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Induction of Pathogenesis Related Proteins and Phenol in Chickpea Plants Treated with Bio-Agents in Response to Infection by *Fusarium oxysporum* f.sp. *ciceri*

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Abstract
Soil and seed treatments with bio-agents, bio root care (BRC), non pathogenic *Fusarium oxysporum* (N*Fo*) and *Rhizobium leguminisarum* (*R.l*) indicated induced acquired resistance against *Fusarium oxysporum* f.sp. *ciceri* (*Foc*) in chickpea plants. This induced resistance was manifested by the accumulation of phenols and pathogenesis related protein in treated plants compared with control treatments (BRC, N*Fo* and *R.l* and plants inoculated with the pathogen alone and untreated plants). Significantly (p=0.05), the highest rate of accumulation of phenols, 286.5µg gfw^{-1} and peroxidase activity, 68.3min^{-1} gfw^{-1} when soil was treated with N*Fo* before inoculation with *Foc* compared with other test treatments. Maximum accumulation of phenols, 228.0 µg gfw^{-1} was at day 5 after *Foc* inoculation, and maximum peroxidase activity 62.3 min^{-1} gfw^{-1} was at day 4 after *Foc* inoculation. The highest rate of phenylalanine ammonia lyase activity was scored when seeds were treated with BRC and N*Fo* with no significant differences. PAL activity was 139.6 for BRC and 141.3 nM cinnamic acid min^{-1} gfw^{-1} for N*Fo* and scored maximum activity of 99.7 nM cinnamic acid min^{-1} gfw^{-1} at day 5 after *Foc* inoculation. The highest rate of glucanase activity was 33.5 µM min^{-1} gfw^{-1} when seeds were treated with BRC compared with other test treatments and scored maximum activity of 29.0 and 29.5 µM $min.^{-1}$ gfw^{-1} at day 4 and 5, respectively, after *Foc* inoculation.

1. Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops worldwide. The global chickpea area was about 11 million ha with production of 8.8 million tons and an average yield of nearly 800 kg ha^{-1} (Gaur, *et al*., 2010). The importance of chickpea is because of its role in human food and domestic animal feed and increase of soil fertility especially in dry lands. Iraq is considered as one of the major chickpea producing countries comprising 1% of world production (Gaur, *et al*., 2010). In Iraq, chickpea cultivation is still limited because of its low yield and not complies to mechanical harvesting and high susceptibility to infection by pathogen.
The wide spread of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matu & Satu (*Foc*) in more than 33 countries is considered as one of the most important diseases which limit chickpea production (Pande, et al., 2012). The high incidence of chickpea Fusarium wilt was often observed in Nineveh province, Iraq and the increased demands for food prompted the use of chemical pesticides and fertilizers which achieved part of this aim. But because of the disadvantage of these chemicals on human and animal health, its high coast and the emergence of resistance in pathogen, research has focused on alternative control methods such as biological control and induced systemic resistance in plant against several diseases (Kaur, *et al*., 2007). Induced systemic resistance was reported against different plant pathogens since the thirties of the last century (Chester, 1933). Increased pathogenesis related proteins (PRP) like chitinases, *β*,1-3 glucanse, peroxidase and Phenylalanin ammonia lyase (PAL) played an important role in inhibition of *F.oxysporum* f.sp.*ciceri* when plants were treated with *Pseudomonas flourescens* (Saikia, *et al.*, 2004). Recent study indicated that treatment of seeds of two chickpea cultivars (ILC-482) susceptible and cv (INRAT87/1) moderate resistance with two isolates of *Rhizobium* before inoculation with *Foc* induced the production of PAL and isoflavon reductase (IFR) and increased phenolic compounds (Arfaoui, *et al.,* 2005). Chickpea seedlings treated with *Rhizobium* isolates before inoculation with *Foc* showed increased accumulation of PAL and production of chalcone and IFR (Arfaoui,et al.,2007).
This study was conducted to determine the effects of BRC, N*Fo* and *R. leguminisarum* (*R.l*) on phenols and PRP accumulation in *F.oxysporum* f.sp. *ciceri* infected chickpea plants.

2. Materials and Methods
2.1. Biological Materials
2.1.1. Chickpea Plants
Chickpea, *Cicer arietinum* L. cv "Marakishi" susceptible to *Foc* was obtained from the local market and was

used in the experiments.

2.1.2. Fusarium oxysporum f. sp. c*iceri*

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

2.1.3. 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 chickpea cv Marakishi.

2.1.4. Rhizobium leguminisarum (*R.l*)

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

2.1.5. Bio-Root Care (BRC)

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

3. Experimental Work

3.1. Chickpea Sowing and Treatments

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 100 ml of 1×10^6 spores ml^{-1} of N*Fo*, 1×10^8 spores ml^{-1} of *R.l* and BRC (5g kg $soil^{-1}$). After 24 hours the pots were treated with suspension of *Foc* 1×10^3 spores ml^{-1}. Soil treated with N*Fo*, *R. leguminisarum*, BRC, *Foc* and intact healthy plant represents control treatment. Chickpea seeds were similarly treated with the bio agents and the pathogen. Phenol content and activities of PAL, chitinase, *β*,1-3-glucansae and peroxidase were estimated at 1, 2, 3, 4, 5, 6, 7and 8 days after *Foc* inoculation.

3.2. Estimation of Phenol and Pathogenesis Related Proteins

3.2.1. Estimation of Phenol

Fresh plant samples, 1g were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70 C (Zieslin and Ben–Zaken, 1993). One ml of the methanolic extract was added

was kept at 25 C. The absorbance of the developed blue color was measured using a spectrophotometer at 725 nm. Catechol was used as a standard. The amount of phenol was expressed as phenol equivalents in µg gfw^{-1}.

3.2.2. Assay of Peroxidase (PO)

Fresh plant samples, 1g were homogenized in 2ml of 0.1M phosphate buffer, pH 6.5 at 4C. The homogenate was filtered through 4-layers of cheese cloth and the filtrate was centrifuged at 6000*g* at 4C for 20 min and the supernatant was used as the enzyme source. Enzyme extract (100µl) and 1.5ml of 0.05M pyrogallol were measured by a spectrophotometer at wave length 420nm. In reference cuvette, contain 100µl of boiled inactivated enzyme extract and 1.5ml of pyrogallol. To initiate the reaction 100µl of (1%, v/v) H_2o_2 was added. The changes in absorbance at 420nm were recorded at 30 sec intervals for 3 min. The enzyme activity was expressed as changes in absorbance min^{-1} gfw^{-1} (Hammerschmidt, et al., 1982).

3.2.3. Determination of Phenylalanine Ammonia Lyase (PAL) Activity

Fresh plant material 1g were homogenized in 5ml of ice cold 0.1M sodium borate buffer (pH 7.0) containing 0.1g of polyvenylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 20000g for 30 min. The supernatant was used to determined PAL activity as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm (Dickerson *et al*., 1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5ml of 0.1M borate buffer (pH 8.8) and 0.5ml of 12mM L-phenylalanine in the same buffer for 30min at 30C. The amount of trans-cinnamic acid was calculated using its extinction coefficient of 9630 M cm^{-1} (Dickerson et al., 1984). Enzyme activity was expressed as n mol trans-cinnamic acid min^{-1} gfw^{-1}.

3.2.4. Assay of β -1, 3-glucanase

*B-1,*3-glucanase activity was assayed by the laminarin dinitrosalicylic acid method (Pen et al., 1991). Chickpea plant samples 1g was extracted with 5 ml of 0.05 M (pH 5.0) sodium acetate buffer and centrifuged at 10000*g* for 15 min at 4C. The supernatant was used in the enzyme assay. The reaction mixture consisted of 62.5µl of 4% laminarin and 62.5µl of the enzyme extract. The reaction was carried out at 40C for 10 min. The reaction was then stopped by adding 375µl of dinitrosalicylic acid and heating for 5 min using a boiling water path, and was diluted with 4.5ml of water, vortexes and its absorbance was measured at 500 nm. The blank was the crude enzyme preparation mixed with laminarin with zero time incubation. The enzyme activity was expressed as µ

mol equivalent glucose released min^{-1} gfw^{-1}

3.3. Statistical Analysis

The data were subjected to analysis of variance and means were separated by the least significant method at (p=0.05) using GenStat 12th Edition.

4. Resuts

4.1. Estimation of Phenol

Significantly (p=0.05), the maximum accumulation of phenols, 286.5µg.g fw^{-1} was observed in chickpea cv Marrakeshi when soil and seeds were treated with N*Fo* before inoculation with *Foc* compared with other test treatments. This was followed by 275.35µg gfw^{-1} for BRC treatment and 196.5µg gfw^{-1} for *R.leguminisarum*. These treatment were significantly outperformed the test control treatment, BRC, N*Fo* and *R. leguminisarum, Foc* alone and untreated plants (Table 1). The accumulation of phenols was higher in all test treatments compared with the untreated control plants which showed no marked change in phenol content. The highest accumulation of phenol, 228.0 µg gfw^{-1} in all treatment was observed on day 5 with a significant difference compared with the other test periods. The highest significant phenol accumulation, 294.7 and 292.2µg gfw^{-1} respectively, when seeds were treated with N*Fo* and BRC respectively compared with *R.l*, 210.6µg gfw^{-1} and outperformed all the test control treatments. The highest average phenol, 204.4µg gfw^{-1} in all treatment was observed on day 5 with a significant difference compared with the other test period except that at day 4 (Table 1). More phenol was significantly accumulated when the bio agent treated plants were challenged with *Foc* compared with pathogen unchallenged treatments and controls.

4.2. Assay of Peroxidase (PO)

S0il treatment with N*Fo* caused significantly (p=0.05) the highest average rate of peroxidase activity, 68.3min^{-1} gfw^{-1} (absorbance) compared with 65.8min^{-1} gfw^{-1} BRC treatment (Table 2). The latter treatment showed significantly more PO activity than *R.l* treatment which recorded 44.7 min^{-1} gfw^{-1}. However, this treatment were superior over all test control treatments (pathogen unchallenged BRC, N*Fo*, and *R.l* and the pathogen alone and healthy plants). Significantly highest average Δ absorbance 62.3min^{-1} gfw^{-1} was reached at 4 days after *Foc* compared with other test periods after *Foc* inoculation. Results of seed treatments were similar to those of soil treatments. Treatment with N*Fo* showed significantly (p=0.05) the highest Δ absorbance of 71.5 min^{-1} gfw^{-1} followed by seed treatment with BRC and *R.l* scoring 70.3 min^{-1} gfw^{-1} and 48.1 min^{-1} gfw^{-1} respectively. Pathogen challenged treatments BRC, N*Fo* and *R.l* showed significant highest PO activity compared to the test control treatments. Significantly highest average activity was recorded at 4 days after *Foc* inoculation compared with the other test period after pathogen inoculation.

4.3. Determination of PAL Activity

Significantly more PAL activity, 141.3 and 139.6 nM cinnamic acid min^{-1} gfw^{-1} was recorded in chickpea plants when the seeds were treated with N*Fo* and BRC respectively followed by 104.4 nM cinnamic acid min^{-1} gfw^{-1} for *R.l* treatment. The enzyme activity was more in the bio agent treatments compared with the other control treatments. Significantly (p=0.05) highest rates of PAL activity was reached at day 5 after *Foc* inoculation for all the test treatments, scoring 99.7 nM cinnamic acid min^{-1} gfw^{-1} compared with other test time periods after *Foc* inoculation.

4.4. Assay of β-1, 3-glucanase

The highest rate of glucanase activity in chickpea plants was when soil was treated with BRC before inoculation with *Foc* reaching 31.1µM $min^{-1}gfw^{-1}$ with a significant difference compared with N*Fo* and R. *l* treatments (Table 4). The enzyme activity in N*Fo* treated plants before inoculation with *Foc*, 27.7µM $min^{-1}gfw^{-1}$ was superior over to *R.l* treatment, 23.3µM min^{-1} gfw^{-1}. These treatments intern were significantly superior over control treatments. The highest rate of glucanase activity, 29.0 and 29.5µM min^{-1} gfw^{-1} were at 4 and 5 days after *Foc* inoculation for all treatment with a significant difference compared with the other test time periods. In the seed treatment experiment, the highest rate of glucanase activity was when chickpea seeds were treated with BRC before inoculation with *Foc* recording 33.5µM min^{-1} gfw^{-1} with a significant difference compared with treatments with N*Fo* and R. *l*. while treatments with N*Fo* before inoculation with *Foc* was 30.0 µM $min^{-1}g$ fw^{-1}, *R.l* scored significantly less enzyme activity, 24.0µM min^{-1} gfw^{-1}. The highest rate of glucanase activity was significantly more, 26.2 and 26.6µM min^{-1} gfw^{-1} in the fourth and fifth days following *Foc* inoculation respectively, compared with other test time periods.

5. Discussion

Results of this study indicated that bio agent treatment when chickpea plants were inoculated with the wilt pathogen *Foc* caused increased phenol accumulation in chickpea plants. Furthermore, the highest rate of phenol accumulation was in day 5 after bio agent treatment. These results were similar to and support previous reports indicated that seedling root treated with *P.flourescens* before *Foc* inoculation induced resistance in chickpea

plants against *Foc*, and the phenolic compound in chickpea plants treated with bio agent before treated with *Foc* inoculation was significant more compared with control treatment (Saikia, *et al.*, 2004). Chickpea seedlings treated with two *Rhizobium* isolates before *Foc* treatment led to increase phenolic compound, peroxidase, and poly phenol peroxidase significantly compared with control treatment (Arfaoui, *et al.,* 2005). The results of this study, however, was different from results of other previous studies indicated that the highest rate of phenol accumulation was in day 6 after chickpea seedling roots were treated with *P. flourescens* before *Foc* inoculation (Saikia, *et al.,* 2004) and 3 days after chickpea root were treatment with *Rhizobium* before *Foc* (Arfaoui, *et al.,* 2005). The reason for these different results is perhaps duo to the different chickpea cultivars, the bio agent used in this study and experiment conditions. The accumulation of phenolic compound and the increased of its concentration in short time in plants led to increase plant resistance to pathogen as it works to inhibit enzymes secreted by plant pathogens. Enzymes such as cellulase, pectinases, laccase, xylanase and others, plays significant role of pathogen-host interaction and inhibit the process of oxidative phosphorylation, deprive minerals, proteins and anti-oxidant in plant tissue (Jersh, et al.,1989; Scalbert,1991).

The increased peroxidase activity in chickpea plants treated with bio agent in this study which reached its highest level in day 4 after *Foc* inoculation were consistent with the results of previous studies indicate that chickpea seedling root treated with *P. flourescens* before *Foc* led to increase peroxidase activity significantly compared with control treatment with maximum activity at day 3 day after *Foc* inoculation (Saikia, *et al.* 2004). Chickpea seedlings root treated with two isolates of *R.leguminisarum* before *Foc* inoculation caused maximum increase in peroxidase activity in day 4 after *Foc* treatment (Arfaoui, *et al.,* 2005). Another study indicated that chickpea seedlings root treated with non pathogenic *F. oxysporum* f.sp.*ciceri* and another isolate of *F.oxysporum* (Chickpea is a non host) before treated with *Foc* led to increase peroxidase activity in chickpea root at day 4 after *Foc* treatment (Cachinero, *et al*., 2002).The results of this study indicated that the maximum increase of peroxidase activity was in day 4 which were different from other previous report indicated that the maximum peroxidase activity was in day 3 after *Foc* treatment. The reason for this difference is probably due to the different test plant and the bio agent isolates used as well as experimental conditions. Peoxidase affects defense mechanisms in plant in response to plant pathogens by deposition and polymerization of proteins, lignin and sobarin in the cell walls and vessels, transmission of an antioxidant during the invasion of pathogens (Mehdy,1994; Bradly, *et al*., 1992; Dicko, *et al*., 2006). It also affects the oxidation of some compounds and converted it into more toxic compounds like oxidation of phenols to quinone (Chen, *et al*., 2000; lavania, 2002) and IAA oxidation (Beffa, *et al*., 1990).

The results of this study also indicated that increased PAL activity as a result of treatement with the bio agents BRC, N*Fo* and *R.leguminisarum* before plant inoculation with *Foc* were consistent with previous results stating that the treatment of the seeds of two chickpea varieties susceptible and resistance to *Foc* with *T. harzianum* led to increased PAL activity (Jayalakshmi, *et al*., 2011). Treatment of chickpea seedlings roots with *P. flourescens* before inoculation with *Foc* led to increase in PAL which reached the highest rate in day 4 after *Foc* treatment (Saikia, *et al.,* 2004). PAL is considered as one of the most important enzymes, because of its responsibility for the manufacture of phenolics, coumarin and cinnamic acid which are associated with resistance to pathogens as well as its role in configure legnin, oxidation of phenols and bio manufacturing of phenyl propanoid and flavonoid (MacDonalds and D'Cunha, 2007).

The increased glucanase activity as a result of treatment with bio agents before *Foc* inoculation and the highest rate of increase in the day 4 and day 5 after *Foc* treatement, may be linked to resistance to disease pathogens. These results were also consistent with results of previous studies which indicated that chickpea seedling root treated with *P. flourescens* before *Foc* led to significant increase in glucanase activity compared with control treatment and the maximum activity was reached at day 3 after *Foc* inoculation (Saikia, *et al.,* 2004). Also chickpea seedling root treated with non pathogenic *F. oxysporum* f.sp. *ciceri* and another isolate of *Fusarium oxysporum* (chickpea is a non host) before treated with *Foc* led to increase glucanase activity in chickpea root at day 4 after *Foc* treatment (Cachinero, *et al*., 2002). The highest rate of PAL activity was in day 4 and 5 after *Foc* inoculation was differed from other study indicated that the highest increased rate of PAL was recorded 3 days after *Foc* treatment (Saikia, *et al.,* 2004; Cachinero,*et al*., 2002). β-1,3-glucanase is considered as one of the PRP Which leads to destruction of β-1,3-glucane which is one of the main components of the various cell walls of pathogen especially in fungi (Leubner-Metzger and Meins, 1999). This enzyme affects plant pathogen directly by hydrolyzing cell walls or indirectly by stimulating the cell to release materials derived from the cell wall which work as inducers of other defense mechanisms (Meins, *et al*., 1992; Boller,*et al*.,1988; Bowles, 1990).

The increased accumulation of phenol and PRP in chickpea plants following soil or seed bio agent treatments in this study was reflected on the significantly ($p=0.05$) low percentages of Fusarium wilt disease incidence (data are not presented) compared with treated but *Foc* challenged chickpea plants.

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Table 1. Phenol accumulation in chickpea, *Cicer arietinum* L. plants grown in pots after induction by bio agents against *Fusarium oxysporum* f.sp.*ciceri*

Soil treatment	Phenol (µg gfw^{-1}) after inoculation with *Foc* (day)								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	88	205	245	360	365	320	318	302	275.3
BRC (Contro)	90	110	125	180	192	180	165	143	147.6
N*Fo* + *Foc*	90	198	250	370	368	355	339	322	286.5
N*Fo* (Control)	92	106	130	182	200	191	168	148	152.1
R. l + *Foc*	87	180	197	230	248	218	211	201	196.7
R. l (Control)	86	94	105	123	137	118	108	103	109.3
Foc (Control)	90	165	192	218	227	198	168	149	175.8
Control	85	88	86	86	87	88	86	88	86.6
Mean	88.5	143.2	166.2	218.6	228.0	208.5	195.3	182.1	
LSD (P = 0.05) for days = 2.7, for treatment = 2.7, for inter action = 7.8									
Seed treatment	Phenol (µg gfw^{-1}) after inoculation with *Foc* (day)								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	104	215	265	375	372	348	338	321	292.2
(Control) BRC	106	105	108	106	107	108	108	107	106.8
N*Fo* + *Foc*	107	208	257	377	375	363	342	329	294.7
Control) N*Fo*(	106	106	107	106	107	107	108	107	106.7
R. l + *Foc*	102	191	208	248	257	237	224	218	210.6
R. l (Control)	103	103	104	103	103	104	104	103	103.3
Foc (Control)	90	165	192	218	227	198	168	149	175.8
Control	85	88	86	86	87	88	86	88	86.6
Mean	100.4	147.5	165.8	202.3	204.4	194.1	184.7	177.6	
LSD (P = 0.05) for days = 2.5, for treatment = 2.5, for inter action = 7.3									

Each number represent three replicate, each replicate is 3 pots with 2 plants. Bio agents was added (100ml kg soil^{-1}) after 30 days of germination as follows: N*Fo* isolate (10^6 spore ml^{-1}), *R. leguminisarum* suspension (10^8 spore ml^{-1}), BRC (5g kg^{-1}). After 24h, spore suspension of *Foc* was added (100ml kg soil^{-1}) with concentration (10^3 spore ml^{-1}). Chickpea seeds cv Marakishi were treated with bio agent and *Foc* as above. Total content of phenol was estimated 1, 2, 3, 4, 5, 6, 7 and 8 days after inoculated with *Foc*. BRC = Biopesticide Bio Root Care,

N*Fo* = Non pathogenic *Fusarium oxysporum* , *Foc* = *Fusarium oxysporum* f.sp. *ciceri* , *R.l* = *Rizobium leguminisarum*.

Table 2. Change in peroxidase activity in chickpea, *Cicer arietinum* L. plants grown in pots after induction by bio agents against *Fusarium oxysporum* f.sp.*ciceri*

Soil treatment	Change in absorbtion ($min^{-1}gfw^{-1}$) after inoculation with *Foc*								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	16	39	81	101	92	80	62	56	65.8
BRC (Contro)	16	30	44	56	50	41	31	25	36.6
N*Fo* + *Foc*	18	44	83	105	94	83	63	57	68.3
N*Fo* (Control)	15	32	47	60	53	47	37	29	40.0
R. l + *Foc*	15	29	51	66	60	50	48	39	44.7
R. l (Control)	15	27	36	46	37	31	28	22	30.2
Foc (Control)	15	24	37	49	43	38	34	27	33.3
Control	15	15	16	16	16	16	15	15	15.5
Mean	15.6	30.0	49.3	62.3	55.6	48.2	39.7	33.7	
LSD (P = 0.05) for days = 1.0, for treatment = 1.0, for inter action = 2.9									
Seed treatment	Change in absorbtion ($min^{-1}gfw^{-1}$) after inoculation with *Foc*								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	23	45	85	105	97	82	68	58	70.3
BRC (Contro)	21	23	22	22	22	21	23	23	22.1
N*Fo* + *Foc*	25	49	86	108	99	84	65	56	71.5
N*Fo* (Control)	21	22	23	22	22	22	22	22	22.0
R. l + *Foc*	20	32	53	72	65	52	49	42	48.1
Control)(*R .l*	19	21	22	21	21	22	22	21	21.5
Foc (Control)	15	24	37	49	43	38	34	27	33.3
Control	15	15	16	16	16	16	15	15	15.5
Mean	20.1	28.3	43.0	51.8	48.0	42.1	36.8	33.0	
LSD (P = 0.05) for days = 1.1, for treatment = 1.1, for inter action = 3.3									

Each number represent three replicate, each replicate is 3 pots with 2 plants. Bio agents was added (100ml kg $soil^{-1}$) after 30 days of germination as follows: N*Fo* isolate (10^6 spore ml^{-1}), *R. leguminisarum* suspension (10^8 spore ml^{-1}), BRC (5g kgl^{-1}). After 24h, spore suspension of *Foc* was added (100ml kg $soil^{-1}$) with concentration (10^3 spore ml^{-1}). Chickpea seeds cv Marakishi were treated with bio agent and *Foc* as above. Total content of phenol was estimated 1, 2, 3, 4, 5, 6, 7 and 8 days after inoculated with *Foc*. BRC = Biopesticide Bio Root Care, N*Fo* = Non pathogenic *Fusarium oxysporum* , *Foc* = *Fusarium oxysporum* f.sp. *ciceri* , *R.l* = *Rizobium leguminisarum.*

Table 3. Change in phenylalanine ammonia- lyase activity in chickpea, *Cicer arietinum* L. plants grown in pots after induction by bio agents against *Fusarium oxysporum* f.sp. *ciceri*

Soil treatment	Cinnamic acid (nM $min^{-1}gfw^{-1}$) after inoculation with *Foc*								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	40	132	138	151	163	148	123	118	126.6
Foc (Control)	44	70	82	90	87	72	60	52	69.6
N*Fo* + *Foc*	42	128	136	138	152	146	132	128	125.2
N*Fo* (Control)	43	68	81	87	82	67	59	49	67.1
R. l + *Foc*	39	88	102	108	123	119	114	109	100.2
R. l (Control)	39	46	57	62	59	51	47	42	50.3
Foc (Control)	41	70	85	93	88	77	62	48	70.5
Control	42	44	44	43	44	42	42	41	42.6
Mean	41.3	80.7	89.9	96.4	99.7	90.2	79.8	73.4	
LSD (P = 0.05) for days = 1.7, for treatment = 1.7 for inter action = 4.9									
Seed treatment	Cinnamic acid (nM $min^{-1}gfw^{-1}$) after inoculation with *Foc*								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	52	140	157	166	175	157	138	132	139.6
BRC (Control)	51	54	52	54	55	53	54	52	53.1
N*Fo* + *Foc*	54	136	158	169	177	162	147	128	141.3
N*Fo* (Control)	49	52	51	54	51	53	51	50	51.3
R. l + *Foc*	44	92	108	122	133	121	112	104	104.4
R. l (Control)	45	47	47	48	47	46	45	45	46.2
Foc (Control)	41	70	85	93	88	77	62	48	70.5
Control	42	44	44	43	44	42	42	41	42.6
Mean	47.2	79.3	87.2	93.0	96.2	88.8	81.3	75.0	
LSD (P = 0.05) for days = 1.8, for treatment = 1.8, for inter action = 5.3									

Each number represent three replicate, each replicate is 3 pots with 2 plants. Bio agents was added (100ml kg $soil^{-1}$) after 30 days of germination as follows: N*Fo* isolate (10^6 spore ml^{-1}), *R. leguminisarum* suspension (10^8 spore ml^{-1}), BRC (5g kgl^{-1}). After 24h, spore suspension of *Foc* was added (100ml kg $soil^{-1}$) with concentration (10^3 spore ml^{-1}). Chickpea seeds cv Marakishi were treated with bio agent and *Foc* as above. Total content of phenol was estimated 1, 2, 3, 4, 5, 6, 7 and 8 days after inoculated with *Foc*. BRC = Biopesticide Bio Root Care, N*Fo* = Non pathogenic *Fusarium oxysporum* , *Foc* = *Fusarium oxysporum* f.sp. *ciceri* , *R.l* = *Rizobium leguminisarum.*

Table 4. Change in glucanase activity in chickpea, *Cicer arietinum* L. plants cultivated in pots after induction by bio agents against *Fusarium oxysporum* f.sp. *ciceri*

Soil treatment	µM min^{-1} gfw^{-1} after inoculation with *Foc*								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	14	28	32	41	47	38	31	18	31.1
Foc (Control)	14	18	22	27	29	22	21	19	21.5
N*Fo* + *Foc*	14	25	31	37	42	31	25	17	27.7
N*Fo* (Control)	14	21	26	27	31	23	22	16	22.5
R. l+ *Foc*	14	19	23	31	32	28	22	18	23.3
R. l (Control)	14	15	17	21	23	20	19	16	18.1
Foc (Control)	14	20	26	25	29	23	21	18	22.0
Control	13	14	13	13	14	14	14	14	13.6
Mean	13.8	20	23.7	29	29.5	24.8	21.8	17	
LSD (P = 0.05) for days = 1.3, for treatment = 1.3, for inter action = 3.7									
Seed treatment	µM min^{-1} gfw^{-1} after inoculation with *Foc*								Mean
	1	2	3	4	5	6	7	8	
BRC+ *Foc*	15	29	34	46	49	41	35	19	33.5
Foc (Control)	16	16	17	16	17	16	16	18	16.5
N*Fo* + *Foc*	16	27	32	41	43	34	29	18	30.0
N*Fo* (Control)	16	17	17	17	18	17	17	18	17.1
R. l + *Foc*	14	18	25	32	32	27	22	16	24.0
R. l (Control)	15	16	16	16	15	16	16	16	15.7
Foc (Control)	14	20	26	25	29	23	21	18	22.0
Control	13	14	13	13	14	14	14	14	13.6
Mean	14.8	19.6	22.5	26.2	26.6	23.5	21.2	17.8	
LSD (P = 0.05) for days = 1.2, for treatment = 1.2, for inter action = 3.4									

Each number represent three replicate, each replicate is 3 pots with 2 plants. Bio agents was added (100ml kg $soil^{-1}$) after 30 days of germination as follows: N*Fo* isolate (10^6 spore ml^{-1}), *R. leguminisarum* suspension (10^8 spore ml^{-1}), BRC (5g kgl^{-1}). After 24h, spore suspension of *Foc* was added (100ml kg $soil^{-1}$) with concentration (10^3 spore ml^{-1}). Chickpea seeds cv Marakishi were treated with bio agent and *Foc* as above. Total content of phenol was estimated 1, 2, 3, 4, 5, 6, 7 and 8 days after inoculated with *Foc*. BRC = Biopesticide Bio Root Care, N*Fo* = Non pathogenic *Fusarium oxysporum* , *Foc* = *Fusarium oxysporum* f.sp. *ciceri* , *R.l* = *Rizobium leguminisarum.*