Studies on integrated management of wilt and root rot complex of chickpea (*Cicer arietinum* L.) caused by *Fusarium* spp. and *Rhizoctonia solani*

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Thesis

Submitted to the
Maharana Pratap University of Agriculture and Technology, Udaipur
In partial fulfillment of the requirements for the Degree of

Doctor of Philosophy in Agriculture

(Plant Pathology)

BY

RAJKUMARI PADAMINI

2014

MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY,

RAJASTHAN COLLEGE OF AGRICULTURE, UDAIPUR

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Studies on integrated management of wilt and root rot complex of chickpea (*Cicer arietinum* L.) caused by *Fusarium* spp. and *Rhizoctonia solani*

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ABSTRACT

The present investigation were undertaken to develop an effective strategy for the integrated management of wilt and root rot complex diseases of chickpea (*Cicer arietinum* L.), which is widely prevalent in moderate to high severity in different parts of Rajasthan.

The pathogens were isolated from disease chickpea plants showing typical wilt and root rot symptoms collected from farmer's field of different chickpea growing areas of Rajasthan *viz.*, Udaipur, Bikaner, Tivari, Pali, Sirohi and Banswara. *Fusarium oxysporum* f.sp. *ciceri* (one isolate), *Fusarium solani* (five isolates) and *Rhizoctonia solani* (six isolates) were isolated and their pathogenicity were confirmed by growing chickpea in pathogen inoculated soil.

The isolates of *F. solani* and *R. solani* exhibited considerable variations in growth, colony character, spore and sclerotial size. The *in vitro* physiological studies revealed that the maximum mycelium growth and sporulation of the isolates of *F. solani*, *Foc* and *R. solani* were recorded at 30^0C temperatures and pH 7 though considerable variations were recorded among isolates of *F. solani* and *R. solani*.

Assessment of losses caused by wilt and root rot complex were studied on field for two years at varied disease levels. Results showed the disease severity increased with combined inoculation of two (*R. solani* + *Foc*) and three (*Foc* + *F. solani* +*R. solani*) pathogens as compared to that of the individual (*R. solani*).

Five fungicides were tested, *in vitro* condition at three concentrations (250, 500 & 1000 ppm). The isolates showed variations in sensitivity to various fungicides but Tebuconazole was highly effective against all the isolates of *Foc, F. solani* and *R. solani* at all the concentrations so it was taken for further field experiment. Neem oil at 0.2 % concentration was effective in suppressing the growth of the pathogens.

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Among the fungal and bacterial biocontrol agents, *T. harzianum* ICRISAT-25 and *T. viride* ICAR-95, *P. fluorescens* UDP Pf-1 and *B. subtilis* Br II showed good suppression of the three pathogens. These were than tested in pot culture and showed good effects. The biocontrol agent *T. harzianum* ICRISAT-25 showed highest efficacy in suppressing the isolates of the pathogens by dual culture method and was further tested in the field.

In order to find host plant resistance to wilt and root rot complex, ten popular varieties were evaluated in pot culture under inoculations of all the three pathogens. The cultivar Avrodhi was highly resistant, GNG-469, RAJ-1581, P-1080 and Pratap Chana-1 were moderately resistant, while BG-1053, BGD-72, BG-391 and RSG-888 were moderately susceptible. The popular cultivar Dahod Yellow was found to be highly susceptible. Alteration of different dates with late sown on 29th Nov showed less incidence of the disease as compared to early sowing on 15th Oct.

The fungicides, botanicals and biocontrol agents found effective *in vitro*, were further evaluated in field for two consecutive seasons as seed treatment individually as well as in combinations for suppression of wilt and root-rot complex of chickpea. It was found that combined treatments were superior in terms of better germination, lower mortality and higher yield as compared to the individual treatments. The most effective treatment was seed treatment with Tebuconazole + *T. harzianum* followed by Vitavax + *T. harzianum* as compared to control as well as other treatments. *T. harzianum* applied as seed treatment effectively established in chickpea rhizosphere and reached high population densities, at 90 DAS while the population of the pathogens was low in the rhizosphere as significant disease suppression was recorded.

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1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown in tropical, subtropical and temperate regions of the world. It is the world's third most important grain legume after common bean and pea (Anwar *et al*., 2009). Asia covers 89.7 per cent of the area in chickpea cultivation followed by 4.3 per cent in Africa, 2.6 per cent in Oceania, 2.9 per cent in Americas and 0.4 per cent in Europe (Gaur, 2010). India ranks first in terms of chickpea production and consumption in the world. About 65% of the global area with 68% of global production is contributed by India (Reddy and Mishra, 2010).

Chickpea is grown as a post monsoon (*Rabi*) crop, between 30^0 4′ to 31^0 35′ N and 71^0 5′ to 76^0 55′ E, both under irrigated and rain fed conditions. It occupies very important position in semi-arid farming system both for human nutrition and restoring the soil fertility (Singh and Sirohi, 2003). It is a protein-rich supplement to all cereal based diets, especially for vegetarians. Its protein is rich in lysine and has low sulphur containing amino acids and hence, it is widely appreciated as health food. On an average, chickpea contains protein (12.4 to 31.5%), carbohydrate (48.2 to 67.6%) and fat (6%) (Anwar *et al*., 2009). The mineral component is high in chickpea as it contains phosphorus (340 mg/100 g), calcium (190 mg/100 g), iron (7 mg/100 g), zinc (3 mg/100 g) and vitamin B in considerable amounts (Anon., 2008).

In India, the total area under chickpea is about 8.21 million hectares, with production of 7.48 million tones, and productivity of 911 kg/ha (FAO, 2012). Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Uttarakhand and Karnataka are major chickpea growing states of India covering 89 per cent of total area and 89 per cent of chickpea production of the country (Anonymous, 2008). MP produces the maximum chickpea from 2.44mha. , followed by Maharashtra (1.35 mha.), Rajasthan (1.23 mha), AP (0.63 mha.) and UP (0.51 mha.). The maximum chickpea production is in MP (1.74 mt.) followed by Maharashtra (0.91 mt.), AP (0.57 mt.), Rajasthan (0.57 mt.) and UP (0.38 mt). (Ministry of Agriculture 2008-09).

In Rajasthan, the major chickpea growing districts are Churu, Jhunjhunu, Sikar, Jaipur and Hanumangarh. The total area and production of chickpea in

Rajasthan are 1.25 million hectares and 1.27 million tonnes, respectively, having productivity of 1019 kg/ha (Ministry of Agriculture, Rajasthan, 2013).

More than 50 pathogens have been reported to infect chickpea crop but only few cause economically important diseases. Wilt and root rot complex caused by several soil borne pathogens is the major yield reducing malady. Among them, Fusarium wilt (*Fusarium oxysporum f. sp. Ciceri* (Pad Wick) Snyd. & Hans), black root rot (*Fusarium solani* (Mart.) Sacc.), root rot (*Rhizoctonia solani*) and dry root rot (*Sclerotia rolfsii*) are of considerable importance (Nene *et al*., 1981). The problem is widespread in several countries of the world like India, Iran, Pakistan, Nepal, Burma, Spain, Mexico, Peru, Syria and USA (Nene *et al*., 1989; Jalali and Chand, 1992). It is one of the important limiting factors of chickpea production in India. Chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceri* was first reported from India by Butler (1918). Black root rot / Wilt caused by *Fusarium solani* (Mart.) Sacc. causes severe yield loss *i.e*., 60-70 per cent under favourable conditions (Tewari and Mukhopadhyay, 2003). The diseases caused by soil borne fungal pathogens *viz*., *Rhizoctonia solani* (root-rot), *Fusarium oxysporum* f.sp. *ciceri* (wilt) and *Sclerotium rolfsii* have been considered as most devastating for the production of chickpea (Singh *et al*., 1986; Khan *et al*., 2002). Early infection of the wilt complex pathogens results in death of plant, *i.e*. in total yield loss (Haware and Nene, 1980). The disease is wide spread in the chickpea growing areas of the world and reported from at least 33 countries (Nene *et al*., 1996). In India, it has been reported from all the chickpea growing states and causes an annual loss of 10% (Singh and Dahiya, 1973). The disease can affect the crop at any stage of growth. Early wilting causes 77- 94% losses while late wilting causes 24- 65% loss (Haware and Nene, 1980). The grain losses due to chickpea wilt and root rot has been estimated to be around 10 percent which amounts to approximately 520 thousand tonnes annually.

Characteristic symptoms of wilt and root rot are sudden drooping of leaves and petioles, no external rotting of roots and black internal discoloration involving xylem and pith (Dubey and Singh, 2004). The disease is characterized by two syndromes, namely vascular wilt and yellowing that can be distinguished by both symptomology and chronological development. The wilt syndrome results in a rapid flaccidity and desiccation of the leaves and stems by 20 days after inoculation. Whereas yellowing syndrome results in a progressive foliar yellowing followed by

necrosis 30- 40 days after inoculation (Trapero-Cases and Jimenez-Diaz, 1985). The pathogens are soil and internally seed borne (Haware *et al*., 1978) and for such pathogens, chemical control is uneconomical and causes groundwater pollution, loss of non-target beneficial flora and evolving fungicidal resistance variants (Sen, 2000).

No single control measure is fully effective against this disease. Management of Fusarium wilt and root rot complex of chickpea is difficult to achieve as the pathogens are soil-borne, surviving through resistant structure *i.e.* chlamydospores and sclerotia in soil for years even in the absence of host and the crop remains susceptible all throughout the growth stages (Kaiser *et al.*, 1994 and Haware *et al.*, 1996). Use of chemical fungicides for effective management of these pathogens is not possible because of the physical heterogeneity of the soil, which might prevent effective concentrations of the chemical reaching the target pathogen (Tewari and Mukhopadhyay, 2001). Soil applications of fungicides are costly and lead to indiscriminate killing of beneficial soil micro flora. The use of resistant cultivars appears to be most practical and economical. However, there is lack of resistance genes and the efficiency of resistant cultivars is reduced due to appearance of new virulent or aggressive strains of the pathogen. Moreover, continuous use of fungicides may lead to development of resistant or tolerant strains of the pathogen towards fungicides. Hence, biological management of root diseases of various field crops including chickpea using microbial antagonists such as *Trichoderma* spp. *Pseudomonas fluorescens, Bacillus* spp. etc. have drawn the attention of growers and researchers throughout the world (Mukhopadhyay *et al*., 1992; Hervas *et al*., 1997; Saikia *et al.*, 2003; Khan and Gangopadhyay, 2008 and Jayalakshmi *et al*., 2009).The disease to some extent can be managed by use of bio-control agents which provide eco-friendly control of the disease (Hervas *et al*., 1997, 1998; Landa *et al*., 2001). Nonpathogenic *Fusarium oxysporum*, *Bacillus* species and *Pseudomonas fluorescens* were identified and found suitable for bio-control of wilt (Hervas *et al*., 1997; Landa *et al*., 2001, 2004). Efficacy of wilt management has been reported to improve when biocontrol agents were combined with cultural practices such as sowing dates (Landa *et al*., 2004). Crop rotation, pathogen free seed, removal of plant debris and fungicide seed treatment are several of the disease management strategies that have been employed for control of wilt and root rot complex disease of chickpea, but have met with limited success (Nene and Reddy, 1987). Therefore, integrated management

strategy is the better solution to maintain plant health. These strategies include minimum use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre and Barhate, 1998).

In sustainable agriculture, diseases of grain legumes need to be managed by integrated disease management (IDM) strategies that involve the use of additive or synergistic combinations of biotic, cultural, and chemical control measures (Conway, 1996 and Jimenez-Diaz *et al*., 1998).The disease is widespread in Rajasthan and at times become severe. A perusal of the available literature revealed that various recommendations are available for management of individual pathogen like *Foc*, *R. solani* and *S. rolfsii*. Since the distribution and prevalence of various pathogens and the efficacy of the management strategies depend on the soil edaphic factors and the prevalent agro-climatic conditions, location specific IDM of the wilt and root rot were desirable. Therefore, the present work was undertaken with the following objectives:

1. To study etiology and distribution of *Fusarium* spp. and *Rhizoctonia solani* causing wilt and root rot complex of chickpea in Rajasthan.
2. To study cultural and pathogenic variability of some selected representative isolates of the pathogens.
3. To assess the yield losses caused by wilt and root-rot complex in chickpea.
4. To develop integrated management module involving cultural practices (date of sowing), chemicals, botanicals, biocontrol agents and host plant resistance.

2. REVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume crop with India accounting for more than 60 per cent of world production. Chickpea wilt and root-rot complex caused by *Fusarium oxysporum* f. sp. *ciceri* (*Foc*) (Pad Wick) Snyd. & Hans, *F. solani* (Mart.) Sacc. , *Rhizoctonia solani* Kuhn and *Sclerotium rolfsii* is one of the serious limiting factors of chickpea production worldwide. Currently the disease is prevalent in several countries. The pathogens are soil-borne and difficult to eradicate as the resting spores (chlamydospores and sclerotia) survive in soil upto several years even in the absence of host plant. Yield losses vary between 10 to 100% depending on varietal susceptibility and agro-climatic conditions. The disease is more severe in light sandy soil than heavy clay (Kotasthane *et al.*, 1979 and Sugha *et al.*, 1994). High soil temperature and deficiency of moisture appear to have a definite bearing on its incidence. The amount of organic matter is directly related to wilt and root rot incidence (Chauhan, 1965)

Padwick for the first time described chickpea wilt caused by *F. o. ciceri* (*Foc*) from India in the year 1940. Westerland (1974) regarded that wilt including flaccidity, yellowing and vascular discoloration induced by *Foc* as the most important in the complex. Nene *et al.* (1985) reported that wilt and root rot complex in chickpea to be caused by several pathogens however, *Foc*, *R. solani* and *S. rolfsii* were the major pathogens. He observed that the disease may appear at any stage of plant growth, symptoms in a highly susceptible cultivar can develop any time between 25 days after sowing till as late as podding stage. The fungus could survive on crop residues in soil for more than 6 years (Haware *et al.*, 1986). Dry root rot of chickpea has also been reported from several countries like, India, Iran, Australia, Ethiopia, Pakistan, Spain and USA, Nene and Reddy (1987).

Demicri *et al.* (1999) isolated *Fusarium* spp. and other soil-borne pathogens from wilt infested chickpea plants and seeds, and found *Foc* was predominant among the different fungi isolated from different plants.

Singh *et al.* (2002) conducted a survey to find out the prevalence of soil-borne diseases in chickpea and recorded that *Foc* was most prevalent in wilted plants. The

development of wilt was favoured by increase in nitrogen. The optimum temperature and pH for pathogen was 25^0C and 5- 6.5 respectively, delay in sowing helped in minimizing disease.

Andrabi *et al*. (2008) carried out extensive surveys in major chickpea growing districts of Jammu division *viz.*, Jammu, Kathua and Udhampur to ascertain the status of chickpea wilt complex and pathogens associated with the disease. During surveys, the wilt complex disease was found to be prevalent in all the three districts with maximum disease incidence 55.0 % recorded with the mean disease incidence of 17.28, 14.20 and 11.57 % in Jammu, Kathua and Udhampur districts, respectively.

Wilt and root rot disease complex of chickpea was reported as a devastating disease causing an estimated annual loss of 12 million rupees in Pakistan (Sattar *et al*., 1953). The disease has been reported from more than 30 countries causing an average of 10-15 per cent losses in grain yield annually (Singh and Dahiya, 1973 and Nene *et al.*, 1996). Attempts were made to estimate loss in yield on per plant basis, and it was found that earlier wilting causes more loss than late wilting. Seeds harvested from wilted plants were lighter and duller than those from healthy plants. Halila and Strange (1996) reported that the annual chickpea yield losses from Fusarium wilt vary from 10 to 15%, but the disease can completely destroy the crop under specific conditions. Under favourable environmental conditions the losses due to Fusarium wilt may reach upto 100 per cent (Halila and Strange, 1996; Navas-Cortes *et al.*, 2000 and Anjaiah *et al.*, 2003).

Survey and surveillance of chickpea wilt in the Latur district by Nikam *et al*. (2008) revealed average wilt complex to the tune of 12.26%. Tashil wise survey report indicated maximum wilt incidence in tashil Ausa (15.4%) followed by Jalkot (14.8%) and Renapur (14.0%). Further study indicated that *Foc* was associated in majority cases. Pathogen was isolated, purified and its pathogenicity was proved in pot culture. Further, on the basis of morphological, cultural characteristics of the pathogen and symptomology, the fungal pathogen was identified as *Foc*.

Pitambar *et al*. (2010) conducted a survey in Nagpur district of Maharashtra in which a total of 617 chickpea wilted samples were collected from 39 villages tested on media and 3 fungi, *Foc, R. bataticola* and *S. rolfsii* were isolated and there showed wilt incidence of 53.48 %, 31.28 and 11.83 % respectively.

The pathogens causing wilt and root rot complex of crops are highly variable. Variability in these has been studied for cultural and morphological traits and pathogenicity.

Eight races of *Foc* (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) have been described (Cabrera *et al.*, 1985; Haware and Nene, 1982; Jimenez *et al.*, 1993; Jimenez *et al.*, 1989), which can be grouped into the wilting and yellowing pathotypes based on the disease symptoms they induce in pathogenicity tests (Trapero-casas and Jimenez 1985). Races 0 and 1B/C are of the yellowing pathotypes, while races 1A and 2 through 6 belong to the wilting pathotypes (Jimenez *et al.*, 1993, Jimenez *et al.*, 2003; Jimenez *et al.*, 2001). Races 2, 3, and 4 have only been reported in India (Haware *et al.*, 1982; Jalali and Chand 1992), whereas races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean and in California (Halila and Strange, 1996; Jimenez-Diaz *et al.*, 1993; Jimenez-Gasco and Jimenez-Diaz, 2003; Jimenez-Gasco *et al.*, 2001).

According to Sharma and Muehlbauer (2007) 8 physiological races of the pathogen *Foc* races 0, 1A, 1B/ C, 2, 3, 4, 5 and 6 are reported so far, though additional races are suspected to be present in India. Pathogen isolates also exhibit differences in disease symptoms. Races 0 and 1B/C cause yellowing syndrome whereas 1A, 2, 3, 4, 5 and 6 lead to wilting syndrome. Genetics of resistance to two races (1B/ C and 6) is yet to be determined. Slow wilting, *i.e.*, slow development of disease has also been reported however, its genetics is not known.

Ram and Pandey (2010) studied four isolates of *F. solani* isolated from four different districts *viz.*, Anand, Arnej, Dahod and Thasra of Gujarat for their pathogenic characteristics on chickpea cv. GG-2 in pots by using soil inoculation method. The isolates caused symptoms of yellowing with characteristic black lesions on roots. The maximum radial growth of 71.6 mm was recorded by Arnej isolate at 8 days of incubation. Also, the isolate from Arnej was found to be highly pathogenic showing 93.3 % disease incidence at 45 days after sowing.

Elewa *et al.* (2000) studied the morphological and cultural characteristics of 53 *R. solani* isolates collected from different host plants in Egypt. The isolates were grouped in eleven anastomosis groups, of which seven were compatible.

The pathogenicity of 53 *R. solani* isolates and their fusion hybrids on bean, broad bean, cotton, squash and tomato was determined in laboratory and pot

experiments. Disease incidence varied among the hosts and depending on the isolate. Results showed no clear correlation between the taxonomic grouping of each isolate under different anastomosis group and their virulence. Fusion between high and low virulence isolates resulted in isolates with lesser degree of virulence compared to the low-virulence parent. Re-isolation of *R. solani* from surface sterilized infected plants yielded isolates that were morphologically similar to resultant fusions between isolates (Mostafa *et al.*, 2000).

In another study, 112 isolates of *R. solani* were collected from soil, root and collar rot or foliage blight infected various plants from several locations in Haryana, India. Of these, 43% belonged to anastomosis group-1 (AG-1 IA, AG-1 IC), 37% to AG-4, 9% to AG-3 and 11% to AG-7. Studies on 20 selected isolates of different AGs revealed considerable variability in their cultural and morphological characters, growth and virulence. Results of pathogenicity tests using mung bean cv. K 851, chickpea cv. C 235, rice cv. Jaya and sugarcane cv. COJ 64 showed that isolates of AG-1, AG-4 and AG-7 were moderate to highly virulent on hypocotyls of chickpea and mung-bean (Sunder *et al.*, 2003).

Yadav and Tiwari, (2005) reported that five isolates (I_1 to I_5) of *R. solani* from fenugreek plants collected from different locations in Chattisgarh varied in the rate and type of growth, colony colour, hyphal width and sclerotial production. Isolate l_3 showed the highest growth on PDA medium and highest pathogenicity.

Kumar *et al*. (2008) conducted an experiment in which a total of 25 isolates of *R. solani* were collected from rice plants from different regions of Uttar Pradesh *viz*., Azamgarh, Basti and Faizabad, and analysed for variability using morphological and virulence characteristics. Virulence diversity was analyzed by inoculation on 10 rice cultivars under greenhouse conditions at 28^0C. In most of the isolates, the disease symptoms appeared at 48 h after inoculation but in some of them the disease appeared at 96 h after inoculation. The disease severity of *R. solani* isolates was analyzed by AUDPC (area under disease progress curve) value on the basis of lesion length recorded at days 4, 8, 12 and 16 after inoculation. A comparative analyses of *R. solani* isolates indicated that fast growing isolates having macro-sized sclerotia were highly virulent compared to slow growers with micro-sized sclerotia.

For management of wilt and root rot complex, use of fungicides and botanicals, host plant resistance, cultural and biological control strategies have been recommended. Adequate characterization of the resistance of chickpea lines and cultivars to specific races of *Foc* is essential for resistance deployment. Earlier reports suggested that resistance to Fusarium wilt in chickpea was conferred by a single recessive gene (Pathak *et al.*, 1975; Kumar and Haware, 1982), but the latter studies revealed that resistance to race1 appeared to be controlled by at least 3 independent loci (Singh *et al.*, 1987). Effective field-screening and laboratory procedure have been developed and wilt resistance sources identified (Nene and Haware, 1980) of which some had additional resistance to dry root rot.

The inheritance of resistance to root rot of chickpea seems to be monogenic with resistance dominant over susceptibility (Rao and Haware, 1987). This diversity in phenotypes and geographic distribution of races of *Foc* makes the identification of races of the pathogen in a given area of chickpea production crucial for development and deployment of host plant resistance. The identification of races 1B/C, 2, 3, and 4 or new races of *Foc* as well as the characterization of resistance reactions in chickpea germplasm, are dependent on traditional pathogenicity tests (Jimenez-Diaz *et al*., 1993; Sharma *et al.*, 2005).

Host plant resistance appears to offer the best practical and economical strategy for control of this disease. Good progress has been made in the identification of sources of resistance to Fusarium wilt (Haware *et al.*, 1990; Jimenez-Dıaz *et al*., 1991) in both desi (small, angular, colored seeds) and kabuli (large, ramhead shaped, beige seeds) germplasm, and kabuli cultivars resistant to Fusarium wilt have been developed. However, the effectiveness of these resistant cultivars may be curtailed because of the occurrence of races in *Foc* pathogenic to them. Crop rotation, pathogen-free seed, removal of plant debris, and fungicide seed treatment are several of the disease management strategies that have been employed for control of Fusarium wilt (Nene and Reddy, 1987), but have met with limited success.

Pande *et al.* (2006) evaluated chickpea mini-core collection composed of 211 germplasm to identify sources of multiple disease resistance against Fusarium wilt (*Foc*), dry root rot (*R. bataticola*), Ascochyta blight (*A. rabiei*) and Botrytis gray mold (*B. cinerea*), under a controlled environment. High levels of resistance were observed to Fusarium wilt, where 21 accessions were asymptomatic and 25 resistant.

Through this study, chickpea germplasm accessions were identified that possess high levels of resistance to more than one fungal disease and would be used in chickpea multiple disease resistance breeding programs.

Iqbal *et al*. (2010) evaluated one hundred and forty five genotypes obtained from various sources. Disease observations were recorded at seedling and reproductive stages. Disease incidence ranged from 0% to 57.2% at reproductive stage and it varied from 0% to 100% at seedling stage. Five genotypes were identified with genes for tolerance against both the diseases which could be tested under wide range of environments and be utilized for developing high yielding cultivars with dual tolerance through building pyramid resistance. Shaban (2011) found that the antagonistic effect between bio-agents and pathogenic fungi can cause more nodule production in different tested legume crops under greenhouse conditions.

Several fungicides have been evaluated against the wilt and root rot pathogens. Viswakarma and Chaudary (1982) evaluated some fungicides against some root disease pathogens of gram under *in vitro* condition. During their study they found that Agrosan GN and RH 893 were found effective against *F. solani* at 5 ppm concentration.

Jimenez and Trapero (1985) studied the efficacy of fungicide seed dressings, foliar sprays, and moderately resistant cultivars alone or in combination for control of the wilt and root rot (WRR) complex of chickpeas in fields with a history of high incidence (≥80%) of the disease. The efficacy of treatments was assessed by their influence on the epidemic development of the disease. They found that seed dressings with each of Captan, Captafol, Thiram, Benomyl, Triadimenol and the mixtures of benomyl with Captan, Captafol or Thiram individually or in various combinations significantly increased seedling emergence of the moderately resistant cultivars.

Christian *et al*. (2007) reported significant effect of five fungicides - Benomyl (1mg/l), Dodine (500mg/l), Mnazate (100mg/l), Cupric sulphate (200mg/l) and Thiobendazole (4mg/l) under *in vitro* conditions on growth of 28 isolates of *Foc*.

Nikam *et al*. (2007) found that chemical seed treatment with Thiram (0.15%) + Carbendazim (0.1%) was the most effective against *Foc.* Mukhtar (2007) reported that Benomyl, Captan and Carbendazim significantly reduced the growth of *Foc* and

at higher concentration, Benomyl and Carbendazim proved to be the most effective fungicides and Captan were least effective in higher concentration.

Madhusudhan *et al*. (2010) tested six fungicides *viz*., Carbendazim (50% WP), Propiconazole (25% EC), Hexaconazole (5% EC). Tridemorph (80% EC), Chlorothalonil (75% WP) and Mancozeb (75% WP) *in vitro by* poisoned food technique for their efficacy on *F. solani* and compatibility with *T. viride* isolates at five different concentrations *viz*., 50, 100,250, 500 and 1000 ppm. Among the six fungicides tested Chlorothalonil (75% WP) was found to be safe to two *T. viride* isolates (T2 and T4) up to 450 ppm as it caused less inhibition of 42.3'5 and 44.4% respectively and effective against *F. solani* as it showed 62.82%, inhibition. It was concluded that Chlorothalonil (75% WP) could be applied along with the *T. viride* isolates lIJl to 250 ppm in integrated management. Mancozeb (75% WP) was found to be safe to *T. viride* isolates and less effective against *F. solani.* Carbendazim (50% WP) Propiconazole (25% EC), Tridemorph and Hexaconazole (5% EC) were found to be effective against *F. solani* and not safe to *T. viride* isolates.

Subhani *et al*. (2011) evaluated six fungicides, namely, Benomyl, Derosal, Ridomil, Cabrio Top, Vitavax and Prevent at four concentrations, 5, 10, 20 and 50 ppm on *Foc.* There was a significant decrease in mycelial growth of the fungus with an increase in fungicidal concentration. The most effective fungicides in inhibiting the growth of the fungus, in descending order were Derosal, Benomyl and Vitavax as they caused 100, 95.81, 93.80 and 70.96% reduction in mycelia growth, respectively at 5 ppm concentration. Derosal and Benomyl were the most effective and exhibited 100% reduction in disease incidence while Vitavax and Cabrio Top exhibited 96.33 and 88.37% in disease incidence respectively.

Ho-Seong *et al.* (1991) reported the antifungal activity of antagonistic bacterium *P. stutzeri* against *F. solani* isolated from a ginseng rhizosphere. In several biochemical tests with culture filtrates of *P. stutzeri* YPL-1 and in mutational analysis of antifungal activities of reinforced or defective mutants, they found that anti- *F. solani* mechanism of the bacterium may involve a lytic enzyme rather than a toxic substance (or) antibiotic.

Nautiyal (1997) developed a procedure for screening chickpea rhizosphere-competent bacteria for suppression of *Foc, R. bataticola* and *Pythium* sp. . Of the 478

bacteria obtained by random selection of the predominant, morphologically distinct colonies, 386 strains that effectively colonized chickpea roots were divided into three different groups, strains from the first group were further screened for their *in vitro* bio-control activity against *Foc, M. phaseolina* and *Pythium* spp. One bacterial strain was selected for further investigation as it inhibited all the three fungi and was a good rhizosphere colonizer.

Larkin *et al*. (1998) showed that combining non-pathogenic *F. oxysporum* strain with either *P. fluorescens* or *B. subtilis* was more effective at suppressing Fusarium wilt than single application of non-pathogenic *F. oxysporum.*

Timothy *et al*. (2000) reported the antifungal activity of 3-(1-hexenyl)-5-methyl-2(5H)-furanone produced by *P. aureofaciens*. The purified furanone showed antifungal activity against *Foc, F. solani, P. ultimum* and *T. basicola.*

Cachinero *et al.* (2002) inoculated germinated seeds of 'kabuli' chickpea cv. ICCV with a conidial suspension of the incompatible race 0 of *Foc* or of non-host *F. oxysporum* resistance inducers, and three days later their seedlings were challenged by root dip with a conidial suspension of highly virulent *Foc* race 5. The extent of disease suppression varied with the nature of the inducing agent; the non-host isolates of *F. oxysporum* were more effective at disease suppression than the incompatible *Foc* race 0. These defense-related responses were induced more consistently and intensely by non-host isolates of *F. oxysporum* than by incompatible *Foc* race 0.

T. viride and *T. harzianum* were reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey 2002, 2003; Poddar *et al*., 2004).

Mujeebur *et al*. (2004) examined the effect of treating seeds of chickpea cv. BG 256 with commercial formulations (2 g/kg seed) of *T. harzianum* and *P. fluorescens,* singly and jointly, to control wilt caused by *Foc* under field condition. On untreated control plants, the wilt fungus caused the characteristic symptoms of wilt and significantly decreased dry weight and the yield of chickpea by 20 and 15 per cent, respectively. On chickpea without wilt, treatment with *P. fluorescens* improved the yield by 36 per cent and *T. harzianum* + *P. fluorescens* by 25 per cent. Both bio-fungicides suppressed wilt severity, the most effective being *T. harzianum* + *P. fluorescens*.

Gupta *et al*. (2006) evaluated the efficacy of *T. viride* in controlling the chickpea wilt complex *Foc, R. solani* and *S. rolfsii* and enhancing the chickpea *Rhizobium* symbiosis. Seeds of chickpea cv. JG-74 were inoculated with Lathyrus local *Rhizobium* isolates no. L4 alone or in combination with one of the test isolates (LS1, LS2, LS5 and LS6) and *T. viride* TNAU and ICAR isolates. Of these, *T. viride* LS2 was superior in enhancing chickpea *Rhizobium* symbiosis, followed by *T. viride* TNAU.

In another study, the efficacy of antagonistic bacteria, *B. thuringiensis* (B-120 from chickpea fields in Karaj, B-22, B-28 and B-32 and *P. fluorescens* (Pf-100 and Pf-19 and CHAO), was investigated against Fusarium wilt under greenhouse conditions. B-120 isolate significantly reduced Fusarium wilt in chickpea and the antagonistic bacteria exhibited a significant positive effect on plant growth factors. B-120, B-28 and B-32 as well as Pf-100 caused an increase in growth factors (Jamali *et al*., 2005).

Rudresh *et al*. (2005) tested nine isolates of *Trichoderma* spp. for their ability to inhibit soil borne fungal pathogens of chickpea *viz., R. solani, S. rolfsii* and *Foc* under both *in vitro* and *in vivo* conditions. Laboratory evaluation of *Trichoderma* Isolates by dual-culture test, inverted plate technique and poisoned food technique revealed *T. harzianum* to be more inhibitory against *R. solani* and *S. rolfsii* followed by *T.* viride. *T. virens* was found to inhibit *Foc* to a greater extent than other isolates. Pot culture evaluations under greenhouse conditions using *T. harzianum, T. viride* and *T. virens* revealed that *T. harzianum* was the most effective biological control agent against Rhizoctonia root rot and Sclerotium collar rot whereas *T. virens* was found effective against Fusarium wilt. Seed inoculation of *Trichoderma* spp. also was found to increase growth and yield of chickpea under greenhouse conditions.

Gupta *et al*. 2006 conducted a field trial in order to evaluate promising isolates of *T. viride* as bio-control agent for wilt complex fungi in chickpea (*Fusarium, S. rolfsii* and *R. solani*). Four selected local isolates of *T. viride* were compared with three national checks. In terms of plant height, plant population, nodulation, wilt incidence and biomass accumulation, performance of *T. viride* local isolate 2 was found superior followed by standard *T. viride* isolate for increasing chick pea *Rhizobium* symbiosis. At farmer's field, about 83 % higher grain yield of chick pea

was recorded by the dual inoculation of *T. viride* local isolate 2 with *Rhizobium* over single inoculation of *Rhizobium* in the wilt complex affected area.

Fungal antagonists *viz., T. viride T. harzianum* and *T. virens* were evaluated against different isolates of *Foc* causing chickpea wilt in which the antagonists inhibited the mycelia growth of *Foc* through the production of volatile substances. *Trichoderma* spp. significantly reduced the wilt incidence in chickpea plants (Dubey, 2006).

Sunitha and Kurundkar (2007) evaluated the efficacy of *Trichoderma* isolates against *Foc* by employing dual culture technique. Results indicated that, in general *Trichoderma* isolate inhibited growth of the pathogen.

Jash and Pan (2007) studied the antagonistic activity and root colonizing behaviour of ten *Trichoderma* isolates collected from different agro-ecological zone of West Bengal. Of these, *T. viride* and *T. roseum* were the most prominent showing superior antagonistic effects and fast growth; both overgrew *R. solani* after three days of incubation in dual culture. The highest growth stimulation of the antagonist by both exudates and extract of chickpea roots was found in *T. harzianum.* This isolate was not only antagonistic to the pathogen, but also colonized the rhizosphere and maintained high population growth of 224 x 10^6 cfu g^{-1} of soil 30 days after sowing.

In vitro evaluation of *Trichoderma* sp. against *Foc* revealed the positive cumulative effect of *T. viride* + *T. harzianum* + *T. hamatum* in respect to the percent inhibition of the test fungus. Pot culture studies revealed that the soil application of *T. viride* @ 25 kg/ha as most effective in reducing the incidence of chickpea wilt. Soil amendment with groundnut cake proved to be effective against *Foc* followed by neem cake. Thus, chickpea wilt incited by *Foc* being soil borne disease could be managed by the integration of various practices like using resistant varieties, seed treatment with chemicals, seed and soil application of bio-agents and amendment of soils with oilseeds cakes (Nikam *et al*., 2007)

Ten isolates of three species of *Trichoderma* (*T. viride*, *T. harzianum*, and *T. virens*) were evaluated against four isolates of the *Foc* representing four different races commonly prevalent in India. *T. viride* isolated from Ranchi followed by *T. harzianum* (Ranchi) and *T. viride* isolated from Delhi inhibited maximum mycelial

growth of the pathogen. They also enhanced seed germination, root and shoot length, and decreased wilt incidence under green house condition (Sunil *et al*., 2007).

Jayalakshmi *et al*. (2009) investigated the induction of plant defense response against *Foc* by inoculating the roots of chickpea cv. JG 62 with the bio-control agent *T. harzianum*. A root extract of chickpea inoculated with *T. harzianum* showed increased activities of phenylalanine ammonia lyase and polyphenol oxidase as well as induction of new trypsin and chymotrysin inhibitors. The *Fusarium oxysporum* protease-2 was inhibited completely by root extract of chickpea inoculated with *T. harzianum* and showed maximum resistance to rotting of roots caused by wilt disease.

Jain and Singh (2009) evaluated two antagonistic fungi, *T. harzianum* and *T. viride* against natural incidence of wilt and root rot of chickpea Cv P-267 caused by *F. oxysporum* and *R. solani* and reported that the soil application of *T. harzianum* gave better seedling emergence and highest vigor index as compared to *T. viride* and fungicide Captan.

Meki *et al*. (2011) collected 32 isolates of *Trichoderma* species from soils grown with chickpea. *In vitro* tests show all *Trichoderma* isolates showed significant differences in their colony growth and in inhibiting the colony growth of race 3 of *Foc*. In potted experiment, among them, four isolates were tested as seed treatment on three chickpea cultivars (JG-62 susceptible, Shasho moderately susceptible and JG-74 resistant) against race 3 of *Foc*. The result showed that *T. harzianum* and unidentified *Trichoderma* sp. significantly reduced wilt severity and delayed disease onset. The degree of wilt severity and delay of disease onset varied with chickpea cultivars.

Andrabi *et al*. (2011) showed that both *T. viride* and *T. virens* were effective against the isolated pathogens by inhibiting the mycelia growth of *Foc*, *F. solani* and *R. solani.*

Shaban *et al*., (2011) found that *Rhizobium* spp. and *Trichoderma* spp. could be used as biological control of some soil-borne fungal diseases causing significant yield losses in legume field crops.

The antagonistic effect of eight antagonistic microorganisms *viz., T. harzianum, P. fluorescens, A. flavus, A. niger, A. ochraceus, Azotobacter* sp., *Penicillium* sp. and *Rhizobium* sp. was determined *in vitro.* All the antagonists

reduced the growth of *Foc* significantly but *T. harzianum* produced larger inhibition zone (6.72 cm) as compared to other. (Subhani *et al*., 2013)

Several plant extracts and plant based formulations have been reported to have good antifungal activities. Chand and Singh (2005) studied that plant extracts, *viz., Calotropis procera, Eucalyptus globulens, Jatropha multifida, Azadirachta indica, Allium sativum* significantly reduced wilt incidence in chickpea.

Chand and Singh (2005) evaluated fungal bio-agents and plant extract for eco-friendly management of *T. harzianum* for chickpea wilt incited by *Foc.* Among the three bio- agents (*T. viride* and *G. virens*) evaluated, seed treatment with *T. viride* was found highly effective, giving 77.8% control. All the plant extracts *viz.*, aak (*Calotropis procera*), eucalyptus (*Eucalyptus globulus*), jatropha (*Jatropha multifida*), neem (*Azadirachta indica*), garlic (*Allium sativum*) tested, except *C. procera*, were significantly superior in reducing wilt incidence in gram compared to control. Seed treatment with bulb extract of *A. sativum* was the most effective against wilt, which reduced disease from 65.9% in the control to 23.6%. Seed treatment with *T. viride* and *A. sativum* bulb extract are identified as important components of integrated management of chickpea wilt.

The antifungal effect of aqueous extracts of four plant species viz., *Azadaracta indica*, *Datura metel*, *Ocimum sanctum* and *Parthenium hysterophorus* at 40 % concentration was determined *in vitro*. All the plant extracts were effective in reducing the mycelial growth of *Foc. A. indica* and *D. metel* inhibited fungal growth by 80% at 10% concentration, both plants extracts had inhibitory effect as compare to other plant extracts (Mukhtar, 2007)

Sitara *et al*. (2008) evaluated essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) for their antifungal activity @ 0.5, 0.1 and 0.15% against eight seed borne fungi *viz., F. oxysporum, A. niger, A. flavus, F. moniliforme, F. nivale, F. semitectum, D. hawiinesis* and *A. alternata*. All the oils extracted, Asafoetida oil @ 0.1% and 0.15% significantly inhibited the growth of all test fungi except *A. flavus. Nigella sativa* oil @ 0.15 was also effective but showed little fungicidal activity against *A. niger* followed by neem and mustard oils.

Ahmed *et al.* (2009) evaluated the effect of seeds powder of *A. indica A. Juss, Adenanthera pavonina , Leucaena leucocephala* and *Eucalyptus* spp., in the control of root rot diseases caused by *Fusarium* spp. , *R. solani* and *M. phaseolina* on mung bean and chick pea in screen house. Application of *A. pavonina, L. leucocephala* and *Eucalyptus* spp., @ 0.1 and 1% w/w showed significant control of the pathogens and enhanced plant growth in terms of shoot length, shoot weight, root length and root weight in both the experiments.

Bhatti (1992) conducted a study to determine the effects of soil moisture on wilt and root-rot of chickpea caused by *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *pisi*, *F. solani*, *P. ultimum* and *T. basicola*. Three soil metric potential regimes (high=-40 to -20 kPa, medium =-260 to -40 kPa, low = - 1060 to -260 kPa) were used . Wilt and root rot increased with decreased soil metric potential; as did rhizosphere populations of each pathogen were present in soil alone or in various pathogen combinations. Chickpea grown in soil infested with equal inoculum densities of two pathogens usually had as much or more disease as plants grown in soil infested with a single pathogen.

Under pot culture conditions a soil temperature range of 24.8-28.5^{0}C and soil moisture above 25 per cent within the water holding capacity of soil was found to be conducive for wilt development. Temperature and soil moisture levels above and below this range delayed the incidence and slowed down the progress of wilt. It was also recorded that, with the advance in the age of host plant the length of time for the disease appearance and disease severity decreased (Sugha *et al.*, 1994).

Navas-Cortes *et al.* (1998) reported that sowing date greatly influenced the Fusarium wilt and yield of chickpea.

Increasing the inoculum density of *F. oxysporum* f.sp. *ciceri* caused an exponential reduction in disease incubation period and an increase of disease incidence and the area under the disease intensity progress curve (Navas-Cortes *et al.*, 2000)

Blanca *et al.* (2004) reported the reduction in disease intensity and increase in chickpea seedling emergence with change in date of sowing, host plant resistant and seed / soil treatment with bio-control agents.

Blanca *et al*. (2006) observed the effect of resistant cultivars and adjustment of sowing dates to be important for management of Fusarium wilt in chickpeas. In this study, the effect of temperature on resistance of chickpea cultivars to Fusarium wilt caused by various races of *Foc* was examined. Greenhouse experiments indicated that the chickpea cultivar Ayala was moderately resistant to *Foc* when inoculated plants were maintained at a day/night temperature regime of $24/21^0$C but was highly susceptible to the pathogen at 27^0C.

In another field trial, micro-plots infested with *Foc* race 5 alteration of sowing date, use of partially resistant chickpea genotypes, and seed and soil treatments with bio-control agents were tested. Advancing the sowing date from early spring to winter significantly delayed disease onset, reduced the final disease intensity and increased chickpea seed yield. The main effects of sowing date, partially resistant genotypes, and bio-control agents were a reduction in the rate of epidemic development over time, a reduction of disease intensity, and an increase in chickpea seedling emergence, respectively. Chickpea seed yield was influenced by all three factors in the study (Blanca *et al*., 2006).

Chand and Khirbat (2009) found that the chickpea root rot complex was more severe in light sandy soil than heavy clay. High soil temperature and deficiency of moisture appeared to have a definite bearing on its incidence. The amount of organic matter was inversely related to wilt incidence. The development of wilt was favoured by increase in nitrogen. The optimum temperature and pH for pathogen was 25^0C and 5-6.5 respectively. Delay in sowing minimized disease. Mixed cropping of chickpea with wheat and berseem gave measurable disease control. Seed treatment with Benlate T (0.15%) destroyed seed borne inoculum completely. Bio-control agents such as *Trichoderma* spp., *Glomus* spp. and fluorescent *Pseudomonas* resulted in considerable reduction in disease.

Integration of biological and chemical control seems to be a promising way of controlling many pathogens with minimum interference in the biological equilibrium in soil (Papavizas, 1973). Since soil is highly complex and biologically active system through which the fungicide act against fungi, fungi toxicants often give variable success in controlling seedling disease of crops in diverse agro-climatic regions of the world (Hans *et al*., 1981).

In a field experiment coating chickpea seeds with bio-control agents, *T. harzianum, T. viride, B. subtilis* and *G. virens* and chemical fungicides like Carboxin and Carbendazim. Carboxin significantly reduced *Foc* wilt by 30-45.8%. Integration of bio-control agents and Carboxin significantly increased seed yield by 25.4-42.6%. Carbendazim was more effective than Carboxin in reducing wilt and increasing seed yield (De *et al*., 1996).

Poddar *et al*. (2004) studied the efficacy of *T. harzianum* singly and in combination with its mutants and Carbendazim against wilt (*Foc*) in chickpea. Carbendazim at 2.5 g/kg as seed treatment was effective against the disease. Propiconazole seed treatment was inhibitory to plant growth and development. Seed treatment with a rhizospheric isolate of *T. harzianum* (TH-1) showed superiority to Kalisena and non-rhizospheric isolates. Integrated seed treatment with *T. harzianum* mutant UM2R + Carbendazim (1.25 g/kg) resulted in the maximum seed yield (4.6 g per plant) and lowest disease incidence (2.5%).

Arfaoui *et al.* (2006) also reported the compatibility of biological control agents (*T. harzianum*, *T. viride* and *P. fluorescens*) with fungicides (Thiram, Captan and Vitavax) against the chickpea wilt. Seed treatment with *T. harzianum* alone recorded the highest seedling emergence and lowest disease incidence, while *T. harzianum* + Captan recorded the highest yield.

Kapoor *et al*. (2006) reported soil amendment with *Lantana camera* (10 t/ha) + bio-agent Tricho guard @ 2.5 kg/62 kg FYM /ha + spray with Carbendazim at pre flowering stage was most effective in managing the root rot-wilt complex disease in pea. Gade *et al.* (2007) reported that soil solarization alone and in combination of seed treatment with Thiram + benomyl 1:1 @ 3g / kg of seed reduced wilting to the extent of 22.8, 22.6 % during first year and 16.3 and 15.7 per cent during second year, respectively in case of pigeon pea.

Andrabi *et al*. (2011) isolated *Foc, F. solani* and *R. solani* from the wilted chickpea plants. To manage the wilt complex cultural practices, use of biocontrol agents and fungicides were tried under *in vitro* and *in vivo* conditions. Sowing of chickpea at different dates revealed that early sowing (10th Oct.) resulted in maximum disease incidence (32.20%), whereas, late sowing (24th Nov.) the minimum (13.35%). Twenty and 50 cm row to row spacing resulted in maximum

(29.17%) and minimum (17.35%) disease incidence respectively. *In vitro* evaluation of biological control agents revealed the superiority of *T. viride* over *T. virens* in controlling the pathogens. Carbendazim at 100, 200, 500 ppm caused maximum per cent inhibition of the pathogens under *in vitro* conditions. Fungicides applied as seed treatment reduced disease incidence significantly. Seed treatment with Carbendazim increased seed germination (71.24%), though it was at par with Carbendazim + Mancozeb (62.21%) and Mancozeb (61.46%). Seed coating with *T. viride* resulted in minimum disease incidence (9.24%), however, it was at par with *T. virens* (9.72%). Maximum yield (10.10 q/ha) was recorded with the application of Carbendazim, followed by Carbendazim + Mancozeb (9.77 q/ha) and *T. viride* (8.10 q/ha)

Fawzy (2011) isolates of eleven fungal biocontrol agents from naturally infected chickpea roots from different locations. Seven bio-control agents, namely *B. subtilis*, *B. megaterium*, *B. cereus*, *T. viride*, *T. harzianum*, *Aspergillus* sp., *Penicillium* sp. isolated from chickpea rhizosphere, were tested for their antagonistic action against the tested pathogens. *B. subtilis* isolate BSM1, *B. megaterium* isolate TVM5, *T. viride* isolate TVM2 and *T. harzianum* isolate THM4 were the most antagonistic ones to the tested fungi *in vitro*, while the other isolates were moderate or weak antagonists and results showed that all tested antagonistic isolates were able to cause significance reduction of damping-off, root and/or stem rot diseases in chickpea plants. *T. viride* (isolate TVM2) and *B. megaterium* (isolate BMM5) proved to be the most effective isolates for controlling the diseases in field condition.

Disease control efficacy of six fungicides and bio-control agent *viz., T. viride* 8g/kg, Vitavax powder 2g/kg (Carboxin37.5 % + Thiram 37.5%), Vitavax powder 1g/kg +*T. viride* @ 4g/kg, Carbendazim 0.75g/ha+ Thiram 1g/kg and soil application of *T. viride* @ 2.5kg/ha were evaluated against collar rot (*S. rolfsii*) of chickpea genotype L 550. The disease mortality was significantly reduced by fungicidal sprays. The minimum per cent disease mortality was recorded in Carbendazim + Thiram (18.90) followed by Vitavax powder + *T. viride* (21.24 %), Vitavax powder 2g/kg (Carboxin37.5 % + Thiram 37.5%) (25.10%). Soil application of *T. viride* (28.12%) and *T. viride* (30.72 %) against control (37.18%). Results showed that two spray with Carbendazim 0.75g/ha+ Thiram 1g/kg at 10 days interval and also enhance grain yield of 2178 kg/ha followed by Vitavax powder 1g/kg + *T. viride* @ 4g/kg 2071 kg/ha as compared to check 1286 kg/ha.(Jain *et al.*, 2012)

From the above reviews, it is clear that many reviews are available on *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea, but there is limited information available on root rot complex caused by *F. solani* and *R. solani*. Hence, the location specific management study for chickpea wilt and root rot complex is required for mitigating the losses caused to this crop.

3. MATERIAL AND METHODS

The study entitled **"Studies on integrated management of wilt and root rot complex of chickpea (*Cicer arietinum* L.) caused by *Fusarium* spp. and *Rhizoctonia solani*"** was undertaken at the Department of Plant Pathology, Rajasthan College of Agriculture (RCA), Udaipur during (2012-13) to (2013-14). The details of techniques followed and the methods and materials used during the course of experiment are described below.

3.1 Glassware

All glassware's used for the experiment were of 'Corning or Borosil make'. These were first thoroughly washed in tap water with cleaning powder and then kept overnight in chromic acid solution ($K_2Cr_2O_7$) 80g, distilled water 300 ml cooled at room temperature and added with constant stirring to concentrated H_2So_4 (400 ml). Soaked glassware were washed in running tap water, finally rinsed with distilled water, dried and sterilized in oven before use.

3.2 Chemicals

All chemicals used for experimental work were of "Analar" quality of British Drug House (Pvt.) limited, Mumbai or "Proanalysis quality of E. Merk ltd. Or Sarabhai Chemicals, Baroda or Central Drug House Pvt. Ltd., Mumbai or Himedia Laboratory Chemicals or S.D. Fine Chem, Ltd., Bosisar or Labo-chemic Industrial co., Mumbai.

3.3 Sterilization

The Petri plates were sterilized at 180^0C for two hours in hot air oven whereas, all the culture media and distilled water were sterilized in an autoclave at 1.045 kg/cm^2 pressure for 20 min. Fresh polythene bags were sterilized with 5% formalin.

3.4 Collection of diseased material and isolation

3.4.1 Collection of diseased samples

The diseased samples of chickpea showing typical wilt and root rot symptoms were collected in *rabi* season of 2012 from farmer's field of different chickpea growing areas of Rajasthan *viz*., Udaipur, Bikaner, Tivari (Jodhpur), Pali, Sirohi and Banswara all from local land races. The main aim was to explore possibility of existence of different species and/or variables of wilt and root rot pathogen. The infected parts of the diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory and subjected to microscopic examination and tissue isolation.

3.4.2 Isolation of pathogen

Isolation from infected root of chickpea plant showing typical root rot symptoms were used to isolate the pathogens by different techniques and pure cultures thus obtained were maintained on potato dextrose agar (PDA) medium for further investigation.

Isolations of the pathogen were attempted from all the samples. These infected aerials parts were thoroughly washed in running tap water to remove the adhering soil. These were then cut into small pieces with the help of a sterilized scalpel, washed in sterilized water, surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) for 2 minutes rinsed thrice in sterilized distilled water and transferred on potato dextrose agar (PDA) medium in Petri plates. The plates were incubated at 28 ± 1^0C for growth. Sub cultures were made from the periphery of the mycelial growth which appeared after five to six days.

3.4.3 Purification

In order to obtain the pure cultures, single spore culture technique for *Fusarium* spp. and single sclerotial isolation for *R. solani* was used. The spore suspension of the respective isolates of *Fusarium* spp. and the sclerotial suspension of *R. solani* isolates was prepared in sterilized distilled water so as to obtain 10-12 spores per microscopic field (10x). The suspension was spread over the surface of sterilized 2 per cent plain agar medium in Petri plates and incubated at 26^0C. After ten hours, the single germinating spore was observed under low power objective and cut through dummy objective. Such pieces containing germinating spores were transferred separately on potato dextrose agar slants with the help of an inoculating needle and incubated at 26^0C for seven days. Single sclerotia were placed under stereo

binocular microscope and transferred on PDA. These cultures were observed under microscope and the stock cultures were kept in refrigerator for further studies.

3.5 Pathogenicity test

To know the pathogenic nature of *F. solani, Foc* and *R. solani* isolated from rotted chickpea root samples and wilted leaves was tested on healthy chickpea plants of Dahod yellow raised in plastic pots. The pots (20 cm diameter) were filled with sterilized soil autoclaved at 1.2 kg cm^{-2} for one hour for three consecutive days. The experiment was carried out by inoculating 20g/pot of pathogens, *F. solani, Foc.* and *R. solani* were separately multiplied on corn meal sand (1:1) medium at 25 ± 1^0C for ten days. The pots filled with inoculated soil were kept in the green house for seven days and were irrigated with sterile water to allow establishment of the pathogen. The healthy and surface sterilized seeds (with 0.1% Hg Cl_2 solution (1g/l) for 30 seconds and three subsequent washing of sterilized distilled water) of susceptible chickpea cultivar 'Dahod Yellow' was sown in inoculated pots at 10 seeds/pot, keeping three pots as three replications for each pathogen. For comparison with un-inoculated control, seeds were sown in sterilized soil, without pathogen. The pots were irrigated on alternate days with sterilized water to provide good moisture. Wilting of leaves developed within 15-20 days and root showed black lesions at the soil surface. From the wilted seedlings showing black lesions of roots, re-isolation of the pathogens (*F. solani*, *Foc* and *R. solani*) was attempted, and the cultures were readily re-isolated. In checks, healthy chickpea plants continued to grow and developed seeds. The cultures were purified and maintained on PDA slants at 4^0C for further studies.

Identification of the pathogens causing wilt and root rot complex of chickpea was carried out by studying the cultural and morphological characters. The cultural characters were recorded right from initiation of mycelial growth up to a period of ten days (Holliday, 1980).The morphological characters *viz.*, mycelial growth colour, conidia and sclerotial formation were studied under low power magnification (10 x) from seven days old culture and tentatively identified as *Fusarium solani*, *Fusarium oxysporium* f.sp. *ciceri* and *Rhizoctonia solani* and were compared with those given in literature. The microphotograph of micro-sclerotia was also taken. The list of the isolates recovered is given in Table: 1

Table 1: Isolates of *Fusarium* spp. and *R. solani* recovered from samples collected from fields in chickpea growing areas of Rajasthan

S.No.	Pathogens isolated	Place of collection	Isolated designation	Isolated code
1.	*Fusarium solani*	Udaipur	UDP	UDP Fs-1
	Fusarium solani	Bikaner	BKN	BKN Fs-2
	Fusarium solani	Tivari (Jodhpur)	TIB	TIB Fs-3
	Fusarium solani	Pali	PAL	PAL Fs-4
	Fusarium solani	Sirohi	SRH	SRH FS-5
2.	*Fusarium oxysporum* f.sp. *ciceri*	Banswara	BNS	BNS Foc-1
3.	*Rhizoctonia solani*	Udaipur	UDP	UDP Rs-1
	Rhizoctonia solani	Bikaner	BKN	BKN Rs-2
	Rhizoctonia solani	Tivari (Jodhpur)	TIB	TIB Rs-3
	Rhizoctonia solani	Pali	PAL	PAL Rs-4
	Rhizoctonia solani	Sirohi	SRH	SRH Rs-5
	Rhizoctonia solani	Banswara	BNS	BNS Rs-6

3.6 Isolation of biocontrol agents using selection media

Isolation of biocontrol agents were attempted from rhizosphere soil of both healthy and diseased chickpea plants. Specific techniques were employed for isolating fungal biocontrol agents and antagonistic micro organisms.

Isolation of fungal biocontrol agents was attempted by using selective media by baiting method and dilution plate method.

3.6.1 Isolation of biocontrol agent by baiting method

A loop full of the dried rhizosphere soil collected from chickpea field was placed in the bottom of the empty sterilized Petri-plates and to this a drop of sterile water was added, thoroughly mixed and allowed to dry on the laminar flow for three minutes. Over this, 20 ml of cool molten PDA amended with 25 ppm chloramphenicol and 2 ml of Triton X-100/litre was poured carefully without disturbing the soil at the bottom. The medium in these plates was allowed to solidify and then 3 mm bit of growing cultures of isolated pathogen was aseptically inoculated on the medium. The plates were incubated at 28 ± 1^0C and examined after two days for growth of the organism under stereo-binocular microscope and hyphae of antagonists which appeared piercing through the hyphae of the pathogen and found growing over the pathogen or causing lyses, were aseptically picked up with the help of sterilized needle and transferred on to fresh poured plates and purified with hyphal tip culture technique and maintained in PDA slants.

3.6.2 Isolation of biocontrol agent by dilution plating method

Dilution plate method (Warcup, 1955) was also employed. For, *Trichoderma* sp. PDA amended with 25 ppm chloramphenicol and 2 ml of Triton X-100/litre (Budge and Whipps, 1991) was used. Since the amount of rhizosphere soil was limited (1g) soil was used and dilution of 10^5 was proposed. One ml of soil dilution was pipetted in sterilized Petri plates and on this 20 ml of molten almost cool medium was poured and the plates were gently rotated to uniformly spread the propagules in the plates and then allowed to solidify. The plates were incubated at 25^0 C for five days and resultant *Trichoderma* colonies were examined under the stereo-binocular microscope and picked up on fresh poured plates. Hyphal tip pure cultures were made and maintained on PDA slants.

For isolation of bacterial antagonists, selective media, King's B media was used (King *et. al*., 1954). Stock soil solution was prepared by taking 10 g soil in 90 ml sterile distilled water in Erlenmeyer flask and shaken gently for 2 to 4 minutes. Serial dilutions were prepared from the stock soil suspension up to 10^8. A soil suspension of 0.2 ml suitable dilution was added on surface of the media in Petri plates and spread

uniformly with the help of glass spreader. The inoculated Petri plates were incubated at 28 ± 1^0 C for 24 hours and the colonies appeared were sub cultured on King's B media for identification and further use.

The list of the isolates both fungal and bacterial bio-control agents recovered is given in Table 2.

Table 2. Isolates of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* recovered from rhizosphere of samples collected from fields in chickpea growing areas of Rajasthan

S.No.	Pathogens isolated	Place of collection	Isolated designation	Isolated code
1.	*Trichoderma viride*	Udaipur	UDP	UDP T-1
2.	*Pseudomonas fluorescens*	Udaipur	UDP	UDP Pf-1
3.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Md
4.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Br II
5.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Pv
6.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Tb-1
7.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Ch

Apart from the biocontrol agents recovered from chickpea rhizosphere, cultures of *Trichoderma* spp. available at the Department of Plant Pathology, RCA, MPUAT and found effective against several soil borne pathogens were also used. (Lodha *et al*., 1994; Sharma *et al.*, 2012 and Tetarwal *et al*., 2011).

3.7 Cultural and morphological variability among the isolates of *Fusarium* spp. and *R. solani*

Twelve isolates, five isolates of *F. solani,* one isolate of *Foc* and six isolates of *R. solani* were studied for their morphological and cultural characters like: studies on growth rate, colony characters (shape and size and color of the colony), effect of temperatures, pH (growth and sporulation), *in vitro* sensitivity to fungicides, botanicals and biocontrol agents.

3.7.1 Studies on cultural and morphological characteristics of *Fusarium* spp. and *R. solani*

Twelve isolates, five isolates of *F. solani,* one isolate of *Foc* and six isolates of *R. solani* were grown on potato dextrose agar (PDA). The autoclaved medium was dispensed in Petri plates and allowed to solidify. Three mm disc of the individual isolates of *F. solani, Foc* and *R. solani* removed from the periphery of seven days old culture was aseptically placed in the centre of the PDA agar plate, keeping three isolate of each plate as three replications for each isolate. The plates were incubated at 28 ± 1^0C. The variations in growth pattern and colony growth (diameter) of fungi in all isolates were recorded. Spore production by isolates of *F. solani, Foc*. and sclerotial production by isolates of *R. solani* was determined by removing agar-plugs (3 mm diameter) from three liner spots across the centre of the colony, which were suspended in 10 ml sterile water in glass taste tube and agitated twice for about ten seconds each time on a vortex shaker to dislodge conidia. The number of conidia and sclerotia in the resultant suspensions was determined using a haemocytometer, and expressed as number of conidia and sclerotia per mm^2 of medium. For spore and sclerotial size (length and width) mounts were prepared in aniline-blue lacto-phenol and measurements were taken by measuring 50 spores of each isolates of *F. solani, Foc* and 50 sclerotia of each isolates of *R. solani* using stage and ocular micrometer.

3.7.2 Effect of different temperature on mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani* on PDA.

Temperature studies were conducted with a view to determine the optimum range of temperature for mycelial growth and sporulation of the isolates of *F. solani, Foc* and sclerotial formation of *R. solani in vitro*. The PDA plates were inoculated with 3 mm disc from one week old pure culture. Three plates for each treatment were kept at different temperatures *viz.*, 15, 20, 25, 30 and 35^0C maintained in different incubators. The observations were recorded by measuring the diameter (Radial

growth) of the colony of the fungus. The sporulation and sclerotial formation was measured by taking 1 cm^2 bit from the mycelial growth from each treatment and dissolved in 10 ml sterile distilled water in test tube. The spore load was observed in each treatment under microscope taking ten observations and counting of conidia of *Fusarium* spp. and sclerotia of *R. solani* was done by using a haemocytometer.

3.7.3 Effect of different pH on mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani* on PDA.

The study was carried out to find out the optimum pH levels for the mycelial growth and sporulation of isolates of *F. solani, Foc* and sclerotial formation of isolates of *R. solani.* Potato dextrose agar was adjusted with pH ranges between 4.0 to 8.0 pH with a narrow fraction of 1 pH was adjusted to these levels by using buffers N/10 HCL and N/10 NaOH solutions. The pH was determined by Pen type digital pH meter. The PDA plates were inoculated with 3 mm disc from one week old pure culture. Three plates for each treatment were kept at different pH *viz.*, 4, 5, 6, 7 and 8 maintained in incubator. The observations were recorded by measuring the diameter (Radial growth) of the colony of the fungus. The sporulation and sclerotial formation was measured by taking 1 cm^2 bit from the mycelial growth from each treatment and dissolved in 10 ml sterile distilled water in test tube. The spore load was observed in each treatment under microscope taking ten observations and counting of conidia of *Fusarium* spp. and sclerotia of *R. solani* by using a haemocytometer.

3.8 Multiplication of *Fusarium* spp. and *R. solani* for soil application in pot as well as field conditions

For the experiment, cultures of *Fusarium* spp. and *R. solani* were multiplied separately on autoclaved corn meal sand (1:1) medium for ten days. To allow establishment of the pathogens before sowing the inoculum was inoculated @ 20g/pot and @ 600 g/plot and distributed evenly three days before sowing to the field. For each treatment three plots as three replications and a control were maintained. All the plots were lightly irrigated immediately after inoculation to allow establishment of the pathogen before sowing.

3.9 Sowing and inter-culture operations

The various treated seeds of chickpea Dahod Yellow were sown in pots and field (10 seeds / pot) and (250 seeds/plot) respectively, keeping three replications

for each treatment. Just before sowing, soil samples were taken from each plot at the depth of 15 cm to determine initial population densities of the two pathogens as well as biocontrol agents.

3.10 Assessment of yield losses due to wilt and root rot complex

The losses caused by a disease vary with the host pathogen combination and the disease severity. Since limited information is available on the losses caused by *F. solani*, *Foc* and *R. solani*, wilt and root rot complex disease of chickpea, field trials were conducted in two consecutive years *rabi* (2012-13) and (2013-14) to assess the germination percentage, per-cent mortality and reduction in grain yield under different disease severity (generated by different inoculum densities).The region has a semi-arid climate. The soil of the experimental fields is sandy-loam in texture, slightly alkaline (pH 7.9), having low organic carbon (0.42) and and electrical conductivity (0.85 dSm ¯1). The experiment was conducted using a local land race host genotype Dahod Yellow and one isolate each of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6) at three inoculum densities, generated by different inoculum densities *i.e*. Mix 1:1:1 inoculum loads of *F. solani, Foc* and *R. solani* (200 g/plot). *F. solani* and *Foc* at 300 g/plot (1:1), *F. solani* and *R. solani* at 300 g/plot (1:1), *R. solani* and *Foc* at 300 g/plot(1:1) and *F. solani , Foc* and *R. solani* at 600 g/plot. Uninoculated plots were maintained as control. The inoculated plots were compared with un-inoculated plots. The seeds were sown in 3×2 m plots, keeping nine rows (30 cm) and 20 plants in each row, with 10 cm plant distance. Recommended agronomical practices for fertilizers (N-80, P-40 & K-40 kg ha^{-1}) and weed management, pre-germination spray of (Atrazine) at 0.5% and mechanical removal were followed, but, no fungicide was used in this trial. The inoculum was multiplied on corn meal sand (1:1) medium. The inoculations were done in the late evening, followed by a heavy irrigation to provide adequate moisture for infection.

Observations for seed germination were recorded at ten days after sowing and observations plant mortality were recorded 90 days after sowing by using the formula,

$$\text{Mortality percentage} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

3.11. Evaluation of fungicides, biocontrol and botanicals against chickpea wilt and root rot complex pathogens (*Fusarium spp*. and *Rhizoctonia solani*) *in vitro*

3.11.1 *In vitro* efficacy of fungicides (Poison food technique)

Relative efficacy of different systemic and non-systemic fungicides was evaluated by using poisoned food technique (Schmitzs, 1930). Five fungicides *viz.*, Thiram 75% WP [tetramethyl thiuram disulphide (TMTD)] Gupta Chemicals (p.) Ltd., Mumbai, Vitavax 75% WP [5,6-Dihydro-2-Methyl-1,4-Oxathiin-3-Carboxamide (carboxin)] Pesticide India Ltd., Udaipur, Tebuconazole 25.9 w/w [1-(4-chlorophenyl) -4,4-dimethyl-3-(1,2,4-triazol-1-methyl) peptan-3-ol (Folicular 250 EC)] Bayer Crop Science, India Ltd., Mumbai, Captan 50% WP [N-trichloromethyl-thio-4-cyclohexane-1-2 dicarboximide] Uni Royal Chemicals, Mumbai and Trifloxystrobin 23% SC [1-[3-(trifluoromethyl) phenyl] ethylidene] Makhteshim Agan India Pvt. Ltd., Hyderabad were tested at three concentrations *i.e.* 250 , 500 and 1000 ppm against each isolate of *F. solani, Foc* and *R. solani.* Desired quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized Petri plates and allowed to solidify. For each treatment, three replications were taken and each plate was inoculated with 3 mm disc of each isolate of *F. solani, Foc* and *R. solani* and incubated at 28 ± 1^0C. The linear growth after seven days was recorded and a check as control was also maintained where medium was not supplemented with any fungicide.

3.11.2 *In vitro* evaluation of botanicals (Poison food technique)

Efficacy of Neem oil (0.2%), developed by Godrej Agrovet Pvt. Ltd., Mumbai, and Karanj oil Mfg. by Shree Aushadh Pratisthan ,Udaipur for commercial purpose. Neem oil and Karanj oil were tested against isolates of *F. solani, Foc* and *R. solani* by poison food method. The formulations were incorporated in PDA at 0.2 per cent and dispersed in sterilized Petri plates. For comparison, plates having PDA without neem and Karanj oil formulations were kept as control. For each treatment, three replications were taken. Three mm bits from isolates of *F. solani, Foc* and *R. solani* removed from the periphery of seven days old cultures and aseptically inoculated in the centre of each plate. The plates were incubated at 28 ± 1^0C for seven days and then colony diameters were measured and compared with control plates.

3.11.3 Evaluation of fungicides and botanicals in pot culture

Cultures of *Fusarium* spp. and *R. solani* were multiplied separately on autoclaved corn meal sand (1:1) medium for ten days. It is then mixed with sterilized garden soil @100g/kg soil, this inoculum mixture was placed on top of non- sterilized soil + FYM mixture (3:1) in fresh earthen pots (30 cm diameter) @ 20 g/pot three days before sowing to allow establishment of the pathogens before sowing. Fungicides and botanicals *viz.*, Thiram, Captan, Vitavax, Trifloxystrobin, Tebuconazole, Neem oil and Karanj oil @ 0.2 % were evaluated individually as seed treatment in pot experiment. All the pots were lightly irrigated immediately after inoculation. Since, only a small quantity of each fungicide was used for seed treatment, the seeds were soaked in Thiram 75 % WP, Captan 50 WP, Vitavax 75% W/P, Trifloxystrobin 23 % SC, Tebuconazole 29.5 W/W, Neem oil and Karanj oil at 0.2 ml / kg seed for 30 minutes. The treated seeds were air dried in shade and then sown. The various treated seeds of chickpea variety 'Dahod Yellow' were sown @ 10 seeds/pot keeping three replications for each treatment and an untreated pot as control. Observations on seed germination and plant mortality were recorded. The experiment was repeated once to confirm the results and the mean of both the runs were subjected to statistical analysis.

3.11.4 *In vitro* efficacy of fungal biocontrol agents (Dual culture technique)

The efficacy of biocontrol agents *i.e. Trichoderma* spp. was tested by using dual culture plate method on PDA medium (Johnson *et al.*, 1959). Antagonistic effect of *Trichoderma* spp. was tested against isolates of *F. solani*, *Foc* and *R. solani*, 3 mm diameter mycelium bit of seven days old culture of *Foc* in center of 1st half of petri plate and *Trichoderma* spp. in centre of 2nd half of petri plate, like wise *F. solani* and *Trichoderma*. spp., *R. solani* and *Trichoderma* spp. were placed separately at some distance on the periphery of Petri plates containing sterilized PDA medium. For each treatment three replications were taken. Inoculated plates were incubated at 28$\pm$1 ^{0}C temperature in incubator. Observations on colony diameter were recorded up to the complete coverage of control plates, which was inoculated with only pathogen. The linear growth after seven days and per cent inhibition were recorded.

3.11.5 *In vitro* efficacy of bacterial biocontrol agents (Dual culture technique)

Dual culture method was used for assessing inhibition of radial growth of the pathogen by bacteria inoculated on King's B agar medium in sterilized Petri dishes. A loopful from the 24 hours old cultures was streaked on two side of each plate and in the centre 3 mm disc from isolates of *F. solani, Foc* and *R. solani* were aseptically inoculated. Control plates were inoculated by only pathogens individually. Three replications were maintained for each treatment and were inoculated at 28 ± 1^0C. The measurement of radial growth of the pathogens were recorded after five days and compared with that in the respective controls.

3.11.6 Preparation of talc based formulations of BCAs

For seed treatment, cultures of fungal biocontrol agent were individually grown on PDA amended with 25 ppm chloramphenicol and 2 ml of Triton X-100/litre while, bacterial biocontrol agents on King's B medium. The spore colonies so developed were harvested by suspending in 20 ml sterilized water in Petri plates and mixed with sterilized fine clay (talc powder) 10 gm to make a slurry. These formulations of the individual BCA's were used for seed treatment @ 6g/kg seed for pot as well as field conditions.

3.11.7 Evaluation of fungal and bacterial biocontrol agents in pot culture

Cultures of *Fusarium* spp. and *R. solani* were multiplied separately on autoclaved corn meal sand (1:1) medium for ten days. It is then mixed with sterilized garden soil @100g/kg soil, this inoculum mixture was placed on top of non- sterilized soil + FYM mixture (3:1) in fresh earthen pots (30 cm diameter) @ 20 g/pot three days before sowing to allow establishment of the pathogens before sowing. All the pots were lightly irrigated immediately after inoculation. Fungal biocontrol agents *viz*., *T. harzianum* isolate ICRISAT-25, *T. viride* isolate ICAR-95 and bacterial antagonists *viz*., *P. fluorescens* isolate UDP Pf-1, *B. subtilis* isolate Br II were evaluated individually as seed treatment in pot experiment. For seed treatment, cultures of fungal biocontrol agent were individually grown on PDA amended with 25 ppm chloramphenicol and 2 ml of Triton X-100/litre while, bacterial biocontrol agents on King's B medium. The seeds were treated with the formulations and the coated seeds were kept overnight in moist chamber so as to enable antagonists to establish on

seeds. The various treated seeds of chickpea variety 'Dahod Yellow' were sown @10 seeds/pot keeping three replications for each treatment and an untreated pot as control. Observations on seed germination, plant mortality and populations of the biocontrol agents and of the two pathogens were recorded. The experiment was repeated once to confirm the results and the mean values of the two runs were subjected for statistical analysis.

3.12 Evaluation of popular chickpea cultivars for resistance to wilt and root rot pathogens

Ten varieties/ genotypes were evaluated under artificial inoculation conditions using soil inoculation technique of spore cum mycelial of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6) causing wilt and root rot complex of chickpea. Varieties/ genotypes *viz.*, Dahod Yellow, Pratap Chana -1, Avrodhi, RSG-888, RAJ-1581, BGD-72, BG-391, BG-1053, GMG-469 and P- 1080 from different districts of Rajasthan. Experiment was laid out in Completely Randomized Design (CRD) with 20 g/ pot and three replications were maintained under cage house conditions. The pots were filled with sterilized soil and were inoculated with 20g/kg inoculum grown on corn meal sand (1:1) medium for ten days, alone and in combination three days before sowing. Ten seeds of chickpea for each variety were sown 5 cm at depth in 9 inch pots. The observations on germination percentage and mortality percentage were recorded after 60 days after sowing by using the formula,

$$\text{Mortality percentage} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

3.12.1 Effect of different sowing dates on incidence of chickpea wilt and root rot complex under field conditions during *rabi* (2012-13) and 2013-14)

For the study of host plant resistance, an experiment was conducted in the field by staggered sowing of susceptible variety of chickpea 'Dahod Yellow' at 15 days interval from 15th October 2012 upto 29th November 2012, 15th October 2013 upto 29th November 2013 in all making four dates of sowing with three replications. The plots were inoculated with mixed (1:1:1) inoculum loads of combinations of wilt and root rot causing pathogens; *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1)

and *R. solani* (isolate BNS Rs-6) at (200 g/plot). The seeds were sown in 3×2 m plots, keeping nine rows (30 cm) and 20 plants in each row, with 10 cm plant distance. The inoculum was multiplied on corn meal sand (1:1) medium. The inoculations were done in the late evening, followed by a heavy irrigation to provide adequate moisture for infection. Observations for seed germination were recorded at ten days after sowing and plant mortality were recorded 90 days after sowing by using the formula, given earlier.

3.13 Evaluation of fungicides and biocontrol agents for suppression of wilt and root rot of chickpea in field.

3.13.1 Seed treatment with fungicides, biocontrol agents (fungal and bacterial) and botanicals in field.

Fungicides (Tebuconazole and Vitavax), biocontrol agents (*T. harzianum* isolate ICRISAT-25) and botanical (Neem oil) were found effective *in vitro* were evaluated individually and in combinations *viz.*, Tebuconazole 25.9 W/W @ 0.2 %, Vitavax75 % WP @ 0.2 %, Neem oil @ 2 %, *T. harzianum* @ 20g/kg seed, Vitavax75 % WP @ 0.2 % + Neem oil @ 2% , Tebuconazole 25.9 W/W @ 0.2 % + Neem oil @ 2%, Vitavax 75 % WP @ 0.2 % + *T. harzianum* @ 20g/kg seed, Tebuconazole 25.9 W/W @ 0.2 % + *T. harzianum* @ 20g/kg seed, Neem oil @ 2% + Tebuconazole 25.9 W/W @ 0.2 % as seed treatment in field as well as pot experiment. For seed treatment, cultures of the fungal biocontrol agents were individually grown on PDA , the colonies so developed were harvested by suspending in 20 ml water in each Petri plates and mixed with sterilized fine clay (talc powder) 10 gm to make a slurry. This formulation of *T. harzianum* was used for seed treatments at 20g/kg seed in plots inoculated in combination of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6) @ 600g/plot. The coated seeds were kept overnight in moist chamber so as to enable the antagonists to establish on seeds.

Since, only a small quantity of each fungicide was used for seed treatment, the seeds were soaked in Vitavax 75 % W/P and Tebuconazole 29.5 W/W at 0.2% / kg seed for 30 minutes. The treated seeds were air dried in shade and then sown.

OBSERVATIONS

The seed germination were recorded ten days after sowing and plant mortality were recorded 90 days after sowing. Seed yield was recorded for each plot after harvesting of the grain.

To determine the population of biocontrol agents and their possible effect on *F. solani, Foc* and *R. solani,* soil samples from chickpea rhizosphere and around from both diseased and healthy plants were collected from each plot carefully by uprooting the plants and lightly shaking these to remove the extra soil. The rhizosphere soil was collected by lightly scrapping with a hard brush. Samples of all the three replications of each treatment were pooled and placed in polythene bags, labeled and brought to the laboratory. Sub-samples from these pooled samples were used for determining population densities of biocontrol agents and the pathogen as per the methods described below:

3.14 Determination of population [C.F.U (colony forming units)] of the biocontrol agents and pathogens

The population C.F.U. (colony forming units) of fungal biocontrol agents, bacterial antagonists, *Fusarium* spp. and *R. solani* were determined by dilution plating (Warcup, 1955) on organism specific media. Soil samples were collected at from rhizosphere of chickpea for determination of population densities of the respective biocontrol agents and the pathogens at initial stage and 90 days after sowing. For estimation from soil, 1g soil was suspended in 100ml water, vigorously shaken and further serially diluted. Dilution of 10^5 was used to determine population of fungi biocontrol agents, 10^6 for *Fusarium spp.* and *R. solani*. For *T. viride* the specific modified PDA + Chloramphenicol + Triton X-100 medium (Budge and Whipps, 1991) was used where Chloramphenicol was used as an antibacterial antibiotic instead of Aureomycin. For *Fusarium* spp. and *R. solani,* PDA medium was used. One ml of soil dilution was pipetted in sterilized Petri plates and on it, almost cooled, molten medium was poured and plate was rotated with hand to disperse the propagules present in the soil, and then medium was allowed to solidify. The plates were incubated at 28 ± 1^0C for five days and the colonies of the organisms were counted with the help of a Quebec colony counter. The populations were computed to get c.f.u. /g soil.

3.15 STATISTICAL ANALYSIS

The data were subjected to analysis of variance and least significant difference (critical deviation) and critical difference (C. D) determined at 5 and 1 per cent probability. Treatments means were compared using C.D. to determine efficacy of the different treatments (Appendix I to XXV).

4. EXPERIMENTL RESULTS

The present investigation "Studies on integrated management of wilt and root rot complex of chickpea (*Cicer arietinum* L.) caused by *Fusarium* spp. and *Rhizoctonia solani*" was carried out with the objectives (i) To study etiology and distribution of *Fusarium* spp. and *Rhizoctonia solani* causing wilt and root rot complex of chickpea in Rajasthan. (ii) To study cultural, morphological and pathogenic variability of some selected representative isolates of the pathogens. (iii) To assess the yield losses caused by wilt and root rot complex in chickpea. (iv) To develop integrated management module involving cultural practices (date of sowing), chemicals, plant extracts, biocontrol agents and host plant resistance. The results of various experiments conducted are presented in this chapter.

4.1 Collection and isolation of pathogens

The disease samples were collected in *rabi* season of 2012 from severely infected plants of six different major chickpea growing districts of Rajasthan, *viz*., Udaipur, Bikaner, Tivari (Jodhpur), Pali, Sirohi and Banswara to explore the possibility of variable populations of the wilt and root rot pathogens.

The cultures of pathogen inciting wilt and root rot in chickpea were isolated from the diseased roots collected from farmer's field of Rajasthan. The isolates of pathogens recovered from different chickpea plant samples and used in present study are listed in Table 1.

The cultures of *Fusarium solani* and *Rhizoctonia solani* were obtained from the samples collected from different chickpea growing areas of Rajasthan *viz*., Udaipur, Bikaner, Ajmer Tivari (Jodhpur), Pali and Sirohi. Samples from only one location- Banswara, yielded *Fusarium oxysporum* f. sp. *ciceri* (*Foc*) and *R. solani*, rest all the samples yielded cultures of *F. solani* and *R. solani*. The cultures of *Foc* from Banswara was designated as BNS Foc-1 while the different *F. solani* isolates were designated as UDP Fs-1 (Udaipur), BKN Fs-2 (Bikaner), TIB Fs-3 (Tivari), PAL Fs-4 (Pali) and SRH Fs-5 (Sirohi). The cultures of *R. solani* isolates were also

designated as UDP Rs-1(Udaipur), BKN Rs-2 (Bikaner), TIB Rs-3(Tivari), PAL Rs-4 (Pali), SRH Rs-5 (Sirohi) and BNS Rs-6 (Banswara).

To purify the cultures of *Fusarium* spp. single spore and for *R. solani* single sclerotia were picked under a stereo-binocular microscope and transferred onto fresh PDA plates. The pure cultures were maintained on PDA slants at 4°C for further studies. The morphological characters, like cultural characteristics, shape and size of spores and chlamydospores of *Fusarium* spp. and sclerotia for *Rhizoctonia* were studied and compared with the standard descriptions of Mordue (1988) for *Rhizoctonia* and Booth (1971) for *Fusarium* spp.

4.2 Pathogenicity test

To prove Koch's postulates, pathogenicity test was conducted for all the six isolates each of *Fusarium* spp. and *R. solani* collected from different places. The pathogenicity of the cultures of *Fusarium* spp. and *R. solani* were tested by growing chickpea plants in soil inoculated with different isolates of *F. solani, Foc* and *R. solani*. A mixture of garden soil: Farm yard manure (3:1) was sterilized in an autoclave at 1.3 kg per square centimeters pressure at 121°C temp for two hours. The culture of the individual pathogen was multiplied on corn meal-sand (1:1) medium at 25 $\pm$ 1°C for ten days and mixed with sterilized soil @ 20 g/kg soil. This inoculated soil was filled in the fresh plastic pots of 20 cm face diameter size. The pots were kept in the cage house for seven days and were irrigated with distilled water to allow establishment of the pathogen. Pots with uninoculated sterilize soil was kept as control.

Apparently healthy chickpea seed of variety 'Dahod Yellow' were surface sterilized (0.1 per cent mercuric chloride solution for two minutes) followed by two rinsing in sterilized distilled water (SDW) and were sown in inoculated pots @ 5 seed / pot, keeping three pots as three replications. For comparison seeds were sown in sterilized soil without pathogen (un-inoculated control). The pots were irrigated on alternate days with SDW to provide good moisture.

The initial symptoms of the disease in the form of yellowing and wilting of leaves were visible on 15^{th} day after sowing and the typical wilt and root rot symptoms developed in 20 days. The collar region and roots showed sunken, elongated dried black lesions. From the diseased roots, re-isolation of the pathogen

was made which yielded the typical cultures of *F. solani, Foc* and *R. solani* identical with the original ones that was inoculated (Plate 4 and 5).

4.3 Isolation of biocontrol agents

Isolation of the biocontrol agents were done from the air dried rhizosphere soil of healthy chickpea leaves and roots using selective media. The studies resulted in recovery of those organisms which are known to act as biocontrol agents of plant pathogens. The isolated cultures of *Trichoderma* spp. and bacteria were identified as *Trichoderma viride* and bacterial antagonists as *Pseudomonas fluorescens* and *Bacillus subtilis* these were used for further *in vitro* screening. The *T. viride* isolate obtained from Udaipur was designated as UDP T-1 while isolate of *P. fluorescens* was designated as UDP Pf-1 and the isolates of *B. subtilis* obtained from Tivari were designated as Md, Br II, Pv, Tb-1 and Ch (Table 2)

4.4 Variability among the isolates of *Fusarium* spp. and *R. solani*

Variability is a natural process in living organisms. In present investigations variability studies in the pathogens *F. solani* (five isolates), *Foc* (one isolate) and *R. solani* (six isolates) were carried out. They were subjected to study the cultural characters, spore morphology, mycelial growth on different temperatures and pH.

4.4.1 Studies on cultural and morphological characteristics of *Fusarium* spp. and *R. solani*

Six isolates each of *Fusarium* spp. and *R. solani* were cultured on PDA and the characteristics of the growth were recorded at 7^{th} day of inoculation.

Fusarium oxysporum f. sp. *ciceri* isolate BNS Foc-1 had colony diameter (90.0 mm) and sporulation (3.6×10^6conidia/mm^2) with velvety, cottony, aerial, suppressed growth with zonations and light violet in colour with irregular margins.

All the isolates of *F. solani* differed in colony characters on 7^{th} day of incubation under uniform environments and medium. Among the isolates, maximum growth and sporulation was in SRH Fs-5 showing 90.0 mm colony diameter with white suppressed mycelium growth with zonations and regular margins and 3.5×10^6 conidia/ mm^2 sporulation. Isolate BKN Fs-2 had 85.0 mm colony diameter with velvety, cottony, suppressed growth, zonations absent and white irregular margins and 3.3×10^6 conidia/ mm^2. 90.0 mm colony diameter with cottony, suppressed growth

with zonations and white irregular margins and 3.5×10^6 conidia/ mm^2 were recorded with TIB Fs-3. PAL Fs-4 had 87.0 mm colony diameter with cottony aerial growth, white to pinkish colour without zonations and regular margins and 3.2×10^6 conidia/ mm^2.UDP Fs-1 showed minimum growth (82.0 mm) with aerial felty mycelial growth with zonations and white regular margins and sporulation (3.0×10^6 conidia/ mm^2) (Table 3 and Plate 1, Fig.1).

All the six isolates of *R. solani* differed in colony characters on 7^{th} day of incubation under uniform environments and medium. Isolate UDP Rs-1 had 90.0 mm colony diameter with 0.6×10^6 sclerotia/ mm^2, and had velvety, suppressed, aerial growth, dull to dark black regular margins. BKN Rs-2 had colony diameter (90.0 mm) with sclerotial formation (0.7×10^6 / mm^2), and had cottony aerial felty growth, dull grey to black with regular margins. Isolate TIB Rs-3 had colony diameter (90.0 mm) with sclerotial formation (0.8×10^6/ mm^2), and showed aerial felty growth, dull grey to black with zonations and irregular margins. Isolate PAL Rs-4 showed 90.0 mm colony diameter with cottony, suppressed growth, dull grey to black without zonations and regular margins with 0.5×10^6 sclerotia /mm^2. Also, SRH Rs-5 showed 90.0 mm colony diameter with cottony, aerial, suppressed, black with zonations and irregular margins and 0.5×10^6 sclerotia /mm^2sporulation. Isolate BNS Rs-6 had colony diameter (90.0 mm) with aerial, felty steel grey to black growth with zonations and irregular margins and produced 0.8×10^6 mm^2/sclerotia (Table 4 and Plate 1, Fig.2).

4.4.2 Variability in Spore and sclerotial morphology

Spore morphology was recorded in five isolates of *F. solani* and one isolate of *Foc*. Morphology of conidia especially varied in terms of length and width among different isolates collected from various locations.

Foc isolate BNS Foc-1 showed macro conidia of size measuring 9.0 (8.0-10.0) × 1.6 (1.4 -1.7) µm and micro conidia 5.5 (4.8 - 6.1) × 1.7 (1.5-1.9) µm.

Among the *F. solani* isolates, macro conidia of SRH Fs-5 were the largest measuring 10.5 (9.3-11.7) × 2.7(2.6-3.8) µm, this was followed by BKN Fs-2 with size 9.5 (8.4-10.6) × 2.4 (2.1-2.6) µm. The macro conidia of PAL Fs-4 measured 9.5 (8.4-10.6) × 2.0 (1.7-2.2) µm. Also, TIB Fs-3 recorded macro conidia of size 9.2 (8.1-

10.3) × 2.2 (1.9-2.4) µm and smallest size macro conidia was recorded with UDP Fs-1 measuring 8.8 (7.8-9.8) × 2.5 (2.2-2.8) µm.

Micro conidia of *F. solani* isolate UDP Fs-1 was the largest in size measuring 5.1 (4.5-5.7) × 1.0 (0.8-1.1) µm. This was followed by the micro conidia of TIB Fs-3 which measured 4.6 (4.0-5.1) × 2.2 (1.9-2.4) µm. Isolate BKN Fs-2 recorded micro conidia of size 4.5 (4.0-5.0) × 1.5 (1.3-1.6) µm and for PAL Fs-4 measuring 3.5 (3.1-3.9) × 1.8 (1.6-2.0) µm. The smallest micro conidia were recorded with that of SRH Fs-5 measuring 3.2 (2.8-3.5) × 1.2 (1.0-1.3) µm. (Table 5 and Plate 2).

Chlamydospores of *Foc* were 5.7 (4.3-6.6) µm in size. The five isolates of *F. solani* did not showed much variations in chlamydospores size, and these ranged from 5.8 (4.7-6.5) µm to 6.3 (5.6-7.2) µm and these matched with the standard description of *F. solani* (Holliday,1980)

Sclerotial formation was recorded in all the six isolates of *R. solani*. Morphology of sclerotia especially varies in terms of length and width among different isolates collected from various locations.

Among the isolates of *R. solani*, sclerotia of TIB Rs-3 were the largest in size measuring 1.8 (1.6 -2.0) × 1.3 (0.1-1.5) mm, followed by sclerotia of isolate PAL Rs-4 which measured 1.6 (1.4-1.8) × 1.0 (0.9-1.1) mm. Isolate SRH Rs-5 recorded sclerotia of size 1.5 (1.3-1.7) × 0.7 (0.6-0.8) mm and those of BNS Rs-6 measured 1.3 (1.2-1.5) × 0.6 (0.5-0.7) mm. The sclerotia of isolates BKN Rs-2 measuring 1.3 (1.2-1.5) × 0.9 (0.8-1.0) mm and the smallest were of UDP Rs-1, measuring 1.0 (0.8-1.1) × 0.8 (0.7-0.9) mm. (Table 6 and Plate 3).

4.4.3 Effect of different temperature on mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani* on PDA.

Growth of different isolates of *Fusarium* spp. and *R. solani* were recorded as described in Materials and Methods, to know the optimum temperature for mycelial growth, sporulation of *Fusarium* spp. and sclerotial formation of *R. solani*.

F. solani and *Foc* isolates showed maximum growth and sporulation at 30^0C followed by 25^0C and minimum growth and sporulation was recorded at 15^0C temperature (Table 7 and Plate 6).

Foc isolate BNS Foc-1 showed good growth (90.0 mm) and sporulation (3.6 $\times 10^6$ conidia/ mm^2) at 30^0C. This was followed by 90.0 mm growth and 3.5 $\times 10^6$/ mm^2 at 25°C. At 35^0C, 80.0 mm growth and 2.8 $\times 10^6$conidia/ mm^2 was recorded and 88.2 mm growth and 3.0 $\times 10^6$/ mm^2 sporulation was observed at 20°C. The minimum growth (62.0 mm) and sporulation (2.8 $\times 10^6$/ mm^2) was recorded at 15^0C.

Among the isolates of *F. solani* the maximum growth (72.4 mm) and sporulation (2.2 $\times 10^6$conidia/mm^2) was recorded isolate TIB Fs-3 at 15^0C. This was followed by SRH Fs-5 with 70.0 mm growth and 2.5 $\times 10^6$conidia/mm^2 sporulation. Isolate PAL Fs-4 showed 68.0 mm growth and 2.2 $\times 10^6$conidia/mm^2 sporulation. This was followed by UDP Fs-1 with mycelial growth (53.0 mm) and sporulation (2.5 $\times 10^6$/mm^2). While significantly the minimum growth (35 mm) and sporulation (2.3 $\times$ 10^6/mm^2) among the isolates was recorded with BKN Fs-2.

At 20^0C temperature, the maximum growth (76.0 mm) and sporulation (2.6 $\times 10^6$conidia/mm^2) was recorded with PAL Fs-4. Followed by UDP Fs-1 with 75.7 mm growth, and 3.0 $\times 10^6$conidia/mm^2. Both the isolates SRH Fs-5 and BKN Fs-2 showed 70.0 mm growth and 2.9 $\times 10^6$conidia/mm^2 and sporulation 2.8 $\times 10^6$conidia/mm^2 respectively. Whereas TIB Fs-3 recorded the minimum growth (65.0 mm) and sporulation (2.5 $\times 10^6$/mm^2).

At 25^0C temperature, isolate TIB Fs-3 recorded the maximum growth (80.0 mm) and sporulation (2.9 $\times 10^6$conidia/mm^2). This was followed by PAL Fs-4 with 78.0 mm growth and 3.0 $\times 10^6$conidia/mm^2 and UDP Fs-1 with 77.0 mm growth and 3.3$\times 10^6$conidia/mm^2. Mycelial growth 76.0 mm and sporulation 3.2 $\times 10^6$conidia/$mm^2$was recorded with SRH Fs-5 and the minimum growth (72.0 mm) and sporulation (3.0 $\times 10^6$conidia/mm^2) were recorded with isolate BKN Fs-2.

At 30^0C isolate UDP Fs-1 showed the maximum growth (80.0 mm) and maximum sporulation (3.5$\times 10^6$conidia/mm^2). This was followed by PAL Fs-4 with 80.0 mm growth and 3.2$\times 10^6$conidia/mm^2 sporulation and SRH Fs-5 with 78.0 mm growth and 3.5$\times 10^6$conidia/mm^2 sporulation. Similar growth (78.0 mm) and lower sporulation (3.3$\times 10^6$conidia/mm^2) was recorded with BKN Fs-2 and TIB Fs-3 showed 78.0 mm growth and 3.0 $\times 10^6$conidia/mm^2sporulation.

At 35^0C temperature, the maximum growth (76.0 mm) and sporulation (2.5$\times 10^6$conidia/ mm^2) was recorded with isolate PAL Fs-4, followed by SRH Fs-5

with 75.0 mm growth and 2.6×10^6conidia/mm^2 sporulation. TIB Fs-3 recorded 75.0 mm growth and 2.3×10^6conidia/mm^2 sporulation and UDP Fs-1 recorded 72.4 mm growth and 2.8×10^6conidia/mm^2 sporulation. The significantly minimum growth (40.0 mm) and sporulation (2.5×10^6conidia/ mm^2) was recorded with BKN Fs-2.

R. solani isolates showed maximum growth at 30^0C and sporulation at 35^0C followed by 25^0C whereas minimum growth and sporulation was recorded at 15^0C temperature (Table 8 and Plate 7).

Among the *R. solani* isolates at 15^0C temperature, BKN Rs-2 recorded the maximum growth (90.0 mm) and 0.1 $\times10^6$sclerotia/mm^2 sporulation. This was followed by TIB Rs-3 with 80.0 mm growth and 0.2×10^6sclerotia/mm^2. SRH Rs-5 recorded 78.0 mm growth with 0.1 $\times10^6$ sclerotia/mm^2, while 74.0 mm and 0.2×10^6 sclerotia/mm^2 was recorded with BNS Rs-6. The mycelial growth (73.0 mm) and lower sclerotial formation (0.1×10^6/mm^2) was recorded with PAL Rs-4. The minimum mycelial growth (68.0 mm) and sporulation 0.1×10^6 sclerotia/mm^2 was recorded.

At 20^0C UDP Rs-1 showed the highest 87.0 mm growth and 0.2×10^6sclerotia/mm^2, followed by BNS Rs-6 with 86.0 mm growth and 0.3×10^6sclerotia/mm^2 BKN Rs-2 recorded similar growth (86.0 mm), but lower sporulation (0.2 $\times10^6$sclerotia/mm^2). This was followed by TIB Rs-3 with 85.0 mm growth and 0.3 $\times10^6$ sclerotia/mm^2. SRH Rs-5 showed 84.0 mm growth and 0.2 $\times10^6$ sclerotia/ mm^2 and the lowest growth (75.0 mm) and only 0.2×10^6 sclerotia/mm^2 was recorded in PAL Rs-4.

At 25^0C the maximum growth (90.0 mm) and highest number of sclerotia 0.5 $\times10^6$ /mm^2 were recorded with TIB Rs-3. Similar growth (90.0 mm) but less number of sclerotia and 0.3 $\times10^6$/mm^2 were recorded with UDP Rs-1, followed by SRH Rs-5 with 87.0 mm growth and 0.3 $\times10^6$ sclerotia/mm^2. PAL Rs-4 also recorded a similar growth (87.0 mm) but lower sporulation (0.2 $\times10^6$sclerotia/mm^2), while BNS Rs-6 showed lesser growth (80.0 mm) but higher sporulation (0.5 $\times10^6$sclerotia/mm^2).

At 30^0C temperature, all the R. solani isolates showed good growth with colony diameter 90.0 mm but they varied in sclerotia formation. TIB Rs-3 recorded the maximum number of sclerotia 0.6 $\times10^6$/mm^2. This was followed by BKN Rs-2

with 0.5 $\times 10^6$sclerotia/mm^2 and BNS Rs-6 recorded 0.5 $\times 10^6$sclerotia/mm^2. This was followed by isolates UDP Rs-1, PAL Rs-4 and SRH Rs-5 with 0.4 $\times 10^6$sclerotia/mm^2.

At 35^{0}C isolate BNS Rs-6 showed the maximum (90.0 mm) growth and 0.8 $\times 10^6$sclerotia/mm^2, followed by UDP Rs-1 with 90.0 mm growth and 0.6 $\times 10^6$sclerotia/mm^2 PAL Rs-4 recorded 86.0 mm growth and 0.5 $\times 10^6$ sclerotia/mm^2 and SRH Rs-5 recorded 85.0 mm growth and 0.5$\times 10^6$ sclerotia/mm^2 sporulation. TIB Rs-3 recorded 84.0 mm growth and 0.8 $\times 10^6$sclerotia/mm^2 while the minimum growth (80.0 mm) and sporulation (0.7 $\times 10^6$sclerotia/mm^2) were recorded with BKN Rs-2.

4.4.4 Effect of different pH on mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani* on PDA

The relationship of pH to the mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani.* was determined at different pH levels *viz.* 4.0 to 8.0 at 20°C for seven days which is described in Materials and Methods.

The only isolate of *Foc*, BNS Foc-1 showed the 90.0 mm colony diameter with 3.0$\times 10^6$conidia/mm^2 at pH 7, and 88.0 mm growth and 2.3 $\times 10^6$conidia/mm^2 at pH 6. At pH 5, it showed 85.0 mm growth and 2.4 $\times 10^6$conidia/mm^2 while less growth (78.0 mm) and sporulation (1.8 $\times 10^6$conidia/mm^2) were recorded at pH4. Interestingly the least growth (70.0 mm) but the highest sporulation (2.8 $\times 10^6$ conidia/mm^2) were recorded at pH 8.

Among the five isolates of *F. solani*, SRH Fs-5 showed the maximum (73.2 mm) growth and 1.5 $\times 10^6$conidia/mm^2 at pH 4, followed by PAL Fs-4 with 68.0 mm growth and 1.2 $\times 10^6$conidia/mm^2 sporulation. UDP Fs-1 recorded 65.0 mm growth and 1.5 $\times 10^6$conidia/mm^2 while 34.0 mm growth and 1.3 $\times 10^6$conidia/mm^2 were recorded with BKN Fs-2. The minimum growth (30.0 mm) and sporulation (1.2 $\times 10^6$conidia/mm^2) were shown by isolate TIB Fs-3.

At pH 5, isolate SRH Fs-5 showed the maximum growth (79.0 mm) and sporulation (2.2$\times 10^6$conidia/ mm^2), followed by BKN Fs-2 showing 78.0 mm colony diameter and 2.3 $\times 10^6$conidia/ mm^2. TIB Fs-3 showed 77.8 mm growth and 2.0 $\times 10^6$conidia/ mm^2 and PAL Fs-4 showed 77.0 mm and 2.0 $\times 10^6$conidia/ mm^2. At pH 5 the minimum growth (76.0 mm) but the maximum sporulation (2.5 $\times 10^6$/ mm^2) were recorded with UDP Fs-1.

At pH 6, SRH Fs-5 recorded the maximum growth (83.5 mm) and sporulation (3.0 $\times 10^6$conidia/ mm^2), followed by BKN Fs-2 with 80.0 mm growth and 2.0 $\times 10^6$conidia/ mm^2. UDP Fs-1 recorded 79.1 mm growth and 3.1 $\times 10^6$conidia/ mm^2 while, PAL Fs-4 recorded 78.0 mm growth and 2.8 $\times 10^6$conidia/ mm^2. The minimum growth (76.0 mm) but good sporulation (2.8 $\times 10^6$conidia/ mm^2) were recorded with TIB Fs-3.

At pH 7 the isolate BKN Fs-2 recorded the maximum growth (88.0 mm) but the lowest sporulation (2.5 $\times 10^6$conidia/ mm^2). SRH Fs-5 had 85.0 mm growth and 3.3 $\times 10^6$conidia/ mm^2while, UDP Fs-1 recorded 80.0 mm growth and 3.2 $\times 10^6$conidia/ mm^2. TIB Fs-3 recorded 80.0 mm growth and 3.0 $\times 10^6$conidia/ mm^2 and PAL Fs-4 recorded 80.0 mm growth and 3.0 $\times 10^6$conidia/ mm^2 sporulation.

At pH 8, isolate BKN Fs-2 showed the maximum growth (70.0 mm) and sporulation (2.1 $\times 10^6$conidia/ mm^2). This was followed by UDP Fs-1 with 68.0 mm growth and 3.0 $\times 10^6$conidia/ mm^2 sporulation.TIB Fs-3 recorded 65.0 mm growth and 2.4 $\times 10^6$conidia/ mm^2 sporulation. SRH Fs-5 recorded 60.0 mm growth and 2.6 $\times 10^6$conidia/ mm^2 sporulation. The significantly less growth (55.0 mm) but good sporulation (2.4 $\times 10^6$conidia/ mm^2) was recorded with PAL Fs-4.

Thus, the maximum growth and sporulation of isolates of *Foc* and isolates of *F. solani* was recorded at pH 7 followed by that at pH 6 whereas significantly less growth and sporulation was observed at pH 4 (Table 9 and Plate 8).

At pH 4, all the isolates of *R. solani* recorded poor growth of 10.0 mm and less sclerotia formation. UDP Rs-1 produced 0.2 $\times 10^6$ sclerotia/ mm^2, BKN Rs-2 produced 0.3 $\times 10^6$sclerotia/ mm^2 sporulation while, TIB Rs-3 recorded 0.3 $\times 10^6$sclerotia/ mm^2. Two isolates PAL Rs-4 and SRH Rs-5 recorded 0.1 $\times 10^6$sclerotia/ mm^2.

At pH 5, isolate UDP Rs-1 recorded 12.0 mm colony diameter and 0.3$\times 10^6$sclerotia/ mm^2, followed by SRH Rs-5 with 12.0 colony diameter growth and 0.2 $\times 10^6$sclerotia/ mm^2 .BNS Rs-6 recorded 11.0 mm colony diameter and 0.4 $\times 10^6$sclerotia/ mm^2 and also, TIB Rs-4 and PAL Rs-4 both recorded 10.0 mm growth but TIB Rs-4 produced 0.5 $\times 10^6$sclerotia/ mm^2, while PAL Rs-4 recorded only 0.2 $\times 10^6$sclerotia/ mm^2.

At pH 6, the isolate BKN Rs-2 showed the 45.0 mm colony and 0.5 $\times 10^6$ sclerotia/ mm^2. This was followed by PAL Rs-4 with 45.0 mm and 0.3 $\times 10^6$sclerotia/

mm^2 and UDP Rs-1 with 40.0 mm and 0.4 $\times 10^6$sclerotia/ mm^2. SRH Rs-5 and BNS Rs-6 recorded 40.0 mm, and 0.3 $\times 10^6$sclerotia/ mm^2. The minimum growth (38.0 mm) and highest sporulation (0.6 $\times 10^6$sclerotia/ mm^2) was observed with TIB Rs-3.

At pH 7, isolate SRH Rs-5 showed the maximum colony diameter (90.0 mm) and sporulation (0.4 $\times 10^6$sclerotia/ mm^2). This was followed by UDP Rs-1 with 88.5 mm colony diameter and 0.4 $\times 10^6$sclerotia/ mm^2. BNS Rs-6 and PAL Rs-4 both recorded 86.0 mm colony diameter, but while the former produces 0.5 $\times 10^6$sclerotia/ mm^2. PAL Rs-4 showed 0.4 $\times 10^6$sclerotia/ mm^2. Isolate TIB Rs-3 showed 84.0 mm growth and 0.5 $\times 10^6$sclerotia/ mm^2. While BKN Rs-2 showed the minimum growth (84.0 mm) and 0.5 $\times 10^6$sclerotia/ mm^2.

At pH 8, isolate SRH Rs-5 showed the 38.0 mm colony diameter and 0.1 $\times 10^6$sclerotia/ mm^2. UDP Rs-1 had 30.0 mm growth and 0.2 $\times 10^6$sclerotia/ mm^2. TIB Rs-3 recorded 28.0 mm growth and 0.2 $\times 10^6$sclerotia/ mm^2 and BNS Rs-6 recorded 25.0 mm growth and 0.2 $\times 10^6$sclerotia/ mm^2. BKN Rs-2 and PAL Rs-4 both recorded 20.0 mm growth but produced 0.2 $\times 10^6$ sclerotia/ mm^2 and 0.1 $\times 10^6$sclerotia/ mm^2, respectively.

Thus, the maximum growth and sclerotial formation among of *R. solani* isolates was recorded at pH 7 followed by pH 6, whereas significantly less growth and sporulation were observed at pH 4 and pH 5 (Table 10 and Plate 9).

4.5 Assessment of losses

To determine the losses caused by the wilt and root rot complex in chick pea, field trials were conducted in the Experimental fields of RCA, MPUAT, for two consecutive years *rabi* (2012-13) and *rabi* (2013-14), by creating different disease levels through varied inoculum densities of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6) individually as well as in different combinations on chickpea variety 'Dahod Yellow'. These were compared with uninoculated protected plots. Observations on seed germination percentage, plant mortality percentage, grain yield and yield losses were recorded. The data are presented in Table 11.

Rabi 2012-13

In *rabi* (2012-13), the uninoculated protected plots (control) showed 74.0% germination. Among inoculation of individual pathogen, plots inoculated with *Foc*

isolate BNS Foc-1 showed significantly low 58.0% germination, as compared to control as well as other two pathogens. Plot inoculated with *R. solani* had 60.8% germination, while with *F. solani* 64.5% germination was observed. In plots inoculated with two pathogens, lesser germination (50.0%) was recorded with combination of *R. solani* and *Foc*, while of *F. solani* and *Foc* showed 56.8% germination. This was followed by combined inoculation of *F. solani* and *R. solani* showing 54.9% germination. Significantly less germination (44.0%) was recorded in plots with mix inoculum of *F. solani*, *Foc* and *R. solani.*

The disease and the resultant mortality also showed increasing trend with mixed inoculations over the individual ones. Among the individual pathogens, *R. solani* recorded the highest mortality (32.5%) followed by *Foc* in showing 30.0% mortality, while the lowest mortality (28.0%) was recorded in plot inoculated with *F. solani*. In plot inoculated with the two pathogens 40.0% mortality was observed in *R. solani* and *Foc* inoculated plot, followed by 38.0% in plot inoculated with *F. solani* and *R. solani*. 34.0% mortality was observed with inoculation with *F. solani* and *Foc*. The highest mortality (44.0%) was recorded with inoculation of *F. solani*, *Foc* and *R. solani*. In the uninoculated control plots, mean mortality was only 11.5%.

The uninoculated control plots yielded 1.03 kg/plot. Among the individual inoculation, lowest 0.76 kg/plot was recorded with *R. solani*, followed by 0.79 kg/plot with *Foc* and significantly high yield 0.83 kg/plot was recorded in plots inoculated with *F solani*. In plots inoculated with the mixed inoculum of two pathogens, 0.69 kg/plot was also recorded with *R. solani* and *Foc*, 0.70 kg/plot in plots inoculated with pathogens *F. solani* and *R. solani* and 0.73 kg/plot with *F. solani* and *Foc*. The lowest grain yield (0.66 kg /plot) was with mix inoculation with all the three pathogens *F. solani*, *Foc* and *R. solani.*

The per cent loss in the yield over the uninoculated plots was the least in plots inoculation of individual pathogens. *R. solani* recorded the highest yield losses (25.7%) followed by inoculation with *Foc* showing 24.4% yield losses while 18.8% reduction in yield was recorded in plot inoculated with *F. solani.* In plot inoculated with the two pathogens in combination, 32.7% loss was observed in *R. solani* and *Foc* inoculated plots, 30.4% loss in those inoculated with *F. solani* and *R. solani* and 27.9% yield loss with *F. solani* and *Foc.* The maximum yield loss over the

uninoculated plots 34.9% was recorded with inoculation of *F. solani, Foc and R. solani* (Table 11).

Rabi (2013-14)

In the trial conducted in *rabi* (2013-14), among the plot, inoculated with the individual pathogens, *Foc* showed minimum germination (52.4%), while those with *F. solani* had 57.8% and those with *R. solani* showed 52% germination. In mixed inoculations, *F. solani* and *R. solani* (48.0%), in those inoculated with *F. solani* and *Foc*, 50% and with *R. solani* and *Foc* 46.0% germination was recorded. 40.0% germination was recorded in plot inoculated with *F. solani, Foc* and *R. solani* in combination, which was significally less over the uninoculated control plots as well as over all the other inoculated plots.

Due to *R. solani* 34.0% mortality was recorded, followed by 32.0% in *Foc* inoculated plots, while 30.0% mortality was recorded in those with *F. solani.* Among the plots inoculated with two pathogens, 43.0% mortality was recorded with *R. solani* and *Foc*, and 40.0% with *F. solani* and *R. solani*. Mortality was 47.0% with inoculation of *F. solani, Foc* and *R. solani*. Lower mortality (37.3%) was recorded in *F. solani* and *Foc* inoculated plot. In the uninoculated plots only 14.0% mortality was observed (Table 11).

The uninoculated plots gave 0.99 kg/plot grain yield, while 0.80 kg/plot was recorded in plots inoculated with *F. solani*, 0.75 kg/plot with inoculation of *R. solani* and 0.73 kg/plot with *Foc*. The plots inoculated with two pathogens- *F. solani* and *Foc* recorded 0.71 kg/plot, those with *F. solani* and *R. solani* yielded 0.68 kg/plot and 0.67 kg/plot yield was recorded with *R. solani* and *Foc*. Plot inoculated with *F. solani, Foc* and *R. solani* together in combination recorded the lowest 0.64 kg/plot grain yield.

Thus in the trial of *rabi* (2013-14) among the plots inoculated with individual pathogens, the highest yield loss 26.9% was recorded with *Foc*, 23.7% in *R. solani* and 19.6% with *F. solani*. Mixed inoculation with two pathogens 33.5% reduction was recorded in plots inoculated with *R. solani* and *Foc* followed by 32.2% yield loss with *F. solani* and *R. solani*. 29.7% yield loss was recorded in *F. solani* and *Foc* inoculated plots. The highest 35.8% yield loss with inoculation of *F. solani, Foc* and *R. solani* (Table 11).

Pooled data revealed that among the individual inoculation, lowest germination (55.2%) was due to *Foc*, followed by 56.4% in *R. solani* and 61.1% in *F. solani*. The reduction in germination due to *F. solani* was statistically ($P \leq 0.05$) significant over the other two pathogens. Among the inoculation of two pathogens in combination, *R. solani* and *Foc* resulted in 48.0%, germination while *F. solani* and *R. solani* showed 51.4%, while 53.4% germination was recorded in plot inoculated with *F. solani* and *Foc*. These differences were also statistically ($P \leq 0.05$) significant. The plots inoculated with all the 3 pathogens *F. solani, Foc* and *R. solani* showed only 42.0% germination, which was significantly less over all the other inoculations as well as the uninoculated control.

Mean mortality in the uninoculated plots was 12.7%. Among individual inoculation, highest 33.2% mortality curved with *R. solani* followed by 31.0% with *Foc* and significantly low 29.0% mortality in *F. solani* inoculated plot. Among the inoculation of two pathogens in combination, *R. solani* and *Foc* resulted in 41.5% mortality while 39.0% mortality was recorded with *F. solani* and *R. solani*. *Foc* and *F. solani* caused 35.6% mortality while 45.5% mortality was recorded with all the three pathogens *F. solani*, *Foc* and *R. solani* (Table 11).

The uninoculated plots yielded 1.01 kg/plot, those inoculated with only *F. solani* yielded 0.82 kg/plot, those with *Foc* yielded 0.76 kg/plot, and those with *R. solani* yielded 0.75 kg/plot.

Inoculation of two pathogens in combination showed 0.69 kg/plot with *F. solani* and *R. solani*, 0.68 kg/plot with *R. solani* and *Foc* and 0.72 kg/plot with *F. solani* and *Foc*. The plots inoculated with all the three pathogens gave the lowest grain yield (0.64 kg/plot).

Thus, the highest yield loss (25.26%) among individual pathogens was with *R. solani*, 24.7% yield loss was observed with *Foc* and lowest 19.2% in *F. solani* inoculated plot. Among the pathogens in combination, 35.4% yield loss was recorded in inoculation with *F. solani, Foc* and *R. solani*. This was followed by 33.1% yield loss with *R. solani* and *Foc* and 31.3% yield loss was recorded with *F. solani* and *R. solani*, while 28.8% yield loss was recorded in inoculation with *F. solani* and *Foc* (Table 11 and Plate 10).

4.6 Evaluation of fungicides, biocontrol and botanicals against chickpea wilt and root rot complex pathogens (*Fusarium spp*. and *Rhizoctonia solani*) *in vitro*

4.6.1 *In vitro* efficacy of fungicides (Poison food technique)

The efficacy of five fungicides was evaluated *in vitro* against *F. solani*, *Foc* and *R. solani* on PDA by poisoned food techniques as given in Materials and Method. Observations on radial mycelial growth were recorded.

With Vitavax at 250, 500 and 1000 ppm, BNS Foc -1 developed 15.0, 10.0 and 10.0 mm colony diameter as compared to 90.0 mm growth in untreated control.

Among the isolates of *F. solani*, at 250 ppm Vitavax, isolate SRH Fs-5, UDP Fs-1, and PAL Fs-4 had colony diameter 22.0, 23.0 and 25.0 mm, while TIB Fs-3 and BKN Fs-2 showed 72.0 mm and 75.0 mm growth respectively. At 500 ppm, the colony diameters in SRH Fs-5, UDP Fs-1, and PAL Fs-4 ranged from18.0 - 20.0 mm, and BKN Fs-2 and TIB Fs-3 recorded 35.0 mm and 50.0 mm growth. At 1000 ppm all the isolates showed the colony diameters ranging from 05.0 -25.0 mm only, as compared to 90.0 mm growth in untreated control (Table 12 and Plate 11).

With Thiram at 250 ppm *Foc* isolate BNS Foc-1 showed 55.0 mm growth, while at 500 and 1000 ppm, colony diameters was 10.0 mm.

With Thiram at 250 ppm, *F. solani* isolates PAL Fs-4 and TIB Fs-3 showed 21.0 mm and 25.0 mm colony diameter, BKN Fs-2 had 55.0 mm and UDP Fs-1 had 58.0, while SRH Fs-5 showed 62.0 mm colony diameter. At 500 ppm, PAL Fs-4 and TIB Fs-3 showed 17.0 mm and 20.0 mm colony diameter, UDP Fs-1 had 45.0 mm, while SRH Fs-5 and BKN Fs-2 showed 47.0 mm and 48.0 mm colony diameter. At 1000 ppm, PAL Fs-4 and TIB Fs-3 showed 12.0 mm and 20.0 mm growth, UDP Fs-1 and BKN Fs-2 had 30.0 mm and 33.0 mm, and SRH Fs-5 showed 45.0 mm colony diameter (Table 12 and Plate 12).

BNS Foc-1 showed highly sensitive to Trifloxystrobin with 12.0, 10.0 and 10.0 mm colony diameter at 250, 500 and 1000 ppm as compared to 90.0 mm growth in the untreated control.

At 250 ppm Trifloxystrobin, *F. solani* isolate SRH Fs-5 showed the maximum sensitivity with 25.0 mm colony diameter, PAL Fs-4 showed 45.0 mm growth, while

TIB Fs-3, UDP Fs-1 and BKN Fs-2 showed colony diameter ranging from 63.0-66.0 mm. UDP Fs-1 showed complete inhibition of growth with Trifloxystrobin at 500 and 1000 ppm. SRH Fs-5 showed 18.0 mm and PAL Fs-4 showed 35.0 mm growth at 500 ppm Trifloxystrobin. The isolates BKN Fs-2 and TIB Fs-3 recorded 55.0 mm and 62.0 mm growth at 500 ppm, respectively. At 1000 ppm, SRH Fs-5 showed the least growth (08.0 mm), PAL Fs-4 had 30.0 mm, while TIB Fs-3 and BKN Fs-2 had 60 and 65 mm, respectively (Table 12 and Plate 13).

With Tebuconazole at 250, 500 and 1000 ppm BNS Foc-1 showed 30.0, 22.0 and 10.0 mm growth as compared to 90.0 mm growth in untreated control.

All the isolates of *F. solani* were found to be sensitive to Tebuconazole, with colony diameter ranging 20.0 – 28.0 mm growth at 250 ppm, 10.0 – 21.0 mm at 500 ppm, but at 1000 ppm PAL Fs-4 and TIB Fs-3 showed 05.0 mm and 10.0 mm colony diameter, respectively. BKN Fs-2 had 12.0 mm and, UDP Fs-1 and SRH Fs-5 had 15.0 mm colony diameter with 1000 ppm Tebuconazole (Table 12 and Plate 14).

BNS Foc-1 was sensitive to Captan at all the three concentrations(250, 500 and 1000 ppm) with 40.0, 30.0 and 20.0 mm growth, respectively, as compared to untreated control with 90.0 mm growth.

At 250 ppm Captan, *F. solani* isolate PAL Fs-4 showed 33.0 mm colony diameter, BKN Fs-2 and SRH Fs-5 showed 65.0 mm, TIB Fs-3 had 68.0 mm and UDP Fs-1 showed 75.0 mm colony diameter. At 500 ppm, PAL Fs-4 showed 33.0 mm, while TIB Fs-3 and UDP Fs-1 showed 43.0 and 45.0 mm, respectively. Isolates SRH Fs-5 showed 50.0 mm and BKN Fs-2 showed 58.0 mm colony diameter. At 1000 ppm, PAL Fs-4 showed 15.0 mm, UDP Fs-1 showed 25.0 mm, TIB Fs-3 showed 28.0 mm, BKN Fs-2 had 45.0 mm growth and SRH Fs-5 had 60.0 mm (Table 12 and Plate 15).

Among the isolates of *R. solani*, at 250 ppm Vitavax, isolate TIB Rs-3 showed complete inhibition, PAL Rs-4 and SRH Rs-5 showed 40.0 and 58.0 mm colony diameter, respectively while BKN Rs-2 and UDP Rs-1 showed 60.0 and 68.0 mm growth respectively. At 500 ppm, complete inhibition of colony growth was recorded with TIB Rs-4, while 43.0 mm and 46.0 mm growth with SRH Rs-5 and PAL Rs-4 respectively, were recorded. Growth ranging from 50.0 – 58.0 mm was observed with UDP Rs-1, BNS Rs-6 and BKN Rs-2 with 500 ppm Vitavax. At 1000 ppm Vitavax,

UDP Rs-1, BKN Rs-2, TIB Rs-3, SRH Rs-5 and BNS Rs-6 showed complete inhibition of growth and PAL Rs-4 showed 10.0 mm colony diameter as compared to 90.0 mm in the untreated control (Table 13 and Plate 11).

At 250 ppm Thiram, *R. solani* isolate PAL Rs-4 showed the maximum sensitivity with 23.0 mm colony diameter, while SRH Rs-5, BKN Rs-2 and TIB Rs-3 showed growth ranging from 40.0 - 43.0 mm. UDP Rs-1 and BNS Rs-6 showed 52.0 and 53.0 mm colony diameter. At 500 ppm Thiram, BNS Rs-6, BKN Rs-2, PAL Rs-4, SRH Rs-5 and TIB Rs-3 showed colony diameter ranging from 33.0 to 38.0 mm and UDP Rs-1 showed 48.0 mm. At 1000 ppm, UDP Rs-1, BKN Rs-2 and BNS Rs-6 showed 10.0 mm colony diameter, TIB Rs-3 showed 12.0 mm while 15.0 mm colony diameter was observed with PAL Rs-4 and SRH Rs-5. The untreated control showed 90.0 mm colony diameter (Table 13 and Plate 12).

With Trifloxystrobin at 250 ppm, isolate UDP Rs-1 showed 78.0 mm colony diameter and BKN Rs-2, TIB Rs-3, SRH Rs-5, PAL Rs-4 and BNS Rs-6 showed colony diameter, ranging 80.0 – 86.0 mm. At 500 ppm, UDP Rs-1, SRH Rs-5 and BKN Rs-2 showed 70.0, 76.0 and 78.0 mm colony diameters respectively. PAL Rs-4 and BNS Rs-6 both showed colony diameter of 85.0 mm and TIB Rs-3 showed 88.0 mm colony growth as compared to 90.0 mm in the untreated control (Table 13 and Plate 13).

Tebuconazole showed to be highly effective to all the isolates of *R. solani* with complete inhibition at 250, 500 and 1000 ppm concentrations as compared to 90.0 mm growth in uninoculated control (Table 13 and Plate 14).

At 250 ppm Captan, BNS Rs-6 showed 50.0 mm colony diameter, while BKN Rs-2, UDP Rs-1, TIB Rs-3, PAL Rs-4 and SRH Rs-5 were less sensitive with colony diameter ranging 86.0 – 90.0 mm. At 500 ppm, BNS Rs-6, PAL Rs-4 and SRH Rs-5 showed 30.0, 38.0 and 42.0 mm colony growth respectively. TIB Rs-3, BKN Rs-2 and UDP Rs-1 showed 75.0, 80.0 and 85.0 mm colony diameter, respectively. At 1000 ppm, SRH Rs-5 and BNS Rs-6 showed 30.0 mm colony growth, while PAL Rs-4 showed 35.0 mm colony growth. BKN Rs-2 had 79.0 mm, UDP Rs-1 and TIB Rs-3 had 80.0 mm colony growth. Untreated control showed 90.0 mm colony diameter (Table 13 and Plate 15).

4.6.2 *In vitro* evaluation of botanicals (Poison food technique)

The efficacy of two botanicals *viz.*, Neem oil and Karanj oil, both @ 0.2% concentration was evaluated *in vitro* against *F. solani, Foc* and *R. solani* on PDA by poisoned food techniques as given in Materials and Method. The radial mycelial growth was recorded (Table 14 and 15).

Foc isolate BNS Foc-1 showed sensitivity to Neem oil with 28 mm colony growth whereas it was less sensitive to Karanj oil with 60.0 mm growth.

F. solani isolates PAL Fs-4 and SRH Fs-5 showed sensitive to Neem oil with 22.0 mm colony diameter, as compared to 90.0 mm growth of untreated control. UDP Fs-1 showed 43.0 mm and BKN Fs-2 showed 60.0 mm colony diameter while TIB Fs-3 showed 90.0 mm colony diameter (Table 14).

Isolates BKN Fs-2 was sensitive to Karanj oil with 56.0 mm colony diameter while PAL Fs-4 and TIB Fs-3 showed 60.0 and 63.0 mm colony diameter. SRH Fs-5 had 70.0 mm and UDP Fs-1 had 77.0 mm colony diameter.

R. solani isolate UDP Rs-1 showed sensitivity to Neem oil with 18.0 mm colony diameter as compared to 90.0 mm in the untreated control. BKN Rs-2 showed 22.o mm and TIB Rs-3 showed 26.0 mm colony diameter while SRH Rs-5 and PAL Rs-4 showed 33.0 mm and 42.0 mm colony growth, respectively.

All the isolates of *R. solani* showed less sensitivity to Karanj oil with colony diameter ranging (75.0 – 85.0 mm) whereas untreated control recorded 90.0 mm growth (Table 15 and Plate 16).

4.6.3 Evaluation of fungicides and botanicals in pot-culture

A pot experiment was conducted in *rabi* (2012-13) to evaluate the efficacy of seed treatment with fungicides *viz.*, Vitavex, Thiram, Trifloxystrobin, Tebuconazole and Captan and botanicals *viz.*, Neem and Karanj oil on chickpea sown on pots with inoculated soil (Table 16 and Plate 17).

All the fungicidal seed treatment showed higher germination over the untreated control which had 52.0% germination. Among the treatments, highest germination (72.0%) was recorded in pots having seed treated with Tebuconazole, followed by 71.0% in those with Vitavax seed treatment. Thiram seed treatment resulted in 69.0% germination, Captan had 66.0% while seeds treated with

Trifloxystrobin showed 65.0% germination. With Neem oil seed treatment, 65.0% germination was recorded, while with Karanj oil it was 62.0%.

In pots without seed treatment (untreated control) the mortality was 70%. Seed treatment with Tebuconazole resulted in 44.0% mortality, which was significantly less over the untreated control as well as over all the other treatments (Table 15 and Plate 17). This was followed by 46.0% mortality with Vitavax and 48.0% mortality with Thiram. 51.0% mortality was recorded in seed treatment with Captan and 53.0% with Trifloxystrobin. Seed treatment with botanicals was less effective than fungicides, 56.0% mortality was recorded in seed treatment with Neem oil and 56.0% mortality with Karanj oil.

4.6.4 *In vitro* efficacy of fungal biocontrol agents (Dual culture technique)

Efficacy of six isolates of the fungal biocontrol agents *Trichoderma* spp. was studied *in vitro* as described in Material and Method, using dual culture technique. Data revealed that all the six isolates were potential antagonist of *Fusarium* spp. and *R. solani,* but the extent of efficacy varied within the isolates.

Fusarium oxysporum f.sp. *ciceri* isolate BNS Foc-1 showed above 90% inhibition due to *T. harzianum* and *T. viride* isolate. It showed 99.5% inhibition with *T. harzianum* isolate Jh-2, 99.5% with *T. viride* isolate ICRISAT-15 and100% with ICAR-95. With ICRISAT-25 it showed 88.9%, while ICRISAT-28 causing 24.5%, and isolate UDP T-1 causing 20.0% inhibition were less effective.

Among the five *F. solani* isolates, PAL Fs-4 showed to be most sensitive to all the isolates of *Trichoderma* spp. showing 100% inhibition due to all the isolates.

Similarly, *F. solani* isolate TIB Fs-3 also was sensitive to all the isolates of *Trichoderma* spp. with 100% inhibition due to *T. harzianum* isolates, ICRISAT-28, ICRISAT-25 and Jh-2 and also to *T. viride* isolates UDP T-1 and ICAR-95, while with ICRISAT-15 it showed 99.5% inhibition.

This was followed by *F. solani* isolate. BKN Fs-2 with 100% inhibition due to ICRISAT-28, ICRISAT-25 and Jh-2, while with *T. viride* isolate UDP T-1 it showed 99.5% and with ICRISAT-15 it showed 88.9% and 86.7% with ICAR-95.

F. solani isolate SRH Fs-5 showed 99.5% inhibition with *T. harzianum* isolates ICRISAT-28 and ICRISAT-25 and 83.3% with Jh-2. It showed 99.5%

inhibition with *T. viride* isolates ICRISAT-15 and ICAR-95, and 88.9% with UDP T-1.

Isolate UDP Fs-1 showed to be least sensitive with *T. harzianum* isolates with 71.1% inhibition due to ICRISAT-28, 64.5% with ICRISAT-25 and 50% with Jh-2. It showed 50- 66.0% inhibition due to various *T. viride* isolates. (Table 17 and Plate 18)

R. solani isolates showed comparatively less sensitivity to fungal biocontrol agents Among the *R. solani* isolates, PAL Rs-4 showed to be most sensitive showing 100% inhibition with *T. harzianum* isolates ICRISAT-28, ICRISAT-25 and Jh-2 and *T. viride* isolates UDP T-1 and ICAR-95 while with ICRISAT-15 it showed 88.3% inhibition.

R. solani isolate BKN Rs-2 was found sensitive sensitive to *Trichoderma* spp. showing 100% inhibition with *T. harzianum* isolate ICRISAT-25, while with ICRISAT-28 it showed 50.0% and with Jh-2 it showed 55.6% growth inhibition. Also 100% inhibition was shown with *T. viride* isolates UDP T-1 and ICAR-95 and 74.5% inhibition was with ICRISAT-15.

R. solani isolate TIB Rs-3 showed sensitivity with 100% inhibition due to *T. harzianum* isolate Jh-2 while 80.0% inhibition was recorded with ICRISAT-25 and 77.8% inhibition due to ICRISAT-28. With *T. viride* isolates ICRISAT-15 it showed 88.9% growth inhibition, with UDP T-1 it showed 66.7%, and with ICAR-95 it showed 58.9%.

With *T. harzianum* isolates ICRISAT-25, isolate SRH Rs-4 showed 90.0% growth inhibition, with Jh-2 it showed 88.9% inhibition was recorded and 72.2% inhibition was with ICRISAT-28. Also, with *T. viride* isolates ICAR-95 it showed 88.9% growth inhibition, while with ICRISAT-15 it showed 66.7% and with UDP T-1 it showed 44.5% inhibition.

R. solani isolate BNS Rs-6 was the least sensitive to *T. harzianum.* With the isolate ICRISAT-25, 61.1% inhibition was recorded with Jh-2 and with ICRISAT-28 55.6% inhibition was recorded. Also, with *T. viride* isolate UDP T-1 it showed 100% inhibition, 51.1% inhibition with ICAR-95 and 38.9% inhibition with ICRISAT-15. (Table 18 and Plate 19).

4.6.5 *In vitro* efficacy of bacterial antagonists (Dual culture technique)

Efficacy of bacterial antagonists, *Pseudomonas fluorescens* isolate UDP Pf-1 and *Bacillus subtilis* isolates Md, Br II, Pv, Tb-1 and Ch was studied *in vitro* as described in Material and Method, using dual culture technique. Data revealed that all the isolates were potential antagonist of *Fusarium* spp. and *R. solani.*

Foc isolate BNS Foc-1 showed 66.7% inhibition of growth due to *P. fluorescens* isolate UDP Pf-1 and with *B. subtilis* isolates Md it showed 66.7%. With Br II it showed 64.5% inhibition of growth, with *B. subtilis* isolate Pv it showed 51.1% inhibition of growth. *B. subtilis* isolate Ch causing 41.1% inhibition and *B. subtilis* isolate Tb-1 caused 37.8% inhibition.

Among the five isolates, *F. solani* isolate UDP Fs-1 showed 50.0% growth inhibition with *P. fluorescens* UDP Pf-1. *B. subtilis* Br II caused 85.6% inhibition. *B. subtilis* Tb-1 caused 55.6% inhibition and *B. subtilis* Md caused 52.2% inhibition of mycelial growth. This was followed by 46.7% and 22.2% inhibition with Ch and Pv, respectively.

F. solani isolate TIB Fs-3 showed 99.5% inhibition with *P. fluorescens* isolate UDP Pf-1. While *B. subtilis* isolate Tb-1 caused 100% growth inhibition, *B. subtilis* Pv showed 71.1%, and *B. subtilis* Ch showed 68.9%. *B. subtilis* Br it showed 56.7% inhibition and with *B. subtilis* Md it showed 44.5 5 inhibition.

F. solani isolate SRH Fs-5 showed 61.1% growth inhibition with *P. fluorescens* isolate UDP Pf-1 and with *B. subtilis* isolates Tb-1 it showed 66.7% inhibition with *B. subtilis* Ch it showed 60.0%. It showed 55.6% inhibition with *B. subtilis* Pv, 55.6% with *B. subtilis* Md and 51.1% inhibition with *B. subtilis* Br II.

F. solani isolate PAL Fs- 4 showed 38.9% inhibition of growth due to *P. fluorescens* isolate UDP Pf-1. With *B. subtilis* isolate Tb-1 showed 78.9%, with *B. subtilis* Pv it showed 73.3% inhibition of growth. It showed 55.6% inhibition with *B. subtilis* Br II, 53.3% inhibition of growth with *B. subtilis* Md and *B. subtilis* isolate Ch it caused 41.1% inhibition.

F. solani isolate BKN Fs-2 showed 50.0% inhibition of growth with *P. fluorescens* isolate UDP Pf-1 and with *B. subtilis* isolates Br II it showed 85.6%. With *B. subtilis* Tb-1 it showed 55.6%, with *B. subtilis* Md it showed 52.2% inhibition of growth. *B. subtilis* Ch showed 46.7% inhibition and *B. subtilis* Pv showed 22.2% inhibition (Table 19 and Plate 20).

Among the six isolates of *R. solani*, isolate PAL Rs-4 showed 100% inhibition of growth with *P. fluorescens* UDP Pf-1 and *B. subtilis* isolates Md, Br II and Ch showed 100% inhibition of growth. *B. subtilis* isolate Pv showed 88.9% inhibition of growth and *B. subtilis* Tb-1 showed 86.7% inhibition.

R. solani isolate SRH Rs-5 showed 75.6% inhibition of growth with *P. fluorescens* isolate UDP Pf-1 and with *B. subtilis* isolate Md it showed 60.0% inhibition of growth. With *B. subtilis* Pv it showed 55.6% inhibition and *B. subtilis* Br II it showed 50.0% inhibition of growth. *B. subtilis* Tb-1 caused 38.9% and *B. subtilis* Ch caused 44.5% inhibition.

R. solani isolate BNS Rs-6 showed 44.5% inhibition of growth with *P. fluorescens* isolate UDP Pf-1.With *B. subtilis* Tb-1 it showed 75.6% inhibition of growth, and with *B. subtilis* Ch showed 47.8% inhibition. *B. subtilis* Ch showed 44.5% inhibition of growth, *B. subtilis* Br II showed 44.5% inhibition of growth, *B. subtilis* Pv showed 41.1% inhibition and *B. subtilis* Md showed 38.9% inhibition.

R. solani isolate UDP Rs- 1 caused 53.3% inhibition of growth with *P. fluorescens* isolate UDP Pf-1. *B. subtilis* isolates Md caused 41.1% inhibition of growth, *B. subtilis* Br II showed 50.0% inhibition of growth, *B. subtilis* Pv showed 44.5% inhibition of growth. With *B. subtilis* Tb-1 it showed 44.5% inhibition of growth and *B. subtilis* Ch it showed 46.7% inhibition.

Also, *R. solani* isolate TIB Rs-3 showed 47.8% inhibition of growth with *P. fluorescens* isolate UDP Pf-1 and with *B. subtilis* isolate Md it showed 53.3% inhibition of growth. *B. subtilis* Br II showed 45.6% inhibition of growth and *B. subtilis* isolates Pv,Tb-1 and Ch it showed 44.5% inhibition.

Isolate BKN Rs-2 showed 44.5% inhibition of growth with *P. fluorescens* isolate UDP Pf-1 and with *B. subtilis* isolate Md it showed 55.6% inhibition of growth. *B. subtilis* Ch showed 44.5% inhibition of growth, *B. subtilis* Pv showed 41.1% inhibition of growth. *B. subtilis* Br II showed 38.9% inhibition of growth and *B. subtilis* Tb-1 showed 33.3% inhibition (Table 20 and Plate 21).

4.6.6 Evaluation of fungal biocontrol agents and bacterial antagonists applied on in pot culture

Efficacy of biocontrol agents found effective *in vitro* namely, *T. harzianum* isolate ICRISAT-25 and *T. viride* isolate ICAR-95, bacterial antagonists *P.*

fluorescens isolate UDP Pf-1 and *B. subtilis* isolate Br II was studied as seed treatment in pots, having soil inoculated with mixture of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6) as described in Materials and Methods. Observations for seed germination and mortality of chickpea, and populations of the biocontrol agents and of the two pathogens in the rhizosphere were recorded.

Highest germination (78.0%) was observed in seeds treated with *T. harzianum* isolate ICRISAT-25 as compared to 55.5% in the untreated control. This was followed by 75.0% germination in seeds treated with *T. viride* isolate ICAR-95, 73.0% with *P. fluorescens* isolate UDP Pf-1 and the lowest germination (70.0%) was recorded with *B. subtilis* isolate Br II.

Pots having untreated seeds showed the highest mortality (80.0%), while those with seeds treated with *T. harzianum* ICRISAT-25 resulted in the lowest 35.0% mortality, followed by 40.0% with *T. viride* isolate ICAR-95. This was followed by 53.0% mortality in seed treatment with *P. fluorescens* isolate UDP Pf-1. Seed treatment with *B. subtilis* isolate Br II showed 55.0%, mortality (Table 21 and Plate 22).

On treated seeds, the mean population of *Trichoderma* isolates ranged from 7.9-8.0 $\times 10^5$ c.f.u./seed, while that of bacteria ranged from 8.0-8.5 $\times 10^5$ c.f.u./seed.

The biocontrol agents applied as seed treatment could establish and multiply in chickpea rhizosphere. At 90 DAS, the highest population density (6.2×10^5 c.f.u./g soil) was recorded rhizosphere of plants with seed treatment of *T. harzianum* ICRISAT-25 and, while in *T. viride* ICAR-95 had 6.2 $\times 10^5$ c.f.u./g soil.

The population density of the bacterial antagonists at 90 DAS was recorded to be the higher in seed treatment with *P. fluorescens* UDP Pf-1 showing 6.7×10^8 c.f.u./g soil as compared to that of *B. subtilis* Br II with 6.5×10^8 c.f.u./g soil.

These BCAs effectively suppressed the population density of the pathogens. At 90 DAS over the untreated control had 8.0×10^6 c.f.u of *R. solani* and 8.3×10^6 c.f.u/g soil of *Fusarium spp.* Seed treatment with *T. harzianum* ICRISAT-25 resulted in 4.4 $\times 10^6$ c.f.u./g soil of *R. solani*, and 4.5×10^6 c.f.u./g soil of *Fusarium* spp. This was followed by 4.5 $\times 10^6$ c.f.u./g soil of *R. solani* and 4.7×10^6 c.f.u./g soil of *Fusarium* spp. in pots treated with *T. viride* ICAR-95. *P. fluorescens* UDP Pf-1

resulted in 4.7×10^6 c.f.u./g soil of *R. solani* and 4.9×10^6 c.f.u./g soil of *Fusarium spp*.. In seed treatment with *B. subtilis* Br II, the population density of *R. solani* was 4.8 $\times10^6$ c.f.u./g soil while of *Fusarium* spp. was 5.0 $\times10^6$ c.f.u./g soil.

As such, while the BCAs significantly suppressed the population densities of the pathogens over the untreated control, the difference among the two fungal pathogens and among the two bacterial pathogens were statistically non-significant. However, suppression due to fungal BCAs was statistically significant over that caused by the two bacterial biocontrol agents.

4.7 Evaluation of popular chickpea cultivar for resistance to wilt and root rot pathogens during *rabi* (2012-13) and (2013-14)

Ten chickpea cultivars were screened to find out the source of resistance against *F. solani, Foc* and *R. solani* causing wilt and root rot complex of chickpea. The experiment was conducted in pots using the varieties Dahod Yellow, Pratap Chana -1, Avrodhi, RSG-888, RAJ-1581, BGD-72, BG-391, BG-1053, GMG-469 and P- 1080 under soil inoculation activity growing virulent cultures of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6). The experiment was conducted in *rabi* (2012-13) and (2013-14).

Rabi 2012-13

The data reveal that in *rabi* (2012-13), the highest germination 85.2% was recorded with variety Avrodhi as compared to other varieties. RAJ-1581 showed 79.0% and GNG-469 showed 76.2% germination, P-1080 showed 76.2% germination and Pratap Chana-1 showed 74.0% germination. This was followed by 73.0% with BG-1053, 71.0% germination was recorded with BGD-72, while 70.0% germination was observed with BG-391.RSG-888 had 64.6% germination and Dahod Yellow showed the minimum (62.0%) germination.

The lowest mortality (21.0%) as compared to other varieties was showed by Avrodhi. This was followed by variety GNG-469 with 27.0%mortality, RAJ-1581 showed 34.0%, P-1080 showed 39.0%, Pratap Chana-1 showed 43.0%, BG-1053 showed 47.0%, BGD-72 showed 52.0% and BG-391 with 55.5% mortality respectively. While, Dahod Yellow recorded the highest percent mortality (63.0%).

Rabi (2013-14)

In the trial conducted in *rabi* (2013-14), the maximum germination (83.0%) was recorded with chickpea variety Avrodhi. This was followed by variety GNG-469 with 82.0% germination, RAJ-1581 with 77.0%, P-1080 with 75.0% and Pratap Chana-1 with 73.0% germination respectively. BG-1053 had 72.0% germination, BGD-72 had 69.0% germination, BG-391 had 67.0% germination and RSG-888 had 61.0% germination respectively. Variety Dahod Yellow showed the minimum germination (60.0%).

The lowest mortality (22.0%) was recorded with variety Avrodhi, GNG-469 showed 30.0% mortality, RAJ-1581 showed 36.0% mortality, P-1080 showed 42.5% mortality and Pratap Chana-1 showed 46.0% mortality. While 50.0% mortality was recorded with variety BG-1053, 57.0% mortality with BGD-72, 58.0% with BG-391 and 61.0% with RSG-888. The highest mortality (65.0%) was recorded with variety Dahod Yellow.

Pooled data revealed among the varieties, the lowest germination (61.0%) was recorded with Dahod Yellow, followed by variety BG-391 with 62.8% germination. BGD-72 showed 68.5% germination, BG-1053 showed 70.0% germination, Pratap Chana-1 showed 72.5% germination, P-1080 showed 73.5% germination, RAJ-1581 showed 75.6% germination and 78.0% germination with GNG-469. The highest germination 82.5% was recorded with Avrodhi.

Among the varieties tested, the highest mortality (64.0%) was recorded with variety Dahod Yellow, followed by 56.8% mortality with BG-391. Variety BGD-72 showed 54.5% mortality, BG-1053 showed 48.5% mortality, Pratap Chana-1 showed 44.5% mortality, P-1080 showed 40.8% mortality, RAJ-1581 showed 35.0% mortality and GNG-469 with 28.5% mortality. The lowest 21.5% mortality was recorded with variety Avrodhi.

4.7.1 Effect of different sowing dates on incidence of chickpea wilt and root rot complex under field conditions during *rabi* (2012-13 and 2013-14)

Effect of sowing dates on incidence of wilt and root rot complex was recorded by sowing chickpea on different dates with 15 days interval from 15th October to 29th November.

Rabi (2012-13)

The data reveals that in *rabi* (2012-13), the minimum 41.0% germination was recorded in plots sown on 15th Oct. showed, as compared to other sowing dates. This was followed by 48.2% germination in plots sown on 30th Oct., 54.8% germination in plots sown on 14th Nov. The maximum germination (62.3%) was recorded with late sown on 29th Nov.

The disease and the resultant mortality also showed higher mortality due to disease in early sowing of chickpea over the late sowing. Plots with seeds sown on 15th Oct. recorded the highest percent mortality (50.3%) followed by 46.4% in those sown on 30th Oct. and 42.7% mortality in those sown on 14th Nov. showing, while the lowest mortality (38.3%) as compared to other sowing dates was recorded in those sown on 29th Nov.

Among the different sowing dates, the lowest 0.67 kg/plot grain was recorded with early sown on 15th Oct., followed by 0.78 kg/plot in plots sown on 30th Oct. and 0.82 kg/plot grain yield was recorded in plots sown on 14th Nov. The highest grain yield (0.92 kg /plot) was with recorded with late sown on 29th Nov.

Rabi (2013-14)

In the trial conducted in *rabi* (2013-14), plots sown on 15th Oct. showed the minimum germination (38.4%), followed by 43.5% in those sown on 30th Oct. Also, 48.1% germination in plots sown on 14th Nov. and the maximum germination (52.8%) with late sown on 29th Nov.

In plots sown on 15th Oct. showed the highest mortality (54.2%), followed by 49.3% with those sown on 30th Oct., 46.4% mortality in plots sown on 14th Nov. showed and the lowest mortality (42.7%) was recorded in plots sown on 29th Nov.

Sowing of chickpea on 15th Oct. yielded the lowest 0.61 kg/plot grain, while 0.74 kg/plot grain yield was recorded with those sown on 30th Oct. In pots sown on 14th Nov. yielded 0.79 kg/plot and the highest grain yield (0.88 kg/plot) was recorded in plots sown on 29th Nov.

Pooled data revealed among the different sowing dates, the lowest germination (39.7%) was with those sown on 15th Oct., followed by 45.9% in plots sown on 30th Oct. and 51.5% in plots sown on 14th Nov. Late sowing of chickpea on 29th Nov. showed highest germination (57.6%).

Among the different dates of sowing, the highest (52.3%) mortality was recorded in plots sown on 15th Oct., followed by 47.9% in plots sown on 30th Oct. Also, 44.6% mortality was recorded in plots sown on 14th Nov. and the lowest mortality (40.5%) was recorded in plots sown on 29th Nov.

Pooled data showed the lowest grain yield (0.64 kg/plot) was recorded in plots sown on 15th Oct., followed by 0.76 kg/plot in plots sown on date 30th Oct. and 0.81 kg/plot in plots sown on 14th Nov. The highest grain yield (0.90 kg/plot) was recorded with late sown on 29th Nov. (Table 22 and Plate 23).

4.8 Evaluation of fungicides, botanicals and biocontrol agents for suppression of wilt and root rot of chickpea in the field

The fungicides, botanicals and biocontrol agents which were found effective *in vitro* and on pot studies were further tested as seed treatment under field conditions individually and in combinations to control wilt and root rot complex of chickpea. For this, an experiment was carried out under field conditions in the years *rabi* (2012-13) and (2013-14).

The plot size was 3×2 meter, total 10 treatments were taken including control and 3 replications, following randomized block design (RBD). Cultures of *F. solani* (isolate SRH Fs-5), *Foc* (isolate BNS Foc-1) and *R. solani* (isolate BNS Rs-6) were multiplied on corn meal-sand (1:1) medium filled in flasks at 28 $\pm$ 1°C for 15 days till good growth occurred. These cultures were then properly mixed together and applied in soil @ 600 g/ plot. All the plots were lightly irrigated immediately after inoculation and to allow establishment of the pathogen before sowing.

At the time of sowing, soil samples were taken from a depth of 15 cm to determine initial population of pathogens. Seeds of chickpea were treated with fungicides, Tebuconazole or Vitavax @ 0.2 per cent, Neem oil @ 2 per cent and the most effective biocontrol agent *T. harzianum* (ICRISAT-25) talc based formulation as seed treatment. The variously treated seeds were planted in the respective plots @ 250 seeds. A light irrigation was given immediately after sowing. Observations on germination percentage, per cent mortality, grain yield and percent yield increase were recorded. After 90 days of sowing, soil samples were collected from rhizosphere

of chickpea for determination of population densities of the respective biocontrol agents and the pathogens.

Rabi (2012-13)

The data reveals that in *rabi* (2012-13), among the individual treatments, seed treated with *T. harzianum* showed the highest 72.0% germination, as compared to only 52.0% in the untreated control as well as other treatments. Seed treated with Tebuconazole had 70.0% germination, while 68.0% germination was observed in seed treated with Vitavax and the lowest germination (66.1%) was observed with Neem oil. In plots with two seed treatments in combination, significantly higher germination (80.0%) was recorded in seed treatment with Tebuconazole + *T. harzianum*, followed by 79.2% with Vitavax + *T. harzianum.* Treatment with Neem oil + *T. harzianum* showed 77.5% germination and treatment with Vitavax + Neem oil showed 76.0% germination. The lowest germination (74.6%) was recorded in plots with seed treatment of Tebuconazole + Neem oil.

The disease and the resultant mortality also showed decreasing trend with combined treatments over the individual ones. In *rabi* (2012-13), among the individual treatments, *T. harzianum* recorded the lowest percent mortality 17.5% followed by 21.0% in Tebuconazole. Seed treatment with Vitavax showed 23.0% mortality and the highest mortality (25.3%) was recorded with Neem oil. in plot with treatments in combination, the lowest mortality (12.0%) was recorded with Tebuconazole + *T. harzianum* as compared to 46.0% in the untreated control plots. Plots with Vitavax + *T. harzianum* treated seeds had 14.0% mortality. 15.2% mortality in those with Neem oil + *T. harzianum*, while 16.0% mortality was observed with Vitavax + Neem oil. Plots having seed treatment with Tebuconazole + Neem oil showed the highest mortality 20.0% among all the combined seed treatments.

In *rabi* (2012-13) the uninoculated control plots yielded 0.65 kg/plot. Among the individual treatments, the highest yield of 1.00 kg/plot grain was recorded in plots with seed treated with *T. harzianum*, followed by 0.98 kg/plot grain yield with Tebuconazole, 0.95% grain yield with Vitavax and the lowest low 0.90 kg/plot grain yield in treatment with Neem oil. In plots with the two treatments in combination, the highest grain yield (1.30 kg/plot) was recorded in treatment with Tebuconazole + *T. harzianum*, 1.25 kg/plot grain yield was recorded in treatment with Vitavax + *T.*

harzianum, 1.20 kg/plot grain yield was also recorded with Neem oil + *T. harzianum* and 1.12 kg/plot grain yield with Vitavax + Neem oil. The lowest grain yield (0.97 kg /plot) was recorded with the combined treatment of Tebuconazole + Neem oil.

The per cent increase in the yield over the untreated plots among the individual treatment was the highest in *T. harzianum* showing 53.9% yield increase. This was followed by seed treatment with Tebuconazole (44.9%) and Vitavax (46.2%), while the lowest (38.5%) yield increase was recorded in plot with seeds treated with Neem oil. In plot with the treatment of seeds in combination, the highest yield increase (100%) was recorded in seed treatment with Tebuconazole + *T. harzianum*, 92.3% yield increase with Vitavax + *T. harzianum* treated seeds and 84.6% yield increase with seed treatment of Neem oil + *T. harzianum* and Vitavax + Neem oil showed 72.3% yield increase. Significantly less yield increase (49.2%) was recorded in plots with seed treatment of Tebuconazole + Neem oil.

Rabi (2013-14)

In the trial conducted in *rabi* (2013-14), the untreated control plots showed 50.0% germination. Among individual treatments, seed treated with *T. harzianum* showed the highest 70.0% germination, as compared to in control as well as other treatments. Seed treated with Tebuconazole had 69.3% germination, while 66.0% germination was observed in seed treated with Vitavax and the lowest germination (64.5%) was observed with Neem oil. In plots with two seed treatments in combination, higher germination (78.0%) was recorded in seed treatment with Tebuconazole + *T. harzianum*, and 76.0% with Vitavax + *T. harzianum*. This was followed by treatment with Neem oil + *T. harzianum* showing 75.0% germination and treatment with Vitavax + Neem oil showing 73.0% germination. The lowest germination (71.0%) was recorded in plots with seed treatment of Tebuconazole + Neem oil.

The lowest mortality (20.0%) among the individual treatments was recorded with individual treatment, *T. harzianum*, followed by 23.0% with Tebuconazole. Vitavax seed treatment showed 25.0% mortality and the highest mortality (27.0%) was recorded in seed treatment with Neem oil. In plot with treatments in combination, the lowest mortality (14.0%) was recorded in treatment with Tebuconazole + *T. harzianum* as compared to 49.0% mortality in untreated control plots. This was

followed by 16.0% mortality in plots with Vitavax + *T. harzianum* treated seeds, and 17.0% in those with Neem oil + *T. harzianum*, 18.0% mortality was observed with Vitavax + Neem oil while treatment with Tebuconazole + Neem oil showed the highest mortality 22.0%.

The uninoculated plots gave 0.64 kg/plot grain yield, while among the individual treatments 0.96 kg/plot grain yield was recorded in plots treated with *T. harzianum*, followed by 0.95 kg/plot grain yield with Tebuconazole, 0.92 kg/plot with Vitavax and the lowest low 0.89 kg/plot grain yield was recorded in treatment with Neem oil. In plots with the two treatments in combination, the highest grain yield (1.20 kg/plot) was recorded in treatment with Tebuconazole + *T. harzianum*, 1.10 kg/plot grain yield was recorded in treatment with Vitavax + *T. harzianum*, 1.00 kg/plot grain yield was also recorded with Neem oil + *T. harzianum* and 0.98 kg/plot grain yield with Vitavax + Neem oil. The lowest grain yield (0.93 kg /plot) was recorded with the treatment Tebuconazole + Neem oil.

In the trial of *rabi* (2013-14) the highest yield increase (51.0%) with individual treatment of *T. harzianum*. Followed by seed treatment with Tebuconazole showed 49.5% and 44.7% yield increase in plots with seed treated with Vitavax, lowest yield increase (39.9%) was recorded in plot with seeds treated with Neem oil. In plot with the treatment of seeds in combination, the highest yield increase (88.5%) was recorded in seed treatment with Tebuconazole + *T. harzianum*, 73.1% yield increase was recorded in plots with Vitavax + *T. harzianum* treated seeds, 57.2% yield increase was recorded with seed treatment of Neem oil + *T. harzianum* and 54.2% yield increase was also recorded with seed treatment of Vitavax + Neem oil. Significantly low yield increase (46.1%) was recorded in plots with seed treatment of Tebuconazole + Neem oil.

Pooled data reveals the untreated control plots had 51.0% germination. Among individual treatments, seed treated with *T. harzianum* showed the highest (71.0%) germination, as compared to control as well as other treatments. Seed treated with Tebuconazole had 69.6% germination, while 67.0% germination was observed in seed treated with Vitavax and the lowest germination (64.5%) was observed with Neem oil. In plots with two treatments in combination, higher germination (79.0%) was recorded in plots having seed treatment with Tebuconazole + *T. harzianum*, those with Vitavax + *T. harzianum* showed 77.6% germination. This was followed by

treatment with Neem oil + *T. harzianum* showed 76.3% germination and treatment with Vitavax + Neem oil showed 74.5% germination. The lowest germination (72.8%) was recorded in plots with seed treatment of Tebuconazole + Neem oil.

The pooled data showed lowest mortality (18.5%) with individual treatment, *T. harzianum,* followed by treatment with Tebuconazole showed 22.0% mortality. Seed treatment with Vitavax resulted in 24.0% mortality and the highest mortality (26.0%) among the individual treatments was recorded in seed treatment with Neem oil. The untreated control showed 50.0% mortality. In plot with treatments in combination, the lowest mortality (13.0%) was recorded in treatment with Tebuconazole + *T. harzianum* as compared to 47.5% mortality in untreated control plots. This was followed by 15.0% mortality in plots with Vitavax + *T. harzianum* treated seeds. Plots having Neem oil + *T. harzianum* showed 16.0% mortality while 17.0% mortality was observed with Vitavax + Neem oil. The seed treatment with Tebuconazole + Neem oil resulted the highest mortality 21.0% among the combined treatments.

The uninoculated plots gave 0.64 kg/plot grain yield, while among the individual treatments highest grain yield (0.98 kg/plot) was recorded in plots treated with *T. harzianum*, followed by 0.95 kg/plot grain yield with Tebuconazole, 0.94% with Vitavax and the lowest grain yield (0.90 kg/plot) was recorded in treatment with Neem oil. In plots with the two treatments in combination, the highest grain yield (1.25 kg/plot) was recorded in treatment with Tebuconazole + *T. harzianum*, followed by 1.18 kg/plot grain yield in treatment with Vitavax + *T. harzianum*, 1.10 kg/plot grain yield with Neem oil + *T. harzianum* and 1.05 kg/plot grain yield with Vitavax + Neem oil. The lowest grain yield (0.95 kg /plot) among the combined treatments was recorded with Tebuconazole + Neem oil.

Pooled data showed the highest yield increase (52.4%) with individual treatment of *T. harzianum,* followed by 47.2% in seed treatment with Tebuconazole. Yield increase of 45.4% in plots with seeds treated with Vitavax. The lowest yield increase (39.2%) was recorded with Neem oil. In plot with combination treatments, the highest yield increase (94.3%) was recorded in seed treatment with Tebuconazole + *T. harzianum*, 82.7% yield increase in plots with Vitavax + *T. harzianum*, 70.9% yield increase with seed treatment of Neem oil + *T. harzianum* and 63.3% yield increase was also recorded with seed treatment of Vitavax + Neem oil. Significantly

low yield increase (47.7%) over the untreated control was recorded in plots with seed treatment of Tebuconazole + Neem oil (Table 24 and Plate 25 and 26).

4.8.1 Determination of population [C.F.U. (colony forming units)] of the biocontrol agents and pathogens

The biocontrol agents (*T. harzianum*) could successfully establish in the chickpea rhizosphere and multiplied to reach high densities counts (c.f.u) of *T. harzianum* seed treated.

The initial counts of *T. harzianum* on various treatments ranged from 6.0- 6.8 $\times 10^5$ c.f.u./seed. Initial population of *Fusarium* spp. in soil was 2.4×10^6 c.f.u /g soil and *Rhizoctonia solani* in soil was 1.8×10^6 c.f.u /g soil.

At 90 DAS the population density of *T. harzianum* was the highest (7.1×10^5 c.f.u./g soil) in plots with individual seed treatment of *T. harzianum*. And among the combination of two treatments, Neem oil + *T. harzianum* showed the highest population counts (7.0×10^5 c.f.u./g soil), Vitavax + *T. harzianum* showed lesser population 6.8×10^5 c.f.u./g soil and the lowest 6.6×10^5 c.f.u./g soil was observed in seed treatment with Tebuconazole + *T. harzianum*.

All the treatments effectively suppressed the population density of *R. solani* over the un-treated control with 7.0×10^6 c.f.u./g soil. Among the individual treatments, in plots having seed treated with *T. harzianum* showed 5.7×10^6 c.f.u./g of *R. solani* and 5.8×10^6 c.f.u./g of *Fusarium* spp. in the rhizosphere at 90 DAS. Seed treated Tebuconazole showed 6.1×10^6 c.f.u./g of *R. solani* and 6.2×10^6 c.f.u./g of *Fusarium* spp. Vitavax seed treatment showed 6.2×10^6 c.f.u./g of *R. solani* and 6.0×10^6 c.f.u./g of *Fusarium* spp. Population density of *R. solani* was 6.4×10^6 c.f.u./g soil and of *Fusarium* spp. was 6.3×10^6 c.f.u./g soil in plots with seed treated with Neem oil.

Among the treatments in combination, the maximum suppression 5.4×10^6 c.f.u./g of *R. solani* and 5.2×10^6 c.f.u./g of *Fusarium* spp. was observed in seed treatment with Tebuconazole + *T. harzianum*. This was followed by seed treatment with Vitavax + *T. harzianum* which showed 5.5×10^6 c.f.u./g of *R. solani* and 5.6×10^6 c.f.u./g of *Fusarium* spp. Population counts of *R. solani* and *Fusarium* spp. both was 5.6×10^6 c.f.u./g soil in plots with Neem oil + *T. harzianum* treatment. Plots with seed treatment with Vitavax + Neem oil showed 5.8×10^6 c.f.u./g of *R. solani* and 5.7×10^6

c.f.u./g of *Fusarium* spp. and the least suppression 6.0 $\times 10^6$ c.f.u./g soil count of *R. solani* and 5.9×10^6 c.f.u./g of *Fusarium* spp. was observed in plots with Tebuconazole + Neem oil seed treatment. Thus, significant suppression of the pathogens due to various treatments resulted in effective suppression of the disease in the field (Table 25 and Plate 27).

5. DISCUSSION

The present investigation were undertaken to develop an effective strategy for the integrated management of wilt and root rot complex diseases of chickpea (*Cicer arietinum* L.), which is widely prevalent in moderate to high severity in different parts of Rajasthan.

The pathogens were isolated from disease chickpea plants collected from six different locations of Rajasthan by following standard isolation procedure. For identification these characters was compared with the standard reference descriptions (Sneh *et al*., 1992 and Mordue, 1988) for *Rhizoctonia solani* and Booth (1971) for *Fusarium* spp. These were identified as *Rhizoctonia solani*, *Fusarium solani* and *Fusarium oxysporum* f. sp. *ciceri*. Involvement of *Sclerotia rolfsii* in chickpea root rot complex is reported by Nene *et al*. (1981), Singh *et al*. (1986) and Khan *et al*. (2002), but none of the samples in our studies yielded *S. rolfsii*. Only one sample yielded culture of *Foc*, showing that root rot complex caused by *F. solani* and *R. solani* is more prevalent. Prevalence of these in causing root rot complex earlier had been reported from other crops like coriander (Yadav *et al*., 2003), soybean (Tetarwal *et al*., 2011) and safed musli (Sharma *et al*., 2012) in Rajasthan.

The culture of each isolate was inoculated in soil in pot culture to test the pathogenicity. Initial symptoms of the disease in the form of yellowing and wilting of leaves were visible on 15-20 days, and the typical root rot symptoms developed in 25 days. Nikam *et al*. (2011) also reported similar symptoms of yellowing, dropping and wilting of leaves of diseased plants caused by *Foc* after 25 days of inoculation in pot culture. From the disease plants re-isolation of the pathogens was made which yielded the fungi, *Fusarium solani, Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* identical with the original one that was inoculated. Similar results have been reported previously by Trapero-Casas and Jimenez-Diaz (1985), Kaur *et al*. (2007) and Khalil (2007).

The one isolate of *Foc* and the five isolates each of *F. solani* and six isolates of *R. solani* were studied for morphological characters like cultural characters, spore and sclerotial morphology, mycelial growth on different temperatures and pH, and *in vitro* sensitivity to fungicides, botanicals and biocontrol agents.

The five isolates of *F. solani* showed variations in growth and in spore morphology. The size of macro conidia ranged from 8.8 × 2.5 µm of isolate UDP Rs-1 to 10.5 × 2.7 µm of SRH Rs- 5. The smallest micro conidia 3.2 × 1.2 µm of SRH Fs-5 and the largest of 5.1 × 1.0 µm of UDP Fs-1.These results are in agreement with Booth (1977), Dubey and Singh *et al* . (2004), Mandhare *et al*., (2011) and Kumar *et al.* (2012) where, they concluded that variability in *Fusarium* spp. exists only with respect to cultural characters.

Chlamydospores of *Foc* measured 4.3-6.6 µm in size. The five isolates of *F. solani* did not show much variation in chlamydospores size, and these ranged from 4.7-6.5 µm to 5.6-7.2 µm.

All the six isolates of *R. solani* differed in colony characters and showed black coloured cultures with 90.0 mm colony diameter on 7^{th} day of incubation under uniform environments and medium. Sclerotial formation was recorded in all the six isolates of *R. solani*. Morphology of sclerotia varied from 1.0 × 0.8 mm of UDP Rs-1 and 1.8 × 1.3 mm of TIB Rs-3. The results showed variations in growth and sclerotial formation among *R. solani* isolates. These results were in similarity with Nikam (2008).

Temperature plays an important role, among the external factors which influenced the growth and reproduction of fungi. The differential impact of temperature on the relative growth rates of the host and pathogen often affects the severity of root rots (Leach, 1947; Bhatti and Kraft, 1992; Chang *et al*. 2004). *In vitro* studies were conducted to determine the optimum range of temperatures for mycelial growth and sporulation of the isolates of *F. solani*, *Foc* and *R. solani.* The results obtained in the present study indicates that out of the five temperatures *viz.*, 15, 20, 25, 30 and 35^0C, the maximum mycelial growth and sporulation of the isolate of *Foc* were recorded at 30^0C and the minimum at 15^0C temperature. Also, all the five isolates of *F. solani* showed the maximum mycelial growth and sporulation at 30^0C and the minimum growth and sporulation were recorded at 15^0C temperature which was similar with the result obtained by Blanca (2006). All the six isolates of *R. solani* showed the maximum mycelial growth at 30^0C and the highest sclerotial formation at 35^0C and the minimum at 15^0C temperature. Similar results were reported by Roseli *et al.*, (2002) on *R. solani*. Qazi *et al*., (1970) reported that temperature range of 24 to 28^0C was the best for growth of *R. solani* and *Fusarium* spp. The same optimum

temperature was recorded many workers, Khare and Nema (1981); Gupta *et al.* (1986); Li *et al*. (1998); Prashanthi and Kulkarni *et al*. (2003) and Bochalya (2010).

Fungi can grow over a wide range of pH but optimum for mycelial growth may differ for each species or even within species. The fungal growth is usually inhibited on acidic side at pH 3 and on alkaline side at pH 8-9 (Lilly and Barnett, 1951). The relationship of pH to the mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani.* was determined *in vitro* at different pH levels *viz*., 4.0 to 8.0 at 20^0C. The present studies revealed that *Fusarium* spp. and *R. solani* grew at wide range of pH (6-8). The maximum growth and sporulation of the isolate of *Foc* was observed at pH 7 while the minimum at pH 4. The five isolates of *F. solani* showed the maximum growth and sporulation at pH 7 and the minimum at pH 4. The isolates of *R. solani* showed the maximum growth and sclerotial formation at pH 7 and the minimum at pH 4. Dubey (1997) reported that pH 6.5 supported maximum growth and excellent sclerotia production of *R. solani.* This level of pH is supported by various other workers who carried out studies on *R. solani* causing root rot on different crops (Li *et al*, 1998).

Field experiments were conducted for two consecutive years to estimate the losses caused by wilt and root rot complex of chickpea with disease generated through inoculation of the pathogens *F. solani, Foc* and *R. solani* individually and in combinations on chickpea variety 'Dahod Yellow' using the most virulent isolate of each pathogens. Results showed the disease severity increased with combined inoculation of two and three pathogens as compared to that of the individual ones. A significant reduction in grain yields at all the severity levels was observed as compared to the uninoculated protected control. Pooled data revealed that among the individual inoculation, lowest germination was due to *Foc,* followed by *R. solani* and *F. solani.* Among the inoculation of two pathogens in combination, plots inoculated with *F. solani* and *Foc* showed highest germination. The plots inoculated with all the three pathogens *F. solani, Foc* and *R. solani* showed only 42.0 % germination, which was significantly less over all the other inoculations as well as the uninoculated control. Similar trends were observed for plant mortality, the maximum mortality was recorded with all the three pathogens *F. solani, Foc* and *R. solani.* Thus, the highest yield loss (25.26%) among individual pathogens was with *R. solani,* and higher losses were recorded from plots inoculated with two pathogens and all the three pathogens

together. These observations suggest that wilt and root rot complex of chickpea has good potential of damaging the crops and may become limiting factor in realization of good yield. Pitambar *et al*. (2010) reported 53.48 % mortality due wilt incidence caused by *Foc*. In our studies, of the three pathogens, *R. solani* caused more losses than *Foc* and *F. solani*. Mostly chickpea is grown as a dry land crop on residual moisture and such conditions helps in proliferation by *F. solani* and *R. solani* (Sneh *et al*., 1992).

The results of current *in vitro* studies indicated that out of five fungicides tested, *viz*., Vitavex, Thiram, Trioxystrobin, Tebuconazole and Captan, *Foc* isolate BNS Foc-1 showed highly sensitive to Trifloxystrobin followed by Vitavax, moderately sensitive to Thiram and Trifloxystrobin and was least sensitive to Captan at all the three concentrations. The different isolates of *F. solani* and *R. solani* showed variations in sensitivity to Vitavax, Thiram, Trifloxystrobin and Captan but all showed sensitivity to Tebuconazole. Similar observations were made by Nikam *et al.* (2007), Mukhtar (2007), Christian *et al*. (2007), Mddhusudhan *et al*. (2010), Subhani *et al*. (2011) and Andrabi *et al*. (2011).

To find out possibilities of use of other eco-friendly products for management of disease, two botanicals *viz*., Neem oil and Karanj oil were evaluated against the pathogens. The result showed Neem oil at 0.2% concentration was quite effective in inhibiting the growth of *Fusarium* spp. and *R. solani.* Karanj oil at 0.2 % concentration was found to be less effective on *Fusarium* spp. and *R. solani*. Manibhusanrao *et al*., (1998) reported that botanicals, particularly Neem have shown good fungicidal potential against several pathogenic fungi including *R. solani* and seemed promising as eco-friendly fungicide and also revealed that Neem leaf extract considerably inhibited mycelium growth of *R. solani in vitro.* Similar results were obtained by Prasad and Ojha (1986), Reddy and Reddy (1987), Eswaramorthy *et al*. (1989), Nwachukewe and Umechuruba (2001), Singh and Chand (2004), Chand and Singh (2005) and Sitara *et al.* (2008) where Neem oil was effective in reducing wilt incidence in chickpea. Result of pot experiment conducted to evaluate the effect of fungicides and botanicals showed that the fungicide, Tebuconazole to be most effective with the maximum germination and the minimum mortality compared to other fungicides and botanicals.

Use of biocontrol agents is an ecologically sound approach to control of soil borne diseases. Kaur and Mukhopadhyay (1992), Datnoff *et al*. (1995) and Hervas *et al*.(1995) have used antagonists individually and in combinations to suppress wilt of chickpea and on other crops. To evaluate the efficiency of some fungal and bacterial biocontrol agents for suppression of the pathogens, *in vitro*, the efficacy of the isolates of *Trichoderma* spp., *P. fluorescens* and *B. subtilis* was evaluated by dual culture method. The *T. harzianum* isolate ICRISAT-25 was found to be highly effective against the pathogens *in vitro* followed by *P. fluorescens* UDP Pf-1 and *B. subtilis* Br II. Chet and Baker (1981) reported similar studies on the parasitic activity of strains of *Trichoderma* spp. on *R. solani*. The control of root rot/wilt diseases by *Trichoderma* spp. might be attributed to the pronounced colonization of rhizosphere by antagonists in advance to the pathogens (Mathew and Gupta, 1998) and also by mycoparasitism (Papavizas and Lewis, 1989). This result was similar with the results obtained by Mujeebur *et al*. (2004) where seeds of chickpea were treated with commercial formulations (2 g/kg seed) of *T. harzianum* and *P. fluorescens*, singly and jointly. Similar results were reported by Meki *et al*. (2011) and Subhani *et al*. (2013). Antibiosis is a very common phenomenon responsible for the activity of many BCAs such as *P. fluorescens, Bacillus* spp., *Streptomyces* spp. *and Trichoderma* spp. A large diversity of molecules produced by various fungal and bacterial biocontrol agents has been described and their role in suppression of several plant pathogens has been documented (Fravel, 1988; Loper and Lindow, 1993; Weller and Thomashow, 1993)

The management of the diseases through host plant resistance is considered as a dependable choice in all the crop improvement programmes. Utilization of resistant cultivars in farming is simple, effective and economical method for management of diseases. The resistant cultivars reduce the cost, time and energy when compared to the other methods of disease management. Screening was done taking ten chickpea cultivars with inoculations of all the three pathogens using soil inoculation technique of spore cum mycelial of *Foc, F. solani* and *R. solani.* The result showed Avrodhi to be highly resistant to wilt and root rot complex of chickpea. Varieties GNG-469, RAJ-1581, P-1080 and Pratap Chana-1 were moderately resistant, while BG-1053, BGD-72, BG-391 and RSG-888 were moderately susceptible. The popular cultivar Dahod Yellow was highly susceptible. Screening of large number of genotypes has been done by several workers Nene, 1980; Nain and Agnihotri, 1984; Nene and

Reddy, 1987 and Bala and Kalia, 2012 and some sources of resistance identified for individual pathogens. In our studies, these evaluations for resistance were done under inoculations of three pathogens. In this screening no variety was immune or highly resistant. Limited success has been achieved in use of resistant varieties for soil borne pathogens, two were found promising in the present study.

The results of field experiment conducted to evaluate the effect of different sowing dates (15th Oct to 29th Nov) with 15 days interval on incidence of chickpea wilt and root rot complex showed that late sown on 29th Nov showed the highest germination, the lowest mortality and the highest grain yield. Singh and Saxena (1996) reported that chickpea winter sowing enables the reproductive phase of the crop to match with more favourable thermal and moisture supply regimes resulting in an increase in yield. Andrabi *et al*. (2011) reported that sowing of chickpea at different dates revealed that early sowing (10th Oct.) resulted in maximum disease incidence (32.20%), whereas, late sowing (24th Nov.) the minimum (13.35%). The reason for this may be the lower temperature during November to February that does not support growth and sporulation of these soil borne pathogens (Holliday, 1980).

The fungicides, botanicals and biocontrol agents found effective *in vitro,* were further evaluated as seed treatment individually as well as in different combinations for suppression of wilt and root-rot complex of chickpea in the field. It was found that combined treatments were superior in terms of better germination, lower mortality and higher yield as compared to the individual treatments. The most effective treatments was seed treatment with Tebuconazole + *T. harzianum* followed by Vitavax + *T. harzianum* as compared to control as well as other treatments. Kovacikova (1970) reported that seed treatment with fungicides gave the best protection against the Fusarium wilt caused by *Foc*. Verma and Vyas (1977) and Sukhla *et al*. (1981) also reported that used of fungicides seed dressings may increase seedling emergence. Arfaoudi *et al*. (2006) also reported the compatibility of biological control agents (*T. harzianum*, *T. viride* and *P. fluorescens*) with fungicides (Thiram, Captan and Vitavax) against the chickpea wilt.

The disease suppression in the treatments seemed to be due to reduction of the inoculum density of the pathogens. It is noteworthy that in the present study the evaluation of the treatments was under rigorous inoculation of the three pathogens. The initial inoculum density of *Fusarium* spp. was 2.4×10^6 c.f.u / g soil and of *R.*

solani was 1.8×10^6 c.f.u / g soil. After 90 DAS in untreated control, these reached to 6.9×10^6 c.f.u / g of *Fusarium* spp. and 7.0×10^6 c.f.u / g of *R. solani* respectively. But in various treatments, the population densities of the pathogens were considerably reduced. Under this inoculum load, significant disease suppression was recorded. In natural field conditions the soil borne pathogens are randomly distributed in soil/ rhizosphere. *T. harzianum* applied as seed treatment effectively established in chickpea rhizosphere and reached high population densities, at 90 DAS while the population of the pathogens was low in the rhizosphere. These BCAs are natural soil inhabitants and once established in the rhizosphere, these are able to suppress the pathogen by active antagonism, mainly antibiosis and competition (Chet, 1989 and Fravel *et. al.,* 1985) and also enhance plant growth by production of volatiles (Chang *et. al.,* 1986). Of the different methods of application of fungicides and biocontrol agents, seed treatments have been most favoured and used, and there are several studies to show that the BCAs applied on seed established in the rhizosphere and provide good suppression of the pathogens and diseases (De and Mukhopadhyay, 1990; Vyas, 2001 and Xue *et. al.,* 2007). The used of BCAs as seed treatment results in their easily establishment in the developing rhizosphere, causing a 'pre-empt' effect, where the established BCAs are able to suppress the activities of the pathogens through active antagonism (competition, exploitation and antibiosis). The used of BCAs with fungicides helps in better establishment of plants at initial stages. While the effect of fungicides may be diminished after a certain period, the BCAs continued to grow and multiply in the rhizosphere. The BCAs are also reported to induced resistance in hosts (Kloepper *et al*., 1993; Van Loon, 1998 and Harman *et al*., 2004 b). In the present studies, various isolates of pathogens were screened *in vitro* against BCAs and only those BCAs that showed good suppression of all the pathogens were used for field trial. In several cases, the BCAs have not been found much effective in field. As is clear from the present study, this is because the prevalent pathogens showed variations in sensitivity to BCAs. Similar results have been reported in some earlier work (Dennis and Webster, 1971; Chet, 1989 and Baker and Cook, 1974). Combination of seed treatment with *T. harzianum* + Neem oil was also effective in suppression of the disease. This treatment may be useful for suppression of soil borne pathogens in organic farming of chickpea as well as for root rot complex in other crops.

6. SUMMARY

Chickpea wilt and root-rot complex caused by *Fusarium solani*, *Fusarium oxysporium* f. sp. *ciceri* and *Rhizoctonia solani* was widely prevalent in moderate to high severity in different parts of Rajasthan. The pathogens were isolated from disease chickpea plants collected from six different locations of Rajasthan by following standard isolation procedure. These were identified as *F. solani*, *Foc* and *R. solani*. Only one sample from Banswara yielded culture of *Foc*, while five isolates of *F. solani* and six isolates of *R. solani* were yielded from the samples collected from Udaipur, Bikaner, Tivari, Pali, Sirohi and Banswara respectively, and showed that wilt and root rot complex caused by *F. solani* and *R. solani* is more prevalent.

The culture of each isolate was inoculated in soil in pot culture to test the pathogenicity. Initial symptoms of the disease in the form of yellowing and wilting of leaves were visible on 15-20 days, and the typical root rot symptoms developed in 25 days.

The one isolate of *Foc* and five isolates each of *F. solani* and six isolates of *R. solani* were studied for morphological characters like cultural characters, spore and sclerotial morphology, mycelial growth on different temperatures and pH, and *in vitro* sensitivity to fungicides, botanicals and biocontrol agents.

The five isolates of *F. solani* showed variations in growth and in spore morphology. The size of macro conidia ranged from 8.8 × 2.5 µm of isolate UDP Rs-1 to 10.5 × 2.7 µm of SRH Rs- 5.The smallest micro conidia 3.2 × 1.2 µm of SRH Fs-5 and the largest of 5.1 × 1.0 µm of UDP Fs-1.

Chlamydospores of *Foc* measured 4.3-6.6 µm in size. The five isolates of *F. solani* did not showed much variation in chlamydospores size, and these ranged from 4.7-6.5 µm to 5.6-7.2 µm.

All the six isolates of *R. solani* differed in colony characters and showed black colored cultures with 90.0 mm colony diameter on 7^{th} day of incubation under uniform environments and medium. Sclerotial formation was recorded in all the six isolates of *R. solani*. Size of sclerotia varied from 1.0 × 0.8 mm of UDP Rs-1 and 1.8

× 1.3 mm of TIB Rs-3. The results showed variations in growth and sclerotial formation among *R. solani* isolates.

In vitro studies were conducted to determine the optimum range of temperature for mycelial growth and sporulation of the isolates of *F. solani, Foc* and *R. solani.* The results obtained in the present study indicates that out of five temperatures *viz.*, 15, 20, 25, 30 and 35^{0}C, the maximum mycelial growth (90.0 mm) and sporulation (3.6 ×10^6 conidia/ mm^2) of the isolate of *Foc* were recorded at 30^{0}C and the minimum 62.0 mm colony diameter and 2.8 ×10^6/ mm^2 sporulation at 15^{0}C temperature. Also, all the five isolates of *F. solani* showed the maximum mycelial growth ranged from 78.0 to 80.0 mm colony diameter and sporulation of 3.0 to 3.5 ×10^6/ mm^2 at 30^{0}C. The minimum growth ranged from 35.0 to 72.4 mm colony diameter and sporulation 2.2 to 2.5×10^6/ mm^2 were recorded at 15^{0}C temperature. All the isolates of *R. solani* showed the maximum mycelial growth (90.0 mm) at 30^{0}C and the highest sclerotial formation ranged from 0.5 to 0.8×10^6/ mm^2 at 35^{0}C, while the minimum growth ranged from 68.0 to 90.0 mm colony diameter at 15^{0}C temperature.

The relationship of pH to the mycelial growth and sporulation of *Fusarium* spp. and sclerotial formation of *R. solani.* was determined *in vitro* at different pH levels *viz.*, 4.0 to 8.0 at 28 ±1^{0}C. The present studies revealed that *Fusarium* spp. and *R. solani* grew at wide ranged of pH (6-8). The maximum growth (90.0 mm) and sporulation (3.0×10^6/ mm^2) with the isolate of *Foc* was observed at pH 7 while the minimum growth (78.0 mm) colony diameter and 1.8×10^6/ mm^2 sporulation at pH 4. The isolates of *F. solani* showed the maximum growth ranged from 80.0 to 85.0 mm and sporulation of 2.5 to 3.3×10^6/ mm^2 at pH 7. The minimum growth ranged from 30.0 to 73.2 mm colony growth and 1.2 to 1.5×10^6/ mm^2 at pH 4. The isolates of *R. solani* showed the maximum growth ranged from 81.0 to 90.0 mm colony diameter and sclerotial formation ranged of 0.4 to 0.6 ×10^6/ mm^2 at pH 7. The minimum growth 10.0 mm colony diameter and sporulation ranged of 0.1 to 0.3×10^6/ mm^2 at pH 4.

Field experiments were conducted for two consecutive years to estimate the yield losses caused by wilt and root rot complex of chickpea with disease generated through inoculation of the pathogens *F. solani, Foc* and *R. solani* individually and in combinations on chickpea variety 'Dahod Yellow' using the most virulent isolate of each pathogens. Results showed the disease severity increased with combined

inoculation of two and three pathogens as compared to that of the individual ones. A significant reduction in grain yields at all the severity levels was observed as compared to the uninoculated protected control. Pooled data revealed that among the individual inoculation, lowest germination (55.2 %) was due to *Foc*, among the inoculation of two pathogens in combination, plots inoculated with *F. solani* and *Foc* showed highest 53.4 % germination. The plots inoculated with all the three pathogens *F. solani*, *Foc* and *R. solani* showed only 42.0 % germination, which was significantly less over all the other inoculations as well as the uninoculated control. Similar trends were observed for plant mortality, the maximum mortality (45.5 %) was recorded with all the three pathogens *F. solani*, *Foc* and *R. solani*. Thus, the highest yield loss (25.26%) among individual pathogens was with *R. solani*, and highest yield losses (35.4 %) were recorded from plots inoculated with all the three pathogens together. These observations suggest that wilt and root rot complex of chickpea has good potential of damaging the crops and may become limiting factor in realization of good yield.

The results of current *in vitro* studies indicated that out of five fungicides tested, *viz*., Vitavex, Thiram, Trioxystrobin, Tebuconazole and Captan, *Foc* isolate BNS Foc-1 showed highly sensitive to Trifloxystrobin with the minimum growth (12.0, 10.0 and 10.0 mm) colony diameter and least sensitive to Captan with the maximum growth (40.0, 30.0 and 20.0 mm) colony diameter at all the three - 250, 500 and 1000 ppm concentrations. The different isolates of *F. solani* and *R. solani* showed variations in sensitivity to Vitavax, Thiram, Trifloxystrobin and Captan but all showed sensitivity to Tebuconazole.

The botanicals *viz*., Neem oil and Karanj oil at 0.2 % concentration were evaluated against the pathogens and the result showed Neem oil was quite effective in inhibiting the growth of the isolate of *Foc* with 28.0 mm colony diameter and showed less effective with Karanj oil with 60.0 mm colony diameter. Neem oil showed highly effective against isolates of *Fusarium* spp. with growth ranged of 22.0 to 90.0 mm colony diameter, while less effective with Karanj oil showed growth ranged of 56.0 to 77.0 mm colony diameter. Isolates of *R. solani* also showed varied sensitivity with growth ranged of 18.0 to 42.0 mm colony diameter, while less effective with Karanj oil showed growth ranged of 78.0 to 85.0 mm colony diameter.

The result of pot experiment conducted to evaluate the effect of fungicides and botanicals showed that the fungicide, Tebuconazole to be most effective with the maximum germination (72.0 %) and the minimum mortality (44.0 %) compared to other fungicides and botanicals.

To evaluate the efficiency of some fungal and bacterial biocontrol agents for suppression of the pathogen, *in vitro*, the efficacy of the isolates of *Trichoderma* spp., *P. fluorescens* and *B. subtilis* was evaluated by dual culture method. The *T. harzianum* isolate ICRISAT-25 was found to be highly effective against the pathogens *in vitro* followed by *P. fluorescens* UDP Pf-1 and *B. subtilis* Br II.

Screening was done taking ten chickpea cultivars with inoculations of all the three pathogens using soil inoculation technique of spore cum mycelial of *Foc, F. solani* and *R. solani.* The result showed Avrodhi to be highly resistant with the maximum seed germination (84.1 %) and the minimum mortality (21.5 %) to wilt and root rot complex of chickpea. Varieties GNG-469, RAJ-1581, P-1080 and Pratap Chana-1 were moderately resistant, while BG-1053, BGD-72, BG-391 and RSG-888 were moderately susceptible. The popular cultivar Dahod Yellow was highly susceptible with the minimum germination (61.0 %) and the maximum mortality (64.0 %). In our studies, these evaluations for resistance were done under inoculations of the three pathogens. In this screening no variety was immune or highly resistant. Limited success has been achieved in use of resistant varieties for soil borne pathogens, two were found promising in the present study.

The results of field experiment conducted to evaluate the effect of different sowing dates (15^{th} Oct. to 29^{th} Nov.) with 15 days interval on incidence of chickpea wilt and root rot complex showed that late sown on 29^{th} Nov. showed the highest germination (57.6 %), the lowest mortality (40.5 %) and the highest grain yield (0.90 kg/plot).

The fungicides, botanicals and biocontrol agents found effective *in vitro,* were further evaluated as seed treatment individually as well as in different combination for suppression of wilt and root-rot complex of chickpea in the field. It was found that combined treatments were superior in terms of better germination, lower mortality and higher yield as compared to the individual treatments. The most effective treatments was seed treatment with Tebuconazole + *T. harzianum* with the highest

79.0 % germination, the lowest mortality (13.0 %) and the highest 1.25 kg/plot grain yield as compared to control as well as other treatments.

It is noteworthy that in the present study the evaluation of the treatments was under rigorous inoculation of the three pathogens. The initial inoculum density of *Fusarium* spp. was 2.4×10^6 c.f.u / g soil and of *R. solani* was 1.8×10^6 c.f.u / g soil. After 90 DAS in untreated control, these reached to 6.9×10^6 c.f.u / g of *Fusarium* spp. and 7.0×10^6 c.f.u / g of *R. solani* respectively. But in various treatments, the population densities of the pathogens were considerably reduced. Under this inoculum load, significant disease suppression was recorded. In natural field conditions the soil borne pathogens are randomly distributed in soil/ rhizosphere. *T. harzianum* applied as seed treatment effectively established in chickpea rhizosphere and reached high population densities, at 90 DAS while the population of the pathogens was low in the rhizosphere with maximum suppression of 5.2×10^6 c.f.u / g of *Fusarium* spp. and 5.4×10^6 c.f.u / g of *R. solani* in plot with seed treatment of Tebuconazole + *T. harzianum.*

In summary, we concluded that management of Fusarium wilt of chickpea in sustainable cropping systems should be based on strategies that integrate several control measures and in that approach sowing date and host resistance are two key measures for a rational management of the disease. Chickpea genotypes should be adapted to winter sowings, have an appropriate level of disease resistance, and a high potential for economic return. Unfortunately, cultivars with high levels of Fusarium wilt resistance are not widely planted because of low economic profitability. Lack of a sufficient level of disease resistance can be compensated to some extent by the use of biocontrol treatments, such as those tested in this study, to maximize grain yield. By following this strategy, chickpeas would maintain their critical role in as a major source of protein and as a contributing factor in agriculture sustainability through improvement of soil fertility.

Table 1: Isolates of *Fusarium* spp. and *Rhizoctonia solani* recovered from samples collected from the fields in chickpea growing areas of Rajasthan

Sl. no.	Pathogens isolated	Place of collection	Isolated designation	Isolated code
1.	*Fusarium solani*	Udaipur	UDP	UDP Fs-1
	Fusarium solani	Bikaner	BKN	BKN Fs-2
	Fusarium solani	Tivari (Jodhpur)	TIB	TIB Fs-3
	Fusarium solani	Pali	PAL	PAL Fs-4
	Fusarium solani	Sirohi	SRH	SRH Fs-5
2.	*Fusarium oxysporum* f.sp. *ciceri*	Banswara	BNS	BNS Foc-1
3.	*Rhizoctonia solani*	Udaipur	UDP	UDP Rs-1
	Rhizoctonia solani	Bikaner	BKN	BKN Rs-2
	Rhizoctonia solani	Tivari (Jodhpur)	TIB	TIB Rs-3
	Rhizoctonia solani	Pali	PAL	PAL Rs-4
	Rhizoctonia solani	Sirohi	SRH	SRH Rs-5
	Rhizoctonia solani	Banswara	BNS	BNS Rs-6

Table 2: **Isolates of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* recovered from rhizosphere of samples collected from the fields in chickpea growing areas of Rajasthan**.

Sl. no.	Pathogens isolated	Place of collection	Isolated designation	Isolated code
1.	*Trichoderma viride*	Udaipur	UDP	UDP T-1
2.	*Pseudomonas fluorescens*	Udaipur	UDP	UDP Pf-1
3.	*Bacillus subtilis*	Tivari	TIB	Md

		(Jodhpur)		
4.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Br II
5.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Pv
6.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Tb-1
7.	*Bacillus subtilis*	Tivari (Jodhpur)	TIB	Ch

Table 3. Radial growth and cultural characters of the isolates of *Fusarium* spp. on PDA

Sl. no.	Pathogens isolated	Location of isolates	Isolates	Colony diameter (mm*)	Sporulation $\times 10^6$ conidia/ mm^2	Growth characters and colony colour
1.	*Fusarium solani*	Udaipur	UDP Fs-1	82.0	3.0	Aerial felty mycelial growth, zonations present, white regular margins but sometimes irregular
2.	*Fusarium solani*	Bikaner	BKN Fs-2	85.0	3.3	Velvety, cottony , suppressed growth, zonations absent, white irregular margins
3.	*Fusarium solani*	Tivari	TIB Fs-3	90.0	3.5	Cottony, suppressed, white, margins irregular sometimes with zonations
4.	*Fusarium solani*	Pali	PAL Fs-4	87.0	3.2	Cottony, aerial growth without zonations, margins regular, white in colour
5.	*Fusarium solani*	Sirohi	SRH Fs-5	90.0	3.5	White suppressed mycelium growth with zonations, white regular margins
6.	*Fusarium oxysporum* f.sp. *ciceri*	Banswara	BNS Foc-1	90.0	3.6	Velvety, cottony, aerial at the centre, suppressed growth, zonations at the centre, light violet in colour, irregular margins
	SEm ±			1.8	0.1	
	CD at 5%			5.5	0.2	
	C.V			3.6	3.7	

*Mean of three replications

Table 4. Radial growth and cultural characters of the six isolates of *Rhizoctonia solani* on PDA

Sl.	Locations	Isolates	Colony	Sclerotial	Growth characters and colony colour

no.			diameter (mm*)	formation × 10^6 sclerotia/ mm^2	
1.	Udaipur	UDP Rs-1	90.0	0.6	Velvety, suppressed at the centre, aerial growth at the margins, margins regular, dull to dark black
2.	Bikaner	BKN Rs-2	90.0	0.7	Cottony, aerial felty growth with regular margins, dull grey to black
3.	Tivari	TIB Rs-3	90.0	0.8	Dull grey to black, aerial felty growth with zonations , margins irregular
4.	Pali	PAL Rs-4	90.0	0.5	Cottony, suppressed dull grey to black, margins regular without zonations
5.	Sirohi	SRH Rs-5	90.0	0.5	Cottony, aerial at centre, suppressed near the margins with zonations and margins irregular , black colour
6.	Banswara	BNS Rs-6	90.0	0.8	Aerial, felty mycelial growth, zonations present, margins irregular, steel grey to black
	SEm ±		2.0	0.0	
	CD at 5%		6.0	0.1	
	C.V		3.8	3.9	

*Mean of three replications

Table 5. Variations in conidial morphology of the isolates of *Fusarium* spp. on PDA

Sl.	Isolates	Conidial morphology/ size of conidia (µm)

no.		Macro conidia				Micro conidia			
		Length (µm*)		Width (µm*)		Length (µm*)		Width (µm*)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	UDP Fs-1	08.8±0.40	7.8-9.8	02.5±0.11	2.2-2.8	05.1±0.26	4.5-5.7	01.0±0.05	0.8-1.1
2.	BKN Fs-2	09.5±0.50	8.4-10.6	02.4±0.12	2.1-2.6	04.5±0.20	4.0-5.0	01.5±0.06	1.3-1.6
3.	TIB Fs-3	09.2±0.47	8.1-10.3	02.2±0.11	1.9-2.4	04.6±0.24	4.0-5.1	02.2±0.11	1.9-2.4
4.	PAL Fs-4	09.5±0.49	8.4-10.6	02.0±0.10	1.7-2.2	03.5±0.18	3.1-3.9	01.8±0.09	1.6-2.0
5.	SRH Fs-5	10.5±0.48	9.3-11.7	02.7±0.37	2.6-3.8	03.2±0.16	2.8-3.5	01.2±0.06	1.0-1.3
6.	BNS Foc-1	09.0±0.47	8.0-10.0	01.6±0.08	1.4-1.7	05.5±0.25	4.8-6.1	01.7±0.07	1.5-1.9
	SEm±	0.0		0.0		0.0		0.0	
	CD at 5%	0.1		0.1		0.1		0.0	
	C.V	0.1		0.1		0.1		0.1	

*Mean no. of 50 conidia and ± S.D. of mean value

Table 6. Variations in sclerotial morphology of the six isolates of *Rhizoctonia solani* on PDA

Sl. no.	Locations	Isolates	Sclerotial morphology			
			Length (mm*)		Width (mm*)	
			Mean	Range	Mean*	Range

1.	Udaipur	UDP Rs-1	1.0±0.05	0.8-1.1	0.8±0.04	0.7-0.9
2.	Bikaner	BKN Rs-2	1.3±0.06	1.2-1.5	0.9±0.04	0.8-1.0
3.	Tivari	TIB Rs-3	1.8±0.09	1.6-2.0	1.3±0.19	0.1-1.5
4.	Pali	PAL Rs-4	1.6±0.08	1.4-1.8	1.0±0.05	0.9-1.1
5.	Sirohi	SRH Rs-5	1.5±0.07	1.3-1.7	0.7±0.03	0.6-0.8
6.	Banswara	BNS Rs-6	1.3±0.06	1.2-1.5	0.6±0.02	0.5-0.7
	SEm±		0.0		0.0	
	CD at 5%		0.0		0.0	
	C.V		0.1		0.2	

*Mean no. of 50 sclerotia and ± S.D. of mean value

Table 7. Effect of different temperatures on growth and sporulation of the isolates of *Fusarium* spp. on PDA

Sl. no.	Isolates	Temperatures (^{0}C)									
		15		20		25		30		35	
		Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2
1.	UDP Fs-1	53.0	2.5	75.7	3.0	77.0	3.3	80.0	3.5	72.4	2.8
2.	BKN Fs-2	35.0	2.3	70.0	2.8	72.0	3.0	78.0	3.3	40.0	2.5
3.	TIB Fs-3	72.4	2.2	65.0	2.5	80.0	2.9	78.0	3.0	75.0	2.3
4.	PAL Fs-4	68.0	2.2	76.0	2.6	78.0	3.0	80.0	3.2	76.0	2.5
5.	SRH Fs-5	70.0	2.5	70.0	2.9	76.0	3.2	78.0	3.5	75.0	2.6
6.	BNS Foc-1	62.0	2.8	88.2	3.0	90.0	3.5	90.0	3.6	80.0	2.8
	S.Em ±	1.3	0.1	1.5	0.1	1.7	0.1	1.7	0.1	1.4	0.1
	CD at 5%	4.0	0.2	4.6	0.2	5.3	0.2	5.3	0.2	4.4	0.2
	C.V	3.7	3.7	3.4	3.7	3.7	3.7	3.8	3.7	3.5	3.7

*Mean of three replications

Table 8. Effect of different temperatures on growth and sclerotial formation of *Rhizoctonia solani* isolates on PDA

Sl. no.	Isolates	Temperatures (^{0}C)									
		15		20		25		30		35	
		Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2
1.	UDP Rs-1	68.0	0.1	87.0	0.2	90.0	0.3	90.0	0.4	90.0	0.6
2.	BKN Rs-2	90.0	0.1	86.0	0.2	86.0	0.4	90.0	0.5	80.0	0.7
3.	TIB Rs-3	80.0	0.2	85.0	0.3	90.0	0.5	90.0	0.6	84.0	0.8
4.	PAL Rs-4	73.0	0.1	75.0	0.2	87.0	0.2	90.0	0.4	86.0	0.5
5.	SRH Rs-5	78.0	0.1	84.0	0.2	87.0	0.3	90.0	0.4	85.0	0.5
6.	BNS Rs-6	74.0	0.2	86.0	0.3	80.0	0.5	90.0	0.5	90.0	0.8
	S.Em ±	1.8	0.0	1.8	0.0	1.9	0.0	2.0	0.0	1.8	0.0
	CD at 5%	5.4	0.0	5.6	0.0	5.8	0.0	6.0	0.0	5.6	0.1
	C.V	3.9	3.9	3.9	3.8	3.8	4.1	3.8	4.0	3.7	4.0

*Mean of three replications

Table 9. Effect of different pH levels on growth and sporulation of the isolates of *Fusarium* spp. on PDA

Sl. no.	Isolates	pH ranges									
		4		5		6		7		8	
		Growth (mm*)	Sporulation $\times 10^6$ conidia/ mm^2	Growth (mm*)	Sporulation $\times 10^6$ conidia/ mm^2	Growth (mm*)	Sporulation $\times 10^6$ conidia/ mm^2	Growth (mm*)	Sporulation $\times 10^6$ conidia/ mm^2	Growth (mm*)	Sporulation $\times 10^6$ conidia/ mm^2
1.	UDP Fs-1	65.0	1.5	76.0	2.5	79.5	3.1	80.0	3.2	68.0	3.0
2.	BKN Fs-2	34.0	1.3	78.0	2.3	80.0	2.0	88.0	2.5	70.0	2.1
3.	TIB Fs-3	30.0	1.2	77.8	2.0	76.0	2.8	80.0	3.0	65.0	2.4
4.	PAL Fs-4	68.0	1.2	77.0	2.0	78.0	2.8	80.0	3.0	55.0	2.4
5.	SRH Fs-5	73.2	1.5	79.0	2.2	83.5	3.0	85.0	3.3	60.0	2.6
6.	BNS Foc-1	78.0	1.8	85.0	2.4	88.0	2.3	90.0	3.0	70.0	2.8
	S.Em ±	1.2	0.0	1.8	0.1	1.6	0.1	1.8	0.1	1.4	0.1
	CD at 5%	3.6	0.1	5.4	0.2	4.8	0.2	5.5	0.2	4.4	0.2
	C.V	3.5	3.7	3.7	3.7	4.0	3.7	3.8	3.7	4.0	3.6

*Mean of three replications

Table 10. Effect of different pH levels on growth and sporulation of the six isolates of *Rhizoctonia solani* on PDA

Sl. no.	Isolates	pH ranges									
		4		5		6		7		8	
		Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation × 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2
1.	UDP Rs-1	10.0	0.2	12.0	0.3	40.0	0.4	88.5	0.4	30.0	0.2
2.	BKN Rs-2	10.0	0.3	10.0	0.3	45.0	0.5	81.0	0.5	20.0	0.2
3.	TIB Rs-3	10.0	0.3	10.0	0.5	38.0	0.6	84.0	0.6	28.0	0.2
4.	PAL Rs-4	10.0	0.1	10.0	0.2	45.0	0.3	86.0	0.4	20.0	0.1
5.	SRH Rs-5	10.0	0.1	12.0	0.2	40.0	0.3	90.0	0.4	38.0	0.1
6.	BNS Rs-6	10.0	0.3	11.0	0.4	40.0	0.3	86.0	0.5	25.0	0.2
	S.Em ±	0.1	0.0	0.6	0.0	0.9	0.0	1.9	0.0	0.5	0.0
	CD at 5%	0.4	0.0	1.9	0.0	2.8	0.0	5.7	0.0	1.7	0.0
	C.V	3.8	4.3	5.0	4.1	3.8	4.3	3.7	4.0	4.2	4.0

*Mean of three replications

Table 11. Yield losses in chickpea cultivar 'Dahod Yellow' due to *Fusarium solani, Fusarium oxysporum* f. sp. *ciceri*. and *Rhizoctonia solani* individually and in combinations during *rabi* (2012-13) and (2013-14)

Sl. no.	Treatments	Seed germination (%)			Plant mortality (%)			Grain yield kg/plot			Yield losses (%)		
		2012-13	2013-14	Pooled	2012-13	2013-14	Pooled	2012-13	2013-14	Pooled	2012-13	2013-14	Pooled
1.	*F. solani* isolate (SRH Fs-5)	64.5 (53.4)	57.8 (49.4)	61.1 (51.4)	28.0 (31.9)	30.0 (33.2)	29.0 (32.5)	0.83	0.80	0.82	18.8 (25.7)	19.6 (25.8)	19.2 (25.7)
2.	*Foc* isolate (BNS Foc-1)	58.0 (49.6)	52.4 (46.3)	55.2 (47.9)	30.0 (33.2)	32.0 (34.4)	31.0 (33.8)	0.79	0.73	0.76	24.4 (29.5)	23.7 (29.1)	24.7 (29.7)
3.	*R. solani* isolate (BNS Rs-6)	60.8 (51.2)	52.0 (46.1)	56.4 (48.7)	32.5 (34.7)	34.0 (35.6)	33.2 (35.2)	0.76	0.75	0.75	25.7 (30.4)	26.9 (31.2)	25.6 (30.4)
4.	*F. solani* (SRH Fs-5) + *Foc* (BNS Foc-1)	56.8 (48.9)	50.0 (45.0)	53.4 (46.9)	34.0 (35.6)	37.3 (37.6)	35.6 (36.6)	0.73	0.71	0.72	27.9 (31.9)	29.7 (33.0)	28.8 (32.4)
5.	*F. solani* (SRH Fs-5) + *R. solani* (BNS Rs-6)	54.9 (47.8)	48.0 (43.8)	51.4 (45.8)	38.0 (38.0)	40.0 (39.2)	39.0 (38.6)	0.70	0.68	0.69	30.4 (32.9)	32.2 (34.3)	31.3 (33.6)
6.	*R. solani* (BNS Rs-6) + *Foc*(BNS Foc-1)	50.0 (45.0)	46.0 (42.7)	48.0 (43.8)	40.0 (39.2)	43.0 (40.9)	41.5 (40.1)	0.69	0.67	0.68	32.7 (34.9)	33.5 (35.)	33.1 (35.1)
7.	*F. solani* (SRH Fs-5) + *Foc* (BNS Foc-1) + *R. solani* (BNS Rs-6)	44.0 (51.5)	40.0 (39.2)	42.0 (40.3)	44.0 (41.5)	47.0 (43.2)	45.5 (42.4)	0.66	0.64	0.65	34.9 (36.2)	35.8 (36.7)	35.4 (36.5)
8.	Control (Uninoculated)	74.0 (49.3)	70.0 (56.8)	72.0 (58.0)	11.5 (19.8)	14.0 (21.9)	12.7 (20.9)	1.03	0.99	1.01	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.Em ±	0.8	0.7	0.6	0.5	0.5	0.4	0.1	0.1	0.0	2.4	3.5	2.5
	CD at 5%	2.5	2.2	1.6	1.6	1.5	1.0	0.1	0.1	0.1	7.1	10.6	7.1
	C.V	2.8	2.7	2.8	2.7	2.3	2.5	8.2	8.1	8.1	23.8	24.8	24.3

Mean of three replications

Figures in parentheses are arcsine √ per cent angular transformed values.

Table 12. Effect of fungicides on growth (colony diameter) of the isolates of *Fusarium* spp. at different concentrations

Sl. no.	Isolates	Colony diameter (mm*)															
		Vitavax			Thiram			Trifloxystrobin			Tebuconazole			Captan			Control (Untreated)
	Conc. (ppm)	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000	
1.	UDP Fs-1	23.0	20.0	05.0	58.0	45.0	30.0	65.0	00.0	00.0	20.0	15.0	15.0	75.0	45.0	25.0	90.0
2.	BKN Fs-2	75.6	35.0	12.0	55.0	48.0	33.0	66.0	55.0	65.0	22.0	10.0	12.0	65.0	58.0	45.0	90.0
3.	TIB Fs-3	72.0	50.0	25.0	25.0	20.0	20.0	63.0	62.0	60.0	28.0	20.0	10.0	68.0	43.0	28.0	90.0
4.	PAL Fs- 4	25.0	20.0	05.0	21.0	17.0	12.0	45.0	35.0	30.0	25.0	21.0	05.0	33.0	33.0	15.0	90.0
5.	SRH Fs-5	22.0	18.0	05.0	62.0	47.0	45.0	25.0	18.0	08.0	23.0	18.0	15.0	65.0	50.0	60.0	90.0
6.	BNS Foc-1	15.0	10.0	10.0	55.0	10.0	10.0	12.0	10.0	00.0	30.0	22.0	10.0	40.0	30.0	20.0	90.0
		SEm ±		CD 5 %	SEm ±		CD at 5 %	SEm ±		CD at 5 %	SEm ±		CD at 5 %	SEm ±		CD at 5 %	

Isolate	0.4	0.5	0.3	1.3	1.5	0.6	0.5	0.4	0.2	1.4	1.2	0.9	0.7	0.8	0.0	2.0	2.2	0.0	0.6	0.8	0.5	1.5	1.8	1.3	0.8	0.6	0.3	2.2	1.8	2.0	-
Concentration	0.3	0.4	0.0	0.8	1.0	0.1	0.3	0.2	0.0	0.9	0.7	0.3	0.5	0.6	0.7	1.3	1.5	0.8	0.4	0.6	0.4	1.0	1.5	0.8	0.5	0.4	0.5	1.5	1.0	1.1	-
I × C	0.8	1.0	0.3	2.2	2.5	0.7	0.8	0.6	0.3	2.3	0.7	2.0	1.2	0.3	1.5	3.4	3.8	0.9	0.9	1.2	0.8	2.7	3.3	2.1	1.4	1.1	1.3	3.8	2.9	2.1	-

* Mean of three replications

Table 13. Effect of fungicides on growth (colony diameter) of the isolates of *Rhizoctonia solani* at different concentrations

Sl. no.	Isolates	Colony diameter (mm*)															
		Vitavax			Thiram			Trifloxystrobin			Tebuconazole			Captan			
	Conc. (ppm)	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000	Control (Untreated)
1.	UDP Rs-1	68.0	50.0	00.0	52.0	48.0	10.0	78.0	70.0	70.0	00.0	00.0	00.0	88.0	85.0	80.0	90.0
2.	BKN Rs-2	60.0	58.0	00.0	42.0	35.0	10.0	80.0	78.0	75.0	00.0	00.0	00.0	86.0	80.0	79.0	90.0
3.	TIB Rs-3	00.0	00.0	00.0	43.0	38.0	12.0	80.0	88.0	80.0	00.0	00.0	00.0	90.0	75.0	80.0	90.0
4.	PAL Rs 4	40.0	46.0	10.0	23.0	35.0	15.0	85.0	85.0	75.0	00.0	00.0	00.0	90.0	38.0	35.0	90.0

5.	SRH Rs-5	58.0	43.0	00.0	40.0	36.0	15.0	84.0	76.0	70.0	00.0	00.0	00.0	90.0	42.0	30.0	90.0
6.	BNS Rs-6	74.0	52.0	00.0	53.0	33.0	10.0	86.0	85.0	65.0	00.0	00.0	00.0	50.0	30.0	30.0	90.0

	SEm ±			CD 5 %			SEm ±			CD at 5 %			SEm ±			CD at 5 %			SEm ±			CD at 5 %			SEm ±			CD at 5 %			
Isolate	0.5	0.4	0.0	1.5	1.2	0.0	0.4	0.5	0.3	1.2	1.1	0.4	0.9	0.8	0.8	2.7	2.6	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.9	0.8	1.9	2.8	2.0	-
Concentration	0.4	0.3	0.0	1.0	0.9	0.0	0.3	0.4	0.3	0.8	0.6	0.5	0.6	0.6	0.5	1.7	1.9	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.7	0.6	1.2	2.0	2.0	-
I × C	0. 9	0.7	0.2	2.6	2.2	0.2	0.8	0.7	0.6	2.1	1.8	1.1	1.6	1.4	1.2	4.6	3.8	2.9	0.0	0.0	0.0	0.0	0.0	0.0	1.1	1.7	1.6	3.2	4.9	4.7	-

* Mean of three replications

Table 14. Effect of botanicals on growth of different isolates of *Fusarium* spp. at 0.2% concentration *in vitro*

Sl. no.	Location	Isolate	Treatments		
			Colony diameter (mm*)		
			Neem oil	Karanj oil	Control
1.	Udaipur	UDP Fs-1	43.0	77.0	90.0
2.	Bikaner	BKN Fs-2	60.0	56.0	90.0
3.	Tivari	TIB Fs-3	90.0	63.0	90.0
4.	Pali	PAL Fs-4	22.0	60.0	90.0
5.	Sirohi	SRH Fs-5	22.0	70.0	90.0
6.	Banswara	BNS Foc-1	28.0	60.0	90.0

	SEm ±	CD at 5%	C.V	SEm ±	CD at 5%	C.V	-
Isolate	0.9	2.5	3.3	0.9	2.7	3.6	-
Botanicals	0.5	1.3	1.8	0.5	1.4	1.9	-
I × B	1.2	3.5	4.6	1.3	3.8	5.1	-

* Mean of three replications

Table 15. Effect of botanicals on growth of different isolates of *Rhizoctonia solani* at 0.2% concentration *in vitro*

Sl. no.	Location	Isolate	Treatments		
			Colony diameter (mm*)		
			Neem oil	Karanj oil	Control
1.	Udaipur	UDP Rs-1	18.0	78.0	90.0
2.	Bikaner	BKN Rs-2	22.0	83.0	90.0
3.	Tivari	TIB Rs-3	26.0	83.0	90.0
4.	Pali	PAL Rs-4	42.0	78.0	90.0
5.	Sirohi	SRH Rs-5	33.0	80.0	90.0
6.	Banswara	BNS Rs-6	36.0	85.0	90.0

	SEm ±	CD at 5%	C.V	SEm ±	CD at 5%	C.V	
Isolate	0.9	2.6	3.5	1.0	2.9	3.8	-
Botanicals	0.5	1.4	1.9	0.5	1.6	2.1	-
I × B	1.3	3.7	5.0	1.4	4.1	5.4	-

* Mean of three replications

Table 16. Evaluation of fungicides and botanicals in pot-culture

Sl. no.	Treatments	Seed germination* (%)	Plant mortality* (%)
1.	Thiram 75 % WP @ 0.2 % seed treatment (ST)	69.0 (56.2)	48.0 (44.7)
2.	Captan 50 WP @ 0.2 % ST	66.0 (54.4)	51.0 (46.6)
3.	Vitavax 75 % WP @ 0.2 % ST	71.0 (57.5)	46.0 (42.3)
4.	Trifloxystrobin 23 % SC @ 0.2 % ST	65.0 (53.2)	53.0 (46.7)
5.	Tebuconazole 25.9 W/W @ 0.2 % ST	72.0 (58.1)	44.0 (42.7)
6.	Neem oil @ 0.2 % ST	62.0 (52.0)	56.0 (48.5)
7.	Karanj oil @ 0.2 % ST	60.0 (50.8)	58.0 (49.0)
8.	Control (Untreated)	52.0 (46.2)	70.0 (56.8)
	SEm±	0.9	0.7
	CD at 5%	2.6	2.0
	C.V	2.8	2.7

*Mean of three replications and two runs of the experiment.
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 17. Effect of fungal biocontrol agents on mycelial growth of *Fusarium* spp. on PDA

Sl. no.	Isolates	Fungal biocontrol agents	Isolate code	Mycelial growth (mm*)		Per cent Growth inhibition*
				Control	Dual culture	
1.	UDP Fs-1	*T. harzianum*	ICRISAT-28	90.0	26.0	71.1 (57.5)
		T. harzianum	ICRISAT-25	90.0	32.0	64.5 (53.4)
		T. viride	UDP T-1	90.0	40.0	55.6 (48.2)
		T. viride	ICRISAT-15	90.0	30.0	66.7 (54.8)
		T. viride	ICAR-95	90.0	45.0	50.0 (45.0)
		T. harzianum	Jh-2	90.0	20.0	77.8 (61.9)
2.	BKN Fs-2	*T. harzianum*	ICRISAT-28	90.0	00.0	100 (90.0)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	05.0	99.5 (76.4)
		T. viride	ICRISAT-15	90.0	10.0	88.9 (70.5)
		T. viride	ICAR-95	90.0	12.0	86.7 (68.6)
		T. harzianum	Jh-2	90.0	00.0	100 (90.0)
3.	TIB Fs-3	*T. harzianum*	ICRISAT-28	90.0	00.0	100 (90.0)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	00.0	100 (90.0)
		T. viride	ICRISAT-15	90.0	05.0	99.5 (76.4)
		T. viride	ICAR-95	90.0	00.0	100 (90.0)
		T. harzianum	Jh-2	90.0	00.0	100 (90.0)
4.	PAL Fs-4	*T. harzianum*	ICRISAT-28	90.0	00.0	100 (90.0)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	00.0	100 (90.0)
		T. viride	ICRISAT-15	90.0	00.0	100 (90.0)
		T. viride	ICAR-95	90.0	00.0	100 (90.0)
		T. harzianum	Jh-2	90.0	00.0	100 (90.0)
5.	SRH Fs-5	*T. harzianum*	ICRISAT-28	90.0	05.0	99.5 (76.4)
		T. harzianum	ICRISAT-25	90.0	05.0	99.5 (76.4)
		T. viride	UDP T-1	90.0	10.0	88.9 (70.5)
		T. viride	ICRISAT-15	90.0	05.0	99.5 (76.4)
		T. viride	ICAR-95	90.0	05.0	99.5 (65.9)
		T. harzianum	Jh-2	90.0	15.0	83.3 (29.6)

6.	BNS Foc-1	*T. harzianum*	ICRISAT-28	90.0	68.0	24.5 (70.5)
		T. harzianum	ICRISAT-25	90.0	10.0	88.9 (26.5)
		T. viride	UDP T-1	90.0	72.0	20.0 (76.4)
		T. viride	ICRISAT-15	90.0	05.0	99.5 (90.0)
		T. viride	ICAR-95	90.0	00.0	100 (90.0)
		T. harzianum	Jh-2	90.0	05.0	99.5 (76.4)
	SEm±			-	0.6	0.4
	CD at 5 %			-	1.6	1.2
	C.V			-	6.7	1.0

*Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 18. Effect of fungal biocontrol agents on mycelial growth of *Rhizoctonia solani* on PDA

Sl. no.	Isolates	Fungal biocontrol agents	Isolate code	Mycelial growth (mm*)		Per cent Growth inhibition*
				Control	Dual culture	
1.	UDP Rs-1	*T. harzianum*	ICRISAT-28	90.0	15.0	83.3 (65.9)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	38.0	57.8 (49.5)
		T. viride	ICRISAT-15	90.0	45.0	50.0 (45.0)
		T. viride	ICAR-95	90.0	20.0	77.8 (61.9)
		T. harzianum	Jh-2	90.0	44.0	51.1 (45.7)
2.	BKN Rs-2	*T. harzianum*	ICRISAT-28	90.0	45.0	50.0 (45.0)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	00.0	100 (90.0)
		T. viride	ICRISAT-15	90.0	23.0	74.5 (59.7)
		T. viride	ICAR-95	90.0	00.0	100 (90.0)
		T. harzianum	Jh-2	90.0	40.0	55.6 (48.2)
3.	TIB Rs-3	*T. harzianum*	ICRISAT-28	90.0	20.0	77.8 (61.9)
		T. harzianum	ICRISAT-25	90.0	18.0	80.0 (63.5)
		T. viride	UDP T-1	90.0	30.0	66.7 (54.8)

		T. viride	ICRISAT-15	90.0	10.0	88.9 (70.5)
		T. viride	ICAR-95	90.0	37.0	58.9 (50.1)
		T. harzianum	Jh-2	90.0	00.0	100 (90.0)
4.	PAL Rs-4	*T. harzianum*	ICRISAT-28	90.0	00.0	100 (90.0)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	00.0	100 (90.0)
		T. viride	ICRISAT-15	90.0	15.0	88.3 (65.9)
		T. viride	ICAR-95	90.0	00.0	100 (90.0)
		T. harzianum	Jh-2	90.0	00.0	100 (90.0)
5.	SRH Rs-5	*T. harzianum*	ICRISAT-28	90.0	25.0	72.2 58.2)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	50.0	44.5 (41.8)
		T. viride	ICRISAT-15	90.0	30.0	66.7 (54.8)
		T. viride	ICAR-95	90.0	10.0	88.9 (70.6)
		T. harzianum	Jh-2	90.0	10.0	88.9 (70.6)
6.	BNS Rs-6	*T. harzianum*	ICRISAT-28	90.0	40.0	55.6 (48.2)
		T. harzianum	ICRISAT-25	90.0	00.0	100 (90.0)
		T. viride	UDP T-1	90.0	00.0	100 (90.0)
		T. viride	ICRISAT-15	90.0	55.0	38.9 (38.6)
		T. viride	ICAR-95	90.0	44.0	51.1 (45.7)
		T. harzianum	Jh-2	90.0	35.0	61.1 (51.4)
	SEm±			-	0.7	0.5
	CD at 5 %			-	1.9	1.3
	C.V			-	5.4	1.2

* Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 19. Effect of bacterial antagonists on mycelial growth of *Fusarium* spp. on King's B medium

Sl. no.	Isolates	Bacterial antagonists	Isolated code	Mycelial growth (mm*)		Per cent Growth inhibition*
				Control	Dual culture	
		P. fluorescens	UDP Pf-1	90.0	45.0	50.0 (45.0)
		B. subtilis	Md	90.0	43.0	52.2 (46.3)

1.	UDP Fs-1	*B. subtilis*	Br II	90.0	13.0	85.6 (67.7)
		B. subtilis	Pv	90.0	70.0	22.2 (28.1)
		B. subtilis	Tb-1	90.0	40.0	55.6 (48.2)
		B. subtilis	Ch	90.0	48.0	46.7 (43.2)
2.	BKN Fs-2	*P. fluorescens*	UDP Pf-1	90.0	46.0	48.9 (44.4)
		B. subtilis	Md	90.0	48.0	46.7 (43.1)
		B. subtilis	Br II	90.0	44.0	51.1 (45.7)
		B. subtilis	Pv	90.0	50.0	44.5 (41.8)
		B. subtilis	Tb-1	90.0	24.0	73.3 (58.9)
		B. subtilis	Ch	90.0	26.0	71.1 (57.5)
3.	TIB Fs-3	*P. fluorescens*	UDP Pf-1	90.0	05.0	99.5 (76.4)
		B. subtilis	Md	90.0	50.0	44.5 (41.8)
		B. subtilis	Br II	90.0	39.0	56.7 (48.8)
		B. subtilis	Pv	90.0	26.0	71.1 (57.5)
		B. subtilis	Tb-1	90.0	00.0	100 (90.0)
		B. subtilis	Ch	90.0	28.0	68.9 (56.1)
4.	PAL Fs-4	*P. fluorescens*	UDP Pf-1	90.0	55.0	38.9 (38.6)
		B. subtilis	Md	90.0	42.0	53.3 (46.9)
		B. subtilis	Br II	90.0	40.0	55.6 (48.2)
		B. subtilis	Pv	90.0	24.0	73.3 (58.9)
		B. subtilis	Tb-1	90.0	19.0	78.9 (62.7)
		B. subtilis	Ch	90.0	53.0	41.1 (39.9)
5.	SRH Fs-5	*P. fluorescens*	UDP Pf-1	90.0	35.0	61.1 (51.4)
		B. subtilis	Md	90.0	40.0	55.6 (48.2)
		B. subtilis	Br II	90.0	44.0	51.1 (45.7)
		B. subtilis	Pv	90.0	40.0	55.6 (48.2)
		B. subtilis	Tb-1	90.0	30.0	66.7 (54.8)
		B. subtilis	Ch	90.0	36.0	60.0 (50.8)
6.	BNS Foc-1	*P. fluorescens*	UDP Pf-1	90.0	30.0	66.7 (54.8)
		B. subtilis	Md	90.0	30.0	66.7 (54.8)
		B. subtilis	Br II	90.0	32.0	64.5 (53.4)
		B. subtilis	Pv	90.0	44.0	51.1 (45.7)
		B. subtilis	Tb-1	90.0	56.0	37.8 (37.9)
		B. subtilis	Ch	90.0	33.0	41.1 (52.7)

SEm±	-	1.0	0.6
CD at 5 %	-	2.7	1.8
C.V	-	4.4	2.2

* Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 20. Effect of bacterial antagonists on mycelial growth of *Rhizoctonia solani* on King's B medium

Sl. no.	Isolates	Bacterial antagonists	Isolated code	Mycelial growth (mm*)		Per cent Growth inhibition*
				Control	Dual culture	
1.	UDP Rs-1	*P. fluorescens*	UDP Pf-1	90.0	42.0	53.3 (46.9)
		B. subtilis	Md	90.0	53.0	41.1 (39.9)
		B. subtilis	Br II	90.0	45.0	50.0 (45.0)
		B. subtilis	Pv	90.0	50.0	44.5 (41.8)
		B. subtilis	Tb-1	90.0	50.0	44.5 (41.8)
		B. subtilis	Ch	90.0	48.0	46.7 (43.2)
2.	BKN Rs-2	*P. fluorescens*	UDP Pf-1	90.0	50.0	44.5 (41.8)
		B. subtilis	Md	90.0	40.0	55.6 (48.2)
		B. subtilis	Br II	90.0	55.0	38.9 (38.6)
		B. subtilis	Pv	90.0	53.0	41.1 (39.9)
		B. subtilis	Tb-1	90.0	60.0	33.3 (35.3)
		B. subtilis	Ch	90.0	50.0	44.5 (41.8)
3.	TIB Rs-3	*P. fluorescens*	UDP Pf-1	90.0	47.0	47.8 (43.7)
		B. subtilis	Md	90.0	42.0	53.3 (46.9)
		B. subtilis	Br II	90.0	49.0	45.6 (42.5)
		B. subtilis	Pv	90.0	50.0	44.5 (41.8)
		B. subtilis	Tb-1	90.0	50.0	44.5 (41.8)
		B. subtilis	Ch	90.0	50.0	44.5 (41.8)
4.	PAL Rs-4	*P. fluorescens*	UDP Pf-1	90.0	00.0	100 (90.0)
		B. subtilis	Md	90.0	00.0	100 (90.0)
		B. subtilis	Br II	90.0	00.0	100 (90.0)
		B. subtilis	Pv	90.0	10.0	88.9 (70.5)
		B. subtilis	Tb-1	90.0	12.0	86.7 (68.6)

		B. subtilis	Ch	90.0	00.0	100 (90.0)
5.	SRH Rs-5	*P. fluorescens*	UDP Pf-1	90.0	22.0	75.6 (60.4)
		B. subtilis	Md	90.0	36.0	60.0 (50.8)
		B. subtilis	Br II	90.0	45.0	50.0 (45.0)
		B. subtilis	Pv	90.0	40.0	55.6 (48.2)
		B. subtilis	Tb-1	90.0	55.0	38.9 (38.5)
		B. subtilis	Ch	90.0	50.0	44.5 (41.8)
6.	BNS Rs-6	*P. fluorescens*	UDP Pf-1	90.0	50.0	44.5 (41.8)
		B. subtilis	Md	90.0	55.0	38.9 (38.6)
		B. subtilis	Br II	90.0	50.0	44.5 (41.8)
		B. subtilis	Pv	90.0	53.0	41.1 (39.9)
		B. subtilis	Tb-1	90.0	22.0	75.6 (60.4)
		B. subtilis	Ch	90.0	47.0	47.8 (43.7)
	SEm±			-	1.5	1.0
	CD at 5 %			-	4.1	2.7
	C.V			-	6.1	3.4

* Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 21. Evaluation of fungal and bacterial biocontrol agents for suppression of wilt and root rot complex of chickpea in pot culture

Sl. no.	Treatments	Initial population of BCA's on seed*	Seed germination (%)	Plant mortality (%)	Population at 90 DAS*		
					BCA's	*Rhizoctonia solani* $\times 10^6$/ g soil	*Fusarium* spp. $\times 10^6$/ g soil
1.	*T. harzianum* (ICRISAT-25) @ 20g/kg seed (ST)	8.0×10^5/seed	78.0 (62.1)	35.0 (44.4)	6.2×10^5/g soil	4.4	4.5
2.	*T. viride* (ICAR-95) @ 20g/kg seed (ST)	7.9×10^5/seed	75.0 (60.1)	40.0 (45.0)	6.0×10^5/g soil	4.5	4.7
3.	*P. fluorescens* (UDP Pf-1) @ 20g/kg seed (ST)	8.5×10^8/seed	73.0 (58.7)	53.0 (46.7)	6.7×10^8/g soil	4.7	4.9
4.	*B. subtilis* (Br II) @ 20g/kg seed (ST)	8.3×10^8/seed	70.0 (56.8)	55.0 (47.9)	6.5×10^8/g soil	4.8	5.0
5.	Control (Untreated)	-	55.0 (47.9)	80.0 (57.5)	-	8.0	8.3
	SEm ±		0.4	1.7		1.0	0.3
0.0		0.0					
	CD at 5%		0.9	5.4		3.2	0.8
0.2		0.2					
	C.V		5.4	7.7		4.5	1.0
3.0		3.2					

* Mean of three replications Initial population of *Fusarium* spp. in soil 2.4×10^6 c.f.u /g soil.

Figures in parentheses are arcsine √ per cent angular transformed values. Initial population of *Rhizoctonia solani* in soil 1.8×10^6 c.f.u /g soil.

Table 22. Evaluation of popular chickpea cultivars for resistance to wilt and root rot pathogens in pot-culture during *rabi* 2012-13 and 2013-14

Sl. no.	Varieties	Seed germination* (%)			Plant mortality* (%)		
		2012-13	2013-14	Pooled	2012-13	2013-14	Pooled
1.	Dahod Yellow	62.0 (51.9)	60.0 (50.8)	61.0 (51.4)	63.0 (52.6)	65.0 (57.8)	64.0 (55.2)
2.	Pratap Chana-1	74.0 (59.4)	73.0 (58.7)	73.5 (59.1)	43.0 (41.0)	46.0 (52.4)	44.5 (46.7)
3.	Avrodhi	85.2 (67.5)	83.0 (65.8)	84.1 (66.6)	21.0 (27.3)	22.0 (52.0)	21.5 (39.7)
4.	RSG-888	64.6 (53.5)	61.0 (51.4)	62.8 (52.5)	59.0 (50.2)	61.0 (50.0)	60.0 (50.1)
5.	RAJ-1581	79.0 (62.9)	77.0 (61.4)	78.0 (62.2)	34.0 (35.7)	36.0 (48.0)	35.0 (41.8)
6.	BGD-72	71.0 (57.4)	69.0 (56.2)	70.0 (56.8)	52.0 (46.2)	57.0 (40.0)	54.5 (46.2)
7.	BG-391	70.0 (56.8)	67.0 (55.0)	68.5 (55.9)	55.5 (48.2)	58.0 (52.4)	56.8 (44.1)
8.	BG-1053	73.0 (58.7)	72.0 (58.1)	72.5 (58.4)	47.0 (43.3)	50.0 (52.0)	48.5 (47.9)
9.	GNG-469	83.0 (65.7)	82.0 (65.0)	82.5 (65.3)	27.0 (31.3)	30.0 (52.0)	28.5 (41.7)
10.	P-1080	76.2 (60.9)	75.0 (60.0)	75.6 (60.5)	39.0 (38.7)	42.5 (70.0)	40.8 (54.3)
	SEm ±	1.5	1.4	1.0	0.7	1.3	0.7
	CD at 5 %	4.3	4.2	2.9			

1.9		3.9	2.1			
	C.V			3.4	3.4	4.2
2.2		3.5	3.9			

* Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 23. Effect of different dates of sowing on incidence of chickpea wilt and root rot complex in the field during *rabi* (2012-13) and (2013-14)

Sl. no.	Treatments	Seed germination * (%)			Plant mortality* (%)			
		2012-13	2013-14	Pooled	2012-13	2013-14	Pooled	2012
1.	15th Oct.	41.0 (39.8)	38.4 (38.3)	39.7 (39.1)	50.3 (45.2)	54.2 (47.4)	52.3 (46.3)	0.0
2.	30th Oct.	48.2 (44.0)	43.5 (41.3)	45.9 (42.6)	46.4 (43.0)	49.3 (44.6)	47.9 (43.8)	0.7
3.	14th Nov.	54.8 (47.8)	48.1 (43.9)	51.5 (45.8)	42.7 (40.8)	46.4 (43.0)	44.6 (41.9)	0.8
4.	29th Nov.	62.3 (52.1)	52.8 (46.6)	57.6 (49.4)	38.3 (39.5)	42.7 (40.8)	40.5 (39.5)	0.9
	S.Em ±	0.8	0.9	0.5	0.7	1.0	0.6	0.
	CD at 5%	2.7	3.1	1.8	2.3	3.5	1.9	0.
	C.V	2.9	3.6	3.3	2.7	4.0	3.4	7.4

* Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values.

Table 24. Effect of different seed treatments on seed germination, plant mortality, grain yield and yield increase in chickpea cultivar 'Dahod Yellow' in the field

Sl. no.	Treatments	Seed germination* (%)			Plant mortality* (%)			Grain yield* kg/plot		
		2012-13	2013-14	Pooled	2012-13	2013-14	Pooled	2012-13	2013-14	Poo
1.	Tebuconazole 25.9 W/W @ 0.2 % ST (Seed treatment)	70.0 (56.8)	69.3 (56.4)	69.6 (56.6)	21.0 (27.3)	23.0 (57.8)	22.0 (42.6)	0.98	0.95	0
2.	Vitavax 75 % WP @ 0.2 % ST	68.0 (55.6)	66.0 (54.4)	67.0 (55.0)	23.0 (28.7)	25.0 (52.4)	24.0 (40.5)	0.95	0.92	0
3.	Neem oil @ 2 % ST	66.1 (54.4)	64.5 (53.4)	65.3 (53.9)	25.3 (28.0)	27.0 (52.0)	26.0 (39.9)	0.90	0.89	0
4.	*T. harzianum* @ 20 g/kg seed	72.0 (58.1)	70.0 (57.5)	71.0 (57.5)	17.5 (51.4)	20.0 (50.0)	18.5 (50.7)	1.00	0.96	0
5.	Tebuconazole 25.9 W/W @ 0.2 % ST + Neem oil @ 2 % ST	74.6 (59.8)	71.0 (56.8)	72.8 (58.6)	20.0 (36.9)	22.0 (48.0)	21.0 (42.4)	0.97	0.93	0
6.	Vitavax 75 % WP @ 0.2 % ST + Neem oil @ 2 % ST	76.0 (60.7)	73.0 (57.5)	74.5 (59.7)	16.0 (49.0)	18.0 (46.0)	17.0 (47.5)	1.12	0.98	1
7.	Tebuconazole 25.9 W/W @ 0.2 % ST+ *T. harzianum* @ 20 g/kg seed	80.0 (63.5)	78.0 (58.7)	79.0 (62.8)	12.0 (49.6)	14.0 (40.0)	13.0 (44.8)	1.30	1.20	1
8.	Vitavax 75 % WP @ 0.2 % ST + *T. harzianum* @ 20 g/kg seed	79.2 (62.9)	76.0 (62.1)	77.6 (61.8)	14.0 (45.0)	16.0 (52.4)	15.0 (48.7)	1.25	1.10	1
9.	Neem oil @ 2 % ST+ *T. harzianum* @ 20 g/kg seed	77.5 (61.7)	75.0 (60.0)	76.3 (60.9)	15.2 (23.0)	17.0 (52.0)	16.1 (37.5)	1.20	1.00	1
10	Control (Inoculated)	52.0 (46.2)	50.0 (45.0)	51.0 (45.6)	46.0 (42.7)	49.0 (70.0)	47.5 (56.4)	0.65	0.64	0
	SEm±	1.1	1.3	0.9	0.6	1.3	0.7	0.1	0.0	0
	CD at 5%	3.2	3.8	2.4	1.7	4.0	2.1	0.1	0.1	0
	C.V	2.6	3.1	3.6	2.0	3.5	3.9	5.4	3.0	5

* Mean of three replications
Figures in parentheses are arcsine √ per cent angular transformed values

Table 25. Population densities of biocontrol agents and pathogens in rhizosphere of chickpea cultivar 'Dahod Yellow' at initial stage and at 90 days after sowing in the field

Sl. no.	Treatments	Rhizosphere population of biocontrol agents and pathogens*			
		Initial population	90 DAS		
		Trichoderma harzianum **$\times 10^5$ / seed**	***Trichoderma harzianum*** **$\times 10^5$ / g soil**	***Rhizoctonia solani*** **$\times 10^6$ / g soil**	***Fusarium*** **spp. $\times 10^6$ / g soil**
1.	Tebuconazole	-	-	6.1	6.2
2.	Vitavax	-	-	6.2	6.0
3.	Neem oil	-	-	6.4	6.3
4.	*T. harzianum* (ICRISAT-25)	6.8	7.1	5.7	5.8
5.	Tebuconazole + Neem oil	-	-	6.0	5.9
6.	Vitavax + Neem oil	-	-	5.8	5.7
7.	Tebuconazole + *T. harzianum* (ICRISAT-25)	6.0	6.6	5.4	5.2
8.	Vitavax + *T. harzianum* (ICRISAT-25)	6.2	6.8	5.5	5.6
9.	Neem oil + *T. harzianum* (ICRISAT-25)	6.5	7.0	5.6	5.6
10.	Control (Untreated)	-	-	7.0	6.9
	SEm±	0.0	0.0	0.3	0.0
	C.D at 5%	0.1	0.1	0.9	0.1
	C.V	1.8	1.8	2.2	1.8

* Mean of three replications.

Initial population of *Fusarium* spp. in soil 2.4×10^6 c.f.u /g soil. Initial population of *Rhizoctonia solani* in soil 1.8×10^6 c.f.u /g soil.

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