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COMPARATIVE EFFICACY OF FUNGICIDES AND BIOLOGICAL CONTROL AGENTS FOR THE MANAGEMENT OF CHICKPEA WILT CAUSED BY F*USARIUM OXYSPORUM* F. SP. C*ICERIS*

Y. Mahmood[1]*, M. A. Khan[1], N. Javed[1] and M. J. Arif[2]

[1]Plant Pathology Department, University of Agriculture, Faisalabad, Pakistan
[2]Department of Entomology University of Agriculture, Faisalabad, Pakistan
*****Corresponding Author: yasir_scout@yahoo.com

ABSTRACT

Efficacy of fungicides and biological control agents were tested side by side both *in vitro* and in glass house against chickpea wilt pathogen. *In-vitro* study showed that Carbendazim was proved to be best by checking the mycelia growth with mean of 83.7 % growth inhibition over control. Almost similar result was obtained in Glass house assay where Carbendazim was proved to be best followed by all other fungicides at different concentrations. In dual culture test of bio control agents with *Foc* showed that *Pseudomonas fluorescens* had more mycelial growth inhibition of pathogen (*Foc*) with 70.94 % inhibition 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 inhibition respectively. In glass house assay *Pseudomonas fluorescens* was proved to be most effective on one moderately resistant variety (Noor 91) and two susceptible varieties (Pb2000 and ICC131-21) showed highly disease reduction percentage with mean 76.78 over un-inoculated control. Comparative study of biological control agents and fungicides showed that both are almost equally effective against chickpea wilt disease. Seed treatment with *Trichoderma harzanium* followed by Carbendazim drenching was proved most effective by increasing the disease reduction percentage up to 93.75%.

Keywords: Chickpea, Carbendazim, *Fusarium oxysporum* f. sp. *ciceris*, *Trichoderma harzianum*, mycelial growth

INTRODUCTION

Wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) is a complex and destructive disease all over the world. The genus *Fusarium* had many soil borne species which were distributed worldwide and known as plant pathogens since a long time ago (Moss and Smith, 1984). It is widely occurred in India (Andrabi *et al.,* 2011), Pakistan (Hameed *et al.,* 2012), Ethiopia (Merkuz and Getachew, 2012a), Mexico (Arvayo-Ortiz *et al.,* 2012), Iran (Moradi *et al.,* 2012), Nebraska, USA (Harveson 2011), Spain (Jimenez-Diaz *et al.,* 1990), Syria (Haware 1990) and Sudan (Ali *at el*., 2002) where ever gram (*Cicer arietinum* L.) is grown.The pathogen of chickpea wilt disease is seed-borne (Pande *et al.,* 2007) as well as soil borne (Jiménez-Fernández *et al*., 2011). It can survive in soil for more than 6 months in the absence of its host and can cause severe damage to crop yield (Hawar*et al.,* 1986). *Fusarium* spreads through the soil to a small extent as mycelium. The spores or sclerotia are carried in the soil water, on farm equipment, transplants, tubers and seeds of some hosts, cuttings of infected plants and might be in some cases by windblown spores or sclerotia (Agrios, 1984). Chickpea crop is affected by several diseases but wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the most serious disease and causes heavy losses up to 10 percent in yield (Dubey*et al.,* 2007). In Pakistan, up to 50 percent crop production is reduced every year due to chickpea wilt disease (Hanif *et al.,* 1999; Khan *et al.,* 2002). A survey was conducted in India by Kumar and Bourai (2012) and estimated 72.16 percent yield losses due to chickpea wilt disease. In Mexico sixty percent reduction in yield were recorded by Gomez (2004), while in Spain the losses were in the ranged from 12-15 percent annually (Landa *et al.,* 2004). General symptoms of chickpea wilt include drooping, yellowing, drying of the leaves and discoloration of vascular system (Luthra *et al.,* 1943). The cultivars which were susceptible to chickpea wilt disease showed symptoms 25 days after sowing. The symptoms of wilt disease might be confused with root rot disease if not observed carefully. Wilted plants showed drooping and yellowing of leaves and lie down on the ground. The fungus attacked the root system, made its way through the epidermis, cortex and finally into xylem vessel of the tap root from where it spread. As a result, the lateral roots might wither off (Murumakr and Chavan 1985; Chavan *et al.,* 2009). Resistance in available germplasm of chickpea is very uncommon and only few lines showed resistance to chickpea wilt disease in 2 years investigation conducted by the author (unpublished data) so it is very important to find out other control measures against wilt pathogen. Among these control measures chemical and biological control are more important (Mahmood *et al*., 2005). Chemical control is widely being used in past and

present to cope with *Fusarium* wilt disease. Subhani *et al.,* (2011) observed the effects of 6 fungicides at four different concentrations through poisoned food technique. There was a significant decrease in mycelial development of *Foc* pathogen with a raise in fungicidal concentration. Natural eco-system of plants also contains many beneficial microbes called symbionts. Those symbionts help plant in getting their nutrition in a better way and improve the resistance ability of the plant. Among these symbionts, two i.e. *Arbuscular mycorrhizal* fungi and *rhizobia* are very important. (Demir and Akkopru 2007).In modern world biological management of diseases is getting more importance than other methods (Mahmood and Khan 2009). For the management of seed and soil borne diseases, antagonists from the rahizosphere region of the host plants were isolated. Those antagonists mainly belonged to *Pseudomonas* spp., *Trichoderma* spp. and *Bacillus* spp. The antagonist not only helped man to cope with plant diseases but also provide batter nourishment to host plants (Glick, 1995; Burr *et al.*, 1998; Perner *et al.,* 2006). These beneficial microbes were abundantly found in forest, rhzosphere soils and herbal compost than common soil (Khan *et al.,* 2004; Tinatin and Nurzat, 2006; Torsvik *et al.,* 2002 and Postma *et al.,* 2003). The objectives of the current research were to successfully manage the disease with the timely use of chemicals and biological control agents against *Fusarium oxysporum* f. sp.*ciceris* when host resistance is not available.

MATERIALS AND METHODS

Isolation of the fungal isolates: The infected roots and stems were cut in to 5-6 cm pieces, washed with tap water and surface disinfected by 2 percent 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 *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) in peteriplates for each isolate collected from different district. All plates were placed at 25±2℃ in an incubator with photo- period of sixteen hours light and 8 hours darkness, for 5-7 days for recovery of pathogen. The colonies of *Foc* with some other colonies of fungi were recovered.

***In-vitro* and glass house assay for evaluation of fungicides:** The efficacy of seven fungicides i.e. Carbendazim 50 WP, Divedent star, Aliette 80 WP, Copper oxychloride, Defeater 20WP, Ridomil gold and Thiowet jet 80 WP in inhibiting the colony growth of *Foc* was tested through poisoned food technique (Falck,1907) on four different concentrations i.e. 10ppm, 20ppm, 30ppm and 50ppm. The prepared concentrations were added in the falcon tubes. PDA medium was prepared; 40 ml of the medium was added in each 50 ml falcon tube for two petri plates. Each treatment was replicated 4 times by following completely randomized design. The middle of each plate was inoculated with a plug of fungus taken from actively growing culture plate. A five mm cork borer was used to cut a round agar plug from actively growing edges of the culture plate and placed hyphae upside down on each fungicide plate with four replications of each concentration. The petri plates were incubated at 25±2 ℃ and observation of mycelial growth were recorded after 12 days of inoculation. The growth of test fungus on non-poisoned PDA served as a control (Nisa *et al.,* 2011). The percent inhibition in growth due to various fungicidal treatments at different concentrations was computed as follows:

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

(PGI = Percent Growth Inhibition, C = Colony growth in Control Plate, T = Colony growth in intersecting plate)

Fungicides proved effective during *in vitro* evaluation by poisoned food technique were further tested in the glass house for control of chickpea wilt disease by pot soil drenching and seed treatment at two different concentrations i. e. 200 ppm and 500 ppm. Soil used in pots was obtained from the research area of Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan having a sandy clay loam with pH 7.9, Phosphorous 7.9 ppm, Nitrogen 0.51% total organic matter 0.78 % and Potassium 190 ppm. The soil was air dried at 2-3 percent moisture level and screened through a 2 mm sieve before use and the pots were filled two third with the soil. The inoculum of 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). The pots with infested soil were kept on glass house bench at 25±2℃ temperature. Four seeds of each chickpea cultivars Pb 2000, Noor 91 and ICC131-21 were treated with fungicides by soaking them in desired fungicides aqueous concentrations for 30 minutes and were sown in each of pots at a depth of 1-2cm (Kamdi *et al.,* 2012). Aqueous suspension of each fungicidewas drenched into soil at each of three concentrations. One liter dilution of each fungicide was used to drench the soil of four pots of each fungicide treatment. Four pots of each of an infested non drenched and non-infested drenched treatment with distilled sterilized water served as a control. All pots were labeled and randomized at glass house benches. Pots were irrigated when required with distillated sterilized water. The data on percent disease incidence was recorded 39 days after sowing by following formula:

$$MKP = \frac{Dc - Dt}{Dc} \times 100$$

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

***In-vitro*and glass house assay for evaluation of bio-control microorganisms against *Foc*:** The pure culture of antagonistic organisms i.e. *Trichoderma harzianum* (Rafai), *Aspergillus niger* (Tiegh.), *Bacillus subtilis* (Cohn) *Pseudomonas fluorescence* (Migula), *Rhizobium spp.*, and *Azospirillium spp.* were collected from Soil Microbiology Department, AARI. The pure culture was multiplied in the test tubes slants and petri plates (Fig. 7) Agar slants of these cultures were placed in refrigerator at 4℃ for use in future experiments. For in vitro assay one millimeter plugs of both the bio-control microorganism and the pathogen were simultaneously inoculated at the opposite ends of the petriplates, 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±2℃ for 7-10 days. The data regarding the fungal hyphal growth were recorded by following percent growth Inhibition formula (already described above).

Fig 7. Effect of micro-organism on mycelia growth of *Focin vitro* assay

For glass house assay the seeds of host crop were dipped in bio-control microorganism methylcellulose suspension (10^8cfu/seed) and dried under laminar flour chamber. Control treatment consisted of non-treated dry seeds which were coated with 1% methylcellulose. The soil was prepared for the experiment as described above fungicide management section.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 ±2 ℃ and the soil moisture was kept at optimum level (Navas-Cortés, 2007). The data were collected by mortality reduction percentageformula (already described above) after 39 days of sowing (Fig. 8).

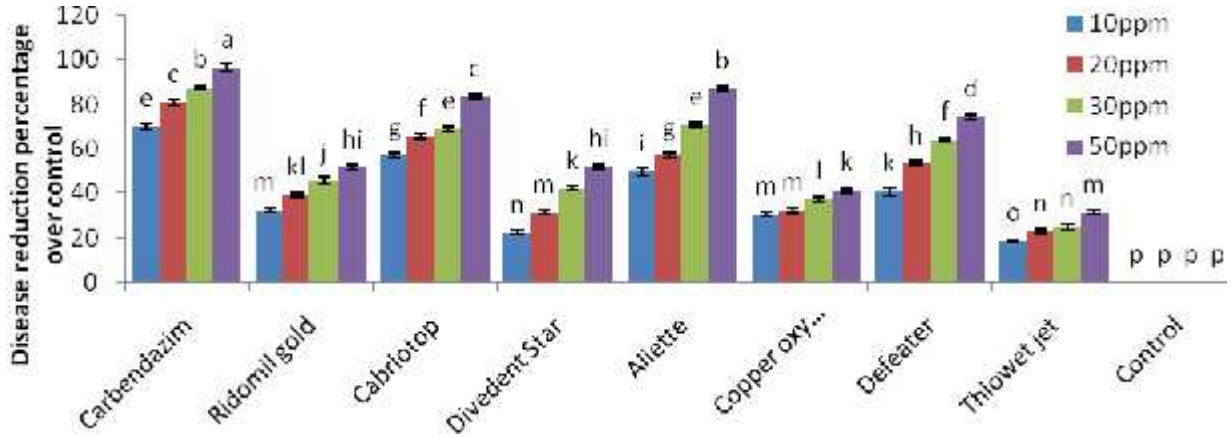
Fig 8. Evaluation of bio-control microorganisms against chickpea wilt disease incidence in glass house on Noor 91

Combined evaluation of bio-control microorganisms and fungicides in glass house assay: Two best treatments of bio-control agents i.e. *Pseudomonas fluorescens* and *Trichoderma harzanium* were selected and seeds of host crop were dipped in bio-control microorganism methylcellulose suspension and dried under laminar flour chamber. Control treatment consisted of non-treated dry seeds which were coated with 1% methylcellulose. Soil for experiment was prepared as described above. In each pot three seeds were sown in a treatment replicated three times in CRD (Kumar and Dube, 1992). The soil temperature during the period of the experiments was maintained at 25 ±2 ℃ and the soil moisture was kept at optimum level (Navas-Cortés, 2007). The soil of un-inoculated control was not inoculated with *Foc* culture. The fungicide which proved best with best concentration was selected and drenched in pots as described above after 14 days of sowing. All the treatments were organized in following order: T1 = *P. fluorescens*, T2 = *T. harzanium*, T3 = Carbendazim, T4 = *P. fluorescens* +Carbendazim, T5 = *T. harzanium* +Carbendazim, T6 = Control inoculated and T7 = Control un-inoculated. Data were collected with mortality reduction percentage formula (already described above) after 39 days of sowing.

Data recording and statistical Analysis: Data of percent growth inhibition and mortality reduction percentage were subjected to analysis of variance (ANOVA) to determine all possible interactions of main factors and sub-factors. The significant difference between treatment means was compared by least significant difference test (SAS, 1990).

RESULTS

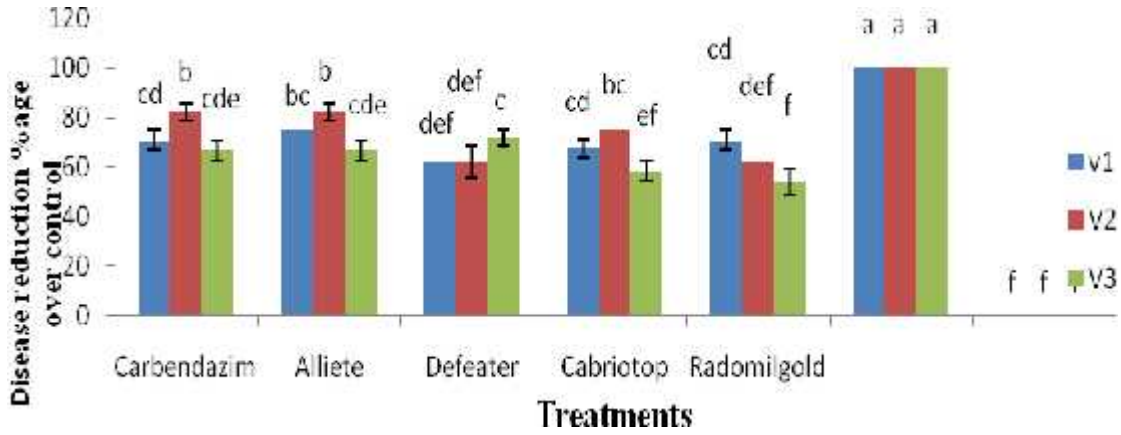
In-vitro* and glass house evaluation of fungicides against *Foc: In-vitro assay showed that Carbendazim was proved to be best by checking the mycelia growth with mean of 83.7 % growth inhibition over control. While Cabriotop proved to be second best followed by Aliette and Defeater with 68.66% 66.15% and 58.15 %. (Fig.1).



CV=1.97, LSD=3.01

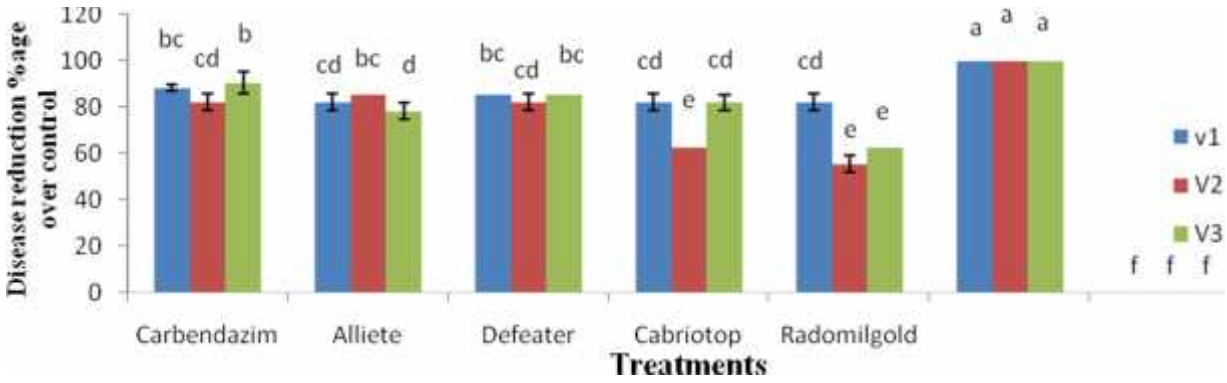
Fig.1 Mean colony mycelia growth percent inhibition of various concentrations of fungicides over control *in-vitro* assay.

The data regarding the fungicidal drenching at different concentration in glass house showed that percent disease reduction over control on all the three varieties was nearly directly proportional to the fungicide concentration i.e. increase in concentration of each treatment were directly proportion to its efficacy to increase in value of percent disease reduction of wilted plants over control plants (Fig 11). Overall effect of treatments on three verities showed that all varieties showed equal defence against disease when treated with fungicides. In case of both concentrations 200ppm and 500ppm fungicides drenching Carbendazim was proved to be best at all genetic source 82.50% and 64.72 % disease reduction over un-inoculated control up. Carbendazim was followed by Alliete (Fig. 2 and 3).



CV 2.01 LSD 8.95

Fig. 2 All treatments effects on disease reduction percentage over control on three different varieties (V1= Pb200, V2= Noor 91 and V3=ICC131-21) @200 ppm concentration.

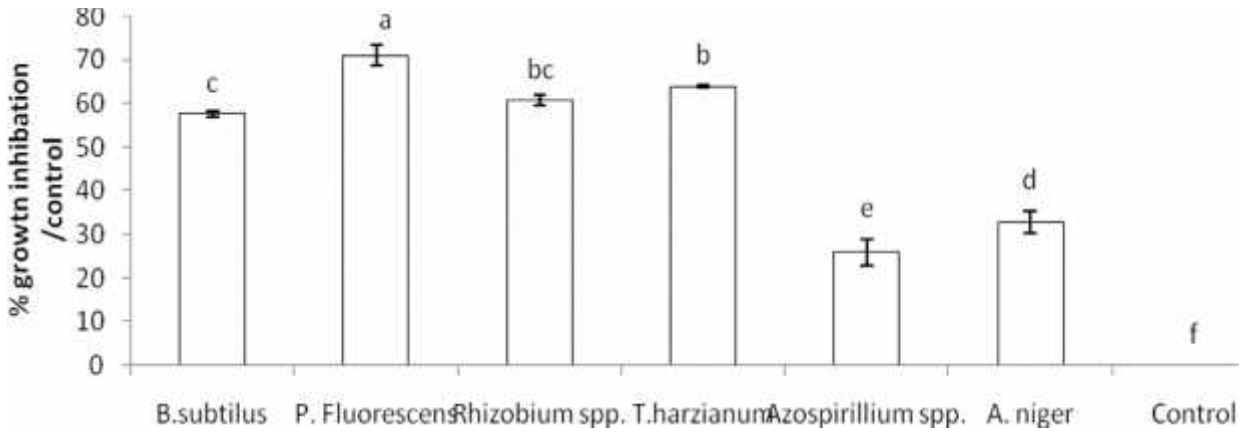


CV 2.01 LSD 7.03

Fig. 3 All treatments effect on disease reduction percentage over control on three different varieties (V1= Pb200, V2= Noor 91 and V3=ICC131-21) @500 ppm concentration

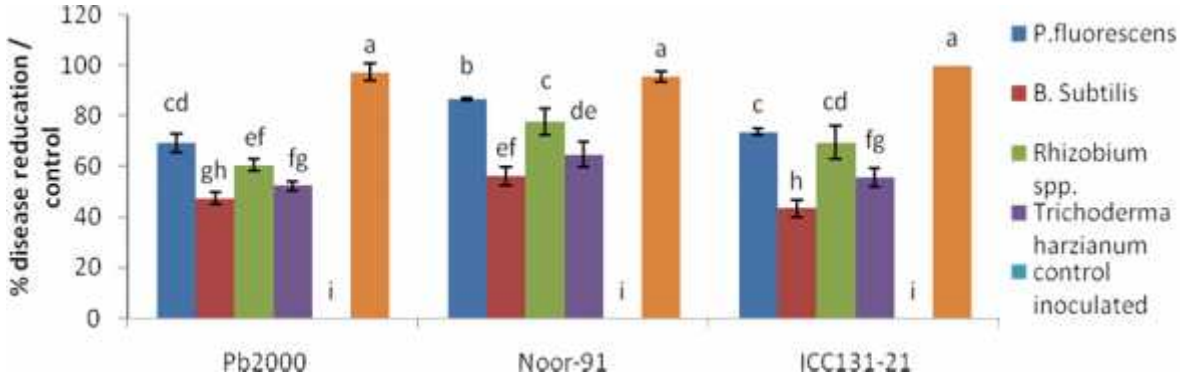
In vitro* and glass house evaluation of bio-control microorganisms against *Foc: In dual culture test of bio control microorganisms with *Foc* showed that *Pseudomonas fluorescens* had more mycelial growth inhibition of pathogen (*Foc*) with 70.94 % inhibition over control. *Trichoderma harzianum* was proved to be second best followed by *Rhizobia spp*. and *Bacillus subtilis* with 63.95%, 60.79% and 57.68% growth reduction over control respectively(Fig 4).In glass house *Pseudomonas fluorescens* was proved to be most effective on all three varieties highly disease reduction percentage with mean 76.78 over inoculated control. While *Rhizobium spp*. was proved to be second best followed by *Trichoderma harzianum*. 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 5).

Combined evaluation of bio-control microorganism and fungicide in glass house assay: Result showed that combined treatment of *Trichoderma harzanium* and carbendazim (T1) was proved effective by increasing the disease reduction percentage up to 93.75%. *Pseudomanas fluorescens* and Carbendazim (T2) were proved to be second best by increasing the disease reduction percentage up to 89.12 %. It was observed that combined treatment of fungicide and bio-control agents were proved more effective than individual one i.e. either fungicide alone or bio-control alone (Fig 6).



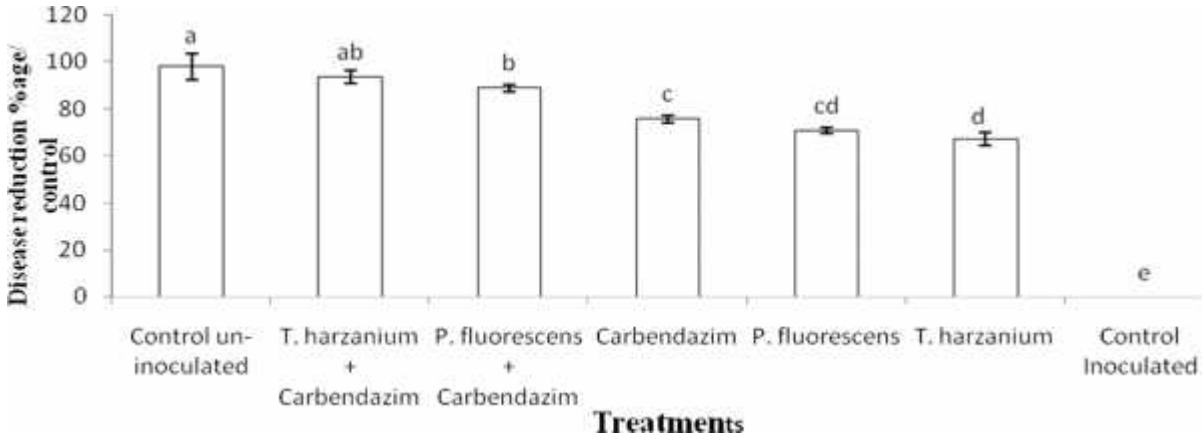
CV 2.05 LSD 5.27

Fig. 4 Efficacy of different bio control agents on percent mycelial growth inhibition over control *in-vitro* assay



C V= 2.03 LSD = 8.70

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



Cv 2.05 LSD 7.31

Fig. 6. Combined evaluations of bio-control microorganism and fungicide in glass house assay.

DISCUSSION

Chickpea wilt disease is found everywhere in the world where ever chickpea crop is grown and caused loss up to 12-15% annually (Landa *et al.*, 2004). The pathogen is both seed and soil borne (Pande *et al.*, 2007) so management of the disease is difficult to achieve. Fungicidal use in the management of disease is widely being used in the world. In current study Carbendazim was proved to be best by checking the mycelial growth.

This study was in complete agreement with Nisa *et al.*, (2011). Sultana and Ghaffar (2010) found that when fungicides viz., Aliette, Benlate and Carbendazim were used @ 100 ppm, complete inhibition of colony growth of *Fusarium solani* was observed *in vitro* conditions. Cabriotop proved to be second best followed by Aliette and Defeater. Five fungicides which proved best in lab were evaluated in glass house. Andrabi *et al.,* 2011 found that seed treatment with Carbendazim increased the disease reduction percentage 86.66 over control. This study was in complete agreement with Subhani *et al.,* (2011). It was observed that Cabriotop and Radomil gold was proved effective in managing the chickpea wilt causal organism both *in vitro* and glass house assay. P. *fluorescens* was almost equally effective as carbendazim dose of 200 ppm in present investigation. The results were in the complete agreement with thought i.e. biological control agents may be effectively used against diseases and considered as a good alternative to fungicides (Fravel, 2005; Guetsky *et al.,* 2001; Maltby *et al.,* 2009). Fungicides are widely being used in the world and most of these chemical are considered to hazardous to life on earth. As these chemical enters in to the food web and cause damage the life directly or indirectly at various points of food web so preference should be given to biological management (Frampton *et al.,* 2006). Biological control of plant diseases can be defined as management of plant disease by reducing the inoculu with the help of beneficial microbes (Campbell, 1994). In the present study *Pseudomonas fluorescens* proved to be best both *in-vitro* and glass house assay while *Trichoderma 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 crop plants were found effective to manage the plant pathogens (Postma *et al.,* 2003, Glick, 1995; Saikia*et al.,* 2003 and Burr *et al.,* 1998). 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 (2012b) 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, phytostimulation and bio-control. Zhang *et al.,* (2004) observed that PGPR effected on plant growth and provided systemic protection against *Perono sporatabacina*, which cause 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 *Trichoderma viride* (Andrabi *et al.,* 2011and Howell 2006). Result showed that when seeds of chickpea were treated with *Trichoderma harzianum*and then infected soil was drenched with Carbendazim, this practice proved to be very effective against chickpea wilt disease incidence. These results were totally in agreement with Podder *et al.,* (2004). It was found that effectively management of chickpea disease might be done through combined use of fungicides and bio-control agents. In the experiments, *Trichoderma harzianum*and and Carbendazim were successfully used against wilt disease. Ainmisha *et al.,* (2011) found that wilt of chickpea incited by *Fusarium oxysporum* f. sp. *ciceris* could be minimize by use of Carbendazim and *Trichoderma viride*. Similar types of results were also observed by Sallam *et al.,* (2008).

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