria and HALEX were tested against the fungus. Flasks were incubated at 30°C for 7 days, and the hyphal mat of each flask was obtained by filtration, dried at 50 °C overnight, and placed in a desiccator. The dry weight was then recorded.

Field experiment :

The experiment was carried out in the experimental farm, College of Agriculture and Veterinary Medicine, Buriedeh, Saudi Arabia during 2000 and 2001 growing seasons. The field plot 3x5 meters was divided into twelve rows. Four plots were used, two plots were received the pathogen, and each plot sown with one cultivar. The other plots were used as a control without the pathogen. Two chickpea cultivars JG 62 and PV 60 were tested. The isolated fungus *F. oxysporum* f. sp*. ciceris* was grown on autoclaved barley grains for 10 days and then applied to the soil at the rate of 4.5 gram dried inocula /row. Seeds of the two cultivars were slightly moistened and completely dressed with the HALEX powder at the rate of 7 g/kg seeds. The treatments were : 1) rows with non- seed treatment, 2) rows with seed coated with HALEX and, 3) rows with seeds coated with vitavax fungicide at the rate of 3 g/kg seeds. Four replicates were used. Rows were sown with seeds at the rate of 10 seeds/row. Fresh, dry weight, disease-severity, number of seeds/pod and weight of 100 seeds to each cultivar were recorded. The obtained data were statistically analyzed using a computer (SAS) Statistical Analysis System (SAS Institute,1988).

RESULTS AND DISCUSSION

Utilization of indigenous microbial populations for protection against soil-borne pathogens has been increasingly investigated. Growth promotion and greater yields cbe achieved by plants escaping the attack of soil-borne pathogens, using the three plant growth promoting N_2 – fixing rhizobacteria and the commercial product HALEX (Reddy & Patrick, 1991). The PDA medium test showed that the 5-day old fungal growth completely covered the control plates, while the area of the fungal growth was restricted in plates streaked with the rhizobacteria used, showing zones of inhibition. Table 1 shows the suppressing effect of rhizobacteria on fungal growth on PDA medium. The effect of rhizobacteria on fungal mycelial mat (dry weight) of *F.*

oxysporum f. sp. *ciceris*, was studied and the data in table 2 showed that the reduction of fungal growth was reduced to 47.31% for *A. brasilens*, 51.75% for *A. chroococcum*, 64.1% for *K. pneumoneae* and 70.72% for mixture rhizobacteria, compared with the control. The reduction of fungal mycelial mat may be due to rhizobacteria products. Khot *et al.,* (1996) suggested that the rhizobacteria produced chitinase β-1,3-glucanse and siderophores which may be involved in suppression of plant diseases and inhibition growthof fungal, which are known to cause chickpea wilt.

In pathogenicity tests and field experiments several symptoms were found affecting chickpea plants, which were designated as wilt. Wilt symptoms included foliar yellowing, dry collor, root rot and dead plants. Simillar symptoms were repoted by Trapero and Jimenez (1985).

Table 1: Suppression effect of rhizobacteria on *F. oxgsporum* f. sp. *ciceris* grown on PDA medium.

Rhizobacteria	Level of suppression
Azospirillium brasilense	(++)
Azotobacter chroococcum	(++)
Klebsiella pneumoneae	(+++)
A.brasilense + A. chroococcum	(++)
A.brasilense + K. pneumoneae	(+++)
A.chroococcum + K. pneumoneae	(+++)
Mixture*	(+++)
Control	(-)

Level of suppression: (-) no suppression, (+) minimal, (++) moderate , (+++) high.
***Mixture = *A. brazilense + A. chroococcum + K. pneumoneae*.**
Control = no rhizobacteria

Table 2: Dry weight of the fungal mycelial mat and reduction precent as affected by rhizobacteria.

Rhizobacteria	Dry weight (g)	Reduction (%)
Control	0.5646 A	0.0
Azospirillium brasilense	0.2975 B	47.31
Azotobacter chroococcum	0.2724 B	51.75
Klebsiella pneumoneae	0.2028 C	64.1
Mixture*	0.1653 C	70.72

Data are based on average of five replicates.
Mixture = *A. brasilense + A. chroococcum + K. pneumoneae
Mean in a column followed by a common letter are not significantly different according o Duncan's multipe range test.

Fusarium wilt caused by *F. oxysporum* f sp. *ciceris* reduced chickpea yield by increasing disease severity and decreasing fresh , dry weight, seed yield and seed weight. Tables 3 and 4 showed that significant decrease in disease severity occurred when chickpea seeds were coated with rhizobacteria (HALEX). Disease severity decreased from 4.0 to 3.2 in a large seeded Kabuli chickpea (cv. PV 60), and in small – seed desi chickpea (cv. JG 62), as a result of fusarium soil infestation.

Table 3: Effect of seed inoculaion with HALEX and *F.oxysporum* f. sp. *ciceris* on plant growth parameters of kabuli type chickpea

plants (cv.PV 60)

Treatment	Disease severity	Fresh weight* (g)	Dry weight* (g)	Number of Seeds/ 100 pod	Weight of 100 Seeds (g)
Control (soil and seed, non treated)	1.2 C	235.44 C	61.180 C	103.3 D	24.008 D
Soil non treated and seed treated with HALEX	1.4 C	344.72 A	79.438 A	126.0 B	28.730 C
Soil non treated and seed treated with fungicide	1.4 C	305.90 B	72.572 B	131.3 A	34.909 A
Soil infested with *F.oxysporum* f.sp. *ciceris*, and seed non treated	4.0 A	164.05 E	38.491 F	87.0 E	17.915 E
Soil infested with with *F.oxysporum* f. sp. *ciceris*, and seed treated with HALEX	3.2 B	176.38 D	45.610 D	121.8 C	23.305 E
Soil infested with with *F.oxysporum* f. sp. *ciceris*, and seed treated with fungicide	2.8 B	172.64 D	43.716 E	119.8 C	31.012 B

***Fresh and sry weight/10 plants after 2 month from sowing.**

- **Mean in a column followed by a common letter are not significantly different according to Duncan's multiple range test**

The obvious, results indicate that rhizobacteria decrease the disease severity of *F. oxysporum* f. sp *ciceris*. Similar results were reported by khot *et al.*, (1996), they found that rhizobacteria reduced the wilt of chickpea caused by *F.oxysporum* f sp. *ciceris* under field condition. Also, Tables 3 and 4 showed the significant increase in fresh and dry weight of chickpea seeds when treated with rhizobacteria (HALEX). The number of seeds/100 pod and weight of 100 seeds increased from 87.0 to 121.8 seeds, from 17.915 to 23.305 g respectively in cv. PV 60, and from 92.0 to 121.25 seeds, from 7.547 to 10.007 g respectively in cv. JG 62 in soil treated with the pathogen. While in the soil nontreated with the pathogen the number of seeds increased from 103.3 to 126.0 seeds and weight of 100 seeds increased from 24.008 to 28.730 g in cv. Pv 60. While in cv. JG 62 the number of seeds increased from 105.25 to 135.75 seeds and the weight of 100 seeds increased from 9.590 to 10.297 g similar results were reported by De *et al*., (1996) they found significant increase in seed yield of chickpea when seed were coated with biocontrol agent and vitavax fungicide. It can be concluded that the mentioned growth promoting N_2- fixing rhizobacteria and HALEX succeeded in controlling the *F. oxysporum* f. sp. *ciceris* by decrease disease severity and increase yield. These results are achieved while hopefully, avoiding the use of ferilizers to produce fresh food uncontaminated with pesticides to encourage propagating and augumenting biocontrol of soil-borne fungi in crop rhizospheres.

Table 4: Effect of seed inoculation with HALEX and *F.oxysporum* f. sp. *ciceris* on plant growth parameters of desi type chickpea plants (cv.JG62)

Treatment	Disease severity	Fresh weight* (g)	Dry weight* (g)	Number of Seeds/ 100 pod	Weight of 100 Seeds (g)
Control (soil and seed, non treated)	1.2 D	152.92C	26.193 C	105.25 E	9.590C
Soil non treated and seed treated with HALEX	1.2 D	278.25A	56.130 A	135.75 A	10.297B
Soil non treated and seed treated with fungicide	1.2 D	195.87B	45.275 B	132.0 B	11.456A
Soil infested with *F.oxysporum* f.sp. *ciceris*, and seed non treated	4.0 A	94.47F	21.068 E	92.0 F	7.547C
Soil infested with *F.oxysporum* f.sp. *ciceris*, and seed treated with HALEX	3.2 B	105.61D	27.136 C	121.25 D	10.007BC
Soil infested with *F.oxysporum* f.sp. *ciceris*, and seed treated with fungicide	2.4 C	101.72E	24.329 D	127.0 C	10.493B

***Fresh and dry weight/10 plants after 2 month from sowing.**

- **Mean in a column followed by a common letter are not significantly different according to Duncan's multiple range test**

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المقاومـة الحيويـة للفطر *Fusarium oxysporum* f. sp. *ciceris* المسبب لمرض ذبول الحمص بواسطة معاملة البذور بالريزوبكتيريا

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استخدمت ٣ عزلات من الريزوبكتيريا و المسماه بالـ *Azospirillium brasilense* و*azotobacter chroococcum* و *klebsilense pneumoneae* وكذلك المنتج التجارى المسمى بالهاليكس (وهو خليط من الثلاث عزلات السابقة) لأختبار قياس زيادة نمو النباتات ومقاومتها للفطر *Fusarium oxysporum* f. sp. *Ciceris* الذى يهاجم نباتات الحمص مسبباً لها مرض الذبول الفيوزاريومى. ومن النتائج المعملية اختزلت عزلات الريزوبكتيريا الوزن الجاف لميسليوم الفطر بمعدل ٤٧,٣-٧٠,٧% وكذلك لوحظ أن الـ *K.pneumoneae* وكذلك خليط الثلاث عزلات السابقة اعطت تأثير مثبط عالى على الفطر تحت الأختبار. ومن النائج الحقلية كان هناك نقص معنوى فى شدة الإصابة عندما عوملت بذور الحمص بالهاليكس. وكذلك حدوث تأثير معنوى بزيادة الوزن الجاف والرطب لكل ١٠ نباتات حمص. فالوزن الجاف زاد من ٦١,١٨ و ٢٦,١٩ إلى ٧٩,٤٣ و ١٦,١٣ جرام وذلك فى التربة الغير معاملة بالفطر الممرض وكذلك زاد الوزن الجاف من ٣٨,٤٩ و ٢١,٠٦ إلى ٤٥,٦١ و ٢٧,١٣ جرام فى التربة المعاملة بالفطر الممرض وذلك لكل من الصنف PV٦٠ والصنف JG ٦٢ على الترتيب. أيضا كان هناك زيادة معنوية فى عدد البذور لكل ١٠٠ قرن وكذلك وزن ١٠٠ بذرة. فوزن ١٠٠ بذرة زاد من ٢٤,٠٠٨ و ٩,٥٩ إلى ٢٨,٧٣ و ١٠,٢٩ جرام فى التربة الغير معاملة بالفطر الممرض وكذلك زاد من ١٧,٩١ و ٧,٥٤ إلى ٢٣,٣٠ و ١٠,٠٠٧ جرام فى التربة المعاملة بالفطر الممرض وذلك لكل من الصنف ٦٠ PV والصنف JG٦٢ على الترتيب.