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Full Length Article

Effectiveness of Resistant Germplasm and Biological Control Agents as a Sustainable Management for *Fusarium* Wilt Disease on Chickpea

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Abstract

Chickpea wilt disease caused by *Fusarium oxysporum* f.sp. *ciceris* (Padwick) is a devastating disease. The best way to control chickpea wilt disease is to find resistant varieties/lines among available germplasm. Accordingly, three hundred and eighteen diverse chickpea genotypes were evaluated within sick plot conditions for disease severity against natural inoculum. The experiment was planted in an augmented design during two year investigating seasons. Disease severity was scored with a disease rating scale and area under disease progressive curve was recorded. A total of just three lines/varieties 5006, k021-10 and k035-10 were possessing high resistance in both investigation seasons. Among six biological control agents evaluated against pathogen *in vitro* by dual culture test showed that *Pseudomonas fluorescens* had more mycelial growth inhibition of pathogen (*Foc*) i.e., 70.94% over control. *Trichoderma harzianum* was proved to be second best followed by *Rhizobia* spp. and *Bacillus subtilis* with 63.95%, 60.79% and 57.68% growth reduction, respectively. In glass house assay, *P. fluorescens* was proved to be most effective, showed high disease reduction percentage with mean 76.78 over inoculated control.

Keywords: Chickpea; *F. oxysporum* f. sp. *Ciceris*; *P. fluorescens*; *Rhizobium* spp.

Introduction

Chickpea (*Cicer arietinum* L.) is an annual legume and the only cultivated species within the genus *Cicer* (Atta and Shah, 2009). Chickpea seed is highly nutritive with protein content and is being used increasingly, as an alternate, for animal protein (Hossain *et al.,* 2010). Chickpea is a major Rabi (winter) pulse crop and can be cultivated between September and November in Pakistan. According to FAO (2010), Pakistan is the second largest chickpea producing country in the world after India. Chickpea crop is mostly cultivated in Punjab followed by Sindh and Kyber Pakhtun Khwa. Punjab contributes about 80% toward total production but the yield is highest in Sindh. In 2009–2010, the chickpea production was anticipated to be 0.571 million tones, and the area under production was 105 million ha (MFALS, 2010). Many factors contribute towards chickpea low yield but the biological constraints are the most important. Chickpea wilt caused by *F. oxysporum* f. sp. *ciceris* (Padwick) is responsible for wilting, flagging and consequently loss in yield of the infected plants (Haqqani *et al.*, 2000; Özer and Bayraktar, 2015). The disease manifests mortality of young seedlings (within 25 to 30 days after sowing) to wilt or death of adult plants (Haware *et al.*, 1978). Fusarium wilt infested seedlings get collapsed, laid down flat on the ground and dried quickly. In Pakistan, disease may induce 10–50% crop loss every year (Khan *et al.*, 2002).

Management of Fusarium wilt of chickpea is difficult to achieve and no single control measure is effective. Considering the nature of damage and survival ability of the fungus, use of resistant varieties is the only economical and practical solution. Most of the resistant varieties are found to be susceptible after some years, because of the breakdown in their resistance and evolution in the variability of the pathogen. The pathogen with high saprophytic ability can survive in soil for a long period of time and during this phase it may undergo through various environmental stresses and biological competition which may lead to the evolution of new physiological races. The pathogen is seed and soil borne (Pande *et al.*, 2007). Most of the chickpea growing countries use resistant cultivars as an effective management tool against chickpea wilt (Gupta and Aneja, 2001). Due to heavy losses caused by chickpea wilt disease globally including Pakistan, it is very important to identify new resistant sources against chickpea wilt disease which is the ultimate goal of present study.

Efficacy of wilt management was improved when bio-control agents were combined with cultural practices such as sowing date (Landa *et al.*, 2004a). Biological control provides an alternative to the use of synthetic pesticides with the advantage of greater public acceptance and reduced environmental impact (Reino *et al.*, 2008). Several researchers have described *Rhizobium leguminosorum* (Singh *et al.*, 2010), *P. fluorescens* (Ramezani, 2009), *T.*

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harzianum (Whipps *et al.*, 2001) and *B. subtilis* (Wulff *et al.*, 2002; Gajbhiye *et al.*, 2010; Chen *et al.*, 2010) as effective bio-control agents against *F. oxysporum* f. sp. *ciceris*. Biological control of plant pathogens using antagonistic bacteria is a promising strategy for plant protection (Kloepper *et al.*, 1999). Strains of *Trichoderma* had gained wide acceptance as effective bio-control agents against several phytopathogens (Benhamou and Chet, 1996; Kay and Stewart, 1994; Bélager *et al.*, 1995; Chakraborty and Chatterjee, 2008; Shanmugam *et al.*, 2008). The objectives of the current research were:

1. To characterize morphological, cultural and pathogenic variability among five isolates of *F. oxysporum* f. sp. *ciceri* isolated from wilt infected fields in various district of Punjab province.
2. To identify level of resistance/susceptibility of chickpea in available germplasm/lines against chickpea wilt in Pakistan.
3. To compare effectiveness of bio-control agents against chickpea wilt disease.

Materials and Methods

Isolation of *F. oxysporum* f.sp. *ciceris (Foc)*

The infected root and stem samples were collected from 6 different district of Punjab and cut in to 5–6 cm pieces, washed with tap water and surface disinfected by 2% sodium hypochlorite for two minutes. The pieces were given two washings in sterilized water and were blotted on sterilized filter paper sheet for drying. The segments were then plated on PDA for the isolation of *Foc* in petri-plates for each isolate collected from different district. All plates were placed at 25±2ºC in an incubator with photo-period of sixteen hours light and 8 h darkness, for 5–7 days for the recovery of pathogen. The colonies of *Foc* with some other colonies of fungi were recovered. *Foc* colonies appeared on PDA medium were sub-cultured again by single spore culture method on PDA at 25±2ºC. After purification, culture of fungal isolate was multiplied on PDA for studying their cultural and morphological characters. After 15 days of incubation at 25±2ºC, colony diameter, sporulation, colony characters and pigmentation were recorded. The isolates were separately cultured and highly virulent isolate based upon its pathogenicity on chickpea plants of Thall-2006 (a susceptible variety) was selected for the management experiments (Sexena and Singh, 1987).

Pathogenic Variation among the Collected Isolates

For the pathogentic variation, test field soil was sterilized with 5% formaldehyde, prepared from 37% commercial formulation (Merck, Germany). The soil was thoroughly mixed with formaldehyde solution (100 mL/kg of soil) and covered with polythene sheets, the edges of which were air-sealed with ordinary soil. Polythene sheets were removed after 48 h and then treated soil was exposed to the air for 3 days and turned over to allow escape of fumes of formaldehyde. The soil was air dried at 2–3% moisture level and screened through a 2 mm sieve before use and the pots were filled two third with the soil. Inoculum of each isolate was multiplied on chickpea seeds (Thall-2006) in polyethylene bags (15 × 20 cm). The soil was inoculated with the identified isolates of *Foc* by mixing sterilized soil band with mass culture. One bag of inoculum was sufficient for the three pots. The inoculum of chickpea was incorporated into the autoclaved soil mixture at the appropriate proportion to achieve an inoculum density of 200 to 500 chlamydospores g^{-1} of soil for each isolate (Landa *et al.*, 2001). All pots were kept in glass house for seven days to allow the fungus to become established before sowing of the seeds. Control pots were not inoculated with any isolate. Three seeds of susceptible variety (Thall-2006) were sterilized with 2.5% sodium hypochlorite solution for 5 mints and sown in the soil at the depth of 1–2 cm. in completely randomized design (CRD) with three replication for each isolate was followed. The experimental pots were placed in the glass house, where temperature was in the range of 25±2ºC and tap water was applied when needed during the growth period. After 21 days the plants were examined for wilt disease symptoms and highly virulent isolate were selected for the management experiments. Data were collected by following formula:

$$\text{Wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

Field Screening of Germplasm against Chickpea Wilt

To catalogue genotypes, chickpea wilt disease screening nursery was established for two seasons during 2010–2011 and 2011–2012 in the PRI, AARI, Faisalabad. Three hundred and eighteen genotypes of chickpea were collected from different sources (i.e. National Agricultural Research Center (NARC), Islamabad, Barani Agriculture Research Institute (BARI), Chakwal and PRI, AARI, Faisalabad) were evaluated in a sick plot established at experimental area of PRI, ARRI. Seeds of these genotypes were not treated with any chemical to increase the chance of primary infection of pathogen. Each test entry was planted with 3 m length and 30 cm row to row distance. The planting was done on 15 October during both seasons. One row of susceptible check (Thall-2006) was planted after every two test lines in addition to two rows of susceptible check all around the experiment and disease nursery was sown by following augmented design with single replication (Hassan *et al.*, 2011**)**. The first data regarding to wilting was recorded 21 days after sowing and then on weekly basis.

The data were recorded after 21 days of sowing the experiment. Progression of disease based on visual symptoms according to disease rating scale was recorded at weekly basis. Plants showing symptoms such as wilting and drooping were quantified on the basis of scale and percent incidence was calculated by following formula:

$$\text{Wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

Disease incidence rating was based on 0–5 arbitrary scale given by (Iqbal *et al.,* 1993). The level of resistance and susceptibility of a test entry against chickpea wilt was determined following this scale: 0–10% mortality = highly resistant, 11–20% = resistant, 21–30% = moderately resistant (tolerant), 31–50% = susceptible and 51–100% = highly susceptible.

Area under Disease Progress Curve

The AUDPC was calculated by the trapezoidal integration of the disease incidence over time, considering the whole period evaluated, as follows:

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{X_i + X_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where, n is number of assessments; X, disease incidence and (*ti*+1 – *ti*), time interval between two consecutive assessments. In order to allow comparison between different treatments that were assessed during different periods of time, the AUDPC integral variable was divided by its respective observation period (*ti*+1 – *ti*), thus AUDPC was the standard area under the chickpea wilt incidence progress curve and interpreted as the mean incidence of disease (Shaner and Finney, 1977).

In vitro* Evaluation of Bio-control Microorganism against Mycelial Growth of *Foc

Six antagonist microorganism namely *B. subtilis*, *P. fluorescens*, *Rhizobium* spp., *T. harzanium*, *Azospirillium spp.* and *A. niger* (isolated from the rhizosphere of chickpea plants) were collected from Soil Microbiology Department, AARI and tested against *Foc*. One milli meter plugs of both, the bio-control microorganism and the pathogen, were simultaneously inoculated at the opposite ends of the petri-plates containing about 20 mL of PDA medium. Three petri-plates were used for each biological control agent and the same number was kept as control. Inoculated plates were incubated at 25±2ºC for 7–10 days. The data regarding the fungal hyphal growth were recorded by following formula (Nikam *et al.,* 2007).

$$\text{PGI} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

(PGI = Percent Growth Inhibition, C = Colony growth in control plate, T = Colony growth in intersecting plate).

Evaluation of Bio-control Microorganism in Glass House

The seeds of host crop were dipped in bio-control microorganism methylcellulose suspension (10^8 cfu/seed) and dried under laminar flow chamber. Control treatment consisted of non-treated dry seeds, which were coated with 1% methylcellulose. The soil was air dried at 2–3% moisture level, screened through a 2 mm sieve before use and the pots were filled two third with the soil and infected with the pathogen Foc-1 by mixing sterilized soil band with mass culture of fungus prepared in polyethylene bags (6 × 8 inches) on chickpea seeds. One bag of inoculum was sufficient for the three pots. The inoculum prepared on chickpea seed was incorporated into the autoclaved soil mixture at the appropriate proportion to achieve an inoculum density of 200–500 chlamydospores g^{-1} of soil (Landa *et al.,* 2001). In each pot three seeds were sown for each treatment, replicated three times in completely randomized design (Kumar and Dube, 1992). The soil temperature during the period of the experiments was maintained at 25±2ºC and the soil moisture was kept at optimum level. The data were collected by following formula after 39 days of sowing;

$$\text{MRP} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100$$

(MRP = Mortality reduction percentage, Dc = Seedling died in control, Dt = Seedling died in treatment)

MRP = Dc-Dt/Dcx100.

(MRP = Mortality reduction percentage, Dc = Seedling died in control, Dt = Seedling died in treatment).

Statistical Data Analysis

All these data recorded from different experiments was analysed by using SAS/STAT statistical software (SAS Institute, 1990).

Results

Cultural, Morphological and Pathological Variation of *Foc* Isolates

Cultural and morphological study of six isolates showed that *Foc-1* collected from Thall region produced slight fluffy thread like spreading at periphery with average colony diameter of 80.10 mm with high sporulation on PDA. Foc-3 showed moderate fluffy growth at the middle with an average 74.25 mm colony diameter. The least colony diameter was observed in case of Foc-2, which was 54.54 mm. Isolate Foc-1, Foc3 produced abundant sporulation followed by Foc-4 and Foc-5. Less sporulation was observed in Foc-2 (Fig. 1).

The plants exhibited typical disease symptoms externally and internally when raised in soil inoculated with all six isolates. It was observed that all the six isolates showed variation in their virulence on Thall-2006 in producing wilt symptoms. Among these Foc-1 was highly virulent and cause 100 percent wilt incidence in 20 days after emergence of chickpea plants. Isolate of *Foc*, from Bhakkar (Foc-3) caused 100% mortality of all chickpea plants in 25 days followed by Foc-2 and Foc-5. Foc-1 was selected for management experiments due to its abundant sporulation and quick virulence pattern (Table 1; Fig. 2).

Table 1: Morphological, cultural and pathogenic variability among five isolates of *Fusarium oxysporum* f. sp. *ciceris* isolated from wilt effected fields

Isolate	Location/ City	Colony diameter (mm)	Sporulation	Colony color	Colony characters	%age wilt incidence	Incubation period	Response
Foc-1	Thall	80.10	Abundant	White	Thread like spreading at periphery	100	20	Vey quick wilting
Foc-2	Muzaffargarh	54.50	Less	Yellowish white	Profuse fluffy growth at middle	100	28	Moderate
Foc-3	Bhakkar	74.25	Abundant	White Pinkish	Moderate fluffy growth at middle	100	25	Quick wilting
Foc-4	Faisalabad	60.70	Moderate	White	Thin flat to fluffy growth with thread like spreading	100	36	Slow wilting
Foc-5	Layyah	65.50	Moderate	Yellowish brown	Fluffy growth at middle	100	32	Slow wilting

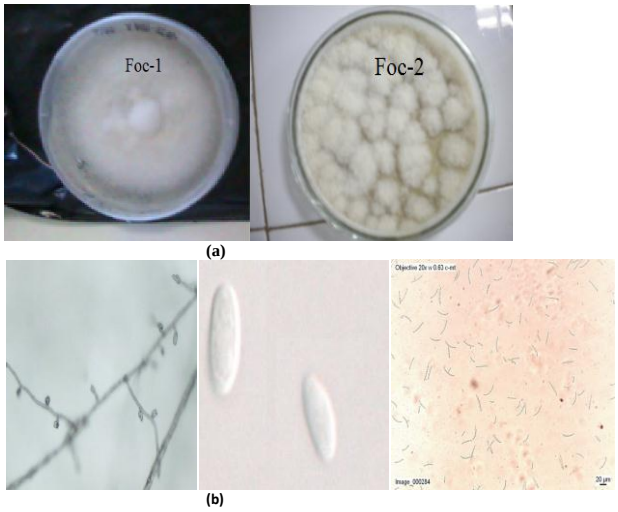

Fig. 1a: Isolates in Plates, b: Microconidia and macroconidia and chlamydospores, microscopy of *Foc*

Fig. 2: Pathogenic variation among isolates of *FOC*

Field Evaluation of Chickpea Germplasm for Resistance against Wilt Disease

Out of 318 germplasm tested only three lines 5006, k021-10 and k035-10 did not show any kind of wilt symptoms and those were graded as highly resistant (HR) during 2010–2011. The remaining showed varying levels of resistance/susceptibility to chickpea wilt disease. Among those, 36 genotypes were graded as resistant (R), 116 lines/varieties were moderately resistant (MR), 120 were susceptible (S) and 40 were highly susceptible (HS) (Table 2).

The response of chickpea germplasm to wilt disease incidence was similar during first year and the second year. During 2011–2012 same three lines 5006, k035-10 k021-10 showed highly resistant response. Thirty three genotypes, were graded as resistant (R) while 119 varieties/line were graded as moderately resistant (MR), 117 varieties/lines showed the susceptible response and 43 were highly susceptible (HS) to *Fusarium* wilt disease on chickpea (Table 3).

Area under Disease Progressive Curve (AUDPC) for Different Varieties/Lines

The area under disease progressive curve (AUDPC) on three hundred and eighteen varieties/lines was low in case of highly resistant varieties while it was high in case of highly susceptible varieties during both years of investigations. The average values of AUDPC for highly resistant varieties were 519.46 and 620.80 during both years of investigation, respectively while it was 4825.0 and 4920.87 for susceptible varieties. The AUDPC values for each type of germplasm showed that disease incidence was high during 1st year of investigation than the 2nd year of investigation (Table 4).

In vitro Evaluation of Bio-control Microorganisms against Mycelia Growth of *Foc*

In dual culture test of bio control microorganisms with *Foc* showed that *P. fluorescens* had more mycelial growth inhibition of pathogen (*Foc*) with 70.94% inhibition over control. *T. harzianum* was proved to be second best followed by *Rhizobia* spp. and *B. subtilis* with 63.95%, 60.79% and 57.68% growth reduction over control, respectively. *Aspergillus niger* and *Azospirillium* spp. were least effective against *Foc* (Figs. 3 and 4).

Table 2: Level of resistance/ susceptibility of chickpea germplasm /lines against chickpea wilt disease during 2010–2011

Disease reaction	%age rating	Varieties/lines
Highly Resistant	0–10	5006, k021-10, k035-10
Resistance	11–20	9023, 09AK050, 2124, 09AK055, 1880, K002-10, k005-10, k008-10, k012-10, k013-10, k015-10, k023-10, k025-10, k026-10, k040-10, k041-10, k043-10, k049-10, k052-10, k053-10, k062-10, k066-10, EM02, 1973, 1975, 09AG019, 04A013, D0072-10, D0074-10, D0088-10, D0097-10, D0100-10, k0030-09, k0054-09, bkk02174, ch47/04
Moderately Resistant	21–30	9013, 9010,9022, 9036, 9044, 9045, 8006, 3019, 5007, 6024, 3009, 8041, 8052, 7021, 7058, cm776/06, cm843/06, cm888/06, cm958/06, cm985-1/06, cm1004/06, cm1012/06, cm1208/08, cm1758/08, cm1333/05, icc5337, 1888, 09AK054, 09AK058, 09AK059, NOORCM2000, 09AK051, 2202, 7119, 09AK061, 7123, AZCM-2, 3103, 09AK053, NOOR 91, 2175, 2186, 1914, 1887, 2174, 2174, 2204, K003-10, k004-10, k006-10, k007-10, k009-10, k010-10, k014-10, k016-10, k017-10, k018-10, k019-10, k022-10, k027-10, k028-10, k030-10, k031-10, k032-10, k034-10, k039-10, k044-10, k045-10, k048-10, k050-10, k051-10, k054-10, k056-10, k057-10, k058-10, k061-10, icc8350, D0071-10, D0073-10, D0075-10, D0076-10, D0077-10, D0078-10, D0086-10, D0089-10, D0090-10, D0091-10, D0092-10, D0093-10, D0094-10, D0095-10, D0099-10, K009-09, k0010-09, k0025-09, k0057-09, k0062-09, k0063-09, k0065-09, k0066-09, k0069-09, bkk17115, bkk17106, bkk02209, bkk02231, bkk02182, ch65/02, ch82/02, ch38/03, FG0901, FG0902, D075-09, D085-09, D094-09, D095-09, D098-09, D0100-09
Susceptible	31–50	9014, 9018,9021, 9024, 9027, 9039, 9041, 9042, 9043, 8010, 8018, 7041, 7045, 7057, 6004, 4004,,7008, 7012, 7045, 7057,pb1, cm709/06, cm848/06, cm877/06, cm1342/05, cm1399/05, cm1380/05, cm1381/05, cm414-1/06, cm579/06, cm601/06, cm687/06, cm759/06, cm770/06, cm795/06, cm1154/06, cm62/06,icc13816,cm3/06, cm4/06, cm112/06, cm114/06, cm115/06, cm116/06, cm1528-14/03, EM06, EM07, 09AK056, 2196, 2200, 09AK052, 1879, 09AK057, 2193, 1888, 2182, 2231, K001-10, k011-10, k020-10, k024-10, k029-10, k033-10, k036-10, k037-10, k038-10, k042-10, k046-10, k047-10, k055-10, k059-10, k060-10, k063-10, k064-10, k065-10, k067-10, k068-10, k069-10, k070-10, EM01, EM03, EM04, ICC13124, EM05, 1968, 1969, 1971, 1974, 1977, 06A017, 06A003, 09AG021, 09AG015, 06A016, 08AG004, 08AG016, 09AG005, 05A020, 09AG006, 08AG015, D0079-10, D0081-10, D0082-10, D0083-10, D0087-10, k0021-09, k0031-09, k0034-09, k0039-09, k0070-09, bkk17124, bkk07151, D080-09, D084-09, D089-09, D090-09,D096-09
Highly Susceptible	51–100	9015, 9028, 9029, 9037, 8016, 8017, 8020, 8025, 8026, 8029, 8030, 8052, 7007, 7008,7009, 7012, 7020, 7021, 7058, 5015, 7020,7041, cm731/06, cm956/06, Pb 2000, 1854, Pb2008, 09AG002,D0080-10, D0084-10, D0085-10, Pb91,bkk07124,bkk02213,k0068-09, k0058-09,k0051-09, k0026-09,D0098-10,D0096-10,Thall 2006

Table 3: Level of resistance/ susceptibility of chickpea germplasm/lines against chickpea wilt disease during 2011–2012

Disease rating	%age rating	Varieties/lines
Highly Resistant	0–10	5006, k035-10 k021-10
Resistance	11–20	9023, 09AK050, 2124, 09AK055, 1880, K002-10, k005-10, k008-10, k012-10, k013-10, k015-10, k023-10, k025-10, k040-10, k041-10, k043-10, k049-10, k052-10, k053-10, k062-10, k066-10, 1973, 1975, k021-10, 04A013, D0072-10, D0074-10, D0088-10, D0097-10, , k0030-09, k0054-09, bkk02174, ch47/04
Moderately Resistant	21–30	9013, 9010, 9022, 9036, 9044, 9045, 8006, 3019, 5007, 6024, 3009, 8052, 7021, 7058, cm776/06, cm843/06, cm888/06, cm958/06, cm985-1/06, cm1004/06, cm1012/06, cm1208/08, cm1758/08, cm1333/05, icc5337, 1888, 09AK054, 09AK058, 09AK059, NOORCM2000, 09AK051, 2202, 7119, 09AK061, 7123, AZCM-2, 3103, 09AK053, NOOR 91, 2175, 2186, 1914, 1887, 2174, 2174, 2204, K003-10, k004-10, k006-10, k007-10, k009-10, k010-10, k014-10, k016-10, k017-10, k018-10, k019-10, k022-10, k027-10, k028-10, k030-10, k031-10, k032-10, k034-10, k039-10, k044-10, k045-10, k048-10, k050-10, k051-10, k054-10, k056-10, k057-10, k058-10, k061-10, icc8350, D0071-10, D0073-10, D0075-10, D0076-10, D0077-10, D0078-10, D0086-10, D0089-10, D0090-10, D0091-10, D0092-10, D0093-10, D0094-10, D0095-10, D0099-10, K009-09, k0010-09, k0025-09, k0057-09, k0062-09, k0063-09, k0065-09, k0066-09, k0069-09, bkk17115, bkk17106, bkk02209, bkk02231, bkk02182, ch65/02, ch82/02,09AG019, ch38/03, FG0901, FG0902, D075-09, D085-09, D094-09, D095-09, D098-09, D0100-09, EM02, k026-10, D0100-10
Susceptible	31–50	9014, 9018,9021, 9024, 9027, 9039, 9041, 9042, 9043, 8010, 8018, 8041, 7041, 7045, 7057, 6004, 4004,,7008, 7012, 7045, 7057,pb1, cm709/06, cm848/06, cm877/06, cm1342/05, cm1399/05, cm1380/05, cm1381/05, cm414-1/06, cm579/06, cm601/06, cm687/06, cm759/06, cm770/06, cm795/06, cm1154/06, cm62/06,icc13816,cm3/06, cm4/06, cm112/06, cm114/06, cm115/06, cm116/06, cm1528-14/03, EM06, EM07, 09AK056, 2196, 2200, 09AK052, 1879, 09AK057, 2193, 1888, 2182, 2231, K001-10, k011-10, k020-10, k024-10, k029-10, k033-10, k036-10, k037-10, k038-10, k042-10, k046-10, k047-10, k055-10, k059-10, k060-10, k063-10, k064-10, k065-10, k067-10, k068-10, k069-10, k070-10, EM01, EM03, EM04, ICC13124, EM05, 1968, 1969, 1971, 1974, 1977, 09AG021, 09AG015, 06A016, 08AG004, 08AG016, 09AG005, 05A020, 09AG006, 08AG015, D0079-10, D0081-10, D0082-10, D0083-10, D0087-10, k0021-09, k0031-09, k0034-09, k0039-09, k0070-09, bkk17124, bkk07151, D080-09, D084-09, D089-09, D090-09,D096-09
Highly Susceptible	50–100	9015, 9028, 9029, 9037, 8016, 8017, 8020, 8025, 8026, 8029, 8030, Pb 2000, 8052, 7007, 7008,7009, 7012, 7020, 7021, 7058, 5015, 7020,7041, cm731/06, cm956/06, Pb2008, 1854, 09AG002,D0080-10, D0084-10, D0085-10, Pb91,bkk07124,bkk12213,k0068-09, k0058-09,k0051-09, k0026-09,D0098-10,D0096-10,06A017, 06A003, Thall 2006

Evaluation of Bio-control Microorganisms against Chickpea Wilt Disease Incidence in Glass House

Four bio-control microorganism, proved effective *in vitro* assay were tested in glass house assay for further evaluation. Analysis of variance indicates that the individual effect of treatments and varieties and combined effect of varieties and treatments were significant. When seeds were treated with bio-control agents, all the antagonists gave satisfactory results in managing the disease. In this case, *P.*

Table 4: AUDPC during the year of 2010–2011 and 2011–2012

Response	AUDPC (2011)	AUDPC (2012)	LSD
Highly resistant	519.46	620.80	570.13E
Resistant	1040.11	1114.82	1077.5D
Moderately resistant	1836.89	1863.60	1850.2C
Susceptible	2848.34	2900.53	2874.5B
Highly susceptible	4825.00	4920.87	4873.0A

CV=2.571

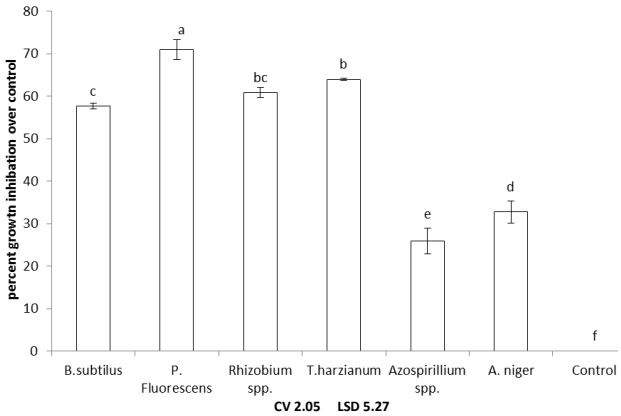

Fig. 3: Efficacy of different bio control agents on percent mycelial growth inhibition over control *in vitro* assay

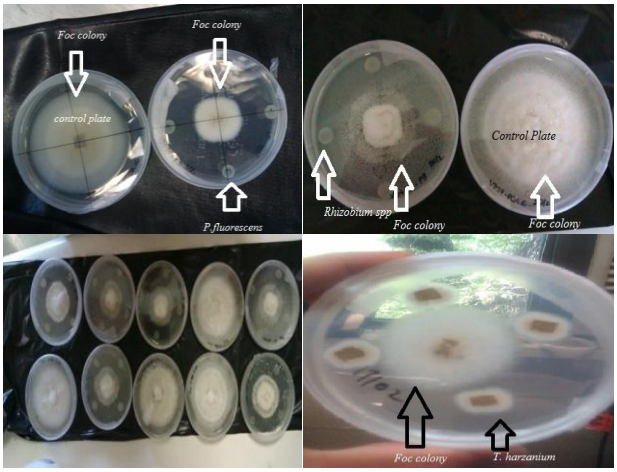

Fig. 4: Effect of micro-organism on mycelia growth of *Foc in vitro* assay

fluorescens was proved to be most effective on one moderately resistant variety (Noor 91) and two susceptible varieties (Pb2000 and ICC131-21) and showed high disease reduction percentage with mean 76.78 over inoculated control. While *Rhizobium* spp. was proved to be second best followed by *T. harzianum* with means disease reduction percentages 69.44 and 57.73, respectively. *B. subtilis* was least effective against the disease (Fig. 5). Overall and individual effect of all treatments was highly significant on moderately resistant variety (Noor 91) as compared to other two and inoculated control (Fig. 6). The individual efficacy of each bio-control agents on percent disease reduction over control showed that all bio-control agents showed effectiveness against chickpea wilt disease incidence (Fig. 7 and 8).

Discussion

Six isolates were collected from different district of Punjab showed variability in pathogenicity and morphological characteristics. All the isolate colonies showed white color with some different shades on PDA. Variability among the isolates is not uncommon (Nanda and Parsad, 1974). Pathogenic and morphological variations have been reported in different isolates of *Foc* (Barhatae *et al.,* 2006; Cunnington *et al.,* 2012).

Genetic resistance is probably the only durable and a long lasting solution of chickpea wilt disease. A possible solution to the disease is the transfer of resistance genes to chickpea varieties, which obviously will require a long time period. The short term solution should be screening of available germplasm for relative susceptibility (Singh and Reddy, 1991; Haware *et al.,* 1992), as in current study and to identify low rating variations for breeding manipulation. A very cheap and economical method to manage the disease is searching for resistance in all the available germplasm. Out of 318 germplasm/lines, only few (3) gremplasm/lines were highly resistant during the both years. Ali *et al.* (2002), screened 330 lines against *Fusarium* wilt disease and found that only four lines were highly resistant to disease incidence. In another study, Arvayo-Ortiz *et al.* (2012) described that through breeding program against *Foc*, new resistant cultivars might be sorted out. Similar kind of work was done by Jamil *et al.* (2002). The study was conducted to find out highly resistant line but no resistant line was found during screening of ten advanced chickpea lines against chickpea wilt disease developed at NIAB. Bajwa *et al.* (2000) found that out of thirty two genotypes evaluated against chickpea wilt, only one line 9701 was found resistant. Ilyas and Chaudary (2007) found that out of one hundred and ninety two lines/cultivars only eighteen varieties were resistant. The sources of resistance to *Fusarium* wilt in chickpea breeding materials were not uncommon and a number of workers reported the occurrence of high level of resistance to *Fusarium* wilt in many cultivars/lines (Pathak *et al.,* 1982; Ahmad *et al.,* 1990; Ahmad and Sharma, 1990; Yu and Su, 1997).

In the present study, the results of screening against chickpea wilt disease was quite similar during the both years except some varieties/lines showed susceptible response toward wilt fungus during the 2nd year than 1st year but this response was so minute that only few varieties/lines moved

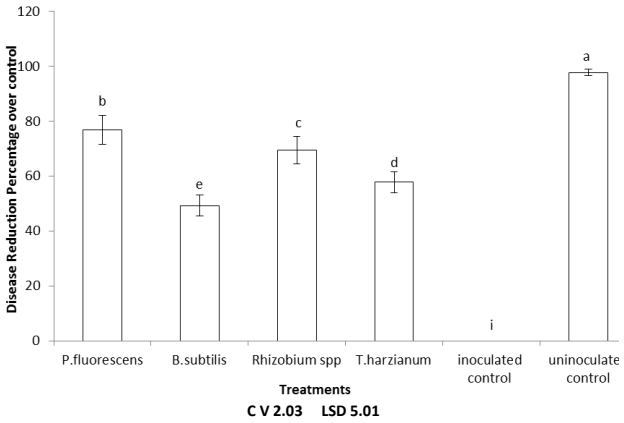

Fig. 5: Overall efficacies of different bio control agents on percent chickpea wilt disease reduction over control in glass house assay on three varieties

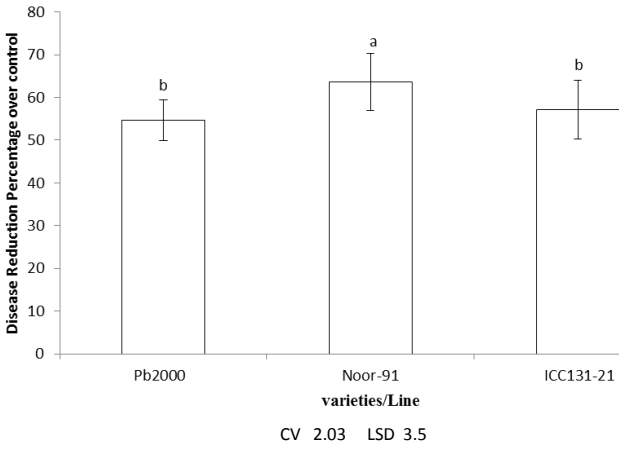

Fig. 6: Disease reduction percentage over control on three varieties when treated with biological control agents

to next disease rating scale (group). Some varieties/lines (i.e., k026-10, EM02, 09AG019 and D0100-10) moved from resistant group to moderately resistant group. 06A017, 06A003 and Pb 2000 moved from susceptible to highly susceptible group due to more susceptibility toward disease from 1st year of investigation. The change in response toward disease during the two years might be due to resistance breaking phenomenon, which may include several factors of host (Landa *et al.*, 2006), pathogen (Navas-Cortés *et al.*, 2007) and environment (ICRISAT, 2010).

Biological control of plant diseases can be defined as management of plant disease by reducing the inoculums with the help of beneficial microbes (Campbell, 1994). In the present study, *P. fluorescens* proved to be best both *in vitro* and glass house assay, while *T. harzianum* was proved to be second best followed by *Rhizobia* spp. and *Bacillus* spp. These findings were completely in agreement with many workers who found that strains of *Pseudomonas*, *Bacillus* and *Trichoderma*, isolated from the rhizospheres regions of host

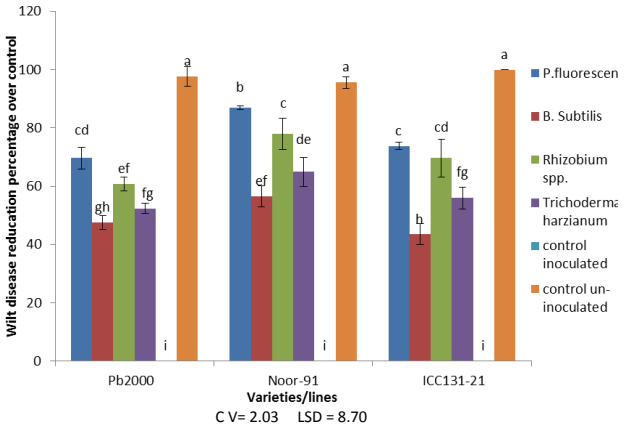

Fig. 7: Individual efficacy of different bio control agents on percent chickpea wilt disease reduction over control in glass house assay on three varieties

Fig. 8: Evaluation of bio-control microorganisms against chickpea wilt disease incidence in glass house on Noor 91

crop plants were found effective to manage the plant pathogens (Glick, 1995; Burr *et al.*, 1998; Postma *et al.*, 2003; Saikia *et al.*, 2003). Karimi *et al.* (2012) evaluated various isolates of *Pseudomonas* and *Bacillus* against *Fusarium* wilt and found that *Pseudomonas* spp. was superior to *Bacillus* spp. in managing the *Fusarium* wilt disease, which was in agreement with our investigation. Merkuz and Getachew (2012) found that isolates of *Trichoderma* were very effective against chickpea wilt disease. Species of *Trichoderma* were found superior to *Bacillus subtilis* and *Aspergillus niger* (Dubey, 2007). Bloemberg and Lugtenberg (2001) worked on plant growth promoting rhizobacteria (PGPR), used as inoculants for bio-fertilization, phyto-stimulation and bio-control. Zhang *et al.* (2004) observed that PGPR effected on plant growth and provided systemic protection against *Peronospora tabacina*, which causes blue mold in tobacco. Antoun *et al.* (1998) studied that *Bradyrhizobia* and *rhizobia* exhibit antagonistic affect towards many plant pathogenic fungi. Less disease incidence were recorded when seed treated with *T. viride* (Howell, 2006; Andrabi *et al.*, 2011).

Conclusion

Three line (5006, k021-10 and k035-10) proved to be highly resistant against *Foc* can effectively be used in breeding program to develop chickpea wilt disease resistant variety. Chickpea seeds treated with *P. fluorescens* and *T. harzanium* showed better results against chickpea wilt disease.

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