BIOLOGICAL CONTROL AND INDUCED SYSTEMIC RESISTANCE IN CHICKPEA *CICER ARIETINUM* L. AGAINST *FUSARIUM OXYSPORUM* F.SP. *CICERI*

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Abstract

Bio-control agents, Bio Root Care (BRC), *Trichoderma harzianum*, *Pseudomonas flourescens*, and Non-pathogenic *Fusarium oxysporum* (N*Fo*) showed clear inhibition of *Fusarium oxysporum* f.sp.ciceri (*Foc*) growth on artificial medium. *T.harzianum* significantly (p = 0.05) scored the highest rate of growth inhibition, 59.7% compared with BRC, N*Fo*, *P.flurescens*, *R.leguminisarum* treatments. Seed or soil treatments with local *T.harzianum* and *P.flurescens* showed clear ability to control chickpea wilt disease by significantly reducing the disease incidence compared with the commercial, *T.harzianum* and *P.flurescens* products and with control. While seed and soil treatment with the local *T.harzianum* and *P.flurescens* caused 7 and 9 disease incidence, 7 and 12 % respectively. The commercial products, *T.harzianum* and *P.flurescens* caused 16 and 19 ,18 , 19 % respectively. Systemic induced resistance in chickpea plants against *Foc* was achieved by N*Fo*, BRC, *R.leguminisarum* with soil and seed treatments. Soil treatments with BRC, N*Fo* and *R.leguminisarum* caused significantly less disease incidence, 11 , 16 and 39 % respectively, and 8, 12 and 41% when seed treatment respectively compared with 69 % for the control treatment(*Foc*). In field experiment, treatment with bio control agents, BRC, local and commercial *T.harzianum*, *P.flurescens* and *R.leguminisarum* significantly reduced wilt disease incidence compared with control treatment. The most effective treatment was BRC, 13 % disease incidence compared with 45 % in control treatment. The test bio control agents increased height, wet and dry weights and yield of chickpea plants. The greatest height of chickpea plant 38.2 and 38.3cm were recorded for BRC and *T.harzianum* + *P.fluprescens* treatments. Seed treatment with BRC scored the highest average plant weights, 41.4 and 13.4 g respectivly. BRC and *T.harzianum* + *P.fluprescens* treatments were significantly superior over *T.harzianum*, *P.fluprescens* (local and commercial product) and *R.leguminisarum* in yield weight 342 and 346 g m^{-2} respectively.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops worldwide. The global chickpea area was about 11 million ha to produce 8.8 million tons with an average yield of nearly 800 kg ha^{-1} (17). Chickpea importance is due to it's nutrition role in human food and soil fertility. Iraq is producing about 1% of the world production of chickpea (17). However, the cultivation of chickpea in Iraq is still limited because of the low yield of the crop, not mechanically harvested and the high susceptibility to pathogens. Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Matu & Satu (*Foc*) was found in more than 33 countries is considered as one of the most important diseases which limit chickpea production (32).The high incidence of chickpea Fusarium wilt was often observed in Nineveh province, Iraq. The increased demands for food prompted the use of chemical pesticides and fertilizers which achieved part of this aim. But because of the negative effects of these chemicals on the environment, human and animal health and its high coast and the emergence of resistance in the pathogen, researchers has focused on alternative control methods such as biological control and induced systemic resistance in plant against many plant pathogens (23).The genus *Trichoderma* was considered as one of the most important bio control fungus for soil, seed born and other pathogens (10,11). Seeds of chickpea treated with *T. harzianum* reduce Fusarium wilt incidence by 25-27% and increased yield by 13% (24). Recent study indicated that *T.harzianum* and *T.viridi* was able to inhibit *Foc* growth and reduce wilt disease incidence under field conditions and increase seed germination, root length and shoot height (14). *Pseudomonas fluorescens* has a major role as bio control of soil born pathogenic fungi, producing several compounds which effects plant pathogens like phenazin (40,18), pyrolnintrin (7), phloroglucinol (20) and chelating agent (35). Recent reports indicated that chickpea seeds treated with *P.fluorescens* before inoculation with *Foc* showed reduced wilt disease incidence and increased yield (24, 22, 41).

Induced systemic resistance was reported against different plant pathogens since the thirties of the last century (9). Numerous stud -ies have confirmed the ability of non pathogenic *Fusarium* to suppress Fusarium wilt (36, 38, 33, 26, 37).Chickpea seedling

treated with *Rhizobium* isolates led to reduce wilt disease incidence and increased plant height, weight and yield of chickpea plant (3, 4, 2, 5).

This study was undertaken to evaluate the bio control and induced systemic resistance ability of different bio agents in chickpea plants against *Fusarium oxysporum* f. sp. *ciceri.*

MATERIALS AND METHODS

Biological Materials

Chickpea Plant

Chickpea, *Cicer arietinum* L. cv "Marakishi" susceptible to *Foc* was obtained from the local market and was used in the experiments.

Fusarium oxysporum* f.sp. *ciceri

This pathogen was isolated from chickpea plants with the characteristic Fusarium wilt symptoms from Nenivah prov- ince (400 km north of Baghdad, Iraq) and propagated on potato dextrose agar medium. The fungus was stored in autoclaved soil at 4 C and used in the experiments.

Non Pathogenic *Fusarium oxysporum*

A non pathogenic *F.oxysporum* isolate (N*Fo*) was isolated from chickpea plants from Nenivah province. This isolate caused no disease symptoms on inoculated susceptible chickpea cv Marakishi.

***Rhizobium leguminisarum* (*R.l*)**

This *R.l* isolate was obtained from the Integrated Management of Plant Production and Prote- ction Center, Plant Protection Directorate, Ministry of Agriculture, Baghdad, Iraq. This isolate was originally isolated from chickpea plants with active bacterial nodules.

Bio-Root Care (BRC)

BRC bio pesticides (Dr. Ragan laboratories, Chennal, India) is a mixture consisting of various bio control agents such as: *P. flourescens, Trichoderma viride, T. harzionum, Bacillus subtilis* and *Paceilomyces lilacinus.*

Pseudomonas fluorescens

Local isolate of *P.fluorescens* was obtained from Dr. Audai Najem (Department of plant protection, college of agriculture, university of Baghdad). Commercial product of this bacterium was obtained from Dr.Ragan laboratories, Chennal, India.

Trichoderma harzianum

local isolate of *T. harzianum* was obtained from DR. Hadi M. Abood (Ministry of technology and sciences, Baghdad, Iraq). Commercial product of *T. harzianum* was optained from Dr. Ragan laboratories, Chennal, India

In vitro* evaluation of bioagents against *Fusarium oxysporum* f.sp. *ciceri

P. fluorescens, *T. harzianum*, *R. leguminisarum*, N*Fo*, and the bio pesticide BRC were used to evaluate their antagonistic activity against *Foc*. Each antagonist and the pathogen were simultaneously inoculated at the opposite ends of 9cm diam. Petri dishes containing Potato Dextrose Agar (PDA). Discs of 5 mm diam from actively growing culture of *Foc* and *T. harzianum* , N*Fo* and 50µl of BRC (5g l^{-1}) was placed at equal distance from the ends of PDA plates. *P. fluorescens* and *R. leguminisarum* were streaked on one side of *Foc* inoculated plates. Each test was replicated three times and inoculation with *Foc* only served as control. Diameter of the pathogen was measured after incubation at 25C (23).

Bio control of *Foc*: Seed treatment with antagonists

Seeds were surface sterilized with 2% sodium hypochlorite solution for 10 min, and washed with sterile distilled water. Seeds (10g) were then thouroghly mixed with 1ml of local *P. fluorescens* ($1x10^8$ CFU ml^{-1}) and local *T.harzianum* (10^6 spores ml^{-1}) and 5g kg $seed^{-1}$ for the commercial products. One % carboxymethyl cellulose was added as sticking agent. Loamy sand soil was used for the experiment. Sterilization of the soil was done at 121C at 1.5 kg cm^{-2} for 30 min 2days prior to sowing and later on was mixed with 100 ml of $1x10^3$ spore ml^{-1} kg $soil^{-1}$ *Foc*. Three treated seeds of chickpea were sown in 18 cm diam pots filled with inoculated soil. Each treatment was replicated three times. Untreated seeds were used as control. Wilt incidence, was recorded 30 days after sowing.

Soil treatment with antagonists

Autoclaved soil in pots were inoculated with 100 ml of $1x10^3$ spore ml^{-1} kg $soil^{-1}$ *Foc* 2 days prior to inoculation with antagonists. The inoculums 100 ml kg $soil^{-1}$ of local *P. fluorescens* ($1x10^8$ CFU g $soil^{-1}$) and *T.harzianum* (10^6 spore.g $soil^{-1}$), 5g kg $soil^{-1}$ for commercial products was mixed with soil. Three treated seeds of chickpea were grown in pots (18 diam) filled with inoculated soil. Treatments were replicated three

times and untreated seeds were used as control. Wilt incidence, was recorded 30 days after sowing.

Induced resistance in chickpea plants against *Foc*

Chickpea seeds were surface sterilized with 2 % sodium hypochlorite solution for 10 min, and washed with sterile distilled water. Seeds then were planted in plastic pots (18cm diam, 3 seeds pot^{-1}) and maintained in a plastic house. When seedlings were 30 days old, the soil was treated with 100ml of 1×10^6 spores ml^{-1} of N*Fo*, 1×10^8 spores ml^{-1} of *R.l* and BRC at 5g kg $soil^{-1}$. After 24 hours the pots were treated with spore 1×10^3 ml^{-1} spores suspension of *Foc*. Soil treated with N*Fo* , *R. leguminisarum*, BRC, *Foc* and intact healthy plant were control treatments.

Bioassay under field conditions

Eight experimental units, 10 × 25 m were chosen in chickpea cultivated field in Alkosh, Nenivah province, known to encounter Fusarium wilt disease in previous seasons. Chickpea seeds, pretreated with 100ml kg $seed^{-1}$ suspension of local *P. fluorescens*, *R. leguminisarum* (10^8 cfu ml^{-1}) and *T. harzianum* (10^6 spore ml^{-1}) and 5g kg $seed^{-1}$ of the commercial products (*P. fluorescens*, *T. harzianum* and BRC) were planted in the experimental units. Wilt incidence, shoot length, wet and dry weight of plant was recorded.

RESULTS

In vitro* evaluation of bio agent against *Foc

All the test biocontrol agents inhibited the growth of the pathogenic fungus *Foc* (Table 1). *T.harzianum* caused significantly (P = 0.05) the highest inhibition percentage, 59.7% of *Foc* compared with other test bio control agent. This was followed by 53.3 and 52.0% inhibition when *P.fluoresens* and N*Fo* were used respectively. The latter treatments were significantly different compared with BRC and *R.leguminisarum* treatments which recorded 32.6 and 35.3 % inhibition of *Foc* respectively.

Table 1. Effect of bio agents on *Fusarium oxysporum* f.sp.*ciceri* (*Foc*) growth on PDA 7 days after inocubation at 25C

Treatment	Grow inhibition (%)
Bio-Root care + *Foc*	32.6
N*Fo* + *Foc*	52
T.harzianum + *Foc*	59.7
P. fluorescens + *Foc*	53.3
R. leguminisarum + *Foc*	35.3
Foc (Control)	0

LSD (P = 0.05) = 4.8

Discs of 5 mm diam. of *Foc* and *T. harzianum* , N*Fo* and 50µl of BRC was placed at equal distance from the periphers of PDA plates. *P. fluorescens* and *R. leguminisarum* were streaked to one side. Each test was replicates three times. N*fo* = non pathogenec *Fusarium oxysporum*.

Bio control of Fusarium wilt

Results revealed the ability of *P.fluoresens* and *T.harzianum* to significantly (P = 0.05) reduce chickpea wilt incidence compared with the control treatment for both seed and soil treatments (Table 2). Treatment chickpea seeds with the local isolates of *P.fluoresens* and *T.harzianum* caused significantly More disease control compared with the commercial product of these isolates. The disease incidence was 7 and 9 % when local *P.fluoresens* and *T.harzianum* were used respectively , while it was 16 and 19 % for commercial product respectively. Treatment of both the local and commercial *P. fluoresens* and *T.harzianum* significantly reduced wilt disease incidence compared with control treatment (*Foc* only) which recorded 62 % disease incidence (Table2). Soil treatment with the local *T.harzianum* and *P. fluoresens* caused significantly (p = 0.05) reduced disease incidence of 7 and 12 % respectively compared with 18 and 19 % for the commercial product of the bio control agent respectively.

Table 2. Bio control of Fusarium wilt in Marakishi chickpea, *Cicer arietinum* L.

Soil treatment	**Disease incidence (%)**
T. harzianum local	7
P. fluorescens local	12
T. harzianum (commercial product)	18
P. fluorescens (commercial product)	19
Control (*Foc* only)	62
LSD (P = 0.05) for treatment = 5.9	
Seed treatment	**Disease incidence (%)**
T. harzianum (local)	7
P. fluorescens (local)	9
T. harzianum (commercial product)	16
P. fluorescens (commercial product)	19
Control (*Foc* only)	62

LSD (P = 0.05) for treatment = 4.8

Numbers represent three replicatess. 100 ml kg soil^{-1} of *Foc* spore suspension was added (10^3 spore ml^{-1}) 2 days after local *P.fluoresens* (10^8 spore ml^{-1}), (100ml kg soil^{-1}), and 50 g kg soil^{-1} of local *T.harzianum* propagated on millet, 5g kg soil^{-1} of commercial products were added.Sowing was 2 days after treatments. Seeds treated with 1ml 10g seed^{-1} of *P.fluoresens* (10^8 spore ml^{-1}) and (10^{-6} spore ml^{-1}) of *T.harzianum* and 5g kg seed^{-1} of commercial products, planted in pots contain soil inoculated with 100ml of *Foc* was added (100ml kg soil^{-1}).

Induced systemic resistance in chickpea plants

Induced systemic resistance (ISR) of chickpea plants was achieved when soil was treated with bio agent BRC, N*Fo* and *R. leguminisarum* before *Foc*. This was indicated as reduced wilt disease incidence in bio agent treatements (Table 3). The disease incidence was 11 and 16% when soil was treated with BRC and N*Fo* respectively with significant (p=0.05) difference compared with 39% for *R. leguminisarum*. Similarly ISR was observed in chickpea when soil was treated with the bio agents (Table 3).

Field experiment

Seed treatments with BRC, *T.harzianum*, *P. fluorescens* local and commercial product, *R. leguminisarum*, was effective in reducing wilt disease incidence in chickpea plants significantly (p =0.05) compared with control treatment (Table 4). BRC was the most effective treatment recorded disease incidence 13% which is significantly difference compared with the other rest of treatment (*T.harzianum*, *P. fluorescens* local and commercial product, *R. leguminisarum*). This was followed by local *T.harzianum*, *P. fluorescens* and commercial

Table 3. Bio control and induced resistance in chickpea, *Cicer arietinum* L. against *Fusarium oxysporum* f.sp.*ciceri* (*Foc*)

Soil treatment	**Disease incidence (%)**
Bio-Root Care	11
Nonpathogenic *Fo* (N*Fo*)	16
R. leguminisarum	39
Foc (Control)	62
LSD (P =0.05) = 7.4	
Seed treatment	**Disease incidence (%)**
Bio-Root Care	8
Nonpathogenic *Fo* (N*Fo*)	12
R. leguminisarum	41
Foc (Control)	62

LSD (P =0.05) = 5.5

Numbers represent three replicates. Bio agents (100 ml kg soil^{-1} as follows: N*Fo* (10^6 spore ml^{-1}), *R. leguminisarum* (10^8 spore ml^{-1}), BRC (5g kgl^{-1}) were added 7 days after sowing. Seeds were similarly treated with the bio agent. When plants were 2 – 3 leaves, 100 ml kg soil^{-1} of spore suspension of *Foc* was added (10^3 spore ml^{-1}). Wilt disease incidence was recorded 15 days after inoculated with *Foc*. Numbers represent four replicate, each replicate is 10 plants in Alkosh, Nenivah province. Chickpea seeds were pre-treated with 100 ml kg seed^{-1} of local *P. fluorescens*, *R. leguminisarum* (10^8 cfu ml^{-1}) and *T. harzianum* (10^6 spore ml^{-1}) and 5g kg seed^{-1} from the commercial product (*P. fluorescens*, *T. harzianum* and BRC). Wilt incidence was recorded at the end of the growing season.

Table 4. Effect of bio agents on Fusarium wilt disease incidence in chickpea, *Cicer arietinum* L. under field conditions

Treatment	Disease incidence (%)
Bio-Root care (BRC)	13
T.harzianum (local)	20
T.harzianum (commercial product)	24
P. fluorescens (local)	21
P. fluorescens (commercial product)	22
T.harzianum + *P. fluorescens*	12
R.leguminisarum	30
Control	45

LSD (p =0.05) = 5.8

product treatment which was recorded significantly difference disease incidence of 20 and 24 % respectively compared with *R. leguminisarum* treatment which reorded infection rate 30%. Treatments with bio agent used in this study caused increased plant growth (Table5). The maximum average of plant height was observed when chickpea seeds treated with BRC, *T.harzianum* + *P. fluorescens* (local and commercial product) and *R.leguminisarum.* The plant height was 38.2 and 38.3cm with BRC and *T.harzianum* + *P. fluorescens* respectively (Table 5). Other treatment with bio agent did not show significant differences which ranged between 34.2 and 36.1cm in commercial product treatment of *P. fluorescens* and *T.harzianum* respectively. All treatments outperformed the control treatment which recorded 28.3cm. All the test bio agent increased the wet and dry weight of the plant. The highest significant increased (p =0.05) of wet and dry weight was 41.4 and 13.4g respectively with BRC treatment compared with other test treatment (Table 5). Other test bio agent treatment did not cause significant differences in wet and dry weight. The wet and dry weight of these treatments ranged between 30.1 and 35.3g for wet weight, and 10.6 to11.4g for dry weight. All treatments outperformed the control treatment which recorded 23.7 and 7.4g for wet and dry weight respectively. The bio agent affected yield, significantly (p = 0.05) maximum yield weight was 342 and 346

g m^{-2} when BRC and *T.harzianum* + *P. fluorescens* treatment were used respectively compared with 292 and 294.3 for local *T.harzianum*, *P. fluorescens* and with 287.4 , 277.2 g for commercial *T.harzianum*, and *P. fluorescens* products and with 289.5 for *R.leguminisarum* treatment (Table 5). No significant differences were observed between *T.harzianum*, *P. fluorescens* (local and commercial product) and *R. leguminisarum* treatment which ranged between 277.2 and 289.5g m^{-2} with *P. fluorescens* and *R.leguminisarum* treatments respectively. All treatment with the bio agent outperformed the control treatment which was 233.8 g m^{-2}.

Table 5. The influence of bio agents on chickpea, *Cicer arietinum* L. growth and yield under field conditions

Treatment	Plant height	Wet weight (g)	Dry weight (g)	Yield weight (g m-2)
Bio-Root Care	38.2	41.4	13.4	342
T.harzianum (local)	35.8	31.6	10.6	292
T.harzianum (commercial product)	35.1	31.1	10.7	287.4
P. fluorescens (local)	36	33.6	11.5	294.3
P.fluorescens (commercial product)	34.2	30.1	10.7	277.2
T.harzianum + *P. fluorescens*	38.3	35.3	11.4	346
R.leguminisarum	36.9	34	11.4	289.5
Control	28.3	23.7	7.4	233.8
LSD (P = 0.05)	1.8	5.5	2.5	45.4

Numbers represent four replicate, each replicate is 10 plants in Alkosh, Nenivah province. Chickpea seeds were pre-treated with 100 ml kg $seed^{-1}$ of local *P. fluorescens*, *R. leguminisarum* (10^8 cfu ml^{-1}) and *T. harzianum* (10^6 spore ml^{-1}) and 5g kg $seed^{-1}$ from the commercial product (*P. fluorescens*, *T. harzianum* and BRC).

DISCUSSION

Result of this study indicated the ability of bio agent used in this study to inhibit *Foc* growth on artificial medium and confirmed, previous studies on the ability of *Trichoderma* isolates to inhibit *Foc* growth (14, 30, 28). Other studies, reported on the ability of *P. fluorescens* and *Rhizobium* to inhibit *Foc* growth for more than 30 - 40% in dual culture test (4, 2, 23, 42). The superiority of the local *T.harzianum* and *P. fluorescens* in reducing wilt disease incidence on chickpea plants compared with the commercial products of these microorganisms may be due to the adaptation of the local isolate to environmental condition or to their directly use after propagation without passing through storage duration. Result of this study support previous studies on the ability of *T. harzianum* to *in vitro* suppress *Foc*, and control of the disease under greenhouse and field conditions (34). Treatment of chickpea seeds with *Trichoderma* isolates under greenhouse condition caused reduced wilt disease incidence, increased plant height and wet and dry weight (28). The disease incidence of chickpea wilt was reported to be reduced to about 40% after treatment with *T. harzianum* (29). Similarly seed treatment with *P.fluorescens* led to control chickpea wilt disease and reduce disease incidence under green- house and field conditions and increased chickpea yield (22, 24, 23, 41). Furthermore, the use of *P.fluorescens* against chickpea wilt disease led to delayed development of the disease symptoms (25). The bio control agents were known to suppress pathogens directly through parasitism, antibiosis and lytic enzymes and indirectly through nutrient competition, element chelating and induced plant resistance (31).

Results of this study confirmed the ability of BRC, N*Fo* and *R.leguminisarum* when exceeded *Foc* inoculation to reduce wilt disease incidence in chickpea plant. This is may be resulted by stimulating the systemic resistance in chickpea plants through the production of enzymes and phenolic compounds, promote plant growth and the strengthening of physical defensive barriers. This study indorse the results of previous studies stating that seeds or seedling of leguminous crop treated with *Rhizobium* caused significant reduction in some root diseases caused by soil born fungi (8, 12). Mechanism of resistance in the plant is activated as a result of *R.leguminisarum* and N*Fo* tratment due to production of phytoallexin by roots, biotoxins and pathogenesis related proteins, PRP (39, 15). Chickpea seedlings treated with *Rhizobium* isolates before *Foc* was found to increase phenylalanine ammonia lyse, chalcon, isoflavon reductase, peroxidase, poly phenol oxidase and phenolic

compound production (4, 2,5). Chickpea seedlings treated with N*Fo* led to reduce wilt disease incidence and disease severity caused by *Foc* (19).

Result of bio control chickpea wilt disease under field condition was similar to the results of the plastic house. The bio agent used in this study BRC, *T.harzianum*, *P.fluorescens*, *T.harzianum* + *P.fluorescens* (local and commercial product) and *R . leguminisarum* were able to bio control chickpea wilt disease. The successful bio control of wilt disease incidence was reflected on the increased plant height, wet and dry weight and yield of chickpea plants. Results of this study confirmed that of previous studies which indicated that chickpea seeds treatment with bio agent caused decreased Fusarium wilt disease incidence (24, 21, 1, 6). Soil treated with *T.harzianum*, *T.koningii*, *T.pseudokoningii* delayed disease appearance and reduce disease severity (27). The increase of some growth and yield criteria could be due to nitrogen fixation by free living associated and symbiotic microorganisms with plant roots. These microorganisms have the ability to transform not available elements to dissolved elements available for plants (43). These microorganisms could regulate the action of hormone and other compounds which are responsible for the growth and development of plant. Bacteria were able to produce plant hormone like auxins, ethylene, gibberellins and cytokinine (16).

Further research is needed to establish a successful and bio control alternatives and decreased the demands on chemicals.

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المكافحة الاحيائية وتحفيز المقاومة الجهازية في الحمص (*Cicer aratinum* L.) ضد الفطر *Fusarium oxysporum* f.sp. *ciceri*

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أظهرت العوامل الإحيائية (Bio-Root Care ,BRC و *Trichoderma harzianum* و *Pseudomonas fluorescens* و *Rhizobium leguminisarum* و عزلة غير ممرضة من *Fusarium oxysporum* ,N*Fo*) فعالية واضحة في تثبيط نمو الفطر *Fusarium oxysporum* f.sp.ciceri (*Foc*) فقد بلغ أعلى معدل لتثبيط نمو *Foc* عند استخدام *T.harzianum* إذ كانت نسبة التثبيط ٥٩.٧% وبفارق معنوي عن باقي المعاملات (BRC وN*Fo* و*P.fluorescens* و*R. leguminisarum*). أظهرت النتائج قدرة *T.harzianum* و *P.fluorescens* المحليين على مكافحة مرض ذبول الحمص من خلال خفض نسبة الإصابة معنويا مقارنة مع معاملة المستحضرين التجاريين *T.harzianum* و *P.fluorescens* ومعاملة السيطرة سواء عند معاملة البذور أو التربة. فقد كانت نسبة الإصابة ٧ و٩% عند معاملة البذور وكانت ٧ و ١٢% عند معاملة التربة على التوالي للعزلتين المحليتين، بينما كانت نسبة الإصابة ١٦ و١٩% عند معاملة البذور و ١٨ و ١٩% عند معاملة التربة على التوالي بالمستحضرين التجاريين. كما أوضحت النتائج مقدرة العوامل الاحيائية N*Fo* وBRC و *R. leguminisarum* في تحفيز المقاومة الجهازية لنبات الحمص عند معاملة التربة والبذور ضد *Foc*. إذ انخفضت نسبة الإصابة بمرض الذبول معنويا فكانت نسبة الإصابة عند معاملة التربة ١١% عند معاملة BRC و١٦% عند معاملة *NFo* و ٣٩% عند معاملة *R. leguminisarum*. بينما كانت نسبة الإصابة عند معاملة البذور ٨% عند معاملة BRC و ١٢% عند معاملة N*Fo* و ٤١% عند معاملة *R. leguminisarum* مقارنة ٦٩% عند معاملة السيطرة (*Foc*). بينت نتائج التجربة الحقلية فعالية عوامل المكافحة الاحيائية BRC و *T.harzianum* و *P. fluorescens* (محلي ومستحضر تجاري) و *R.leguminisarum* في خفض نسبة الإصابة بمرض الذبول معنويا مقارنة بمعاملة السيطرة. وكان أكثرها فعالية معاملة BRC التي سجلت نسبة إصابة ١٣% مقارنة بنسبة إصابة ٤٥% لمعاملة السيطرة. وأدت معاملات المكافحة الاحيائية إلى زيادة ارتفاع النباتات والوزن الرطب والجاف ووزن الحاصل لنباتات الحمص. إذ كان اعلى ارتفاع للنبات٣٨.٢cm في معاملة BRC و٣٨.٣cm في معاملة *T.harzianum* + *P.fluorescens* . وسجلت معاملة BRC لبذور الحمص أعلى متوسط للوزنين الرطب و الجاف وبفارق معنوي عن باقي المعاملات إذ بلغ الوزن الرطب ٤١.٤g و الجاف ١٣.٤g. وتفوقت المعاملتان BRC و *T.harzianum* + *P.fluorescens* معنويا على المعاملات *T.harzianum* و *P.fluorescens* (محلي ومستحضر تجاري) و *R.leguminisarum* في وزن الحاصل $g\ m^{-2}$ ٣٤٢ و ٣٤٦على التوالي.