5. DISCUSSION

Chickpea is one of the most important pulse crops of India. India has a distinct position covering about 76 percent of the total area and production of chickpea in the world. At national level, Karnataka stands sixth position in area and eighth in production. The inevitable importance of essential nutrients available in chickpea seeds is well recognized especially in the predominantly vegetarian population of India.

Several factors responsible for low production of chickpea has been recognised. High incidence of diseases namely Fusarium wilt, Ascochyta blight, root rot, Alternaria alternata etc., chickpea wilt incited by Fusarium oxysporum f. sp. ciceri is catastrophic disease resulting ultimately in the death of the plants which could cause severe loss to the farmers. The present investigations include symptomology, isolation, pathogenicity, cultural, nutritional, morphological, physiological, biochemical studies, In vitro and In vivo evaluation of different fungicides and bioagents. The results of the studies mentioned above are discussed in this chapter.

5.1 Survey on disease incidence of foliar fungal diseases of chickpea (Cicer arietinum. L)

Maximum disease incidence of Fusarium oxysporum was observed with positive presence of disease in all the chickpea (Cicer arietinum. L) growing areas and recorded highest in Hiriyur (8.39- 12.66 %) followed by Hebbal (7.12- 8.11 %), GKV (6.25-7.79 %) and Chamrajnagar (3.21- 4.79 %). Presence of Ascochyta rabie incidence was observed only in Chamrajnagar and Hiriyur district, whereas Alternaria alternata was observed in Chamrajnagar (2.99- 3.45 %) followed by GKV (2.12 - 3.33 %). Similar

The pathogen was isolated repeatedly from the infected plants collected from the different districts during survey. The fungus isolated from infected plants was identified as *Fusarium oxysporum*, *Ascochyta rabie* and *Alternaria alternata* based on the morphological and cultural characters as described by Butler (1910), Padwick (1940) and Booth (1971).

5.2 *In vitro* studies of foliar fungal pathogens of chickpea (*Cicer arietinum*. L.)

The fungus was isolated by using tissue isolation technique. The medium used was Potato Dextrose Agar. After 7 days of incubation the cultural characters were studied. The colony was fluffy with effused growth having abundant aerial mycelium. The fungus produced both macro and microconidia. The isolated fungus is similar as described by Booth (1971). The method used to estimate soil fungi using acid rose and streptomycin in plate count method was done earlier by Martin., 1959.

Differences due to the carbon sources, isolates and interactions were found significant. Among the carbon sources tested, glucose recorded significantly high growth and dry mycelial weight *viz.*, 502.26 mg in *Fusarium oxysporum*, 494.90 mg in *Ascochyta rabie* and 498.70 mg in *Alternaria alternata* compared to other carbon sources.
5.3 Evaluation of fungicides on growth inhibition of foliar fungal pathogens of chickpea (*Cicer arietinum*. L.)

All the fungicides differed significantly with respect to inhibition. Among the fungicides, carbendazim 50 % WP was found to be superior in inhibiting the radial growth of the three fungal pathogens followed by mancozeb 75 % WP in *Fusarium oxysporum* (78.86 %), *Ascochyta rabiei* (75.66 %) and *Alternaria alternata* (75.63 %). Significant differences were found among fungicides and *Fusarium oxysporum*. High percent germination of seeds was observed with carbendazim in all the three pathogens i.e., *Fusarium oxysporum* (71.24 %), *Ascochyta rabiei* (68.25 %) and *Alternaria alternata* (65.25 %) followed by mancozeb treated seeds *viz.*, *Fusarium oxysporum* (62.22 %), *Ascochyta rabiei* (60.22 %) and *Alternaria alternata* (58.22 %). Similar observations were made by Radhakrishan and Sen (1985), Baayen and DeMaat (1987), Mohammed Amen and Fufa Melkamu (2014) in management of foliar fungal pathogens of muskmelon and chickpea was reported.

Fungicides play an important role in checking the fungal growth. In the present study, *in vitro* evaluation of fungicides namely, carbendazim, mancozeb, zynab, hexaconazole and propiconazole each at 250, 500, 1000, 2000 and 3000 ppm were evaluated against the pathogens. Among the fungicides evaluated carbendazim showed 100 per cent inhibition of mycelial growth at all concentrations, followed by mancozeb fungicide at 3000 ppm (86.7 %) and the least percent inhibition was noticed in zynab at 250 ppm (37 %). In general carbendazim was the best, which gave considerable inhibition at all concentrations tested. These results are in agreement with the work of Ghosh and Sinha (1981) who reported that bavistin appeared to be the most toxic among seven fungicides against *Fusarium udum* and inhibited mycelia growth at 10 and 25 ppm. Futher, Gupta *et al.*, (1983) reported among seven fungicides tested benlate

Antagonists were evaluated *in vitro* to manage three selected fungal pathogens of chickpea. Two fungal antagonists namely, *Tricoderma harzianum* and *Tricoderma viridae* and two bacterial antagonists namely, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested for their antagonistic effect against the pathogen by dual culture test. All isolates of antagonists successfully inhibited the growth of fungal pathogen. Further, the fungal antagonists inhibited the pathogen better than bacterial antagonists. Among fungal antagonists the maximum inhibition was observed in *T. viridae* (83.33%) followed by *T. harzianum* (75.66%). Among bacterial bioagents, the maximum inhibition was observed in *B. subtilis* (76.66 %), least inhibition was observed in *P. fluorescens* (68.51 %).

The inhibitory effect of these bioagents against the pathogen was probably due to competition, antibiosis and mycoparasitism (Cook and Baker.,1983, Papavizas., 1985). Semeoni *et al.*, (1987) reported that significant suppression of chlamydospore germination of *F. oxysporum* f. sp *cucumaraniun* by the use of *P. fluorescens*. Antagonistic activity of *T. harzianum* and *T. viridae* against soil borne fungal pathogens were reported by Dhedhi *et al.*, (1990) where *T. harzianum* and *T. viridae* were antagonists in vitro to *F. oxysporum* f. sp *lycoperisci* and Patel (1991) found that *T. harzianum* initially showed 2mm inhibition zone to *Fusarium* spp and later it over grew the colony of the pathogen where the similar results were observed in present study.
5.4 Effect of biocontrol agents, fungicides and foliar fungal pathogens on chickpea under greenhouse and field condition

The effect of selected bioagents on growth and yield parameters were observed on plant height at 45, 90 and 135 DAS which showed highest in *i.e.* combination of *T.v + B.s + P.f* with *F. oxysporum, A. rabie* and *A. alternata* respectively (87.33 %, 85.33 % and 80.10 %) and the least was observed in control. Among the fungicides maximum height was observed in *T.v + carbendazim* (73.12 % for *Fu.o*), *T.v + carbendazim* (70.12 % for *As.r*) and *T.v + mancozeb* (53.44 % for *Al.a*) respectively in greenhouse experiment and also revealed the same results in the field experiments where the plant height at 45 days after sowing was observed highest in the treatment inoculated with foliar fungal pathogens and triple inoculation of biocontrol agents and *Glomus fasciculatum*. *Fu.o + T.v + B.s + P.f + Gl.f* recorded the highest plant height (52.80 cm) which was significantly different compared to all the other treatments and on par in the treatment inoculated with *As + T.v + B.s + P.f + Gl.f* (48.47 cm) and *Al.a + T.v + B.s + P.f + Gl.f* (46.73 cm). Similar observations were made on *Fusarium* wilt disease of Tomato plant using *Tricoderma harzianum* and salicylic acid under greenhouse condition by Houssien, *et al.*, 2010.

Plant height at 90 days after sowing and 135 DAS the same trend was continued with highest plant height in the treatments inoculated with *Fu.o + T.v + B.s + P.f + Gl.f* (56.47 cm), *As.r + T.v + B.s + P.f + Gl.f* (52.60 cm) and *Al.a + T.v + B.s + P.f + Gl.f* (51.57 cm).

The effect of bioagents and fungicides on shoot length, root length and total biomass for *Fu.o, As.r* and *Al.a* was recorded highest with combination of *Tv + Bs + Pf* each (15.95 %, 14.925 % and 13.8 % respectively) and the least was found in control. The no.
of pods/plant, weight of 5 pods and yield/m² was also tabulated where among three pathogens *Fu.o + T.v + B.s + P.f* showed the maximum of 140.5 % and the less in control. The effect of bioagents and fungicides on seed protein, carbohydrates and polyphenols of chickpea after harvest with respect to the three pathogens was highest in *Fu.o + B.s + P.f + Gl.f* (19.5 %, 42 % and 258 %) and among fungicides *Fu.o +* carbendazim recorded the maximum when compared with control. The effect of shoot NPK, root NPK, total NPK and 100 seed weight was maximum observed in *Fu.o + Ty + Bs* in bioagents and among fungicides *Fu.o + T.v +* carbendazim (17.5 kg/ha) showed highest when compared with control. The studies were conducted in recent past by Sobita Simon., and Anamika., (2011), where *Pseudomonas fluorescens* and *Trichoderma viridae* were tested against *M. incognita* and *Fusarium oxysporum* f. sp. *Ciceri* in pots and in infested field. In the pots *Trichoderma viridae* were applied on seed, soil and foliar, Hesamedin Ramezani (2009) also investigated the efficacy of four fungal bioagents viz., *Trichoderma hamatum, Trichoderma harzianum, Trichoderma viridae, Gliocladium virens* and two bacterial bioagents namely *Pseudomonas fluorescence* and *Bacillus subtilis* were evaluated against the chickpea vascular wilt pathogen, *Fusarium oxysporum* f.sp. *Ciceri* in *in vitro* condition using Dual Culture Technique. Similarly effect of coinoculation of *Mesorhizobium ciceri* and *Ascochyta rabie* with PGPR on *Cicer arietinum*. L was observed (Labdi, M., 1995 and Ladwal, A., et al., 2012).

Shoot and root length per plant was found to be highest in the treatment inoculated with biocontrol agents along with the *Fu.o+T.v+B.s+P.f* (35.77 cm and 15.73 cm) per plant respectively which was significantly different compared to all the other treatments.
Fresh shoot and root biomass per plant was found to be significantly highest in the treatment inoculated with $Fu.o + T.v + B.s + P.f$ (6.77 and 5.60 g/plant) which was on par with the treatment inoculated with $Fu.o + T.v + B.s$ (6.60 and 4.70 g/plant) respectively under green house condition and also shoot and root length per plant was found to be highest in the treatment inoculated with biocontrol agents along with the $As.r + T.v + B.s + P.f$ (33.27 and 15.50 cm) per plant respectively which was significantly different compared to all the other treatments. Fresh shoot and root biomass per plant was found to be significantly highest in the treatment inoculated with $As.r + T.v + B.s + P.f$ (6.53 and 4.40 g/plant) which was on par with the treatment inoculated with $As.r + T.v + B.s$ (6.07 and 4.50 g/plant) respectively. Whereas similar trend was also observed in field condition i.e., shoot and root biomass per plant was found to be significantly highest in the treatment inoculated with $Fu.o + T.v + B.s + P.f + Gl.f$ (7.20 g and 5.70 g respectively) which was on par with the treatment inoculated with $Fu.o +$ carbendazim in shoot and root biomass observed in the treatment inoculated with individual pathogen control of $Fu.o$, $As.r$ and $Al.a$ (6.1 g, 5.2 g and 5.0 g respectively) with the shoot biomass where as in the root biomass (1.8 g, 1.5 g and 1.5 g respectively). This results evidences previous research which was conducted by Pradeepkumar et al., (2000) reported that the seed treatment with *Trichoderma viridae* improved the seed germination, emergence and shoot length of pigeonpea and Sobita Simon, et al., (2011) on chickpea. Perspective of plant promoting rhizobacteria containing ACC deaminase in stress related plants was observed by Saleem, M., et al., 2007.

In greenhouse studies shoot and root length per plant was found to be highest in the treatment inoculated with biocontrol agents $Al.a + T.v + B.s + P.f$ (31.50 and 14.50 cm) per plant respectively which was significantly different compared to all the other treatments. Fresh shoot and root biomass per plant was found to be significantly highest
in the treatment inoculated with *Al.a + T.v + B.s + P.f* (5.47 and 3.50 g/plant) which was on par with the treatment inoculated with *Al.a + T.v + B.s* (5.63 and 4.07 g/plant) respectively. Under field conditions similar trend of results enhanced the shoot and root biomass per plant was found to be significantly highest in the treatment inoculated with *Fu.o + T.v + B.s + P.f + Gl.f* (7.20 g and 5.70 g respectively) which was on par with the treatment inoculated with *Fu.o + carbendazim* on shoot and root biomass was observed in the treatment inoculated with individual pathogen control of *Fu.o, As.r* and *Al.a* (6.1 g, 5.2 g and 5.0 g respectively) with the shoot biomass. whereas, in the root biomass 1.8 g, 1.5 g and 1.5 g was recorded respectively. The same trend of observations were also recorded by the earlier researchers Ratul Saikia *et al.*, (2003) and Arfaoui *et al.*, (2006).

Maximum number of pods were observed under green experiments in the treatment inoculated with *Fu.o + T.v + B.s + P.f* (39 pods/plant) followed by the treatments inoculated with *Fu.o + T.v + P.f* (37 pods/plant) and *Fu.o + T.v + B.s* (35 pods/plant) fresh weight of 5 pods and yield/m² triple biocontrol agents inoculated treatment (T₁₀) along with the pathogen *Fu.o* (4.3g and 140.5 g/m²) followed by dual biocontrol agents inoculated treatments *Fu.o + T.v + P.f* and *Fu.o + T.v + B.s* (3.8g and 3.7g ; 135.75 g/m² and 132.5 g/m²) and similar observations also recorded in field conditions, where data on yield parameters revealed significant differences among the treatments. Highest number of pods were recorded in the treatment *Al.a + T.v + B.s + P.f + Gl.f* (43.10) which was on par with the treatments *Fu.o + T.v + B.s + P.f + Gl.f* (42.0), *Al.a + T.v + P.f* (14.1), *As.r +T.v + B.s + P.f + Gl.f* (40.2) and *Fu.o + T.v + P.f* (40.0).
Maximum weight of 5 pods observed in the treatment \( Al.a + T.v + B.s + P.f + Gl.f \) (4.9 g) followed by \( Al.a + T.v + B.s \) (4.8 g). Hossain et al., 2012 and Hesamedin Ramezani (2009) were studied extensively integrated management of Fusarium wilt of chickpea treated with microbial antagonist, botanical extract and fungicide and results of the study were greatly anticipated the same results.

Maximum number of pods were observed in the treatment inoculated with \( As.r + T.v + B.s + P.f \) (38.3 pods/plant) followed by the treatments inoculated with \( As.r + T.v + P.f \) (35.6 pods/plant) and \( As.r + T.v + B.s \) (34.3 pods/plant) which were significantly different compared to all the other treatments. Fresh weight of 5 pods and yield/m² with triple combination of biocontrol agents inoculated treatment along with the pathogen \( As.r \) (4.1 g and 130.8 g/m²) followed by dual biocontrol agents inoculated treatments \( As.r + T.v + P.f \) and \( As.r + T.v + B.s \) (4g and 3.8g ; 130.8 g/m² and 126.6g/m²). Maximum number of pods were observed in the treatment inoculated with \( Al.a + T.v + B.s + P.f \) (40.1pods/plant) followed by the treatments inoculated with \( Al.a + T.v + P.f \) (38.1 pods/plant) and \( Al.a + T.v + B.s \) (36.1 pods/plant) which were significantly different compared to all the other treatments. Fresh weight of 5 pods and yield/m² triple biocontrol agents inoculated treatment (T10) along with the pathogen \( Al.a \) (4.5g and 135.7 g/m2) followed by dual biocontrol agents inoculated treatments \( Al.a + T.v + P.f \) and \( Al.a + T.v + B.s \) (4.3g and 4.1g ; 129.75 g/m2 and 124.5 g/m2). The research studies of Benzohra et al.,(2011) and Mohammed Amin and Fufa Melkamu (2014) exhibited the use of biocontrol mode of control in fungal pathogenicity in plants.

Both in greenhouse and field experiments seed protein was recorded high in the triple inoculated biocontrol agents along with control pathogens \( Fu.o + T.v + B.s + P.f + Gl.f \) (21.6 %), \( As.r + T.v + B.s + P.f + Gl.f \) (20.5 %) and \( Al.a + T.v + B.s + P.f +
Gl.f (18.6 %) and Highest carbohydrates recorded in the treatment Fu.o + T.v + B.s + P.f + Gl.f (44.20 %) which was significantly different compared to all the other treatments followed by Fu.o + carbendazim (46.2 %) and As.r + T.v + B.s + P.f + Gl.f (40.50 %). Highest polyphenol content was observed in the treatment inoculated with Fu.o + T.v + P.f (263.8 mg) which was significantly different from all the other treatment followed by the treatment inoculated with Fu.o + T.v + B.s + P.f + Gl.f (261.1 mg). Similar kind of observations revealed in previous studies conducted by Agarwal et al., (2011).

The protein, carbohydrates and polyphenol content of chickpea (Cicer arietinum. L) seeds was maximum (18.4 %) in the treatment inoculated with As.r + T.v + B.s + P.f which was significantly higher compared to all the other treatments followed by the treatment T11. pathogen control + carbendazim (16.2 %) treated with chickpea (Cicer arietinum. L). Seeds and the lowest per cent seed protein which was observed are recorded in the treatment inoculated with only pathogen (11.8 %) followed by the treatment As.r + B.s (13.2 %). This may be due to the integrated beneficial role of bioagents and plant growth promoting substances secreted by beneficial microorganisms. Similar kind of analysis releaved on ethylene biosynthesis for plant development (Jouyban, Z., 2012, and Schaller, G.E., 2012).

The synergetic effect of biocontrol agents with bacterial plant growth promoters has enhanced the protein content in chickpea seeds were maximum (15.7 %) in the treatment inoculated with Al.a + T.v + B.s + P.f and pathogen control + carbendazim treated with chickpea seeds and maximum percent carbohydrates and polyphenols recorded in the triple inoculation with biocontrol agents along with Al.a (36.5 % and 243.5 mg/g) respectively in these experiments. Similarly induction of resistance in
chickpea against *Ascochyta rabie* by applying chemicals and plant extracts was reported (Usman Ghazanfar, *et al.*., 2011). Highest shoot and root total Nitrogen, Phosphorous and Potassium uptake was recorded in the triple inoculated bioagents treatment *Fu.o* + *T.v* + *P.f* + *Gl.f* (192 kg/ha, 11.9 kg/ha and 339 kg/ha respectively) which was significantly different compared to all the other treatments which was on par with the treatments *As.r* + *T.v* + *B.s* + *P.f* + *Gl.f* (190, 11.5 and 337 respectively) and *Al.a* + *T.v* + *B.s* + *P.f* + *Gl.f* (188, 11.3 and 335 respectively). This may be due to the reason that combined inoculation effects of biocontrol agents and plant growth promoting rhizo microorganisms enhanced the root proliferation and uptake of mineralized nutrients in the rhizosphere of chickpea.

Increasing trend of total number of microorganisms observed in all the treatments at different day of sowing with respect to all the groups at 45 DAS and significantly decreasing at the decreasing rate of trends observed with respect to all the treatments till the harvest i.e., at 135 DAS. Highest total number of bacteria was recorded in the treatment *Fu.o* + *T.v* + *B.s* (49 × 10^4 CFU/mL) followed by *Al.a* + *T.v* + *B.s* + *P.f* + *Gl.f* (47 × 10^6 CFU/mL). Highest total population was observed in the treatment *Fu.o* + *T.v* + *B.s* + *P.f* + *Gl.f* (64.60 × 10^4 CFU/mL) which was significantly different compared to all the other treatments followed by *Fu.o* + *T.v* + *B.s* (62.70 × 10^4 CFU/mL) and *As.r* + *T.v* + *B.s* + *P.f* + *Gl.f* (61.3 ×10^4 CFU/mL) at 45 DAS and same trend was observed with respect to 135 DAS. Highest population of actinomycetes was found in the treatment *Fu.o* + *T.v* + *B.s* + *P.f* + *Gl.f* (64.30 ×10^3 CFU/mL) followed by *Fu.o* + *T.v* + *B.s* (63.50 ×10^3 CFU/mL) which were significantly different compared to all the other treatments at 45 DAS and the same trend of decreasing rate was observed till the harvest. This evidences synergistic role of different group of microorganisms activity in the
rhizosphere of chickpea and also succession of microorganisms during different intervals of crop growth.

Effect of biocontrol agents, fungicides and foliar fungal pathogens of chickpea (Cicer arietinum. L) on total population of biocontrol agents (CFU/mL) at different intervals under field condition was observed and highest population of Tricoderma viridae in the treatment of Al.a (17.11 × 10^5 CFU/mL) was observed significantly higher compared to all the other treatments followed by Fu.o (13.77 × 10^5 CFU/mL) at 45 DAS and the increasing trends of the same was observed at 90 DAS (19.33 × 10^5 CFU/mL) followed by As.r (13.11 × 10^5 CFU/mL). This is because of micro parasitism and highest growth rate of T. viridae under rhizosphere which dominated the population of other microorganisms. Psuedomonas florencens was highest in the treatment Fu.o + T.v + B.s (24 × 10^6 CFU/mL) followed by Fu.o + T.v + B.s (23.22 × 10^6 CFU/mL) which was significantly higher and Bacillus subtilis was highest in the treatment Al.a + T.v + B.s (13.66 × 10^6 CFU/mL) at 45 DAS followed by As.r + T.v + B.s (12.33 × 10^6 CFU/mL) and Al.a + T.v + B.s (12.11 × 10^6 CFU/mL) and the same trend at increasing rate was observed during 90 DAS and 135 DAS. The antagonistic and synergistic activity of bacterial bioagents inoculated in those treatments increased the population of both Psuedomonas florencens and Bacillus subtilis in rhizosphere at different intervals of plant growth. Similar work was conducted on host parasite relationships and associated physiological changes on Meloidogyne by Hussey, R.S., 1985.

Influence of biocontrol agents, fungicides and foliar fungal pathogens of Chickpea (Cicer arietinum. L) on mycorrhizal spore count and percent root colonization under field condition revealed the highest in treatment inoculated with AM fungi i.e., As.r + T.v + B.s + P.f + Gl.f (83.52 / 50 g soil) followed by As.r + T.v + B.s (82.67 / 50
g soil) and \( Fu.o + T.v + B.s + P.f + Gl.f \) (82.00 / 50 g soil) which was significantly higher compared to all the other treatments and also highest percent root colonization was observed in the treatment \( As.r + T.v + B.s + P.f + Gl.f \) (73.40 %) followed by \( As.r + T.v + P.f \) (71.70 %) and \( Fu.o + T.v + B.s + P.f + Gl.f \) (71.33 %). This observation clearly indicates combined interactive inoculation effect of \textit{Glomus fasciculatum} with \textit{Psuedomonas florencens}, \textit{Bacillus subtilis} and many studies in different crops with the combined effect of mycorrhizae and plant growth promoting microorganisms evidenced by earlier research reviews. Similar increase in plant growth parameters in \textit{P. fluorescens} treated plants was reported by Jaya Kumar et al., (2004). Further, they reported application of \textit{P. fluorescens} through seed and soil as single and split application, either singly and in combination reduced the larvae population of parasitic nematodes in soil and root as compared to control. Effective reduction in nematode population due to split applications in different intervals might be due to maximum building up of bacterial population/ root colonization (Eapen et al., 2005 and Devi and Dutta 2002) by bacterium inoculation. Similarly effect of microbial inoculation on wheat growth and phyto-stabilization was conducted of contaminated soil with chromium and determination of \textit{Fusarium oxysporum} f. sp. \textit{ciceri} isolates by biological pathotyping and vegetative compatibility was made (Khan, et al., 2013 and Khan, et al., 2002).