Fusarium wilt management and growth stimulation of chickpea (*Cicer arietinum* L.) by inoculation with antagonistic *Bacillus* species

BY

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CERTIFICATE - I

This is to certify that this dissertation entitled, **“Fusarium wilt management and growth stimulation of chickpea (*Cicer arietinum* L.) by inoculation with antagonistic *Bacillus* species”** submitted for the degree of **Doctor of Philosophy** in the subject of **Microbiology** to Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms. Anju Sehrawat**, Admn. No. **2013BS14D** under my supervision and that no part of this dissertation has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that this dissertation entitled, **“Fusarium wilt management and growth stimulation of chickpea (*Cicer arietinum* L.) by inoculation with antagonistic *Bacillus* species”** submitted by **Ms. Anju Sehrawat**, Admn. No. **2013BS14D** to Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in the subject of **Microbiology** has been approved by the Student’s Advisory Committee after an oral examination on the same.

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It is certified that the research work report submitted by **Ms. Anju Sehrawat,** Admn. No. **2013BS14D**, a Ph. D. student of the Department of Microbiology has been checked and found as per specifications of the format circulated by Dean, PGS vide memo no. PGS/A-1/09/6926-90 dated 26.08.2009.

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CHAPTER I

INTRODUCTION

Legumes are the second most important crop for humans. Grain and forage legumes are grown in about 15% of the world's cultivated land and account for 27% of world's primary crop production, and provide 33% of dietary nitrogen requirement, apart from providing a natural fertilizer (Graham and Vance, 2003). Chickpea (*Cicer arietinum* L.) ranks third after common bean (*Phaseolus vulgaris*) and peas (*Pisum sativum*) in grain legume production and grown in over 50 countries with 90% of its area in developing countries. Chickpea has been thought to originate in south-eastern Turkey around 7,500 B.C., from where it has spread to other countries of the world. *Cicer arietinum*, the cultivated species of *Cicer*, has been domesticated from *C. reticulatum* Ladizinsky, a closely related wild species. After its domestication in Middle East, this crop progressed further throughout the Mediterranean region, India and Ethiopia (van der Maesan, 1987). Lately, it has been introduced in Mexico, Argentina, Chile, Peru, Australia and the US (Duke, 1981). According to FAO, 2014 chickpea is cultivated on area of 14.81 million hectares with 14.24 million tonns production around the world. Chickpea is also produced in eastern Africa, western Asia, Mediterranean Basin, Australia and in some parts of the European and American continents.

Chickpea is high protein legume grown in India and an important source for human and animal nutrition (Yadav *et al.*, 2011). It also improves soil fertility, particularly in dry areas. It is grown in about 9.21 million ha with production of 8.88 million tonnes with productivity of 995 Kg ha^{-1} (Singh and Sewak, 2013). India is one of the the major producing countries of chickpea, contributing more than 75% of total production of chickpea in the world and nearly 42 to 47% of total pulse production in India. Nearly 90% of the area and production is from six states viz., Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh. The Indian subcontinent is the principal chickpea-producing and -consuming region, contributing almost 70% of the world's total production (FAOSTAT, 2009). Chickpea is a rich source of protein and starch for the predominantly vegetarian population in the developing countries. Moreover, being a grain legume it plays an integral part in diversifying the cereal-based cropping system because of its ability to fix atmospheric nitrogen and breaks disease cycles (Arnon, 1972). However, the chickpea area marginally declined in India from 9.3 million hectares to 9.0 million hectares during the triennium 1975-77 to 2004-2006. There is also a regional shift in chickpea area from Northern India to Central and Southern India.

About 172 pathogens including fungi, bacteria, viruses and nematodes have been reported to infect the crop, out of which 89 have been reported from India alone (Cother, 1977). Ascochyta blight (*Ascochyta rabies*), wilt (*Fusarium oxysporum* f. sp. *ciceris*), black root rot (*Fusarium solani*) and wet root rot (*Rhizoctonia solani*) are amongst the serious fungal diseases of chickpea (Nene and Reddy, 1987). Wilt complex, which manifests itself by wilting or root rots, is one of the most devastating and challenging diseases and can damage the crop at any stage. Wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* (FOC) is a complex and destructive disease all over the world. The pathogen is seed-borne (Pande *et al.,* 2007) as well as soil borne (Jiménez-Fernández *et al.,* 2011a). The spores of fungus enter in the plants passing through the roots. When the spores reach in the vascular system, they produce certain enzymes that degrade the cell walls and obstruct the plant's transport system. The plant shows pale colour drooping of leaves and the seedling collapses, and lie flat on ground. Such plants when uprooted show shrinkage of stem. The roots do not show any external symptom and apparently look healthy. When split vertically, they clearly show internal discolouration of pith and xylem (Nene *et al*., 1979). Adult plants show typical wilting symptoms. Early wilting causes more loss than late wilting. Yellowing and wilting of the foliage occur and finally there is necrosis (Leslie and Summerell, 2006). *F. oxysporum* survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for up to 6 years (Haware *et al*., 1996).

Eight races have been reported for *Fusarium oxysporum* worldwide, out of which six (1A, 2, 3, 4, 5 and 6) cause wilting; whereas the races 0 and 1B/C cause yellowing symptoms (Gowda *et al*., 2009). Fusarium wilt is prevalent in almost all chickpea-growing areas of the world and its incidence varied from 14 to 32% in the different states of India (Dubey *et al*., 2010). Chickpea crop is affected by several diseases but wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the most serious disease and the losses due to chickpea wilt may hover around 10-15% each year. In the years of severe epidemics, crop losses may become as high as 60-72 (Jamali *et al*., 2004; Dubey *et al*., 2007). The disease causes yield losses up to 100% under favorable conditions in chickpea (Pandey *et al*., 2010). It widely occurs in chickpea growing region of India, Pakistan, Ethiopia, Mexico, Iran, Nebraska, USA, Spain, Syria and Sudan. Recent investigations showed that *F. redolens* and *F. oxysporum* f. sp. *pisi* cause diseases to chickpea crop with symptoms similar to those produced by *F. oxysporum* f. sp. *ciceris* (Jiménez- Fernández *et al.,* 2011b).

The most efficient method for the management of disease is by using resistant cultivars (Jimenez-Gasco and Jimenez-Diaz, 2003) but resistance in available germplasm of chickpea is very uncommon and only few lines show resistance to chickpea wilt disease. Therefore, it is very important to find out other control measures against wilt pathogen. Conventional strategies, such as crop rotations, avoiding the infected field to grow chickpea and the use of chemical fungicides are being used to manage the disease. However, they have not been successful in controlling the disease (Haware and Nene, 1982). Usually, fungicide sprays are done for controlling the disease. Seed dressing with 0.15% benlate T (30% benomyl + 30% thiram) destroyed the seed borne *Fusarium oxysporum* fungus completely

(Haware *et al.,* 1986). Kaur and Mukhopadhyay (1992) reported that seed treatment with Vitavex-200 (carboxin + thiamine) and ziram gave 29% wilt disease control in chickpea in the field. This control increased to 63.3% when *Trichoderma harzianum* was also applied. Considering the importance of wilt complex disease in terai area, effect of integrated managements (bioagents + fungicides + *Rhizobium*) on chickpea wilt complex was studied. Fungicides such as Thiram and Vitavax, bioagents such as *Trichoderma* sp. and *Rhizobium* sp. of chickpea were used as seed treatments (Trivedi and Rathi, 2016). Subhani *et al.* (2011) observed the effect of 6 fungicides at four different concentrations through poisoned food technique. There was a significant decrease in mycelial development of FOC pathogen with an increase in fungicidal concentration. Furthermore, application of fungicides does not always prove economic against soil borne pathogens and it leads to environmental pollution, pathogen resistance, and increased risk to human and animal health (Li *et al*., 2012; On *et al*., 2015). In addition, excessive use of fungicides creates an imbalance in the microbial community in soil (On *et al*., 2015). Hence, scientists are steadily looking out for eco-friendly measures for plant disease management. Integrated management strategies should include minimum use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculums, modification of cultural practices and use of resistance varieties (Moradi *et al.*, 2012). The use of microbial biocontrol agents is an alternative to fungicides application for the management of plant disease because biological control is one of the most environmentally viable and health-friendly approaches for replacing fungicides in management programmes to control fungal pathogens (Li *et al*., 2012; Jiménez-Díaz *et al*., 2015). Several bacterial species such *Bacillus* and *Pseudomonas* are being currently examined as alternative to fungicides due to their perceived level of safety and minimal environmental hazards.

Natural ecosystem of plants also contains many beneficial microbes called symbionts. These symbionts help plant in getting their nutrition in a better way and improve the resistance ability of the plant. Among these symbionts, arbuscular mycorrhizal fungi and rhizobia are very important. (Demir and Akkopru, 2007). *Rhizobium* is most extensively explored microbe with N_2 fixing capacity on root of more than 20,000 species of family *Fabaceae* (Sharma and Gill, 2010). Introduction of specific strains for chickpea nodulation is of particular importance since *Mesorhizobium* symbiosis is highly host specific. *Mesorhizobium* promotes plant growth either directly through N_2 fixation, or indirectly as an antagonist against *Fusarium* wilt of chickpea. The biocontrol effect of rhizobia is also attributed due to the production of secondary metabolites *viz.* cell wall degrading enzyme production (protease and cellulose), Fe chelating siderophores and HCN or to the accumulation of phytoalexins leading to induction of systematic resistance (Deshwal *et al.*, 2003; Arfaoui *et al.*, 2006). *Mesorhizobium* spp. has been investigated for their antagonistic activity against *Fusarium oxysporum* sp. *ciceris* in chickpea under *in vitro* condition (Bhagat *et al*., 2014). Besides, *Mesorhizobium*, many rhizobacteria such as *Pseudomonas* and *Bacillus* strains, have been identified to suppress seed and soil-borne fungal diseases in various legumes (Manikandan *et al*., 2010). Seed bacterization with species of *Azotobacter*, *Rhizobium, Bacillus* and *Pseudomonas* was found to inhibit growth of soilborne root infecting fungi and suppression of the disease and enhancement of the plant growth and yield under green house and field conditions (Siddiqui *et al*., 2000b). Similarly, the antagonistic effect of antagonistic microorganisms' viz., *Aspergillus flavus, Aspergilus niger, Aspergilus ochraceus, Azotobacter* sp., *Penicillium* sp., *Pseudomonas fluorescens*, *Rhizobium* sp. and *Trichoderma harzianum* was determined *in vitro* for the management of chickpea wilt caused by *Fusarium oxysporum* sp. *ciceris* (Subhani *et al*., 2013). Moreover, coinoculation of the rhizobacteria along with an effective strain of *Mesorhizobium* sp. *Cicer* strain Ca181, caused reduction in wilt incidences and the nodulation was also enhanced (Sindhu and Dadarwal, 2001; Mohan, 2010). Different mechanisms have been implicated in the suppression of plant diseases and inhibition of plant pathogens by rhizobacteria, such as the competition for nutrients and the production of biocontrol metabolites (extracellular antibiotics, volatile substances, siderophores, lytic enzymes, hydrogen cyanide, etc.) (Das *et al*., 2008; Sindhu *et al*., 2009; Erdogan and Benlioglu, 2010).

Various chemical and biocontrol agents have been employed for the control of *Fusarium* but rhizobacteria offer great antagonistic effects on soil borne pathogens (Arfaoui *et al.,* 2006; Khalequzzaman and Hossian, 2008). Although, numerous microorganisms have been reported antagonistic to soil borne plant pathogens, relatively few of these antagonistic microbes have been commercialized as biocontrol agents (BCAs) (Meyer and Roberts, 2002). Among them, *Bacillus* spp. are considered to be the safe microorganism that hold remarkable abilities for synthesizing a vast array of beneficial substances (Stein, 2005). *Bacillus* spp. having potent plant growth promoting traits such as IAA production, phosphate solubilization, nitrogen fixation and biocontrol attributes like production of HCN, siderophore, hydrolytic enzymes and antibiotics have been isolated from soybean (Senthilkumar *et al*., 2009). Moreover, *Bacillus* species are often regarded as ideal candidates for commercial BCA due in part to their ability to form heat- and desiccation- resistant endospores (Kumar *et al*., 2011). The use of *Bacillus* species for plant disease biocontrol includes field crops, fruits, vegetables and horticultural plants. *Bacillus* species can protect plants from pathogens via multiple mechanisms, including induction of systemic resistance (Kloepper *et al*., 2004), competition for space and nutrients (Chen *et al*., 2012) and by secretion of antifungal compounds, like the lipopeptide antibiotics, surfactin, iturin and fengycin (Kim *et al*., 2009; Kumar *et al*., 2011). *Bacillus subtilis* isolated from citrus fruit surface was successfully evaluated for control of citrus green and blue moulds caused by *Penicillium digitatum* and *Penicillium italicum*, respectively (Obagwu and Korsten, 2003). *Bacillus licheniformis* was reported as an effective biocontrol agent against tomato gray mould caused by *Botrytis cinerea* (Lee *et al*., 2006). *Bacillus amyloliquefaciens* produced antibiotics that might be useful as multiple control agents against various plant diseases (Yoshida *et al*., 2001) and extracellular antifungal metabolites produced by *Bacillus pumilus* inhibited mycelial growth of many species of *Aspergillus, Penicillium* and *Fusarium* (Munimbazi and Bullerman, 1998). In addition to their biocontrol potential, it is also well documented that *Bacillus* species enhance plant growth, mainly by modulating the plant hormone levels, enhance the availability of phosphate in soil (Pane *et al*., 2012) and promoting nutrient uptake during plant growth (Kumar *et al*., 2011; Li *et al*., 2012).

Growth promotion and disease control by *Bacillus* sp. are complex interrelated processes involving direct and indirect mechanisms that include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of 1-amino cyclopropane-1-carboxylate (ACC) deaminase, production of siderophore, antibiotics, hydrogen cyanide (HCN) and volatile compounds. Other mechanisms include mineral solubilization (e.g. phosphorus), competition and induced systemic resistance (Abbasdokht and Gholam, 2010). *Bacillus pumulius* can be used as a bioinoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia (Hafeez *et al*., 2006). Dileep Kumar (1999) showed that seed bacterization of chickpea with a siderophore- producing *Pseudomomas* strain RBT13 and an antibiotic producing *Bacillus subtilis* strain AF1 resulted in a significant reduction in wilt disease. In wilt sick nursery tests, *Pseudomomas* isolate CRSM8 and *Bacillius* isolate CRSM18 reduced wilt by 48.6 and 31.5%, respectively (Khot *et al*., 1996). Chickpea *Fusarium* wilt severity caused by *Fusarium oxysporum* was reduced from 99 to 60% in the susceptible cultivar ILC 482 treated with antagonistic *Bacillus* sp. (Rb29, Rb6, Rb12, Rb4, and Rb15) in pot assays and by 98, 81, 68, 64, 57.2%, respectively in the field trials (Zaim *et al*., 2013).

Therefore, use of biocontrol agents isolated from rhizosphere or soil holds a great promise to control various plant diseases without disrupting the ecological balance (Weller, 2007). Despite the potential of biocontrol in agriculture, it is one of the poorly understood areas of plant-microbe interactions. Due to better survival ability of *Bacillus* species in the soil under abiotic stress conditions, rhizosphere bacteria having antagonistic activity were isolated from the rhizosphere soil of chickpea. The present research project was proposed with the following objectives:

1. Isolation of *Bacillus* isolates from rhizosphere of chickpea and testing of their antagonistic interactions with *Fusarium oxysporum* under *in vitro* conditions.
2. Characterization of other beneficial activities of the antagonistic *Bacillus* isolates for plant growth promotion.
3. Evaluation of their effectiveness either as single or as coinoculant with *Mesorhizobium* strains for disease control activity and plant growth promotion in chickpea under pot house conditions.

CHAPTER-II

REVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is one of the world's most important food crops mostly grown in dry lands and contributes 3.1% to the world grain legume production. In developing countries, chickpea is a rich complement to the cereal diet since it has a high nutritive value. It is mostly grown for its' highly proteinated edible seeds and used for both seed and forage production (Yadav *et al*., 2011). Chickpea has been grown in India together with the Middle East and parts of Africa from ancient period (Upadhyaya *et al*., 2008). Despite its economic importance, its productivity is low owing to biotic stress caused by many soil-borne as well as seed-borne pathogens. The crop also suffers from other serious diseases that affect it in all growth stages. The pathogens that affect chickpea include fungi, bacteria, viruses, nematodes and mycoplasma, which results in severe economic losses globally. Among these, fungi are the largest and perhaps most important group affecting roots, stems, leaves, flowers and pods of chickpea. Fungal plant pathogens cause serious losses to agricultural products annually (Ekundayo *et al.*, 2011). *Fusarium* is a well distributed large genus of filamentous saprophytic fungus affecting plant, animal and human health as they enter the food chain (Smith *et al.,* 1988). They produce toxins, fumonisins and trichothecenes. *F. oxysporum* has a variety of hosts that include chickpea, garden beans, cowpeas, potatoes, banana, watermelon, tomato, cucumber, pepper, muskmelon, tobacco, sweet potatoes, strawberry and cotton (Nikam *et al*., 2011; Zaim *et al*., 2016). The vascular wilt fungus *Fusarium oxysporum* f. sp. *ciceris* (FOC) causes *Fusarium* wilt of chickpea which is one of the most important and destructive vascular diseases of chickpea whereby losses are estimated with the rate at 10% in India and Spain, 40% in Tunisiaand 17% in Iran (Dileep kumar, 1999; Jamali *et al*., 2004).

Management of fungal diseases using antagonistic microorganisms, known as biological control, has been the focus of intense research worldwide (Kilani *et al.*, 2011). Biological control of plant pathogens is considered as a viable alternative method to chemical control. Nonpathogenic soil bacteria and the plant growth promoting rhizobacteria (PGPR) living in association with roots of higher plants have been reported to enhance the adaptive potential of the hosts and increase their growth through a number of mechanisms (Kilani *et al.*, 2011; Grobelak *et al*., 2015). Among them, *Bacillus* sp. are recognized as a powerful biocontrol agents as they form heat- and desiccation-resistant spores (Emmert and Handelsman, 1999). Species of *Bacillus* are able to synthesize more than 60 different types of antibiotics which also act as plant growth promoter (Shobha and Kumudini, 2012; Anusuya and Manimekalai, 2016). *Bacillus* sp. have shown significant inhibitory activity against many plant pathogens including *Ceratocystis ulmi*, *Puccinia pelargonii-zonalis*, *Euthypa lata*, *Fusarium* *moniliforme*, *Phytophthora capsici*, *P. cinnamomi*, *Botrytis cinerea*, *Colletotrichum musae* and *F. oxysporum.* Species of *Bacillus* have also been known to produce compounds which promote plant growth either directly or indirectly via production of hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), phosphorous solubilization and through inhibition of fungal growth (Verma *et al*., 2014; Syamala and Sivaji, 2017). In many instances, a correlation has been established between antagonist *in vitro* and protection of crop against pathogens in the field (Pal and Gardener 2006).

2.1. Fusarium wilt of chickpea

Fusarium species are the major soil-borne as well as seed-borne pathogens causing wilt and rot diseases in more than 80 plant species including chickpea. It causes up to 100% yield loss in chickpea worldwide (Alves-Santos *et al*., 2002).

2.1.1. Fusarium nomenclature

McKerral (1923) described that *Fusarium* wilt is a soil-borne disease that belongs to the genus *Fusarium*. McRae (1932) reported FOC to be pathogenic to chickpea crop which is now accepted worldwide as the causal agent of *Cicer* sp. (Kaiser *et al*., 1994). *Fusarium* wilt of chickpea is caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato. The fungus was first named *Fusarium orthoceras* Appel & Wollenw. var. *ciceri* by Padwick, and later Chattopadhyay and Sen Gupta renamed the pathogen *F. oxysporum* Schl. f. sp. *ciceri* (Padwick) Snyder & Hansen. This was accepted as the correct name of the pathogen until revised by Holliday in 1980 (Nene and Reddy, 1987).

2.1.2. Symptoms of Fusarium wilt

Fusarium wilt has been found to cause 10–15% yield losses annually depending upon the environmental conditions. *Fusarium* wilt reduces chickpea production by decreasing both seed yield and seed weight (Navas-Cortés *et al*., 2000). The FOC is more prevalent in the Indian subcontinent, USA, Tunisia, Turkey, Ethiopia, Spain and Mexico (Halila and Strange, 1996). FOC is a primarily soil-borne pathogen; however, it can be transmitted through seeds (Haware *et al*. 1978). Pathogens survive in soil and seed in the form of chlamydospores for many years. Mycelia enter the epidermal tissues invading through roots, extend to the vascular bundles and form spores in plants (Chehri *et al*., 2010). The pathogen causes seed abortion and rot, necrosis, reduction or elimination of germination capacity as well as plant damage at later stages of plant growth resulting in the development of the disease as systemic or local infection (Khanzada *et al*., 2002). Upon pathogen attack, adult plants show typical wilt symptoms that involve drooping of petioles, rachis and leaflets (Fig. 1). The roots of the wilting plants do not show any external rotting, but when split open vertically, dark brown discoloration of internal xylem is seen (Nene *et al*., 1991). Pods from the wilted plants look normal but seeds are generally smaller, wrinkled and discolored. Symptoms of the disease develop at any stage of plant growth and affected plants may be grouped in patches or appear spread across a field (Nene and Reddy, 1987) (Fig. 1.).

Fig. 1. Typical Fusarium wilt symptoms of chickpea

(Nene and Reddy, 1987; McTaggart, 2007)

2.1.3. Occurrence of *Fusarium oxysporum*

FOC exhibited two pathotypes and eight pathogenic races on chickpea. The yellowing pathotype induces progressive foliar yellowing and vascular discoloration with plant death within 40 days, whereas the wilt-causing pathotype induces severe and fast chlorosis, flaccidity, and vascular discoloration with plant death within 20 days after inoculation (Jorge *et al*., 2005). The eight races of FOC were identified as 0, 1A, 1B/1C, 2, 3, 4, 5 and 6, by reaction on a set of differential chickpea cultivars (Jiménez-Gasco and Jiménez-Diaz, 2003). Besides, races 0 and 1B/1C which cause yellowing other races 1A, 2, 3, 4, 5 and 6 induce wilting (Jiménez-Gasco *et al*., 2001). Race 1A has been reported from India, California, Morocco and Spain, while race 6 has been found in California, Spain, Israel and Morocco (Jimènez-Gasco *et al*., 2001). Out of these, races 2, 3 and 4 have been reported only in India, while races 0, 1B/1C and 5 have been found mainly in the Mediterranean region and in California, USA (Jimènez-Gasco and Jimènez-Diaz, 2003).

2.2. Control methods of *Fusarium oxysporum*

Once introduced and established, pathogenic strains of *F. oxysporum* can survive in soil for extended periods, even in the absence of crop hosts, making eradication a long term process.

2.2.1. Cultural control

Fusarium wilt can be managed through the use of disease prevention strategies, such as rotation with non-host crop species. Soil amendment with organic matter, such as wheat or barley straw, has been found to enhance antagonism by other soil microorganisms. Delayed sowing can reduce disease incidence but late sowing can dramatically reduce yield potential and its effect on disease development can differ over locations and seasons (Kannaiyan and Nene, 1975). However, as the causal pathogen, it can survive in soils for long periods of time

(Lange and McLaren, 2002). Hence, eradication can become a long term process. Since Fusarium wilt of chickpea is seed borne, the use of clean seed from disease-free crops is very important.

2.2.2. Host plant resistance

Development of plant lines resistant to Fusarium wilt is the most effective approach to the management or eradication of the disease. Breeding of resistant lines and identification of DNA markers for resistance to Fusarium wilt has been achieved in chickpea (Sharma *et al*., 2005). In some cases, resistant plant varieties are only suitable for use against certain Fusarium wilt races (Jiménez-Gasco *et al*., 2004). Currently, all Australian commercial varieties of lupin and chickpea are susceptible to Fusarium wilt. However, there are resistant chickpea breeding lines available in Australia that can be utilized in the event of a Fusarium wilt incursion. The breeding program is actively screening advanced breeding lines in Syria annually for evaluation in the Fusarium wilt sick plot.

2.2.3. Chemical control

Seed applied and foliar fungicides and crop rotation offer some control of Fusarium wilt but are generally not as effective as the use of resistant varieties. Lentil seed treatment with thiram + pentachloronitrobenzene or thiram + carboxin reduced the incidence of the disease (Bayaa and Erskine, 1998). Fusarium wilt control with seed-applied fungicides can be difficult in chickpea and lupin because the disease appears to be most aggressive late in the growing season, long after seed-applied fungicides can reasonably be considered effective. However, seed treatments may reduce losses by eliminating or reducing seed-borne inoculum sources. Foliar fungicides tend to be expensive and must be applied as protective sprays well before symptoms become apparent (Lange and McLaren, 2002).

2.2.4. Mechanical control

Deep ploughing over summer and removal of infected trash can reduce inoculum levels of Fusarium wilt of chickpea (Haware, 1998). Solarization of soil by covering the soil with transparent polythene sheets for 6-8 weeks during the summer months has been shown to effectively control Fusarium wilt of chickpea and improve plant growth and yield (Chauhan *et al*., 1988). However, this method of control is not practical for broad-acre farming systems.

2.2.5. Biological control

The application of chemicals helped in the increase of yields obtained, but the major problems with the constant use of chemicals are the resistance induced in target organisms and contamination of the environment with very toxic substances. It is extremely difficult to control soil-borne fungi by employing conventional strategies that may include the use of synthetic fungicides. Upon the continuous use of chemicals, fungal spores survive for many years in the soil due to their resistance and hence, biological control strategies are selected and handled in an eco-friendly way instead of using chemical fungicides (Okigbo, 2004).

Biological control of plant diseases by microorganisms is a very promising alternative to an extended use of pesticides, which are often expensive and accumulate in plants or soil, having adverse effects on humans. Nonpathogenic soil bacteria living in association with roots of higher plants enhance their adaptive potential and they can be beneficial for their growth (Nagórska *et al*., 2007). In the absence of effective chemical control methods, there is renewedinterest in biological control based on application of populations of antagonisticmicroorganisms.

Numerous microorganisms have been reported as potential biocontrol agents of *F. oxysporum* f. sp. *ciceris* including *Rhizobium* (Arfaoui *et al.*, 2006), *Pseudomonas* sp. (Anjaiah *et al*., 2003) and *Bacillus* sp. (Jamali *et al*., 2004). Many microbial antagonists have been reported to possess antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis, B. cereus*, *B. amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, *Saccharomyces* sp. and *Gliocadium* sp. Biological modification of soil microflora has proved successful in reducing disease severity (Mazzola, 2002). Enrichment of soils with the beneficial mycorrhiza fungi (Goswami *et al*., 2007) or *Rhizobium leguminosarum* bacteria (Essalmani and Lahlou, 2003) may provide an effective management strategy against pathogenic fungal species. In addition, prior inoculation of chickpea with nonpathogenic isolates of *F. oxysporum* significantly reduced disease incidence and severity (Hervas *et al*., 1995). Thus, biological control of plant pathogens using antagonistic bacteria is a promising strategy for plant protection wherein plant growth-promoting bacteria (PGPB) specifically plant growth-promoting rhizobacteria (PGPRs) have been shown to improve plant health and increase yield (Maheshwari, 2010).

2.3. Preference of *Bacillus* species over other rhizobacteria as biocontrol agent

Bacillus species are widely distributed in soils, have high thermal tolerance, rapid growth in liquid culture and readily form resistant spores. They are considered to be safe and their potential as biocontrol agents is considered high (Kim *et al*., 2003). *Bacillus* sp. produce spores that are resistant to various physical and chemical treatments such as desiccation, heat, UV irradiation and organic solvents (Leelasuphakul *et al*., 2008). These attributes and ability to produce antibiotics, cell wall-degrading enzymes and antifungal volatiles indicate that *Bacillus* sp. could act excellent biological control agents against a wide range of plant pathogens (Kim and Chung, 2004).

2.4. Application of *Bacillus* species as biocontrol agent

Microorganisms that reduce the incidence or severity of plant diseases are often referred to as biocontrol agents, whereas those microbes that exhibit antagonistic activity toward a pathogen are defined as antagonists. The main antagonistic bacteria are representatives of the genera *Pseudomonas, Bacillus, Alcaligenes*, *Agrobacterium* and *Streptomyces*. Different mechanisms are involved in the actions of these bacteria against plant pathogens such as parasitism (production of lytic enzymes which hydrolyze cell wall components of pathogenic fungi such as glucanases, proteases, cellulases and chitinases), cross protection, antibiosis, competition and induced systemic resistance (Beattie, 2006).

Bacillus megaterium strains JUMB1, JUMB2, JUMB3, JUMB4, JUMB5, JUMB6 and JUMB7 revealed significant inhibitory effects on mycelial radial growth of *F. oxysporum* by all the seven isolates. Among seven isolates, JUBM5showed highest inhibition of 3.25, 0.22 and 0.21 cm in well diffusion, streak and point inoculation method, respectively (Shobha and Kumudini, 2012). Seven bacterial isolates screened from rhizosphere of common bean growing at Uttarakhand showed potential plant growth promoting (PGP) and antagonistic activities (Kumar *et al*., 2012). Based on 16S rRNA gene sequence, the isolate BPR7 was identified as *Bacillus* sp. BPR7. The BPR7 strain produced IAA, siderophore, phytase, organic acid, ACC deaminase, cyanogens, lytic enzymes, oxalate oxidase and solubilized various sources of organic and inorganic phosphates as well as potassium and zinc. Strain BPR7 strongly inhibited the growth of several phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Colletotricum* sp. *in vitro*. Cell-free culture filtrate of strain BPR7 also caused colony growth inhibition of all test pathogens. These PGP and antifungal activities of *Bacillus* sp. BPR7 suggested that it may be exploited as a potential bioinoculant agent for *P. vulgaris* (Kumar *et al*., 2012). Indigenous rhizospheric *Paenibacillus polymyxa* strain CTS-B19 and *Bacillus subtilis* strain CTS-G24 exhibited antagonistic activity against *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and against a panel of phytopathogens (Patil *et al*., 2015). *In vitro* detection for fungal wall degrading enzymes revealed that both isolates produced chitinases, β-1, 3-glucanases, proteases and cellulases. Siderophores and catalase activities were observed only in *Bacillus subtilis* strain CTS-G24.*In vitro* detection for fungal wall degrading enzymes revealed that both isolates produced chitinases, β-1, 3-glucanases, proteases and cellulases. While siderophores and catalase activities were observed only in *Bacillus subtilis* (CTS-G24). Additional to antagonism, plant growth promoting traits exhibited by these natural biocontrol agents may suppress plant diseases and might be applied in agriculture as an alternative to chemical pesticides and fertilizers (Patil *et al*., 2015).

Rhizobacteria from rhizosphere of chickpea (*Cicer arietinum* L.) plants were isolated (Khot *et al*., 1996) and were found to inhibit the growth of plant pathogenic fungi *Fusarium oxysporum* sp. *ciceri* and *Rhizoctonia bataticola*, under cultural conditions on agar plates as well as in liquid broth. Two isolates CRSM8 (identified as *Bacillus* sp.) and CRSM18 (identified as *Pseudomonas fluorescens*) reduced the wilt incidence by 48.6 and 31.5%, respectively under field conditions. Inam ul Haq *et al*. (2003) found that among 36 rhizosphere bacteria, isolate no. 3, 6, 12, 16, 18, 21, 22, 23, 27, 29 and 33 inhibited the growth of *Fusarium oxysporum* under *in vitro* studies. In water culture test-tube assay, isolate no. 1, 2, 4, 5 and 9 showed antagonistic activity against *Fusarium oxysporium* f. sp. *ciceri* by reducing symptoms of Fusarium wilt upto 91.67, 94.33, 94.37, 96.0 and 96.0%, respectively. Jamali *et al*. (2004) studied effect of bacterial control of *Fusarium* wilt under green house conditions, with seven antagonistic bacteria. Isolates B-120, B-32, B-28 and B-22 were identified as *Bacillus subtilis* and isolates Pf-100, Pf-10 and CHA0 as *Pseudomonas fluorescens*. Only isolate B-120 reduced Fusarium wilt of chickpea in both seed and soil treatments. Moradi *et al*. (2012) reported that *Bacillus subtillis* and *Trichoderma harzianum* suppressed the *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *ciceris*. Increased protein content and β-1, 3-glucanase enzyme activity might have contributed to induce systemic resistance after treatment with biocontrol agents. Five antagonistic *Bacillus* sp. Rb29, Rb6, Rb12, Rb4, and Rb15 showed the most inhibitory effect against *Fusarium oxysporum* 1 (from 25.63 to 71.11%) mycelial growth and *Fusarium oxysporum* 2 (from 28.43 to 60.65%) *in vitro*.

Zaim *et al*. (2013) reported chickpea Fusarium wilt severity caused by FOC1 was reduced from 99 to 60% in the susceptible cultivar ILC 482 treated with antagonistic *Bacillus* sp. (Rb29, Rb6, Rb12, Rb4 and Rb15) in pot assays and by 98, 81, 68, 64, 57.20%, respectively, in the field trials. An *in vitro* experiment was carried out using *Bacillus cereus* as a biological agent to control *Colletotrichum lindemuthianum* and *F. oxysporum* (Bosah, 2015). The percentage inhibition for *C. lindemuthianum* was low and the range was 22 to 38.09%, which indicates a slight inhibition. For *F. oxysporum,* the percentage inhibition was high and ranged from 62.50 to 66.90%.

2.5. Mechanism of fungal growth inhibition

Many antagonists inhibit the growth of fungi by various mechanisms, e.g., secretion of lytic enzymes, siderophores and antibiotics. *B. subtilis* has as an average of 4–5% of its genome devoted to antibiotic synthesis and has the potential to produce more than two dozen structurally diverse antimicrobial compounds (Stein, 2005). Small organic volatile compounds (VOCs) emitted from bacterial antagonists negatively influence the mycelial growth of the soil-borne phytopathogenic fungus *Rhizoctonia solani* (Kai *et al*., 2007). *Bacillus amyloliquefaciens* NJN-6 produces volatile compounds (VOCs) that inhibit the growth and spore germination of *Fusarium oxysporum* f. sp. *cubense.* Among the total of 36 volatile compounds detected, 11 compounds completely inhibited fungal growth (Yuan *et al*., 2012).

Torres *et al*. (2017) confirmed the antifungal effects of the lipopeptides produced by *B. amyloliquefaciens* PGPBacCA1, which caused damage to chlamydospores and sclerotia of *Fusarium* and *Sclerotinia*, respectively. Nihorimbere and Ongena (2017) screened activities of *Bacillus* strains to investigate their potential traits to suppress plant-pathogenic and to stimulate plant growth. Out of 14, 8 isolates showed their highest ability to inhibit growth of some phytopathogenic fungi such as *Botrytis cinerea*, *Aspergillus niger*, *Fusarium* *oxysporum* and *Cladosporium cucumerinium*. They produced protease, cellulase and a wide variety of lipopeptides homologous i.e., fengycin (A and/or B) C-14 to C-20, iturin A C-14 to C-17 and surfactin C-12 to C-17. Three out of six *Bacillus* isolates produced antifungal agents and among them *Bacillus cereus* strain SB15 showed the most significant antifungal effects against a wide range of fungi (Motamedi *et al*., 2017)

2.5.1. Production of lytic enzymes

The inhibitory effect of *Bacillus* strains EU07, QST713 and FZB24 on *Fusarium* was investigated and bacterial cell cultures, cell-free supernatants and volatiles displayed varying degrees of suppressive effect (Baysal *et al*., 2013). Proteomic analysis of secreted proteins from EU07 and FZB24 revealed the presence of lytic enzymes, cellulases, proteases, 1, 4-β-glucanase and hydrolases, all of which contribute to degradation of the pathogen cell wall. The process parameters influencing the submerged fermentation of chitinase by *Bacillus pumilus* JUBCH08 and determination of antagonistic activity of the bacterium against *Fusarium oxysporum* was highlighted (Bhattacharya *et al*., 2016). When subjected to dual plate assay, the bacterium showed 45% antagonism against *F. oxysporum*. Thus, it could be inferred that cultural conditions strongly affected the chitinase production by *B. pumilus* JUBCH08. Inhibition of *F. oxysporum* by the culture through lytic mechanism indicates its potentiality as a biocontrol agent.

Among nine chitinase-producing strains isolated from Tunisian soil, one isolate called S213 exhibited a potent chitinolytic activity (Slimene *et al*., 2015). S213 strain was identified as *Bacillus licheniformis* by API 50CH system and sequence analysis of its partial 16S ribosomal DNA. Chitinolytic activity was induced either by colloidal chitin or fungal cell walls, and the highest chitinase activity reached at the late stationary phase exhibiting optimal temperature and pH of 50–60°C and pH 6.0, respectively. SDS-PAGE analysis of the secreted colloidal chitin induced proteins showed a major protein of about 65 kDa. This protein was identified as chitinase on the basis of its peptide sequences which displayed high homology with chitinase sequence of *B. licheniformis* ATCC 14580. Moreover, chitinolytic activity containing supernatant inhibited the growth of several phytopathogenic fungi including *Phoma medicaginis*. S213 strain reduced efficiently the damping-off disease caused by *P. medicaginis* in *Medicago truncatula* and may be envisaged in enzyme-based biopesticides against phytopathogen application.

Alamri (2015) tried to increase the production of fungal cell wall degrading enzymes by *Bacillus subtilis* JF419701 to enhance its efficiency in the biological control process. In dual culture, *B. subtilis* JF419701 showed the highest antagonistic effect out of the 256 bacterial strains tested against six soil-borne pathogens, *Alternaria alternata*, *Exserohilum rostratum*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Pythium ultimum* and *Rhizoctonia solani*. The production potentiality of the enzymes α-1, 3-glucanase, β-1, 3-

glucanase, chitinase and protease by *B. subtilis* JF419701 was studied *in vitro*. The maximum production of enzymes by this bacterium was achieved after a two-day incubation period at a slightly alkaline pH (8.0). The addition of colloidal chitin or S-glucan to the growth media enhanced the production of all the enzymes except protease, which was stimulated by casein.

2.5.2. Production of antifungal lipopeptides

Bacillus lipopeptides, specifically lipopeptide homologues exhibiting antifungal efficacy, offer an alternative environmentally benign protocol for reduction of postharvest phytopathogens. *Bacillus amyloliquefaciens* DSM23117 was identified with superior potential for lipopeptide production (Pretorius *et al*., 2015). Efficacy of *B. amyloliquefaciens* lipopeptides against *Botrytis cinerea* substantiated appropriateness of this *Bacillus* species. Subsequent process modification of *B. amyloliquefaciens* cultures demonstrated that the nitrate and oxygen availability as key parameters defining optimal lipopeptide production. Discrete *B. amyloliquefaciens* cultures supplied with 4, 8, 10 and 12 g/L NH_4NO_3 demonstrated optimal lipopeptide concentration, yield and productivity with respect to both total and antifungal lipopeptides, in the culture containing 8 g/L NH_4NO_3.

The antibacterial activity against bacterial plant pathogens and its relationships with the presence of the cyclic lipopeptide (CLP) biosynthetic genes *ituC* (iturin), *bmyB* (bacillomycin), *fenD* (fengycin) and *srfAA* (surfactin), and their corresponding antimicrobial peptide products was studied in 64 strains of *Bacillus* sp. isolated from plant environments (Mora *et al*., 2015). The most frequent antimicrobial peptide (AMP) genes *bmyB*, *srfAA* and *fenD* were found in 34-50% of isolates. Most isolates (98.4%) produced surfactin isoforms, 90.6% isolates produced iturins and 79.7% produced fengycins. The antibacterial activity was very frequent and 75% of the isolates were active against at least 6 of the 8 bacterial plant pathogens tested. Hierarchical analysis confirmed the presence of two clearly differentiated groups. One group consisted of *Bacillus* strains that showed a strong antibacterial activity, presented several *clp* genes and produced several isoforms of CLPs simultaneously, mainly composed of *B. subtilis* and *B. amyloliquefaciens*. Another group was characterized by strains with very low or none antibacterial activity that showed one or none of the *clp* genes and produced a few or none of the corresponding CLPs, and was the most heterogenous group including *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. cereus* and *B. thuringiensis*, although the last two were exclusive to this group.

The antifungal activity of novel peptides isolated from *Bacillus amyloliquefaciens* PT14 was reported (Kim *et al*., 2015). Reverse-phase high-performance liquid chromatography revealed that *B. amyloliquefaciens* PT14 produced five peptides (PT14-1, -2, -3, -4a, and -4b) that exhibited antifungal activity but are inactive against bacterial strains. In particular, PT14-3 and PT14-4a showed broad-spectrum antifungal activity against *Fusarium solani* and *Fusarium oxysporum*. PT14-4a displayed strong fungicidal activity with minimal inhibitory concentrations of 3.12 mg/L (*F. solani*) and 6.25 mg/L (*F. oxysporum*), inducing severe morphological deformation in the conidia and hyphae. On the other hand, PT14-4a had no detectable hemolytic activity and suggested that PT14-4a has the potential to serve as an antifungal agent in clinical therapeutic and crop-protection applications (Kim *et al*., 2015). *Bacillus amyloliquefaciens* is also known for lipopeptide and polyketide production for biological control activity and plant growth promotion activity against soil borne pathogens (Ongena and Jacques, 2008).

2.5.3. Production of antibiotics

The production of antibiotics is considered to be one of the most effective biocontrol mechanisms of plant growth promoting rhizobacteria against phytopathogens over the past two decades (Shilev, 2013). Soil bacteria have been reported to produce antibiotic-like substances in culture media under laboratory conditions, which are effective in inhibiting the growth of fungi (Fernando *et al.*, 2006). The antimicrobial compounds may act on plant pathogenic fungi by inducing fungistasis, inhibition of spore germination, lysis of fungal mycelia or by exerting fungicidal effects. A variety of antibiotics have been identified, including compounds such as amphisin, 2,4-diacetylphloroglucinol (DAPG), oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone and cyclic lipopeptides produced by pseudomonads (Loper and Gross, 2007) and oligomycin A, kanosamine, zwittermicin A and xanthobaccin produced by *Bacillus*, *Streptomyces* and *Stenotrophomonas* sp. to prevent the proliferation of plant pathogens (Compant *et al*., 2005).

Two strains of *B. substilis* (Bs10, CBs4) and two strains of *P. fluorescence* (Pf1, CPf5) were evaluated to check the potential of super-strains on reduction in the fungal growth of *Macrophomina phaseolina* (Reetha *et al*., 2014a). The antibiotics produced by all the four bacterial isolates were effective against *M. phaseolina* and reduction in growth of the pathogen ranged between 61.13 to 69.62% over control. Antibiotics produced by Pf1 showed maximum per cent reduction in the fungal growth (69.92%) followed by CPf5 (65.59%). The *Bacillus* isolates Bs10 and CBs4 showed efficacy against the pathogen with 63.36 per cent and 61.13 per cent reduction over control.

2.5.4. Production of Fe-chelators siderophores

Iron is among the bulk minerals present on the surface of the earth, yet it is unavailable in the soil for plants. Iron is commonly present in nature in the form of $Fe^{3+,}$ which is highly insoluble (Rajkumar *et al*., 2010). PGPR secrete siderophores to solve this problem. Siderophores are low molecular weight iron binding protein compounds involved in the process of chelating ferric iron from the environment. When Fe is limited, microbial siderophores provide plants with Fe, enhancing their growth. Rhizobacteria produce different types of siderophores, which are involved in disease suppression and plant growth promotion (Pathma *et al.,* 2011). The various categories of siderophores produced by PGPR include catechol, hydroxamate, pyoverdine, pyochelin, cepabactin, schizokinen and some other types like azotochelin, rhizobactin, anthranilic acid and azotobactin. Production of siderophore and antifungal activity were simultaneously exhibited by free-living rhizospheric isolates of *Azotobacter* (16.22%), fluorescent *Pseudomonas* (11.11%) and *Bacillus* (10%) (Ahmad *et al*., 2008). Several studies have demonstrated that production of siderophore by PGPR was most effective in controlling the plant root pathogens (Dey *et al.*, 2004). *B. subtilis, B. amyloliquefaciens* and *B. pumilus* have have been reported to act as biological control agents against diverse soil pathogens (Liu *et al.*, 2008). Siderophore production under iron stress conditions confers upon *Bacillus* sp. an added advantage, in inhibiting pathogen growth or metabolic activity (Henry *et al.,* 2008).

Kloepper *et al.* (1980) were the first to demonstrate the importance of siderophores production in biocontrol of plant pathogens with pseudobactin, a siderophore produced by plant growth promoting *Pseudomonas* strain B10. Moores *et al.* (1984) constructed a gene bank from plant growth promoting *Pseudomonas* sp. strain B10. Non-fluorescent mutants of *Pseudomonas* sp. strain B10 were obtained by mutagenesis with nitrosoguanidine (NTG), ethyl-methanosulphonate (EMS) or UV light that were defective in the biosynthesis of yellow-green fluorescent siderophore pseudobactin. Complementation analysis showed that a minimum of 12 genes, arranged in four gene clusters were required for the biosynthesis of pseudobactin. Sindhu *et al.* (1999) reported that *Pseudomonas* isolates inhibited the growth of phytopathogenic fungi (*Aspergillus* sp., *Curvularia* sp., *Fusarium oxysporum* and *Rhizoctonia solani*). The rhizobacterial isolates produced siderophores in Fe-deficient succinate medium. It was found that different antagonistic *Pseudomonas* isolates grown in either Luria Bertani or King's B medium were not limiting in ferric ions and suggested that besides siderophores, other antifungal compounds produced by these rhizobacteria were also involved in antagonism of pathogenic fungi.

Soil samples collected from rhizosphere soil, cultivable field soil and rhizoplane soil of paddy plants were used to isolate bacteria by culture methods of which thirty two bacterial cultures were identified with diverse colony characteristics on nutrient agar medium. Sixteen bacterial isolates were further tested against *Fusarium* and *Rhizoctonia* species. Two *Pseudomonas* strains (RB15, RB30) and *Bacillus cereu*s (RB13) were cyanogen producers. Both strains of *Pseudomonas* RB15 and RB30 and *Bacillus cereus* (RB13) were also siderophore producers. Only three isolates *Serratia marcesens* (RB24), *Bacillus cereus* (RB13) and *Enterobacter cancerogenus* (RB17) showed chitinolytic activity (Usha Rani *et al*., 2016). *Acinetobacter rhizosphaerae, Pantoea dispersa, Rahnella* sp., *Bacillus megaterium*, and various *Pseudomonas* sp. were found to form halo zone in CAS agar, indicative of siderophore production at lower temperature (Mishra *et al*., 2009; Vyas *et al*., 2010). Gull and hafeez (2012) isolated 28 *Pseudomonas* bacterial strains and eight strains showed siderophore production and best antagonism against *Rhizoctonia solani*. MS3y, Mst8.2 and Mst7.4 strains were found most effective with more than 70% disease reduction in wheat plant.

Under conditions of iron limitation *P. fluorescens* ATCC 17400 produced two siderophores, pyoverdine and a second siderophore quinolobactin, which itself results from the hydrolysis of the unstable molecule 8-hydroxy-4-methoxy-2-quinoline thiocarboxylic acid (thioquinolobactin). *P. fluorescens* ATCC 17400 also displayed a strong *in vitro* antagonism against the Oomycete *Pythium*, which is repressed by iron, suggesting the involvement of a siderophore(s) (Matthijs *et al*., 2006). While a pyoverdine-negative mutant retains most of its antagonism, a thioquinolobactin-negative mutant only slowed-down *Pythium* growth and a double pyoverdine-, thioquinolobactin-negative mutant, which does not produce any siderophore, totally lost its antagonism against *Pythium*. The siderophore thioquinolobactin was purified and identified from spent medium and showed anti-*Pythium* activity, but it was quickly hydrolysed to quinolobactin, which showed no antimicrobial activity. Analysis of antagonism-affected transposon mutants revealed that genes involved in heme biosynthesis and sulfur assimilation are important for the production of thioquinolobactin and the expression of antagonism.

2.5.5. Production of antifungal metabolite HCN

The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules (Ahmad and Kibret, 2014). Hydrogen cyanide effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at pico-molar concentrations. Hydrogen cyanide is a toxic gas having the ability to form metal complexes with functional groups of various enzymes; inhibiting processes like CO_2 and nitrate assimilation; disruption or reduction of oxygen in the cytochrome respiratory chain and electron transport in photosynthesis. The possible phytotoxic mechanism leading to significant growth reduction in plants has been reported in *Lactuca sativa* and *Echinochloa crus-galli* (Zeller *et al*., 2007). Wani *et al.* (2007b) tested rhizosphere isolates for HCN producing ability *in vitro* and found that most of the isolates produced HCN and stimulated the plant growth. The bacterium *Pseudomonas entomophila* produced HCN with biocontrol properties and pathogenicity (Ryall *et al.*, 2009).

The bacterial isolate KC1 was isolated from the rhizosphere of castor plants (*Ricinus communis*) indigenous to agricultural fields of Bihar (Lakshmi *et al*., 2015). The strain was validated as *Pseudomonas aeruginosa* (HM195190) based on sequencing of 16S rDNA. The strain KC1 was found to produce cyanide (4.78 nmol L^{-1}) over a period of 36 h. Seed bacterization exhibited reduction in root length and shoot length of weed seedlings (*Amaranthus spinosus* and *Portulaca oleracea*), which was significant in both laboratory and glasshouse experiments. Biomass was significantly reduced for the weed seedlings in glass house experiments. However, KC1 inoculated crop seedlings (*Triticum aestivum*) were found to be less inhibitory as compared to weed seedlings. The observations are significant to establish that the secondary metabolites producing *P. aeruginosa* KC1 could be exploited as a weed biocontrol agent.

Fifty-six bacterial strains were isolated from the rhizosphere soil samples of healthy tomato fields, collected from different locations of Faridabad district, Haryana, India (Prashar *et al*., 2013). Out of these, ten isolates were found to be antagonistic against the tested fungal pathogen i.e. *Fusarium oxysporum,* under *in vitro* conditions. On the basis of percentage inhibition of radial growth of *Fusarium oxysporum,* isolate TNAM5 was found to be the most effective antagonistic rhizobacteria and it was identified as a *Bacillus* sp., with close nucleotide identity to *Bacillus subtilis* group. Average percentage inhibition given by this isolate was 47.77% and it was found to produce diffusible and volatile antifungal metabolites along with hydrogen cyanide and ammonia. A total of nine species of PGPR were identified from maize roots (Agbodjato *et al*., 2015). Those PGPR include five *Bacillus* species (*B. polymyxa*, *B. pantothenticus*, *B. anthracis*, *B. thuringiensis* and *B. circulans*), three *Pseudomonas* species (*P. cichorii, P. putida* and *P. syringae*) and *Serratia marcescens*. All *Serratia* strains produced ammonia, whereas 80% of *Bacillus* and 77.77% of *Pseudomonas* strainsproduced HCN. The hydrogen cyanide was produced by all isolates (100%) independent of their genus.

2.5.6. Induction of systemic resistance

Application of mixtures of different PGPR strains to the seeds or seedlings of certain plants has resulted in increased efficiency of induced systemic resistance (ISR) against several pathogens (Ramamoorthy *et al*., 2001). Various non-pathogenic PGPR strains have the ability to induce systemic resistance in plants against broad spectrum phytopathogens (Elbadry *et al*. 2006). Interactions between *Bacillus* sp. and plants with special reference to induced systemic disease resistance have been elicited (Choudhary and Johri, 2009). Several strains such as *B. amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides* and *B. sphaericus* were recorded to elicit significant reduction in disease incidence on diversity of hosts (Ryu *et al*., 2004). Activation of various defence-related enzymes like chitinases, β-1, 3-glucanase, peroxidise (PO), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) in different PGPR strains can induce systemic resistance in plants (Bharathi *et al*., 2004).

2.6. *Bacillus* sp. as plant growth promoting agent

Plant growth promoting rhizobacteria (PGPR) are capable of promoting plant growth either directly or indirectly. There are several ways the PGPR may directly facilitate the proliferation of their plant hosts: (i) solubilize minerals like phosphates in a form that can be used by the plant (ii) synthesize phytohormones like auxins or change the concentration of plant growth regulators that trigger plant cell growth and proliferation (iii) synthesize enzymes that can modulate plant hormone levels (iv) fix atmospheric nitrogen and supply it to the plant. The indirect promotion of plant growth occurs when PGPRs prevent the deleterious effects of phytopathogenic organisms either through antibiosis and it can be either due to the depletion of a scarce resource, required by the pathogen, or to the production and release of a compound that impedes the growth of the phytopathogenic organism (Smitha *et al*., 2015). The list of indirect mechanisms used by PGPR is substantial: (i) synthesis of enzymes able to hydrolyze fungal cell walls (ii) synthesis of hydrocyanic acid (HCN) and volatile compounds which suppresses growth of fungal pathogens (iii) production of antibiotics that kill the phytopathogenic fungus (iv) induction of systemic resistance (ISR) and (v) antagonism against phytopathogenic microorganisms by production of siderophores. *Bacillus pumulius* can be used as a bioinoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia (Hafeez *et al*., 2006).

Seven isolates of *Bacillus megaterium* JUMB1, JUMB2, JUMB3, JUMB4, JUMB5, JUMB6 and JUMB7 were screened *in vitro* for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia, HCN, phosphate, siderophore and evaluated for the ability to suppress fusarial growth (Shobha and Kumudini, 2012). All the isolates were found to produce IAA, ammonia, HCN and siderophore but none of the isolates solubilized phosphorous. Production of IAA and siderophore was highest in the isolate JUMB3 (127 µg/ml and 124% respectively) and lowest in JUMB7 (35 µg/ml and 44%, respectively).

2.6.1. Utilization of stress hormone precursor ACC (1-aminocyclopropane-1-carboxylate)

Some rhizosphere bacteria possess the enzyme ACC deaminase that reduces the level of ethylene production (Jacobson *et al.,* 1994; Khandelwal and Sindhu, 2012; 2013). Ethylene has been reported to increase the severity of fungal infection and some ethylene synthesis inhibitors have been found to significantly decrease the severity of a fungal infection (Robison *et al.,* 2001). These ACC deaminase-containing bacterial strains were found more effective than biocontrol strains that did not possess this enzyme (Wang *et al.,* 2000). A portion of the ACC exuded from plant roots is then taken by the bacteria and subsequently converted by the enzyme, ACC deaminase, to ammonia and α-ketobutyrate, both of which are readily metabolized by most soil bacteria (Glick, 2004). Currently, bacterial strains exhibiting ACC deaminase activity have been identified in wide range of genera such as *Acinetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Ralstonia, Serratia* and *Rhizobium* etc. (Kang *et al*., 2010). Ghosh *et al*. (2003) recorded ACC deaminase activity in three *Bacillus* species namely, *Bacillus circulans* DUC1, *Bacillus firmus* DUC2 and *Bacillus globisporus* DUC3 that stimulated root elongation in *Brassica campestris*.

Plant growth promoting rhizobacteria which possess the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant growth and development by decreasing ethylene levels, inducing salt tolerance and reducing drought stress in plants (Zahir *et al*., 2008). Several forms of stress are relieved by ACC deaminase producers, such as effects of phytopathogenic microorganisms (viruses, bacteria, and fungi etc.) and resistance to stress from polyaromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, draft, extremes of temperature, high light intensity, and flooding (Lugtenberg and Kamilova, 2009; Glick, 2012). The major effects of seed/ root inoculation with ACC deaminase-producing rhizobacteria are the plant root elongation, promotion of shoot growth and enhancement in rhizobial nodulation and N, P and K uptake as well as mycorrhizal colonization in various crops (Glick, 2012). Dey *et al*. (2004) reported that inoculation of peanut with fluorescent pseudomonad isolates *viz.* PGPR1, PGPR2 and PGPR4 containing ACC deaminase activity significantly enhanced the pod yield (23-26, 24-28 and 18-24%, respectively), haulm yield and nodule dry weight over the control in field trials.

Recently, ACC utilizing rhizobacteria have been found to lower the level of nodulation inhibitor ethylene leading to improved nodulation and plant growth of legumes. Khandelwal and Sindhu (2012) demonstrated that ACC containing rhizobial isolates GSA6, GSA11 and GSA110 formed 42-48 nodules per plant and caused 80.7–205.3% gain in plant dry weight in comparison to uninoculated plants in sterilized chillum jar assembly conditions at 60 days of plant growth. Whereas, ACC deaminase lacking bacterial strains GSA114 and GSA115 caused only 35.7 and 3.6% increase in plant dry weight, respectively. The coinoculation of ACC^{+} *Pseudomonas* isolate CPA123 with ACC^{+} *Bradyrhizobium* isolate GSA11 caused 220.7% increase in plant dry weight in comparison to control uninoculated plants at 60 days of growth (Khandelwal and Sindhu, 2013). In comparison to coinoculation with ACC^{-} *Pseudomonas* isolate CPA152, the coinoculation of *Pseudomonas* isolate CPA123 showed more enhancements in symbiotic parameters of clusterbean.

Soil inoculation with ACC deaminase containing *Variovorax paradoxus* 5C-2 promoted the growth of ethylene-overproducing *Arabidopsis* mutant eto1-1 but not in ethylene-insensitive mutants (etr1-1 and ein2-1) and indicated the importance of ACC deaminase on plant growth promotion under stressed environment (Chen *et al*., 2013). ACC deaminase producing bacteria not only directly promote plant growth but also protect plants against flooding, drought, salt, flower wilting, metals, organic contaminants and both bacterial and fungal pathogens (Glick, 2014). Two ACC deaminase producing bacterial strains, *Bacillus subtilis* IIPR-ACC-3 and *Pseudomonas stutzeri* IIPRACC-79 isolated from chickpea rhizosphere were found to reduce negative effects of stress ethylene and to improve plant growth (Senthilkumar *et al*., 2016). Gnotobiotic root elongation assay with bacterized chickpea recorded higher root length and biomass up to 113% over stressed seedlings. Root biomass of rainfed chickpea was improved by seed bacterization with IIPR-ACC-79 and its combined inoculation with *Mesorhizobium ciceri* strain CR-13 up to 31.59 and 38.34% respectively over uninoculated control. Enhanced root nodulation at early vegetative stages of chickpea due to combined inoculation of bacterial strains helped the plant to develop 47.91% higher shoot biomass at 90 days post inoculation. ACC deaminase producing bacteria interact synergistically with *M. ciceri* strain CR-13 and enhanced plant growth and grain yield of chickpea under rainfed as well as irrigated field.

2.6.2. Aminolevulinic acid (ALA) production

5-Aminolevulinic acid (ALA), a nonprotein amino acid, is extensively found in bacteria, algae, plants and animals (Sasaki *et al*., 2002). ALA has been found as one of the potential plant growth regulators. ALA is a key intermediate in the biosynthesis of tetrapyrroles, such as porphyrins, vitamin B12, chlorophyll (bacteriochlorophyll) and heme. In plants, the ALA concentration is strictly controlled at less than 50 nmol g^{-1} fresh weight (Stobart and Bukhari, 1984). However, low ALA concentrations, within the range of 0.06-0.6 mM, promoted the plant growth rather than damage by increasing nitrate reductase activity, increasing fixation of CO_2 in the light and suppressing the release of CO_2 in darkness (Hotta *et al.,* 1997). ALA has been reported to increase stress resistance of plants (Zhang *et al.,* 2008). ALA has recently drawn increasing attention as a photodynamic chemical, which can be used as a favorable biodegradable insecticide, which is harmless to crops, humans and animals (Kang *et al*., 2012).

One hundred sixty two bacterial isolates were obtained from mustard rhizosphere soil and significant ALA production (> 15 μg ml^{-1}) was observed in bacterial isolates HMM21, HMM22, HMM80, HMM86, HMM92, HMM97, HMM115, JMM11, JMM15 and JMM35 (Phour, 2016). Other eighty four isolates produced ALA ranging from 10.1 to 15.0 μg ml^{-1}. Rhizobacterial isolates obtained from the rhizosphere of wheat and mustard WSA38, MSA57, WSA68, WSA56, MSA42, MSA39, WHA98 and MSA11 showed >11.0 μg ml^{-1} production of δ-aminolevulinic acid (Khandelwal, 2016).

The concentration of phytohormones IAA and aminolevulinic acid (ALA) exert differential response on higher plants in relation to plant biomass and yield. Therefore, inoculation tests under field conditions are essential for evaluating the impact of allelochemicals secreted by rhizosphere microorganisms. Moreover, the interactions between plants and rhizobacteria usually enhance the establishment of specific beneficial rhizospheres and such associations between different crop species can also be cultivar-specific (Merharg and Killham, 1995). Screening of rhizosphere bacteria for production of phytohormones such as IAA and ALA as well as their inhibitory effect on growth of weeds or phytopathogenic fungi, could be a better approach for selection of effective growth promoting rhizobacterial strains for their application as biofertilizers and bioherbicides (Sindhu *et al*., 2014; Harding and Raizada, 2015).

ALA can be used as a favorable biodegradable herbicide and insecticide which is harmless to crops, humans and animals (Kang *et al*., 2012). In addition, ALA could increase plants' tolerance to cold temperature and high salt concentration, and it might also be utilized as an efficient promoting factor for several crop yields even at low concentrations (Zhang *et al*., 2006). A few studies have revealed that organic acids, ALA, proline and nodulation factors produced by *Ochrobactrum* (Paulucci *et al*., 2015), *Rhodopseudomonas* (Nunkaew *et al*., 2014), *Bacillus* (Tiwari *et al*., 2011), and *Bradyrhizobium* (Munoz *et al*., 2014), respectively stimulate salt tolerance in plants. Koh and Song (2007) obtained two strains of *Rhodopseudomonas* sp. KL9 and BL6, which produced an efficient growth enhancement of tomato seedlings under axenic conditions, together with the production of IAA and ALA. Solubilization of insoluble phosphate may also be responsible for the growth promotion of tomato seedlings. Lee *et al*. (2008) reported that application of *Rhodopseudomonas* sp. KL9 enhanced growth, fruit formation, yield and the quality of fruit in tomato plants in greenhouse. Nunkaew *et al.* (2014) assesed the amelioration of NaCl stress by determining the growth of rice seedlings treated with culture supernatants containing 5-aminolevulinic acid (ALA) secreted by strains of *Rhodopseudomonas palustris* (TN114 and PP803) and compared to the effects of synthetic ALA (positive control) and no ALA content (negative control). The relative root growth of rice seedlings was determined under NaCl stress (50 mM NaCl), after 21 d of pretreatment. Pretreatments with 1 µM commercial ALA and 10X diluted culture supernatant of strain TN114 (2.57 µM ALA) gave significantly better growth than 10X diluted PP803 supernatant (2.11 µM ALA). The strain TN114 has potential as a plant growth stimulating bacterium that might enhance rice growth in saline paddy fields at a lower cost than commercial ALA. Liu *et al*. (2014) reported that 5-aminolevulinic acid (ALA), an important intermediate in tetrapyrrole biosynthesis in organisms, could be widely applied in many fields, such as medicine, agriculture and food industry due to its biochemical characteristics.

2.6.3. Production of plant growth regulator hormone indole-3-acetic acid

Phytohormones are organic substances that influence the biochemical, physiological and morphological processes in plants. Phytohormones that are not naturally synthesized by the plants but are synthesized exogenously by natural and synthetic means are known as plant growth regulators (Davies, 2013). A few notable plant hormones such as auxins, ethylene, gibberellins, abscisic acid (ABA) and cytokinins may regulate plant growth and development (Somers *et al*., 2004). Among plant growth regulators, indole acetic acid (IAA) is the most common natural auxin found in plants which showed positive effect on root growth (Miransari and Smith, 2014). Up to 80% of rhizobacteria can synthesize indole acetic acid (IAA) which may act in conjunction with endogenous IAA in plants to stimulate cell proliferation and enhance the host's uptake of minerals and nutrients from the soil (Hayat *et al.*, 2010). Ahmed and Hasnain (2010) reported that auxin-producing *Bacillus* sp. inflicts a positive effect on *Solanuntuberosum* growth. The processes of seed germination and emergence, floral induction, flower and fruit development, and steam and leaf growth include the involvement of phytohormone gibberellin (GA) (Bottini *et al*., 2004).

The production of phytohormones i.e., indole-3-acetic acid (IAA) in chemically defined media has been reported in many PGPR strains including *Azotobacter chroococcum*, *Azospirillum*, *Rhizobium* species, *Bacillus polymyxa*, *Pseudomonas fluorescens*and *Pseudomonas putida*. IAA biosynthesis has been correlated with stimulation of root proliferation by rhizosphere bacteria (Spaepen *et al*., 2007), which enhanced uptake of nutrients by the associated plants. The effect of IAA has been found to depend on the concentration, that is, low concentrations of exogenous IAA can promote, whereas high concentrations can inhibit root growth (Arshad and Frankenberger, 1992). Moreover, inoculation with an *Azospirillum brasilense* Sp245 mutant strain strongly reduced in auxin biosynthesis or addition of increasing concentrations of exogenous auxin to the plant growth medium, indicated that the response to *A. brasilense* Sp245 among the common bean (*Phaseolus vulgaris* L.) genotypes is related to the bacterial produced auxin (Remans *et al*., 2008a). The role of bacterial IAA in different microorganism–plant interactions suggest that bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defense mechanisms.

Population of IAA-producing microorganisms range as high as 80% of total soil bacteria, highlighting the enormous contribution potential of these organisms to plant's endogenous pool of IAA. Natural auxins have modes of action similar to many herbicides that interfere with plant growth such as 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) (Patten and Glick, 1996). *Bacillus megaterium* has also been reported to produce IAA at various growth temperatures in tryptophan supplemented medium (Trivedi and Pandey, 2008). Mouloud *et al*. (2015) isolated actinobacteria (*Streptomyces*) from rhizosphere of *Ononis angustissima* Lam. growing in extreme environment in southern of Algeria. Four isolates i.e., 21, 2A26, 1B10 and 2C34 showed potent antagonism against both pathogenic bacteria and fungi. The four *Streptomyces* sp. produced extracellular fungal cell-wall degrading enzymes (chitinase and protease), solubilized phosphate and also produced relatively high levels of IAA. *In vivo* biocontrol assays revealed that the *Streptomyces* strains significantly promoted the growth of the chickpea plants and showed greater suppression of chickpea wilt disease caused by *Fusarium oxysporum*.

Liu *et al*. (2013) reported that oriental Thuja seedlings inoculated with cytokinin-producing *Bacillus subtilis* strains were more resistant to stress due to drought. Several plant growth promoting rhizobacteria *Azotobacter* sp., *Rhizobium* sp., *Pantoea agglomerans*, *Rhodospirillum rubrum, Pseudomonas fluorescens, Bacillus subtilis* and *Paenibacillus polymyxa* were found to produce cytokinins or gibberellins or both can produce either cytokinins or gibberellins or both for plant growth promotion (Kang *et al*., 2010). Some strains of phytopathogens can also synthesize cytokinins. However, it appears that plant growth promoting rhizobacteria produce lower cytokinin levels compared to phytopathogens so that the effect of the plant growth promoting rhizobacteria on plant growth is stimulatory while the effect of the cytokinins from pathogens is inhibitory.

2.6.4. Abiotic stress tolerance in plants

Abiotic stresses are considered to be the main sources of agricultural yield reduction. However, the intensity of abiotic stress varies depending on the type of soils (deficiency of hormonal and nutritional imbalances) and plant factors (physiological disorders such as being susceptible to diseases) (Nadeem *et al*., 2010). Pishchik *et al*. (2002) reported that PGPR could be attenuated by the toxic effect of cadmium pollution on barley plants due to the ability of the bacteria to cadmium ions from the soil by binding mechanisms, thereby decreasing the availability of cadmium in the soil. Marulanda *et al.* (2010) reported that *Bacillus megatertum* strains inoculated into maize roots increased the ability of the root to absorb water under the salinity conditions. Inoculation of pepper with *Bacillus* sp. TW4 led to relief from osmotic stress, which is often manifested as salinity (and/or drought) stress (Sziderics *et al*., 2007). In these plants, genes linked with ethylene metabolism under abiotic stress such as *caACCO* (encoding ACC oxidase) and *caLTPI* (an abiotic stress-inducible gene encoding a lipid transfer protein (Jung *et al*., 2003) were down-regulated (Sziderics *et al*., 2007). Because *Bacillus* sp. TW4 showed ACC deaminase activity, it can be speculated that the enzyme may be involved in the lower expression of these genes.

Bacillus species were isolated from rhizosphere and screened *in vitro* for their PGPR traits like production of IAA, phosphate solubilization, siderophore, ammonia, HCN, catalase, organic acid production and observed for heavy metal and salinity tolerance (Rayavarapu and Padmavathi, 2016). Thus, *Bacillus* can enhance the plant growth and also combat with heavy metal and salinity stress of soil. The stomatal conductance of plant leaf was higher in PGPR inoculated plants than non-PGPR inoculated plants under drought conditions (Ahmad *et al*., 2013) which proved that PGPR-inoculated plants tend to improve the water-use efficiency of plants and could be beneficial to the environment in terms of reducing excessive usage of water.

2.6.5. Nutrient availability for plant uptake

Rhizobacteria possess the ability to increase the availability of nutrient concentration in the rhizosphere (Choudhary *et al*., 2011) by mineralization of nutrients, thus preventing these nutrients from leaching out of soil. *Bacillus* is the most abundant genus in the rhizosphere and the PGPR activity of some of these strains has been known for many years. There are a number of metabolites that are released by these strains (Charest *et al*., 2005) which strongly affect the environment by increasing nutrient availability of the plants. *Bacillus subtilis* is able to maintain stable contact with higher plants and promote their growth. In a micro-propagated plant system, bacterial inoculation at the beginning of the acclimatization phase can be observed from the perspective of the establishment of the soil microbiota rhizosphere. *Bacillus licheniformis* when inoculated on tomato and pepper showed considerable colonization and can be used as a biofertilizer without altering normal management in green houses (Garcia *et al*., 2004). *Bacillus* species used as biofertilizers probably have direct effects on plant growth through the synthesis of plant growth hormones (Amer and Utkhede, 2007). Phosphate solubilizing *Bacillus* sp. stimulates plant growth through enhanced P nutrition and increasing the uptake of N, P, K and Fe. *Bacillus* species could enhance plant growth by increasing the efficiency of biological nitrogen fixation and the availability of iron (Fe) and zinc (Zn) through production of plant growth promoting substances.

2.6.5.1. Phosphate-solubilizing *Bacillus* strains

Some rhizobacteria have the ability to solubilize insoluble bound phosphate (Wani *et al*., 2007a), resulting in an increased availability of phosphate ions in the soil, which can be easily taken up by the plants. Phosphorus is the most important key element in the nutrition of plants, next to nitrogen (N). It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Khan *et al*., 2010). It is abundantly available in soils in both organic and inorganic forms. Plants are unable to utilize phosphate because 95-99% phosphate is present in the insoluble, immobilized and precipitated form (Pandey and Maheshwari, 2007). Plants absorb phosphate only in two soluble forms, the monobasic (H_2PO_4) and the diabasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). Plant growth promoting rhizobacteria present in the soil employ different strategies to make use of unavailable forms of phosphorus and in turn also help in making phosphorus available for plants to absorb. The main phosphate solubilization mechanisms employed by plant growth promoting rhizobacteria include: (i) release of complexing or mineral dissolving compounds e.g. organic acid anions, protons, hydroxyl ions, CO_2, (ii) liberation of extracellular enzymes (biochemical phosphate mineralization) and (iii) the release of phosphate during substrate degradation (biological phosphate mineralization) (Sharma *et al*., 2013).

Strains from genera of *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers, while tricalcium phosphate and hydroxyl apatite seem to be more degradable substrates than rock phosphate (Banerjee *et al*., 2006). Bacterial strains *Azotobacter vinelandii* and *Bacillus cereus* when tested *in vitro* were found to solubilize phosphate and thus help in the growth of plant (Husen, 2003). *Bacillus* strains SG08-09 and SG09-12 demonstrated many characteristics that are beneficial for plants, such as production of ammonia, indole-3-acetic acid, and siderophores; cellulase and protease activity and phosphate solubilization (Xu *et al.*, 2016). In the greenhouse, both strains controlled *B. cinerea* and promoted growth of tomato seedlings, as measured by growth increases of 10-42% for roots and shoots. SG08-09 and SG09-12 reduced disease severity by 67 and 66%, respectively, relative to an untreated control. Thus, both *Bacillus* strains were found useful as potential biocontrol agents in tomato, due to their effects on plant growth and antifungal activities. Ehsan *et al*. (2016) evaluated the potential of *Pseudomonas* strains as bioinoculants in wheat on the basis of plant growth promotion and physiological characterization. These strains showed phosphorus solubilization and indole acetic acid production.

2.6.5.2. Potassium solubilizing *Bacillus* strains

Inoculation of rhizobacteria increased uptake of nutrient elements like Ca, K, Fe, Cu, Mn and Zn by plants through stimulation of proton pump ATPase (Mantelin and Touraine, 2004). The coinoculation of *Bacillus* and *Microbacterium* inoculants to improve the uptake of the mineral elements by crop plants has been reported (Karlidag *et al*., 2007). *Bacillus* species help in solubilization of mineral phosphates, potassium, zinc and other nutrients in the soil–plant system, thus reducing the need for fertilizers and enhancing the release of the nutrients from indigenous or mineral sources, for the economic and environmental sustainability (Meena *et al*., 2016).

Norkina and Pumpyanskaya (1956) reported that the silicate solubilizing bacteria *B. mucilaginosus* subsp. *siliceus* liberated potassium from feldspar and alumino-silicates. Heinen (1960) reported the ability of *Bacillus caldolyticus* and *Proteus* sp. to grow and solubilize quartz. Jones and Handrecht (1967) reported that bacteria solubilized the insoluble silicates by production of CO_2, organic acids and exopolysaccharides. Sheng and He (2006) reported that a wild-type strain of *Bacillus edaphicus* and its mutants solubilized potassium-bearing minerals and increased potassium uptake by wheat. Coinoculation of waste mica with potassium solubilizing *B. mucilaginosus* and N_2-fixing *A. chroococcum* A-41 resulted in highest biomass production and nutrient acquisition by sudan grass (Basak and Biswas, 2010).

Zhang *et al.* (2004) reported the beneficial effect of potassic bacteria on sorghum, which resulted in increased biomass and increased contents of P and K in plants than the control. The beneficial effect of silicate solubilizing *Bacillus* sp. was observed on grain yield and silica content of rice and available silica in soil (Raj *et al*., 2004). Wu *et al.* (2005) found that inoculation of K solubilizer *B. mucilaginosus* along with P solubilizer *B. megaterium* and N_2-fixer *Azotobacter chroococcum* increased the growth and nutrient uptake significantly in maize crop. Supanjani *et al.* (2006) reported that integration of P and K rocks with inoculation of phosphorus and potassium solubilizing bacteria increased P availability from 12 to 21 per cent and K availability from 13 to 15 per cent, in the soil as compared with control and subsequently improved N, P and K uptake in *Capsicum annuum*.Inoculation of maize and wheat plants with *Bacillus mucilaginosus, Azotobacter chroococcum* and *Rhizobium* could be used to mobilize potassium from waste mica, which in turn served as a source of potassium for plant growth (Singh *et al*., 2010).

Potassium solubilizing plant growth promoting rhizobacteria such as *Acidothiobacillus ferrooxidans, Bacillus edaphicus, Bacillus mucilaginosus, Burkholderia, Paenibacillus* sp. and *Pseudomonas* has been reported to release potassium in accessible form from potassium bearing minerals in soils (Liu *et al*., 2012). Thus, application of potassium solubilizing plant growth promoting rhizobacteria as biofertilizer for agriculture improvement can reduce the use of agrochemicals and support ecofriendly crop production.

2.6.6. Production of volatile compounds

Effmert *et al*. (2012) reported 300 bacteria and fungi as volatile organic compounds (VOCs) producers, and nearly 800 VOCs were recorded in the database of volatiles emitted by microorganisms. Among these, 671 VOCs belong to 212 bacterial species, and 335 belong to 96 species of fungi. Bacterial VOCs play a beneficial role in three ways: promoting plant growth, inhibiting the growth of plant pathogens and inducing systemic resistance. Several bacterial species, from diverse genera including *Bacillus*, *Pseudomonas*, *Serratia*, *Arthrobacter* and *Stenotrophomonas*, produce VOCs that influence plant growth (Ryu *et al*., 2003). Acetoin and 2, 3-butanediol synthesized by *Bacillus* are the best known of these compounds and are responsible for significant improvements in plant growth. Some PGPR strains namely *Bacillus subtilis* GB03, *B. amyloliquefaciens* IN937a, *B. megaterium* XTBG34, *Pseudomonas fluorescens* SS101 and *Enterobacter cloacae* JM22 that released a blend of volatile components, particularly, 2, 3-butanediol and acetoin, 2-pentylfuran, 13-tetradecadien-1-ol, 2-butanone and 2-methyl-n-1-tridecene which promoted growth of *Arabidopsis thaliana*, suggested that synthesis of bioactive VOCs is a strain-specific phenomenon (Ryu *et al*., 2004). VOCs produced by the rhizobacterial strains can act as signaling molecule to mediate plant–microbe interactions. Farmer (2001) identified low-molecular-weight plant volatiles such as terpenes, jasmonates and green leaf components as potent signal molecules for living organisms in different trophic levels.

The microbial activity of VOCs produced by *Bacillus* strains has been widely studied. In the nineties, Fiddaman and Rossall (1994) reported the importance of substrate on the production of antifungal volatiles from *B. subtilis* and the response of fruit pathogens to the volatile organic compounds produced by *B. amyloliquefaciens* strains has been described during recent year (Asari *et al*., 2016). Gotor-Vila *et al*. (2017) focussed on the antifungal effect of volatile organic compounds (VOCs) produced by *Bacillus amyloliquefaciens* CPA-8 against *Monilinia laxa*, *M. fructicola* and *Botrytis cinera*, three postharvest fruit pathogens of sweet cherry fruit. Thiophene was found the most effective VOC, showing more than 82% suppression of mycelial growth.

2.7. Contribution of *Bacillus* sp. as biocontrol and plant growth promoting agent

Fusariumwilt caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the main threats to chickpea production. To combat the phytopathogens, successful measures the so-called "biocontrol" are developed over the years wherein numerous plant growth-promoting rhizobacteria have been investigated for their capacities to protect plants from pathogens and stimulate plant growth. Antagonistic effects of 6 isolates of *Pseudomonas* and 6 isolates of *Bacillus* genera isolated from rhizosphere of chickpea were evaluated against *F. oxysporum* f. sp. *ciceris* as potential biocontrol agents *in vitro* and *in vivo* (Karimi *et al*., 2012). According to phenotypic properties, selected isolates were identified as *Bacillus subtilis* (B1, B6, B28, B40, B99 and B108), *Pseudomonas putida* (P9 and P10) and *P. aeuroginosa* (P11, P12, P66 and P112). The ability of bacterial isolates varied in production of hydrogen cyanide, siderophore, protease and indole acetic acid (IAA). Biocontrol activity and plant growth promotion of bacterial strains were evaluated under greenhouse conditions, in which *P. aeuroginosa* (P10 and P12), *B. subtilis* (B1, B6, B28 and B99) and *P. aeuroginosa* (P12 and B28) provided better control than untreated control (15.8-44.8%) in seed treatment and soil-inoculation, respectively. The growth parameters (plant height, fresh and dry weight of plants) were significantly increased by B28, P12 and P112 isolates in both tests compared to the untreated control.

Pseudomonas and *Bacillus* species were collected from several chickpea growing areas in Algeria to control *Fusarium oxysporum* f. sp. *ciceris* (Abed *et al*., 2016). According to API 50 CH test, six *Bacillus* species (*B. subtilis*, *B. circulans*, *B. lentus*, *B. aneurinilyticus*, *B. firmus*, *B. licheniformis*) and with API 20NE test, three *Pseudomonas* species (*P. aeruginosa*, *P. luteola*, *P. fluorescens*) were identified. The ability of bacterial isolates was varied in production of protease, gelatinase, amylase, cellulase, indole acetic acid, lipase, catalase and hydrogen cyanide. This is reflected in different rate of growth inhibition due to production of various extracellular compounds, where strain B61 (*Bacillus aneurinilyticus)* and P39 (*Pseudomonas luteola*) and P70 (*Pseudomonas fluorescens)* were found most efficient with 77 and 55.5% growth inhibition of the fungus, respectively, while B39 (*Bacillus firmus*) and P41 (*Pseudomonas luteola*) were the most efficient by volatile compounds with 70.5 and 77.5%, respectively. It was concluded that these bacterial isolates can be used in the biocontrol of *Fusarium oxysporum* f. sp. *ciceris*.

2.8. Effect of *Bacillus* sp. on seed germination of chickpea

The effect of inoculation of five isolates of PGPR designated as *Pseudomonas aeruginosa* strain BHUPSB02, *Pseudomonas putida* strain BHUPSB04, *Bacillus subtilis* strain BHUPSB13, *Paenibacilluspolymyxa* strain BHUPSB17 and *Bacillus boronophilus* strain BHUPSB19 was studied on the growth of chickpea plant (Yadav *et al*., 2010). PGPR isolates produced plant hormones indole acetic acid, solubilized phosphate and produced ammonia to enhanced plant growth. Most of isolates resulted in a significant increase in shoot length, root length of chickpea seedlings. The highest shoot length 15.6 cm plant^{-1} was recorded in treatment of *P. putida* BHUPSB04 isolate followed by statistically at par values due to inoculation of isolate *P. aeruginosa* BHUPSB02 (14.5 cm plant^{-1}). *B. subtilis* BHUPSB13, *P. polymyxa* BHUPSB17 and *B. boronophillus* BHUPSB19 showed significantly higher shoot length over control. Root length ranged from 3.87 to 9.2 cm plant^{-1}. The isolate BHUPSB02 produced the highest root length (9.2 cm plant^{-1}) followed by BHUPSB04 and BHUPSB17 in comparison to control and other isolates. BHUPSB13 and BHUPSB19 isolates also showed significant increase in root length, respectively over control.

Sivaramaiah *et al*. (2007) studied ten *Bacillus* strains for their antifungal activity, effect on seedling emergence and plant growth promotion. Two *Bacillus* strains CBS127 and CBS155 inhibited the growth of all the four pathogenic fungi *Alterneria* sp., *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* on nutrient agar medium plates *in vitro*. Seed inoculation with different *Bacillus* strains showed stimulatory effect on root and shoot growth at 10th day of observation in comparison to control, whereas four *Bacillus* strains CBS24, CBS127, CBS129 and CBS155 caused retardation of shoot growth at 10th day. Five *Bacillus* strains i.e. CBS17, CBS106, CBS127, CBS153 and CBS155 caused inhibition of root growth of the seedlings at 5th day of observation. *Bacillus* strain CBS20 showed significant stimulation of root and shoot growth at both 5th and 10th day of seedling growth. Inoculation with *Bacillus* strain CBS155 stimulated lateral root formation. The inhibition of seedling growth in some of the treatments may be due to synthesis or secretion of some toxic metabolites in the growth media as well as in root exudates of chickpea.

Mishra *et al*. (2010) studied efficiency of plant growth promoting rhizobacteria for the enhancement of *Cicer arietinum* growth and germination under salinity. Ten isolates PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2 resulted in significant increase of shoot length, root length and dry matter production of shoot and root of *Cicer arietinum* seedlings. Nelson (2004) observed that PGPR strains were able to exert beneficial effects on plant growth and increased the germination rate. Seven strains of bacteria (*Pseudomonas plecoglossicida* SRI-156, *Brevibacterium antiquum* SRI-158, *Bacillus altitudinis* SRI-178, *Enterobacter ludwigii* SRI-211, *E. ludwigii* SRI-229, *Acinetobacter tandoii* SRI-305 and *Pseudomonas monteilii* SRI-360 did not show any influence on germination as 100% germination was found in both bacteria-treated as well as untreated control plants in both chickpea as well as pigeonpea (Gopalakrishanan *et al*., 2016). However, the shoot height and root lengths of seedlings were increased significantly up to 17 and 30%, respectively for chickpea and up to 29 and 22%, respectively for pigeonpea. Among the bacterial treatments, SRI-229 and SRI-305 in chickpea and SRI-158 and SRI-211 in pigeonpea were found to increase both shoot height and root length significantly over the uninoculated control. Other bacterial treatment showed either increased shoot height or root length. As a whole, the bacterial treatments significantly increased the shoot height rather than root length in both the legumes over the control.

2.9. Symbiotic effectiveness of *Rhizobium* on legume plants

Rhizobium-legume symbiosis is one of the most important interactions for increasing the nitrogen (N) pool of both legumes and soils. This relationship between rhizobial species and their cognate host legume plant is invariably advantageous since it not only provides N in fixed forms to the host-plants but also nurture and maintain the N status, and turnover of the soil ecosystem (Wani *et al.*, 2008).

2.9.1. Effect of *Mesorhizobium* strains on chickpea plant

Chickpea is one of the most widely grown legumes (Romdhane *et al*., 2007) and forms a highly specific symbiosis with its rhizobial partner (*Mesorhizobium ciceri*) (Khan *et al*., 2006). Besides providing nitrogen to legumes, rhizobia exhibit antifungal activity, synthesize phytohormones and siderophores (Wani *et al*., 2007a, 2008) and solubilize mineral phosphate (Wani *et al*., 2007b) (Table 1). The multifaceted properties of rhizobia in turn lead to a substantial increase in legume productivity. The bioremediation and plant growth-promoting potentials of *Mesorhizobium* isolate (MRC4) was assessed using chickpea as a test crop grown under fipronil- and pyriproxyfen-stressed soils. *Mesorhizobium* isolate MRC4 used in fipronil- and pyriproxyfen-treated soil increased symbiotic properties (nodulation and leghaemoglobin content), root N, shoot N, root P, shoot P, seed yield and seed protein compared to the uninoculated but treated solely with insecticide (Ahmad *et al*., 2008; Ahemad and Khan, 2009).

Table 1. Growth promoting substances released by *Rhizobium* sp.

***Rhizobium* strains**	**Plant growth promoting traits**	**References**
Rhizobium sp.	IAA, siderophores, HCN, ammonia	Wani *et al*. (2007a,b)
Rhizobium sp. (pea)	IAA, siderophores, HCN, ammonia, exopolysaccharides	Ahemad and Khan (2009)
Rhizobium sp. (lentil)	IAA, siderophores, HCN, ammonia, exopolysaccharides	Ahemad and Khan (2011)
R. meliloti	Siderophores	Arora *et al*. (2001)
R. leguminosarum	Cytokinin	Noel *et al*. (1996)
R. phaseoli	IAA	Zahir *et al*. (2010)
Rhizobium, Bradyrhizobium	HCN, siderophore, IAA, P-solubilization	Deshwal *et al*. (2003)
Bradyrhizobium sp.	IAA, siderophore, HCN, ammonia, exopolysaccharides	Wani *et al*. (2007a)
Bradyrhizobium sp. 750	Heavy metal mobilization	Dary *et al*. (2010)
Bradyrhizobium sp., *Mesorhizobium* sp.	Siderophores	Khan *et al*. (2002)

Mesorhizobium sp.	Antifungal activity, HCN	Ahmad *et al*. (2008)
Mesorhizobium sp.	IAA, siderophore, HCN, ammonia, exopolysaccharides	Wani *et al*. (2008)
M. ciceri	IAA, siderophores	Wani *et al*. (2007c)

2.9.2. Factors affecting nodulation in chickpea

Nodulation in legumes is affected by a series of physical, nutritional and biological factors in the environment. Additional constraints in all grain legumes including chickpea are three processes namely photorespiration, nitrogen fixation and photosynthetic energy relationships, which operate against high grain yields. Photorespiration occurs in the light and consumes about 30% of the products of photosynthesis in all grain legumes. Secondly, the symbiotic relationship between the legume plant and the *Rhizobium* to fix nitrogen diverts the carbohydrates from the plant to the bacteria and reduces potential grain production by about 10%. Lastly, the plant needs more energy to produce a given amount of oil and protein than starch. This energy intensive process ultimately manifests itself in lower yields in grain legumes (Hymowitz, 1990).

2.9.2.1. Physical factors

Physical factors include water status, soil salinity, soil pH, root and shoot temperature, photoperiod and light intensity. Slattery *et al.* (2004) found that communities of resident rhizobia in Southern Australia are capable of effective nodulation of pulse crops over a range of soil environments. These populations from soil at 50 sites in Southern Australia were evaluated for nitrogen-fixing effectiveness in association with *Pisum sativum*, *Vicia faba*, *Lens culinaris*, *Vicia sativa*, *Cicer arietinum* and *Lupinus angustifolius*. It was found that 33% of Paddocks had sufficient resident population of *Rhizobium leguminosarum* bv. *viciae* for effective nodulation of faba bean, 54% for lentils, 55% for field pea and 66% for the effective nodulation of the vetch host plants. *Mesorhizobium* sp. *Cicer* population was very low in 7% of Paddocks surveyed.

2.9.2.2. Nutritional factors

One of the nutritional factors affecting nodulation is the availability of combined and free nitrogen. For sustainable organic farming, there is need to be self-sufficient in nitrogen (N) available through the fixation of atmospheric dinitrogen, recycling of combined nitrogen as in form of crop residues and application of farmyard manure, compost and biofertilizer. In chickpea, host-*Rhizobium* interaction lead to successful nodule formation by *nod* gene activation and subsequently nitrogen fixation will provide nutrient to host and after symbiosis to next rotating crop in soil. Rhizobia can grow using ammonium (NH_4^+) and nitrate (NO_3^-) as the sole nitrogen source. Bacteroids release the ammonia produced by nitrogenase to the plant for assimilation by glutamine synthetase (GS) and glutamate synthase (GOGAT), which are highly expressed in root nodule cells. The assimilated nitrogen is transported systemically within the plant, predominantly as amides (glutamine and asparagine) in temperate legumes such as pea and alfalfa, or as ureides (the purine derivatives allantoin and allantoic acid) in tropical legumes such as soybean.

2.9.2.3. Biological factors

Biological factors which affect nodulation include both the host and the bacterial factors. Host factors are determined by plant genotype, as within the same species different genotypes may respond differently to an inoculated *Rhizobium* strain. Bacterial factors affecting nodulation include survival of the inoculated strain on seeds sown in soil and its competitive ability (Sindhu and Dadarwal, 2000; Sindhu *et al.*, 2003). Microbial antagonism may prevent normal colonization of rhizosphere by the *Rhizobium* strain inoculated on the seeds, thereby decreasing nodulation (Kumar Rao *et al.*, 1974). Removal of toxic compounds from the seeds by soaking the seeds for a definite time increased rhizobial survival on the seeds and nodulation in bengal gram, green gram, pea, berseem, soybean, urid, lucerne, arhar and cowpea on inoculation (Sen *et al*., 1972). Biotic and abiotic stresses represent the most important factors causing low production of chickpea and a number of pathogens affecting chickpea have shown a threefold increase during last 15 years (Nene *et al.*, 1996).

2.10. Inoculation effect of rhizobacterial (*Bacillus* + *Mesorhizobium*) strains on nodulation and plant growth of chickpea

Rhizobacteria as well as mycorrhizal fungi have been found to enhance the production of flavonoid-like compounds or phytoalexins in roots of several crop plants (Goel *et al*., 2001) that induce the transcription of rhizobial nodulation (*nod*) genes. The localized plant hormone auxins have also been shown to participate in the fundamental responses of nodule morphogenesis. Nodule-promoting effects of *Pseudomonas* and *Bacillus* sp. on coinoculation with *Rhizobium* strains have been reported in soybean (Zhang *et al*., 1996), green gram (Sindhu *et al*., 1999) and chickpea (Kumari and Khanna, 2014). A total of 74 bacteria were isolated from herbal vermicomposts and rhizosphere soils of chickpea, and screened for their antagonistic potential against soil-borne fungal pathogens of chickpea (Sreevidya and Gopalakrishnan, 2017). Four bacterial isolates (VBI-4, VBI-19, VBI-23 and SBI-23) were identified as *Bacillus* species by 16S ribosomal DNA (rDNA) sequence analysis. Under greenhouse and field conditions on chickpea, all the isolates increased growth parameters including nodule number, plant growth and yield parameters when compared to uninoculated control.

Valverde *et al*. (2006) reported that coinoculation with *Pseudomonasjessenii* and *Mesorhizobium ciceri* showed significant enhancement of nodulation, growth and yield of chickpea at field experiments. Coinoculation with P-solubilizing bacteria and *Rhizobium* stimulated plant growth more profoundly than their separate inoculations (Zaidi *et al*., 2003). Positive interaction of *Rhizobium* with P-solubilizing sp. of *Bacillus* has translated into significant yield increases of legumes (Toro *et al*., 1998). A positive influence of plant growth promoting rhizobacteria and *Mesorhizobium* sp. strain BHURC03 on nodulation, plant biomass and yield related parameter were recorded in both year of field experiment (Verma *et al*., 2009). Significant increase in nodule number, dry weight of nodule, root and shoot were recorded in coinoculation of *Mesorhizobium* sp. and *Pseudomonas fluorescens* followed by coinoculation of *Mesorhizobium* sp., *Azotobacter chroococcum* and *Bacillus megaterium* over uninoculated control in both year of field study. The *Mesorhizobium* sp. and *P. fluorescens* have shown significant increase in all parameter due to higher nitrogen fixation by *Mesorhizobium* sp. and strong phosphate solubilizer, higher production of plant growth promoting hormones like indole acetic acid by *P. fluorescens*. Therefore, coinoculation of *Mesorhizobium* sp. strain BHURC03 and *P. fluorescens* may be highly effective bioformulation for chickpea production.

Halverson and Handelsman (1991) observed that seed treatment with *Bacillus cereus* UW85 increased nodulation of soybean in three field seasons and in three different sterilized soils in the growth chamber. Huang and Erickson (2007) treated legume seeds with *Rhizobium* sp. and observed improved seedling growth, nodulation and root-shoot biomass. Similarly, Yuming *et al*. (2003) reported the synergistic effect of *Bacillus* inoculation on crop plants. Sivaramaiah *et al*. (2007) studied the improvement in symbiotic efficiency of chickpea (*Cicer arietinum*) by coinoculation of *Bacillus* strains with *Mesorhizobium* sp. *Cicer*.Maximum nodule-promoting effect was observed with *Bacillus* strains CBS106, CBS127 and CBS155. The symbiotic effectiveness of *Mesorhizobium* sp. *Cicer* strain Ca181 was further improved on coinoculation with six *Bacillus* strains i.e. CBS9, CBS17, CBS20, CBS106, CBS127 and CBS155 at 80 d of plant growth under sterile (chillum jar) conditions and shoot dry weight ratios increased 1.62 to 1.74 times those of *Mesorhizobium*-inoculated treatments, suggesting the usefulness of introduced rhizobacteria in improving crop productivity.

The efficacy of *Bacillus* sp. to enhance nodulation, plant dry matter and grain yield on coinoculation with rhizobia has also been reported for other legumes like white clover, pigeon pea and peanut. PGPR isolated from the maize rhizosphere including *Bacillus, Pseudomonas* and *Serratia* were reported to improve the yield by 9–14%. Significant increase in the root dry mass of rapeseed was observed due to inoculation with *Proteus*, *Klebsiella* and *Bacillus* (Bertrand *et al*., 2001). Sturz and Nowak (2000) found that rhizobacteria from the local rhizosphere soil could be exploited for use as microbial inoculants to improve nodulation (in legumes) and crop productivity of both cereals as well as legumes. Qureshi *et al*. (2009) conducted a pot experiment to evaluate the effect of coinoculation with *Mesorhizobium ciceri* and *Bacillus megaterium* on growth and yield of chickpea at different fertilizer levels (0-15, 15-30, 30-60 kg NP ha^{-1}). Results revealed that *Mesorhizobium* and *Bacillus* significantly increased the yield of chickpea as compared to control. However, coinoculation of *M. ciceri* and *B. megaterium* further enhanced pod yield in comparison with uninoculated controls. Coinoculation produced 25.77 g pot^{-1} pod and 29.07 g pot^{-1} straw yield, whereas rhizobial inoculation produced 24.77 g pot^{-1} pod yield and 28.57 g pot^{-1} straw yield at full fertilizer level. Similarly, coinoculation produced higher root fresh weight (181.4 g), root length (58.4 cm) and number of nodules per pot (61) as compared to uninoculated control (94.7 g, 52.1 cm and 14). *Mesorhizobium* and *Bacillus* inoculation also increased the NP contents in chickpea straw and grain but this effect was more with coinoculation. Inoculation significantly enhanced postharvest soil N and P. Results suggested that coinoculation (*M. ciceri* and *B. megaterium*) could be an effective approach at recommended NP fertilizer than single strain inoculation.

Plant growth promoting traits of 26 non-rhizobial endophytes isolated from *Vigna radiata* root nodules were assessed for IAA and siderophore production, phosphate solubilization and hydrolytic enzymes production (Pandya *et al*., 2015). Endophytes Ml, M10 and M15 were most influential in improving seedling vigor index. Three endophytes having multiple PGP traits with maximum siderophore production: 46.77 µg ml^{-1} (*Bacillus anthracis*; Ml), IAA production: 10.81 µg ml^{-1} (*Paenibacillus taichungensis*; M10) and phosphate solubilization: 134.483 µg ml^{-1} (*Paenibacillus xylanilyticus*; M15) significantly increased root length (RL), shoot length (SL), number of lateral roots (NLR) and plant dry weight (DW) when inoculated/coinoculated with *Ensifer adhaerens* (native rhizobia) to *V. radiata* in a small field trial. M10 inoculation produced longest RL while Ml when coinoculated with *E. adhaerens* produced highest SL and NLR. Ml inoculation or coinoculation was most effective in improving dry weight of mature plants. Most of the endophytes coinoculated with *E. adhaerens* improved growth parameters. Non-rhizobial endophytes with PGP traits in combination with native rhizobia can be prospective candidates for use as biofertilizer.

The coinoculation of *Bradyrhizobium japonicum* and *Bacillus subtilis* stimulated the growth of the whole soybean plant by 125%, 100% aerial part, 235% root, 20% number of leaves and 88% nodule number compared to control (Sarti and Miyazaki, 2013). Coinoculation of rhizobacteria with an effective strain of *Rhizobium* sp. *Cicer* strain Ca181, enhanced the nodulation along with reduction in wilt incidences. Rhizobacteria belonging to *Bacillus* sp. isolated from the rhizosphere of green gram (*Vigna radiata*) were found to enhance the plant growth and nodulation (Sindhu *et al*., 2002b). Fluorescent *Pseudomonas* sp. were studied to determine their effects on seedling growth, nodulation and plant growth in chickpea (*Cicer arietinum* L.). Seed inoculation with the rhizobacteria caused stunting of root growth of chickpea seedlings at 5 days in comparison to the control, whereas the same rhizobacteria caused stimulation of shoot growth of both green gram (*Vigna radiata* L.) and chickpea seedlings at 10 days of plant growth under aseptic conditions.

Coinoculation of some *Bacillus* strains with effective *Bradyrhizobium* strain S24 resulted in enhanced nodulation and plant growth of green gram (Valverde *et. al*., 2006). Maximum gains in nodulation, nitrogenase activity and plant growth were observed with *Bacillus* strains MRS12, MRS18, MRS22 and MRS27 after 40 d of plant growth. Wani *et al.* (2007b) have reported the synergistic effect of nitrogen-fixing and phosphate-solubilizing rhizobacteria on plant growth, yield, grain protein and nutrient uptake of chickpea plants. Effect of coinoculation with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacteria) and *Mesorhizobium ciceri* C-2/2 strains enhanced the growth and seed yield of chickpea under greenhouse and field conditions.

Pseudomonas isolates obtained from the rhizosphere of chickpea (*Cicer arietinum* L.) and green gram (*Vigna radiata*) were found to produce significant amount of indole acetic acid (IAA) (Malik and Sindhu, 2011). Seed bacterization of chickpea cultivar C235 with different *Pseudomonas* isolates showed stunting effect on the development of root and shoot at 5 and 10 days of seedling growth except the strains MPS79 and MPS90 that showed stimulation of root growth, and strains MPS104 and MRS13 that showed shoot growth stimulation at 10 days. Exogenous treatment of seeds with IAA at 0.5 and 1.0 µM concentration caused similar stunting effects on root and shoot growth compared to untreated control both at 5 and 10 days of observation, whereas higher concentration of IAA (10.0 µM) inhibited the growth of seedlings. Coinoculation of chickpea with IAA-producing *Pseudomonas* strains increased nodule number and nodule biomass by *Mesorhizobium* sp. *cic*er strain Ca181.

Salinity stress has been reported to adversely affect the growth, nodulation and yield of chickpea. However, inoculation with *Mesorhizobium* strains containing ACC deaminase improved the plant growth by reducing the level of ethylene induced by salt stress. Six salt tolerant *Mesorhizobium* isolates were checked for their role in plant growth promotion under pot house conditions in chillum jar assembly (Chaudhary and Sindhu, 2015). *Mesorhizobium* strains having ACC utilization ability caused an increase in the nodule number, nodule weight and shoot dry weight at 50 and 80 days of plant growth, both with or without NaCl. *Mesorhizobium* isolate MBD26 showed 294 mg/plant shoot dry weight without salt condition after 50 days of plant growth. *Mesorhizobium* isolate MBD26 increased shoot dry weight by 49.52% (without salt) and 41.53% in the presence of salt (40 mM NaCl) after 80 days of plant growth. It was observed that inoculation with *Mesorhizobium* isolates containing ACC deaminase improved nodulation and plant growth of chickpea over ACC deaminase lacking isolates. Thus, inoculation with *Mesorhizobium* strains possessing ACC utilization ability could be a sustainable approach to improve plant growth under salinity stress.

2.11. Inoculation effect of *Bacillus* species on Fusarium wilt incidence

Parmar and Dadarwal (2001) reported that the fluorescent pseudomonads and spore forming *Bacillus* group predominantly colonise the rhizosphere of healthy chickpea plants. Yadav *et al*. (2010) found that population of *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia* and *Serratia* dominated the chickpea rhizosphere. Joseph *et al.* (2007) reported that out of 150 isolates tested, 40 belonged to genus *Bacillus*, 35 to *Pseudomonas*, 40 to *Azotobacter* and 35 to *Rhizobium*. Kumar *et al.* (2010) observed that *Sinorhizobium fredii* KCC5 and *P. fluorescens* LPK2 inhibited the growth of plant pathogenic fungus *Fusarium udum* in dual culture and increase in fungal inhibition corresponded to incubation period. Strains KCC5 and LPK2 inhibited *F. udum* by 56 and 83% after 6 days of incubation.

The antagonistic activities of 21 *Rhizobium* isolates were tested using two cultivars (the susceptible ILC482 and the moderately resistant INRAT 87/1) of chickpea (*Cicer arietinum*), *in vitro* in dual culture, and *in vivo* under greenhouse and field conditions against *Fusarium oxysporum* f.sp. *ciceris* (Foc) race 0 (Arfaoui *et al*., 2006). *Rhizobium* isolates Rh8, Rh11, Rh16 and PchSOM inhibited fungal growth more than 50%. Among the 14 *Rhizobium* isolates tested for volatiles, cyanide production and phosphate solubilization, 8 isolates significantly inhibited fungal growth by producing volatiles, 6 were positive for cyanide production and only three were able to solubilize phosphate. Isolate Rh8 produced the highest levels of volatiles gave more than 10.7% fungal inhibition and was the only one positive for both cyanide production and phosphate solubilization. Green house experiments revealed the effectiveness of five isolates PchDMS, Pch 121, Rh5, Rh17 and Pch43 (Jayantibhai, 2014). These isolates reduced the percentage of wilted plants in both susceptible and moderately resistant cultivars. The percentage reduction of wilted plant ranged from 12.5 to 54.6% in the susceptible cultivar ILC482 and from 8.3 to 29.1% in the moderately resistant cultivar INRAT 87/1. The best disease control was achieved by isolate PchDMS. Field experiments showed that none of the 14 *Rhizobium* isolates significantly reduced the percentage of wilted plants of the susceptible cultivar ILC482, although with the moderately resistant cultivar INRAT 87/1, eight of the isolates significantly reduced wilt incidence. Inoculation of seeds with these isolates reduced the percentage of diseased plants from more than 48.6% in infected control plants to less than 20.3% in plants inoculated with the bacteria and infected with the pathogen. The best protection against disease was obtained with isolates Pch43 and Rh4, which reduced the percentage of wilted plants to less than 8%. Besides their beneficial effects on disease control, studies showed that rhizobia may improve plant growth and yield, and can reduce the deleterious effects of Fusarium wilt.

Twelve rhizobacteria were isolated from rhizosphere of chickpea and identified as *Bacillus subtilis* (B1, B6, B28, B40, B99, and B108), *Pseudomonas putida* (P9 and P10) and

P. aeruginosa (P11, P12, P66 and P112) on the basis of phenotypic properties (Karimi *et al*., 2012). All were evaluated against *Fusarium oxysporum* f. sp. *ciceris* as potential biocontrol agents *in vitro* and *in vivo*. The ability of bacterial isolates varied in production of cyanide hydrogen, siderophore, protease and indole acetic acid (IAA). Biocontrol activity and plant growth promotion of bacterial strains were evaluated under green house conditions, in which *P. aeruginosa* (P10 and P12) and *B. subtilis* (B1, B6, B28 and B99) provided better control than untreated control (15.8-44.8%) in seed treatment and soil-inoculation, respectively. The growth parameters (plant height, fresh and dry weight of plants) were significantly increased by B28, P12 and P112 isolates in both tests compared to the untreated control.

Among 131 rhizobacteria isolates tested, five antagonistic *Bacillus* sp. Rb29, Rb6, Rb12, Rb4, and Rb15 showed the most inhibitory effect against FOC1 (from 25.63 to 71.11%), mycelial growth and FOC2 (from 28.43 to 60.65%) *in vitro* (Zaim *et al*., 2013). Results also revealed that production of volatile metabolites and inhibition of the test pathogen by volatile metabolites, varied among different antagonistic rhizobacteria. Isolates Rb29, Rb6, Rb12, Rb4, and Rb15 produced more volatile metabolites which inhibited mycelial FOC growth by 40%. Chickpea *Fusarium* wilt severity caused by FOC1 was reduced from 60 to 99% in the susceptible cultivar ILC 482 treated with antagonistic *Bacillus* sp. (Rb29, Rb6, Rb12, Rb4 and Rb15) in pot assays and by 98, 81, 68, 64, 57.20%, respectively in the field trials.

The antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris* was determined for 40 chickpea rhizobacteria (Kumari and Khanna, 2014). Twenty eight isolates showed antagonistic activity against test fungus ranging from 18.2 to 41.8%. Characterization of the antagonistic attributes showed that all the antagonistic isolates produced diffusible and volatile antifungal metabolites in terms of growth inhibition, maximum being with the isolates 39P (77.8%) and 15B (64.2%), respectively. Nineteen isolates showed catechol and hydroxamate type siderophore production. All the isolates produced ammonia and twelve showed HCN production. On the basis of their antagonistic and PGP functionality traits, five isolates (2B, 7B, 28P, 34P and 38P) were selected for glass house studies on two chickpea varieties (JG-62 and GPF-2). Isolates 28P, 34P and 38P were found to be most promising for wilt control and plant growth promotion. Isolate 38P reduced the wilt incidence to 44.6%, which was at par with fungicide treatment (55.5%) and had a significant edge over negative control (85%) in the chickpea variety JG-62. Similar trend of wilt incidence was observed in GPF-2 variety. Green house experiments on two varieties of chickpea JG-62 and GPF-2 showed that seed treatment with plant growth promoting rhizobacteria + mesorhizobiahad a synergistic effect in terms of disease control and growth promotion as compared to use of single bioinoculants, thus positively influencing plant-microbe interaction.

Sixteen native isolates *Mesorhizobium* sp. along with reference *Mesorhizobium* sp. (LGR 33) were tested as biocontrol agent *in vitro* against the causative agent of Fusarium wilt of chickpea (Bhagat *et al*., 2014). All *Mesorhizobium* sp. inhibited the growth of *Fusarium oxysporum* f. sp. *ciceris*, with growth inhibition varied from 34.2 (LGR 1) 59.3% (LGR 16), whereas variation in production of volatile antifungal compounds was from 20 (LGR 9) - 46.3% (LGR 16). Out of 17 *Mesorhizobium* sp., 88% were able to produce siderophores and protease, and 64% were able to produce HCN and cellulase. Three native isolates of *Mesorhizobium* sp. LGR 14, LGR 15 and LGR 16 were able to produce HCN, siderophore, cell wall degradation enzymes and volatile antifungal compounds. Two plant growth promoting rhizobacteria *Pseudomonas putida* NBRIRA and *Bacillus amyloliquefaciens* NBRISN13 with ability to tolerate abiotic stress along with multiple PGP traits like ACC deaminase activity, minerals solubilization, hormones production, biofilm formation and siderophore activity were evaluated for their synergistic effect to ameliorate drought stress in chickpea (Kumar *et al*., 2016). *In vitro* results clearly demonstrated that both the PGPR strains are compatible to each other and their synergistic growth enhanced the PGP attributes. Greenhouse experiments were conducted to evaluate the effect of inoculation of both strains individually and consortia in drought tolerant and sensitive cultivars BG362 and P1003. The growth parameters were observed significantly higher in consortium as compared to individual PGPR. Colonization of both PGPR in chickpea rhizosphere has been visualized by using *gfp* labeling. It was found that synergistic application has better potential to improve plant growth promotion under drought stress conditions.

Black root rot (*Fusarium solani*) is known to cause great yield losses in faba bean (*Vicia faba* L.), especially in the highlands of Ethiopia. Study was conducted to evaluate the biological control ability of native *Bacillus* species on the basis of their antagonistic effects against *F. solani in vitro* and in a green house (Belete *et al*., 2015). All tested *Bacillus* isolates significantly ($P<0.05$) reduced radial mycelial growth of the pathogen. Seven bacterial isolates restricted growth of the pathogen to <14 mm diameter, and showed 39-44% efficacy over the positive control. Isolate BP048 was the most effective, with 43.6% efficacy. The lowest inhibitory effects, 15.5 and 27.8%, were recorded from isolates BS083 and BS0102, respectively. The culture filtrate of the bacterial isolates also inhibited *F. solani* spore germination. *In vivo*, the isolates significantly reduced severity of black root rot on artificially inoculated faba bean seedlings. The antagonist *Bacillus* isolates kept black root rot severity low with more than 50% disease suppression, compared to the untreated control.

Bacillus amyloliquefaciens subsp. *plantarum* strain FZB42 is used commercially as biofertilizer and biocontrol agent in agriculture. Genome analysis of FZB42 revealed that nearly 10% of the FZB42 genome is devoted to synthesizing antimicrobial metabolites and their corresponding immunity genes (Chowdhury *et al*., 2015). However, recent *in planta* investigations demonstrated that except surfactin, the amount of such compounds found in vicinity of plant roots is relatively low. These metabolites have also been suspected to induce changes within the rhizosphere microbial community, which might affect environment and plant health. On the other hand, adding FZB42 to plants compensate, at least in part, changes in the community structure caused by the pathogen, indicating an interesting mechanism of plant protection by beneficial bacilli. Sub-lethal concentrations of cyclic lipopeptides and volatiles produced by plant-associated bacilli trigger pathways of induced systemic resistance (ISR), which protect plants against attacks of pathogenic microbes, viruses, and nematodes. Stimulation of ISR by bacterial metabolites is likely the main mechanism responsible for biocontrol action of FZB42. *Bacillus* strains EU07, QST713 and FZB24 were tested for their inhibitory effect on *Fusarium* (Baysal *et al*., 2013). Bacterial cell cultures, cell-free supernatants and volatiles displayed varying degrees of suppressive effect. Proteomic analysis of secreted proteins from EU07 and FZB24 revealed the presence of lytic enzymes, cellulases, proteases, 1, 4-β-glucanase and hydrolases, all of which contributed to degradation of the pathogen cell wall.

The antagonistic and inhibitory activity of 71 *Bacillus* sp. strains were tested against several phytopathogen fungi, *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium avenaceum*, *Bipolaris* sp. and *Alternaria* sp. (Sansinenea *et al*., 2016). From the antagonism study, the strain ELI149 showed a marked inhibition of growth against all tested fungi. Therefore, crude metabolites from this strain were extracted using ethyl acetate and amberlite resin, and probed against the same fungi as well as strains of *Mucor* sp., *Penicillium* sp. and *Paecilomyces* sp. The results indicated that amberlite was more suitable for extraction of secondary metabolites with antifungal activity. Finally, observation of cell damage in the tested pathogenic fungi showed marked morphological changes on reproductive structures in all tested fungi indicating that antibiosis was the mechanism of the antagonistic effect.

Three *Bacillus* isolates 1, 2 and 3 were screened *in vitro* for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia, HCN, phosphate and siderophores, and were evaluated for the ability to suppress fusarial growth (Anusuya and Manimekalai, 2016). All the isolates produced IAA, ammonia, HCN and siderophore but none of the isolates solubilized phosphorous. Production of IAA and siderophore was highest in the *Bacillus* isolates 3 (127 µg/ml and 124%, respectively) and lowest in *Bacillus* isolates 2 (35 µg/ml and 24%, respectively). *In vitro* screening for antagonism against *F. oxysporum* revealed significant inhibitory effects on mycelial radial growth by all the three isolates. Isolate 3 showed highest inhibitions of 0.21, 0.20 and 3.24 cm in well diffusion, streak and point inoculation method, respectively.

Kumari *et al*. (2016) conducted an experiment to study the influence of antagonistic *Bacillus* sp. isolate, B-I, as seed bioinoculant alone and in consortium with *Mesorhizobium* *ciceris* on seedling emergence, growth parameters and wilt incidence in pathogen inoculated chickpea plants. Seed bacterization with isolate B-I reduced the wilt incidence in bacterized pots (36%) as compared to non-bacterized plants (74%). Coinoculation with *Mesorhizobium ciceris* further reduced the wilt incidence (26%) and provided defense against the pathogen which was significantly higher than that recorded with fungicides (46%). Inoculation with isolate B-I alone and in coinoculation with *Mesorhizobium* recorded 94 and 96% seedling emergence, respectively as compared to 89% in control. Chlorophyll content affected by B-I inoculation alone and in combination with *Mesorhizobium* was also at par to control (0.87 and 0.90 mg/g leaf tissue, respectively). In pathogen challenged plants, *Bacillus* sp. isolate alone increased the fresh weight by 29.2% and in combination with *Mesorhizobium* by 49.9% over uninoculated control. Maximum increase in root and shoot length was also recorded with dual inoculation. Coinoculated treatment (B-I + *Mesorhizobium)* recorded maximum shoot and root length (21.5 cm and 15.9 cm, respectively).

Effect of *Rhizobium* (RE10 and RM02) and *Bacillus* (B02 and B15) strains was evaluated to reduce the incidence of disease caused by *Sclerotinia sclerotiorum* as well as to improve the yield of common bean cv. Centenary plants in field trial (Memenza *et al*., 2016). At harvest time, all the inoculated treatments showed the lowest percentage of plants infected by the disease caused by *S. sclerotiorum* with significant difference compared with untreated plants (14%). The best treatment was RE10 + B02 (2%), followed by RM02 (3%) and B02 (4%) without significant difference compared with the chemical control (2%). The same strains also showed the highest percentage of germination. Treatment RE10 + B02 also gave the highest yield (16 pods/plant and grain yield of 1717.8 kg/ha) with significant differences compared with untreated plants (11 pods/plant and 883.2 kg/ha). On the other hand, plants inoculated with *Rhizobium* strains formed between six and ten nodules per plant, while untreated plants formed less than four.

On-farm demonstration on use of resistant variety JG-16, summer deep ploughing, *Trichoderma viride,* PSB, *Rhizobium* and fungicides was conducted during Rabi (October-February) 2011-12 and 2012-13 for controlling chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* in two villages of Datia district (M.P) in Bundelkhand region (Singh *et al*., 2016). In treatments module 1 (T1), where summer deep ploughing (SDP) + resistant variety (JG-16) were used, wilt incidence was recorded 8.7% at village Sitapur and 9.04% at village Kakraua. In treatments module 2 (T2), where treatments module 1 (T1) + seed treatment with the combination of Carbendazim (1.0g) + Thiram (2.0 g) per kg seed followed by *Trichoderma viride* @ 5.0 gram/kg were applied as seed treatment, wilt incidence was 5.2% at village Sitapur and 5.46% at village Kakraua. In treatments module 3 (T3), where treatments module 1 (T1) was applied followed by seed treated with PSB and *Rhizobium*, wilt incidence was 7.7% at village Sitapur and 8.10% at village Kakraua. Highest seed germination per cent (87.22), 100 seed weight (17.65 g), yield (22.35 q/ha), benefit cost ratio (3.61) and reduction in wilt incidence (69.26%) was found in treatments module 4 (T4) at village Sitapur where integration of the treatments module 1 (T1), treatments module 2 (T2), and treatments module 3 (T3) followed by *Trichoderma viride* 4.0 kg/ha with 200 kg farm yard manure (FYM) was applied as basal application at the time of field preparation. Singh *et al*. (2017) evaluated fifteen *Bacillus subtilis* strains which produced antifungal substances against plant pathogens i.e., *Fusarium oxysporum* f. sp. *melonis* and *Rhizoctonia solani* (RRS1), and screened *in vitro* for their plant growth promoting traits as indole acetic acid (IAA), siderophore and hydrogen cyanide (HCN) production, as well as their antagonistic activity. In dual culture assays *B. subtilis* strains decreased growth of *F. oxysporum* (up to 85.5%) causing muskmelon wilt, *R. solani* (RRS1) (up to 73.7%) causing sheath blight of rice and *R. solani* (PRS1) (up to 82.5%) causing black scurf of potato. The production of indole acetic acid (IAA) by all rhizobacteria was investigated as important mechanism for plant growth stimulation. Maximum *in vitro* IAA values were produced by *B. subtilis* FDK-21L (7.0 µg/ml) by addition of L-tryptophan in the culture medium. *B. subtilis* FDK-21L gave high activity of siderophore production. Diameter of halo zone varied from 0.4 to 3.2 cm on CAS agar plates. FDK-21L showed significant reduction of muskmelon wilt incidence (65.7%), disease severity of sheath blight of rice (70.8%) and black scurf of potato (97.2%).

2.12. Influence of PGPR and *Mesorhizobium* species in stimulation of chickpea plant growth

It has been reported that application of *Rhizobium* isolates significantly reduced the wilting index and increased the plant growth (Hussain and Ghaffar, 1990). These rhizobia also increased nitrogen content and dry weight of roots and shoots. Siddiqui and Singh (2004) reported better plant growth, higher transpiration, better root nodulation and lower wilting index in chickpea plants infected with Foc and inoculated with rhizobia. Coinoculation of PGPR and *Rhizobium* resulted in further significant increase in shoot length as rhizobiaare reported to produce plant growth regulators such as auxins, cytokinins and gibberellins like substances that stimulate and enhance plant growth (Hemissi *et al*., 2011). Hahm *et al.* (2012) also reported that PGPR treatments resulted in the taller peppers as compared to negative control treatments. Coinoculation of PGPR and *Rhizobium* sp. increased root and shoot dry weight, plant vigour, nodulation and nitrogen fixation in various legumes. The presence of PGPR in the root vicinity may also improve ability of rhizobia to compete with indigenous populations for nodulation. Srinivasan *et al*. (1997) reported that *Bacillus* sp. enhanced the nodulation in common bean when coinoculated with *Rhizobium etli*. Nitrogen fertilization improved dry matter production but depressed nodulation. Phosphate mitigated the depressive effect of nitrogen on nodulation and further enhanced its stimulatory effect on dry matter production (Mahdi and Mustafa, 2005). Single *Rhizobium* inoculation of *P. vulgaris* showed significant difference for different symbiotic parameters (Ahmed *et al*., 2009).

Yahalom *et al.,* (1987) reported that coinoculation of legumes with *Rhizobium* and free-living IAA producers such as *Azospirillum brasilense* and several *Bacillus* species significantly increased nodule fresh weight and nitrogenase activity compared to inoculation with *Rhizobium* alone. The combined inoculation of white clover with *R. leguminosarum* bv. *trifolii* and *A. lipoferum* enhanced the number of nodules by 2-3 times and acetylene reduction activity by 2.3 to 2.7 times (Tchebotar *et al.,* 1998). Coinoculation of *Rhizobium* sp. and *Azospirillum* sp. was found to enhance the number of root hairs, the amount of flavonoids exuded by the roots and the number of nodules formed as compared to single *Rhizobium* inoculation (Remans *et al.,* 2008b). In contrast, similar experiments using mutants of *Bacillus megaterium* with altered IAA production level (overproducers and underproducers) had a negative effect on these symbiotic parameters (Srinivasan *et al.,* 1996).

Groppa *et al*. (1998) found that coinoculation with *B. japonicum* caused significant differences in total dry matter production and nitrogen content of coinoculated plants was significantly increased (by 23%) as compared with plants inoculated only with *B. japonicum*. Accordingly, a strong stimulation of acetylene reduction activity and a significant increase (39%) in leghemoglobin content were observed using this treatment. Rondon *et al*. (2007) reported that BNF by common bean increased with addition of *Rhizobium* CIAT 899 and biochar (charcoal, biomass-derived black carbon) and, hence bean yield increased by 46% and biomass production by 39% over the control at 90 and 60 g kg^{-1} biochar, respectively. Figueiredo *et al*. (2008) assessed the potential of PGPR on nodulation, biological nitrogen fixation and growth of *P. vulgaris* cv. *tenderlake*. The disinfected seeds were inoculated with *Rhizobium tropici* alone and in combination with PGPR strain. Beans coinoculated with *R. tropici* and *Paenibacillus polymyxa* had higher concentrations of leghemoglobin, nitrogenase activity and N-fixing efficacy, and thus formed associations of greater symbiotic efficiency. Inoculation with *Rhizobium* and *P. polymyxa* strain stimulated nodulation as well as nitrogen fixation (Lee *et al*., 2005). *B. subtillis* DLA coinoculated with *R. leguminosarum* bv. *phaseoli* increased growth and yield of bean plants under nitrogen-free conditions in comparision to plant inoculated with *R. leguminosarum* bv. *phaseoli* alone.

Greenhouse and field experiments were carried out to test the effect of single and dual inoculations on chickpea growth (Velverde *et al*., 2006). Under greenhouse conditions, plants inoculated with *Mesorhizobium ciceri* C-2/2 alone had the highest shoot dry weight. The inoculation treatment with *Pseudomonas jessenii* PS06 yielded a shoot dry weight 14% greater than the uninoculated control treatment. Under field conditions, plants inoculated with *M. ciceri* C-2/2, in single or dual inoculation, produced higher nodule fresh weight, nodule number and shoot N content than the other treatments. However, the coinoculation treatment ranked the highest in seed yield (52% greater than the uninoculated control treatment) and nodule fresh weight. Data suggested that *P. jessenii* PS06 can act synergistically with *M.*

ciceri C-2/2 in promoting chickpea growth. Sindhu *et al*. (2002b) studied coinoculation effect of the rhizobacteria with *Mesorhizobium* strain Ca181 and reported a significant increase in nodule weight and shoot biomass, when grown in sterilized chillum jar conditions. The shoot dry weight gains at different stages of plant growth varied from 1.05 to 1.57 times that in *Mesorhizobium*-inoculated treatments and 2.28–3.38 times that in uninoculated controls at 75 days of plant growth. Three *Pseudomonas* strains (MRS13, CRS55b and CRS68) caused maximum gains in plant dry weight ratios i.e., 1.92, 1.84 and 1.98 times those of *Mesorhizobium*-inoculated and 4.08, 3.92 and 4.20 times those of uninoculated control plants, respectively, at 90 days of plant growth. Under pot house conditions, seed inoculation with rhizobacteria, singly or in coinoculation, significantly increased root and shoot biomass, even in wilt sick soil amended with fungal pathogens.

Coinoculation of *Pseudomonas* sp. with rhizobia has been reported to enhance nodulation, plant dry matter and grain yield in other legumes like alfalfa, clover, pea and soybean Malik and Sindhu (2011) isolated *Pseudomonas* sp. producing high indole acetic acid and also studied coinoculation effect of isolated *Pseudomonas* sp. with *Mesorhizobium* sp. *Cicer* on plant growth of chickpea (*Cicer arietinum*). It was found that plant dry weights of coinoculated treatments showed 1.10 to 1.28 times increase in comparison to *Mesorhizobium*-inoculated plants alone and 3.62 to 4.50 times over uninoculated controls at 100 days of plant growth. Goel *et al.* (2002) showed that coinoculation of chickpea with *Pseudomonas* strains MRS23 and CRP55b, and *Mesorhizobium* sp. *Cicer* strain Ca181 resulted in the formation of 68.2–115.4% more nodules at 80 and 100 days after planting as compared to single inoculation with *Mesorhizobium* strain under sterile conditions. The shoot dry weight ratios of coinoculated treatments at different stages of plant growth varied from 1.18 to 1.35 times that of *Mesorhizobium*-inoculated and 3.25 to 4.06 times those in uninoculated controls.

Sindhu *et al.* (2002a) found that coinoculation of *Bacillus* strains with effective *Bradyrhizobium* strain S24 caused enhancement in shoot dry mass ranging from 1.28 to 3.55 at 40 days of plant growth. Nodule promoting effect and increase in nitrogenase activity was also observed with majority of *Bacillus* strains at 40 days of plant growth. Similarly, coinoculation studies with PGPR and *Rhizobium* or *Bradyrhizobium* sp. showed increase in root and shoot weight, plant vigor, nitrogen fixation and grain yield in various legumes (Valverde *et al*., 2006; Yadegari *et al*., 2008). Phosphate-solubilizing *Bacillus* sp. stimulated plant growth through enhanced P nutrition and increased the uptake of N, P, K and Fe (Biswas *et al*., 2000). Combined inoculation of *Rhizobium* with *Pseudomonas striata* or *Bacillus polymyxa* and with *Bacillus megaterium* showed increased dry matter, grain yield and phosphorus uptake over the uninoculated control in different legumes (Elkoca *et al*., 2008).

2.13. Perspective and future aspects of *Bacillus* species and application in sustainable agriculture

The rhizospheric community is highly complex and it comprises of a myriad of organisms interacting in various ways, acting upon each other and reacting to the external environment. Several isolates of *Bacillus* spp. have been developed as biocontrol agents (BCAs) of plant pests and pathogens. However, for their use as successful BCAs', a greater understanding of their ecology is desired. In this context, greater knowledge of the diversity, distribution and physiology of Gram-positive species will be helpful for identification of new strains compatible with the cropping systems. Paramount to success of PGPR is a need to better understand the ecology of rhizobacteria either indigenous or introduced within the rhizosphere. Exploration and identification of traits involved in the ability of certain bacteria to establish *Bacillus* as PGPR into the rhizosphere to exert effects on plant growth, effectively compete with the indigenous microflora, cooperatively interact with other beneficial members of rhizospheric biota and understanding of the mechanisms (signaling, growth promotory actions, disease suppression etc.) that occur between plants and bacteria are also required. The understanding of taxonomic and physiological diversity of *Bacillus* sp. is required for reducing the disease incidence or severity, and promoting plant growth.

The molecular mechanisms involved in the root colonization and advancement in the molecular and genomic tools offers new possibilities for improving the selection, characterization and management of biological control. Development of proteomics and functional genomics will be helpful to determine and follow expression of crucial genes of BCAs during mass production, formulation and application. Transformation of BCAs by inserting genes that improve the tolerance of antagonists to abiotic stresses such as increased tolerance or resistance to cold, heat, drought, high salinity, heavy metal rich soils or acidic soils etc., could be another exciting and challenging task and may provide with better opportunities to implement the concept of biocontrol in the field under the dynamic natural environments.

CHAPTER-III

MATERIALS AND METHODS

The present investigations were carried out on isolation of antagonistic *Bacillus* sp. from rhizosphere soil and to study their antagonistic effect on the growth of *Fusarium oxysporum*, and to determine their inoculation effect on the growth of chickpea (*Cicer arietinum* L.). *Bacillus* sp. were isolated on the basis of their morphological characteristics. Selected *Bacillus* sp. were characterized for other beneficial activities for plant growth promotion and further examined for coinoculation studies with *Mesorhizobium* strains for their effect on nodulation and plant growth in chickpea under chillum jar conditions as well as for disease control of wilt caused by *Fusarium oxysporum* under pot house conditions. The materials used in the study, procedures used and the techniques adopted during the present investigations are described in this chapter.

3.1. Materials

3.1.1. Chemicals

Various chemicals and medium ingredients used in the investigation were obtained from Hi media company. Other chemicals used were obtained from BDH, Sarabhai chemicals, E-Merck and SRL company.

3.1.2. Host species

Seeds of chickpea (*Cicer arietinum* L.) variety HC-1 were obtained from Department of Plant Breeding, CCS Haryana Agricultural University, Hisar.

3.1.3. Microorganisms used

One hundred ninety three *Bacillus* isolates were obtained from the rhizospheric soil of chickpea by serial dilution plate method using Luria Bertani (LB) medium (Sambrook *et al*., 1989). The *Bacillus* isolates were maintained by periodic transfer on LB slants. Fungi *Fusarium oxysporum* was maintained on potato dextrose agar medium slants. These bacterial and fungal cultures were stored at 4°C in refrigerator for further use.

3.1.4. Preparation of media

Different media were prepared in single distilled water and autoclaved at 15 lbs pressure for 20 minutes. The following media were used during the present investigations.

(i) Luria Bertani medium (Sambrook *et al.*, 1989)

Composition	**g/L**
Tryptone	10.0
Yeast extract	5.0
NaCl	3.0
Glucose	1.0

Agar agar	20.0
pH	7.0

(ii) Modified Luria-Bertani medium

Composition	**g/L**
Tryptone	5.0
Yeast extract	5.0
NaCl	2.0
Glucose	5.0
Peptone	1.0
Agar agar	20.0

(iii) Soil extract agar medium (0.8%)

Components	**g/L**
Soil extract	100 ml
Agar-agar	8.0 g

(iv) Aleksandrov medium (Hu *et al*., 2006)

Composition	**g/L**
Glucose	5.0
$MgSO_4.7H_2O$	0.5
$CaCO_3$	0.1
$FeCl_3$	0.006
Ca_3PO_4	2.0
Mica powder	2.0
Agar agar	20.0

(v) Minimal medium (Dworkin and Foster, 1958)

Composition	**g/L**
KH_2PO_4	4.0
Na_2HPO_4	6.0
$MgSO_4.7H_2O$	0.2
$FeSO_4$	1.0 mg
Boric acid	10 µg
$MnSO_4$	10 µg
$ZnSO_4$	70 µg
$CuSO_4$	50 µg
Molybdenum oxide	10 µg
Glucose	2.0
Gluconic acid	2.0
Citric acid	2.0

Ammonium sulfate	2.0
Agar agar	20.0

In ACC supplemented plates, ammonium sulphate was replaced by 3 mM ACC.

(vi) Pikovskaya medium (Pikovskaya, 1948)

Composition	**g/L**
Glucose	10.0
$(NH_4)SO_4$	0.5
NaCl	0.2
$MgSO_4$. $7H_2O$	0.1
KCl	0.2
Yeast extract	0.5
$MnSO_4$. H_2O	0.002
$FeSO_4$. $7H_2O$	0.002
$Ca_3(PO_4)_2$	5.0
Agar agar	20.0
pH	7.0

(vii) Yeast extract mannitol agar medium

Composition	**g/L**
Mannitol	10.0
K_2HPO_4	0.5
NaCl	0.1
$MgSO_4.7H_2O$	0.2
Sodium glutamate	0.32
$CaCO_3$	0.5
Yeast extract	0.5
Congo red	10.0 ml
Agar agar	20.0

(viii) Sloger's nitrogen-free mineral salt solution (Sloger, 1969)

Composition	**g/L**
K_2HPO_4	0.25
$MgSO_4.7H_2O$	0.2
NaCl	0.2
H_3BO_3	3.0 mg
$CoCl_2$	0.25 mg
$MnCl_2$	4.0 mg
$CuSO_4$	0.20 mg
Na_2MoO_4	0.25 mg

Na_2EDTA (Fe)	29.0 mg
$CaHPO_4$	1.0

(ix) Potato dextrose agar medium

Composition	**g/L**
Potato extract	450 ml
Distilled water	450 ml
Dextrose	9.0
Peptone	1.0
Agar agar	20.0

100 g of sliced potatoes were boiled in 1L of H_2O for about 1 hour and filtered twice through a cloth. The volume was made to 1 liter.

(x) King's B medium (King *et al.*, 1954)

Composition	**g/L**
Peptone	20.0
Glycerol	10.0
Glucose	1.0
K_2HPO_4	1.5
$MgSO_4.7H_2O$	1.5
NaCl	5.0
Agar agar	20.0
pH	7.0

3.2. Methods

3.2.1. Isolation of *Bacillus* sp. from the rhizosphere of chickpea

Soil samples were collected from the rhizosphere of chickpea plants grown in different fields of chickpea in CCS Haryana Agricultural University, Hisar at 60 and 75 days of plant growth. The serial dilutions of soil samples were made in 9.0 ml sterilized water blank (up to 10^{-5}) and 0.1 ml of diluted soil suspension was plated on Luria Bertani medium plates. The plates were incubated at 28±2°C in BOD incubator for 3 to 5 days. *Bacillus* colonies were selected based on typical morphological characteristics. Isolated and purified colonies of *Bacillus* sp. were transferred on LB medium slants (Sambrook *et al.*, 1989) and stored in the refrigerator for further studies.

3.2.2. Study of the antagonistic interactions of selected *Bacillus* sp. with *Fusarium oxysporum, Aspergillus niger, Neovossia indica* and *Rhizoctonia solani* under *in vitro* conditions

Interactions of selected *Bacillus* cultures with pathogenic fungi *Fusarium oxysporum* and *Aspergillus niger, Neovossia indica* and *Rhizoctonia solani* were studied by spot test method (Sindhu *et al.,* 1999) on modified LB medium plates. Fungal cultures were grown on PDA slants for 3 days at 28 ± 2^0C and spore suspension was harvested in 2 ml sterilized water. Spore suspension of each fungus was spreaded (0.1 ml) on modified LB medium agar plates. A loopful of 48 hrs old growth of the *Bacillus* isolate was spotted on modified LB medium agar plates. The inhibition of the growth of phytopathogenic fungi around the spotted growth of *Bacillus* isolates was recorded after 4 days of incubation at 28 ± 2^0C. The *Bacillus* cultures showing efficient antagonistic activity were selected for further studies.

3.2.3. Characterization of other beneficial activities from the antagonistic *Bacillus* cultures for plant growth promotion

Bacillus isolates showing antagonistic activity against the phytopathogenic fungi were studied for different beneficial activities having plant growth promotion attributes.

3.2.3.1. Screening of *Bacillus* cultures for siderophore production

Siderophore production was studied by using CAS (Chrome azurol S) assay (Mayer and Abdallah, 1978; Schwyn and Neilands, 1987).

Solutions

Chrome azurol S (CAS) agar medium

(i) Dye solution (Solution A): Fifty ml of dye solution containing 60.5 mg CAS was prepared in distilled water and mixed with 10 ml of iron (III) solution containing 1 mM $FeCl_3.6H_2O$ in 10 mM HCl. The mixture was slowly added to a solution containing 72.9 mg hexadecyltrimethyl-ammonium bromide (HDTMA) in 40 ml of distilled water. The resultant dark blue liquid was autoclaved at 15 lbs pressure for 10 minutes. (For 100 ml medium, take 10 ml of this solution).

(ii) Solution B: A basal medium containing deferrated 1M sucrose (3 ml), deferrated 1M $CaCl_2$ (0.4 ml), deferrated 1M $MgSO_4.7H_2O$ (0.8 ml), 2% K_2HPO_4 (10 ml), 10% NaCl (2 ml), 5% Na_2MoO_4 (0.1 ml), PIPES buffer (30.42 g) and agar (15 g) in 800 ml of distilled water was prepared. pH of the medium was adjusted to 6.8 by the addition of 50% (w/w) NaOH and autoclaved at 15 lbs pressure for 20 minutes.

(iii) After cooling to 50ºC, 30 ml of 10% mannitol solution was added as a carbon source and then dye solution (solution A) was added to it with gentle mixing to avoid the formation of air bubbles. The molten medium was distributed in sterilized petri plates. Five µl inoculant of each log phase grown culture was spotted on the plates and incubated at 28+2ºC for 3-4 days. The presence of iron chelator (siderophore) is indicated by the decolourization of the blue-coloured ferric dye complex and resulting in formation of yellow halo zones around the colonies.

3.2.3.2. Screening of *Bacillus* isolates for utilization of 1-aminocyclopropane-1- carboxylate

The minimal medium (Dworkin and Foster, 1958) supplemented with 3 mM ACC was prepared (Penrose and Glick, 2003). A loopful of 48 hrs old growth of *Bacillus* isolate was spotted on medium plates. Growth of *Bacillus* isolates on ACC supplemented medium plates was recorded after 5 days of incubation at 28 ± 2^0C. The *Bacillus* isolates showing good growth on ACC supplemented medium plates indicated high efficiency of utilization of ACC as nitrogen source and were selected for further studies. Minimal medium plates containing ammonium sulfate as nitrogen source were used as control plate for growth comparison of different *Bacillus* isolates.

3.2.3.3. Screening of *Bacillus* cultures for hydrogen cyanide production

Hydrogen cyanide production by the *Bacillus* isolates was determined qualitatively by the method of Alstrom and Burns (1989). The selected *Bacillus* cultures were grown on King’s B medium broth supplemented with 4.4 g/l glycine. Three millimeter strips of Whatman no. 42 filter paper were sterilized and soaked in 0.5% picric acid and 2% sodium carbonate solution. These strips were hanged in the test tube inoculated with *Bacillus* cultures. These tubes were pluged with cotton and incubated at 28 ± 2^o C for 5 days. The change in the color of the strips from yellow to orange red was recorded for hydrogen cyanide production. Hydrogen cyanide production by the *Bacillus* isolates was determined quantitatively by the method of (Sadasivam and Manickam, 1992). The colour of strips was eluted by placing the filter paper in a test tube containing 10 ml of distilled water and its absorbance was read at 625 nm.

3.2.3.4. Screening of *Bacillus* cultures for δ-aminolevulinic acid (ALA) production

Bacillus isolates were tested for their ability to produce δ-aminolevulinic acid. Cultures were inoculated in 10 ml LB broth supplemented with 15 mM glycine and succinate, and incubated at 28 ± 2^oC for 48 hrs under stationary conditions of growth. Culture samples were withdrawn and centrifuged at 10,000 rpm for 15 min (Remi Instruments, Mumbai, India). ALA was determined in the culture supernatant by the method as described by Mauzerall and Garnick (1955).

Reagents:

(i) Modified Ehrlich reagent:

p-dimethylaminobenzaldehyde	1.0 g
Perchloric acid (70%)	8 ml
Glacial acetic acid	42 ml

(ii) Buffer:

Acetate buffer (1M; pH 4.6) was made by adding 57 ml of glacial acetic acid (1M) to 136 gm of sodium acetate trihydrate (1M) and diluted to 1 liter. To 0.5 ml of culture supernatant, 50 µl of acetylacetone and 0.5 ml of 1M sodium acetate buffer were added. The mixed solution in the tubes was boiled in a water bath for 15 min. After cooling, 3.5 ml of modified Ehrlich reagent was added. The absorbance of the mixture was measured at 556 nm wavelength after 20 min at room temperature. The concentration of ALA in the culture supernatant was determined by comparing with standard curve.

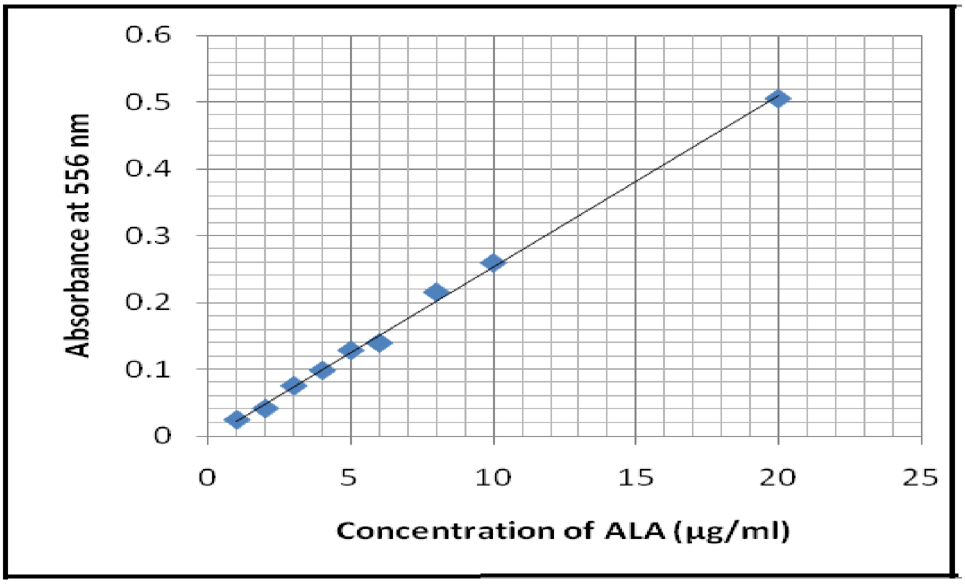


Fig. 2. Standard graph for determination of ALA concentration

3.2.3.5. Screening of *Bacillus* cultures for indole acetic acid (IAA) production

Bacillus isolates were tested for their ability to produce indole acetic acid. Cultures were inoculated in 30 ml LB broth supplemented with DL-tryptophan @ 100 µgml^{-1} (Hartman *et al.*, 1983) and incubated at 28±2°C for 72 hrs under stationary conditions of growth. Culture samples were withdrawn after 72 hrs and centrifuged at 10,000 rpm for 15min (Remi Instruments, Mumbai, India). IAA was determined in the culture supernatant as described by Gordon and Weber (1951).

Reagents:

Salkowski reagent:

Ferric chloride	0.05 M
Perchloric acid (35%)	1000 ml

Two ml of Salkowski reagent was added to two ml of culture supernatant, mixed and allowed to stand for 30 min for the development of pink colour. Colour intensity was estimated at 500 nm wavelength using spectrophotometer (UV-VIS spectrophotometer 117, Systronics Ahemadabad, India) against a reagent blank. Indole acetic acid (100 µg ml^{-1}) was used as standard and results were expressed as µg IAA produced ml^{-1} of culture supernatant.

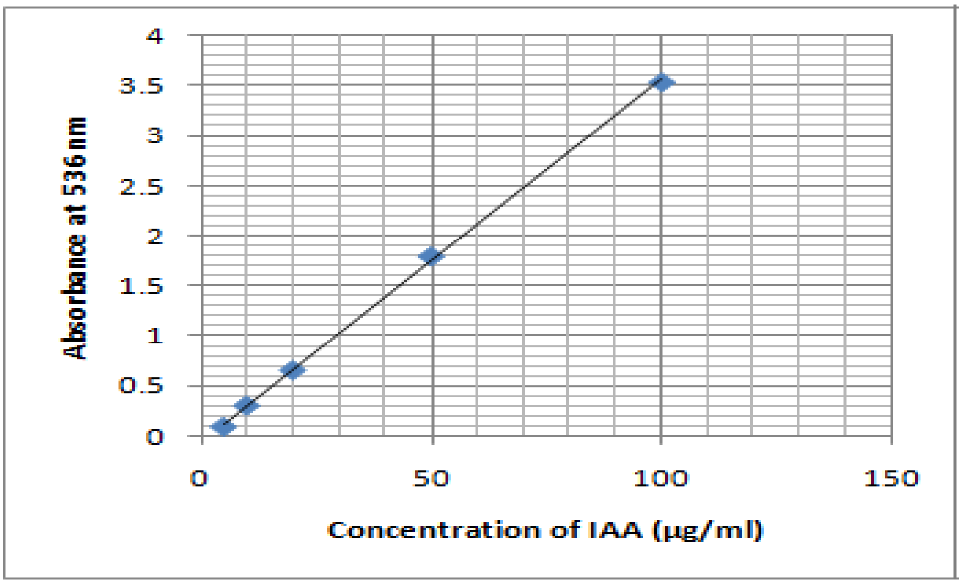


Fig. 3. Standard graph for determination of IAA concentration

3.2.3.6. Phosphorous solubilization by *Bacillus* cultures

Phosphorous solubilization ability of *Bacillus* cultures was determined by the spot test method on Pikovskaya medium (Pikovskaya, 1948) plates. Plates of Pikovskaya medium having tricalcium phosphate and acidic dye bromophenol blue were prepared. A loopful of 48-hr old growth of the *Bacillus* culture was spotted on above prepared plates. Five cultures were spotted on each plate. Plates were incubated at 28±2°C for 2-3 days. Detection of phosphorous solubilization by *Bacillus* cultures was based upon the ability of solubilization zone formation.

3.2.3.7. Potassium solubilization by *Bacillus* cultures

Potassium solubilization by *Bacillus* cultures was studied by the spot test method (Sindhu *et al.*, 1999) on Aleksandrov medium (Aleksandrov *et al.*, 1967) plates. Plates of Aleksandrov medium having mica powder (insoluble form of potassium) and with/without acidic dye bromothymol blue were prepared. A loopful of 48-hr old growth of the *Bacillus* culture was spotted on above prepared plates. Five cultures were spotted on each plate. Plates were incubated at 28±2°C for 2-3 days. Detection of potassium solubilization by *Bacillus* isolates was based upon the ability of solubilization zone formation and change of color from greenish blue to yellow. K-solubilizing activity was determined by formation of solubilization zone and solubilization index (K-SI) was calculated for each selected isolate by the following formula:

$$\text{Potassium-solubilizing index (K-SI)} = \frac{\text{Colony diameter + Clear zone diameter}}{\text{Colony diameter}}$$

3.2.4. Effect of antagonistic *Bacillus* isolates on seed germination of chickpea

Selected antagonistic *Bacillus* isolates were studied for their effect on seed germination of chickpea on agar plates. Surface sterilized seeds were used for germination on soil extract agar (0.8%) medium plates after inoculation with different selected antagonistic *Bacillus* isolates. The seeds were grown at 28 ± 2°C in the B.O.D. incubator and growth of seedlings was recorded after 5 and 10 days of growth. The observations for retardation/stimulation of root and shoot growth of chickpea seedlings were recorded.

3.2.5. Isolation of *Mesorhizobium* isolates from nodules of chickpea

Plant roots of chickpea (bearing nodules) were collected from CCS HAU, Hisar farm. Plants samples collected from different locations were brought in the laboratory and washed with tap water to remove adhering soil particles. Healthy pink nodules were surface sterilized by immersing in 0.1% $HgCl_2$ solution for 5 minutes. After $HgCl_2$ treatment, the nodules were washed with sterilized water 3 to 5 times to get rid of the sterilizing agent. The surface sterilized nodules were crushed in sterilized plates and suspension was streaked on congo red containing-yeast extract mannitol agar (YEMA) medium plates. The plates were incubated at $28+2^0$C for 3 to 8 days. Isolated colonies of mesorhizobia were transferred on YEMA medium slants and stored in refrigerator at 4^0C for further studies.

3.2.6. Growth inhibition of *Fusarium oxysporum* by *Mesorhizobium* isolates for antagonistic activity

Growth inhibition of pathogenic fungi *Fusarium oxysporum* by *Mesorhizobium* isolates was studied by spot test method (Sindhu *et al.*, 1999) on modified YEMA-LB plates. *F. oxysporum* cultures were grown on PDA slants for 3 days at 28 ± 2^0C and spore suspension was harvested in 2 ml sterilized water. Spore suspension was spread (0.1 ml) on modified YEMA-LB medium agar plates. A loopful of 48 hrs old growth of the *Mesorhizobium* isolates was spotted on agar plates. The inhibition of the growth of phytopathogenic fungi around the spotted growth of *Mesorhizobium* isolates was recorded after 4 days of incubation at 28 ± 2^0C. The isolates showing zone of inhibition were selected and used for further studies.

3.3. Screening of *Bacillus* and *Mesorhizobium* isolates for growth at different salt concentrations

Bacillus and *Mesorhizobium* isolates were checked for their ability to grow at different concentrations of NaCl, i.e., 1, 2, 4, 6 and 8% (w/v), on LB and YEMA medium, respectively containing 20 mM HEPES (N-2-hydroxyethane-sulphonic acid) buffer (Marsudi *et al.*, 1999). Medium plates were spotted with a 20 µl growth suspension of different *Bacillus* isolates. The plates were incubated for 3-4 days at 28±2°C in a B.O.D. incubator. The susceptibility or tolerance to NaCl was recorded by observing the growth and colony size in different salt concentrations.

3.4. Effect of isolated *Mesorhizobium* isolates from nodules on nodulation and plant growth of chickpea under chillum jar conditions

Isolated *Mesorhizobium* isolates alongwith two reference *Mesorhizobium* strains MBD26 and KR48 were studied for their effect on nodulation and plant growth of chickpea under chillum jar conditions. Surface sterilized seeds of chickpea were inoculated with 10 ml of each cultures having 10^7 to 10^8 cell/ml of growth suspension. Inoculated seeds were sown in autoclaved chillum jar assemblies (with two replications for each treatment).

S. No.	Inoculation with bacterial cultures
T1	Control
T2	*Mesorhizobium*strain MBD26
T3	*Mesorhizobium*strain KR48
T4	*Mesorhizobium* isolate MCA1
T5	*Mesorhizobium* isolate MCA2
T6	*Mesorhizobium* isolate MCA3
T7	*Mesorhizobium* isolate MCA4
T8	*Mesorhizobium* isolate MCA5
T9	*Mesorhizobium* isolate MCA6
T10	*Mesorhizobium* isolate MCA7
T11	*Mesorhizobium* isolate MCA8
T12	*Mesorhizobium* isolate MCA9
T13	*Mesorhizobium* isolate MCA10
T14	*Mesorhizobium* isolate MCA11
T15	*Mesorhizobium* isolate MCA12
T16	*Mesorhizobium* isolate MCA13
T17	*Mesorhizobium* isolate MCA14
T18	*Mesorhizobium* isolate MCA15
T19	*Mesorhizobium* isolate MCA16
T20	*Mesorhizobium* isolate MCA17
T21	*Mesorhizobium* isolate MCA18
T22	*Mesorhizobium* isolate MCA19
T23	*Mesorhizobium* isolate MCA20
T24	*Mesorhizobium* isolate MCA21
T25	*Mesorhizobium* isolate MCA22
T26	*Mesorhizobium* isolate MCA23
T27	*Mesorhizobium* isolate MCA24

The observations for the nodulation and plant dry weight were recorded at 40 and 80 days of plant growth.

3.5. Coinoculation studies of selected *Bacillus* isolates with *Mesorhizobium* strains for their effect on nodulation and plant growth of chickpea under chillum jar conditions

Selected *Bacillus* isolates were used for coinoculation with symbiotically effective *Mesorhizobium* strains in chickpea under chillum jar conditions. Surface sterilized seeds of chickpea were inoculated with 10 ml of each cultures having 10^7 to 10^8 cell/ml of growth suspension. The inoculated seeds were sown in autoclaved chillum jar assemblies (with three replications for each treatment).

S. No.	Inoculation with bacterial cultures
T1	Control
T2	*Mesorhizobium* strain MBD26
T3	*Mesorhizobium* strain KR48
T4	Antagonistic *Bacillus* isolate HCA61
T5	Antagonistic *Bacillus* isolate RCA3
T6	Antagonistic *Bacillus* isolate RCA7
T7	*Bacillus* isolate (control) SYB101
T8	*Bacillus* isolate (control) HCA76
T9	*Mesorhizobium* strain MBD26 + antagonistic *Bacillus* isolate HCA61
T10	*Mesorhizobium* strain MBD26 + antagonistic *Bacillus* isolate RCA3
T11	*Mesorhizobium* strain MBD26 + antagonistic *Bacillus* isolate RCA7
T12	*Mesorhizobium* strain MBD26 + *Bacillus* isolate (control) SYB101
T13	*Mesorhizobium* strain MBD26 + *Bacillus* isolate (control) HCA76
T14	*Mesorhizobium* strain KR48 + antagonistic *Bacillus* isolate HCA61
T15	*Mesorhizobium* strain KR48 + antagonistic *Bacillus* isolate RCA3
T16	*Mesorhizobium* strain KR48 + antagonistic *Bacillus* isolate RCA7
T17	*Mesorhizobium* strain KR48 + *Bacillus* isolate (control) SYB101
T18	*Mesorhizobium* strain KR48 + *Bacillus* isolate (control) HCA76

The observations for the nodulation and plant dry weight were recorded at 60, 75 and 90 days of plant growth. Completely randomized design (CRD) was used for experimental data analysis.

3.6. Coinoculation studies of *Mesorhizobium* and selected *Bacillus* isolates for disease control of *Fusarium oxysporum* and plant growth of chickpea under pot house conditions

Selected three antagonistic *Bacillus* isolates alongwith *Bacillus* isolates SYB101 and HCA76 (control) were tested for disease control using wilt sensitive chickpea variety under pot house conditions with the following treatments. Inoculated chickpea seeds were grown in pots (with three replications of each treatment) containing 10 kg of soil prepared with river sand and soil (mixed in the ratio of 30:70). Fungal spore suspension was inoculated in the soil in T9 to T26 treatments.

S. No.	Inoculation with microbial cultures
T1	Soil + Control
T2	Soil + *Mesorhizobium* strain MBD26
T3	Soil + *Mesorhizobium* strain KR48
T4	Soil + *Bacillus* isolate HCA61
T5	Soil + *Bacillus* isolate RCA3
T6	Soil + *Bacillus* isolate RCA7
T7	Soil + *Bacillus* isolate SYB101
T8	Soil + *Bacillus* isolate HCA76
T9	Soil + *Fusarium oxysporum*
T10	Soil + *F. oxysporum* + *Mesorhizobium* strain MBD26
T11	Soil + *F. oxysporum*+ *Bacillus* isolate HCA61
T12	Soil + *F. oxysporum*+ *Bacillus* isolate RCA3
T13	Soil + *F. oxysporum* + *Bacillus* isolate RCA7
T14	Soil + *F. oxysporum* + *Bacillus* isolate SYB101
T15	Soil + *F. oxysporum* + *Bacillus* isolate HCA76
T16	Soil + *F. oxysporum* + *Mesorhizobium* strain MBD26 + *Bacillus* isolate HCA61
T17	Soil + *F. oxysporum* + *Mesorhizobium* strain MBD26 + *Bacillus* isolate RCA3
T18	Soil + *F. oxysporum* + *Mesorhizobium* strain MBD26 + *Bacillus* isolate RCA7
T19	Soil + *F. oxysporum* + *Mesorhizobium* strain MBD26 + *Bacillus* isolate SYB101
T20	Soil + *F. oxysporum* + *Mesorhizobium* strain MBD26 + *Bacillus* isolate HCA76
T21	Soil + *F. oxysporum* + *Mesorhizobium* strain KR48
T22	Soil + *F. oxysporum* + *Mesorhizobium* strain KR48 + *Bacillus* isolate HCA61
T23	Soil + *F. oxysporum* + *Mesorhizobium* strain KR48 + *Bacillus* isolate RCA3
T24	Soil + *F. oxysporum* + *Mesorhizobium* strain KR48 + *Bacillus* isolate RCA7
T25	Soil + *F. oxysporum* + *Mesorhizobium* strain KR48 + *Bacillus* isolate SYB101
T26	Soil + *F. oxysporum* + *Mesorhizobium* strain KR48 + *Bacillus* isolate HCA76

Observations for shoot and root growth, nodule number, nodule weight and control of plant disease were recorded at 60, 75 and 90 days of plant growth.

3.6.1. Plant fresh and dry weight

Plants were carefully uprooted and washed under tap water. Roots were separated from shoots. Shoot and root portions of the plants were weighed. Then, they were dried in oven at 90°C for 24 hrs and weighed again to determine root and shoot dry weight.

3.6.2. Disease incidence and reduction in disease

On the basis of symptoms observed per pot, percent disease incidence and percent disease control were calculated by following formulae.

$$\text{Disease incidence (DI) \%} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

$$\text{Disease control \%} = \frac{\text{No. of infected plants in control - No. of infected plants in treatment}}{\text{No. of infected plants in control}} \times 100$$

Disease control and disease incidence were recorded at 60, 75 and 90 days of sowing. It was calculated on the average of five plants grown per pot.

CHAPTER-IV

RESULTS

Certain bacteria isolated from the rhizosphere soil possess useful properties that allow them to exert beneficial effects on plants either by promoting plant growth or by reducing the damages caused by plant pathogens/pests. Some of these rhizosphere bacteria such as *Pseudomonas* and *Bacillus* have emerged as important biological inputs in agricultural soils in which *Bacillus* is more prominent due to its unique characterstics. During the present investigations, one hundred ninety three *Bacillus* isolates were obtained from the rhizosphere soil of chickpea. The effect of *Bacillus* cultures was studied on growth inhibition of fungal pathogen *Fusarium oxysporum* and their inoculation effect on seed germination of chickpea was tested under culture conditions. The coinoculation effect of antagonistic *Bacillus* isolates with *Mesohizobium* strains was investigated for control of Fusarium wilt disease and growth promotion of chickpea under chillum jar and pot house conditions. Observations for nodule number, nodule dry weight, shoot dry weight and root dry weight were recorded after 60, 75 and 90 days of plant growth.

4.1 Isolation of *Bacillus* sp. from chickpea rhizosphere soil

One hundred ninety three bacterial isolates were obtained from soil samples collected from the rhizosphere of chickpea grown in CCS Haryana Agricultural University, Hisar and farmers' field in Alwar District of Rajasthan at 75 and 90 days of plant growth. The serial dilutions of rhizosphere soil samples were made up to 10^{-5} and dilutions were plated on Luria Bertani medium. *Bacillus* isolates were selected based on morphological characterstics (Table 2, 3, Fig. 4). One reference strain of *Bacillus subtilis* and culture of pathogenic fungus *Fusarium oxysporum* were obtained from Department of Microbiology, CCS H.A.U., Hisar.

Table 2. List of *Bacillus* isolates recovered from rhizosphere of chickpea fields in Hisar, Haryana

Bacterial sp.	Source	Location	*Bacillus* isolates
Bacillus isolates	Chickpea field- 1	Farm area of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar	HCA1, HCA2, HCA3, HCA4, HCA5, HCA6, HCA7, HCA8, HCA9, HCA10, HCA11, HCA12, HCA13, HCA14, HCA15, HCA16, HCA17, HCA18, HCA19, HCA20, HCA21, HCA22, HCA23, HCA24, HCA25, HCA26, HCA27, HCA28, HCA29, HCA30, HCA31, HCA32, HCA33, HCA34, HCA35, HCA36, HCA37, HCA38, HCA39, HCA40, HCA41, HCA42, HCA43, HCA44, HCA45, HCA46, HCA47, HCA48, HCA49, HCA50

Bacillus isolates	Chickpea field- 2	Farm area of Department of Agronomy, CCS Haryana Agricultural University, Hisar	HCA51, HCA52, HCA53, HCA54, HCA55, HCA56, HCA57, HCA58, HCA59, HCA60, HCA61, HCA62, HCA63, HCA64, HCA65, HCA66, HCA67, HCA68, HCA69, HCA70, HCA71, HCA72, HCA73, HCA74, HCA75, HCA76, HCA77
Bacillus isolates	Chickpea field- 3	Farm area of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar	HCA78, HCA79, HCA80, HCA81, HCA82, HCA83, HCA84, HCA85, HCA86, HCA87, HCA88, HCA89, HCA90, HCA91, HCA92, HCA93, HCA94, HCA95, HCA96, HCA97, HCA98, HCA99, HCA100, HCA101, HCA102, HCA103, HCA104, HCA105
Reference strain	Deptt. of Microbiology, CCS H.A.U., Hisar	-	SYB101

Table 3. List of *Bacillus* isolates recovered from rhizosphere of chickpea fields in Alwar, Rajasthan

Bacterial sp.	**Source**	**Location**	***Bacillus* isolates**
Bacillus isolates	Chickpea field- 4	Alwar, Rajasthan	RCA1, RCA2, RCA3, RCA4, RCA5, RCA6, RCA7, RCA8, RCA9, RCA10, RCA11, RCA12, RCA13, RCA14, RCA15, RCA16, RCA17, RCA18, RCA19, RCA20, RCA21, RCA22, RCA23, RCA24, RCA25, RCA26, RCA27, RCA28, RCA29, RCA30, RCA31, RCA32, RCA33, RCA34, RCA35
Bacillus isolates	Chickpea field- 5	Alwar, Rajasthan	RCA36, RCA37, RCA38, RCA39, RCA40, RCA41, RCA42, RCA43, RCA44, RCA45, RCA46, RCA47, RCA48, RCA49, RCA50, RCA51, RCA52, RCA53, RCA54, RCA55, RCA56, RCA57, RCA58, RCA59, RCA60, RCA61, RCA62, RCA63
Bacillus isolates	Chickpea field- 6	Alwar, Rajasthan	RCA64, RCA65, RCA66, RCA67, RCA68, RCA69, RCA70, RCA71, RCA72, RCA73, RCA74, RCA75, RCA76, RCA77, RCA78, RCA79, RCA80, RCA81, RCA82, RCA83, RCA84, RCA85, RCA86, RCA87, RCA88

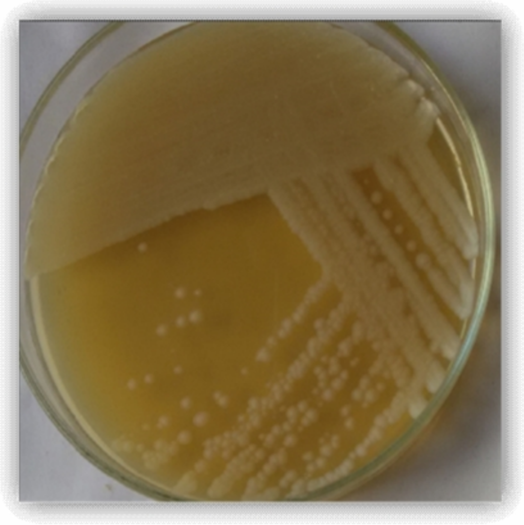


Fig. 4. Isolated colonies of *Bacillus* species

4.2. Antagonistic interactions of *Bacillus* isolates with *Fusarium oxysporum* under *in vitro* conditions

All the 193 *Bacillus* isolates were screened for their antagonistic interactions using spot test method (Sindhu *et al*., 1999) against *Fusarium oxysporum* on modified LB medium plates. Growth suspension (2 µl) of *Bacillus* cultures was spotted on fungus preseeded plates and plates were incubated at 28±2ºC for 2 days. Antagonistic activity was determined on the ability of bacteria to inhibit fungal growth under culture conditions. Out of 193 *Bacillus* isolates tested, only 87 isolates inhibited the growth of *F. oxysporum* (Table 4). The fungal growth inhibition zone varied with different isolates tested (Fig. 5a, b, 6). Thirty one isolates showed more than 10 mm inhibition zone, whereas eleven isolates i.e., HCA3, HCA10, HCA26, HCA29, HCA41, HCA44, HCA53, HCA61, RCA3, RCA7 and RCA9 showed large inhibition zones measuring more than 20 mm. Out of 87 antagonistic *Bacillus* isolates, 24 isolates showing variable effect on inhibition of fungus were selected for further experiments (Table 5).

Table 4. Growth inhibition of *F. oxysporum* by *Bacillus* isolates

Sr. no.	***Bacillus* Isolates**	**Inhibition of *F. oxysporum* (Halo zone size) (mm)**	**Sr. no.**	***Bacillus* isolates**	**Inhibition of *F. oxysporum* (Halo zone size) (mm)**
1.	HCA1	13.3	**47.**	RCA3	27.0
2.	HCA3	25.4	**48.**	RCA4	19.8
3.	HCA6	7.1	**49.**	RCA7	20.4
4.	HCA7	6.3	**50.**	RCA9	25.5
5.	HCA8	7.1	**51.**	RCA10	5.1
6.	HCA9	6.2	**52.**	RCA11	19.5
7.	HCA10	20.6	**53.**	RCA12	4.8
8.	HCA11	13.2	**54.**	RCA14	9.1

9.	HCA12	6.1	**55.**	RCA15	4.5
10.	HCA19	12.6	**56.**	RCA17	4.8
11.	HCA20	6.4	**57.**	RCA18	4.7
12.	HCA21	5.9	**58.**	RCA19	11.0
13.	HCA22	6.0	**59.**	RCA22	5.1
14.	HCA23	12.8	**60.**	RCA23	4.7
15.	HCA24	6.0	**61.**	RCA24	12.2
16.	HCA26	25.1	**62.**	RCA25	4.6
17.	HCA28	7.2	**63.**	RCA29	4.5
18.	HCA29	21.1	**64.**	RCA30	4.5
19.	HCA32	5.7	**65.**	RCA31	0.0
20.	HCA34	5.9	**66.**	RCA38	8.9
21.	HCA35	12.8	**67.**	RCA39	8.7
22.	HCA36	13.9	**68.**	RCA40	5.2
23.	HCA40	14.6	**69.**	RCA41	13.2
24.	HCA41	20.2	**70.**	RCA42	19.4
25.	HCA43	6.3	**71.**	RCA46	4.4
26.	HCA44	21.3	**72.**	RCA48	5.0
27.	HCA45	6.0	**73.**	RCA50	4.3
28.	HCA46	7.2	**74.**	RCA51	4.3
29.	HCA48	6.4	**75.**	RCA56	9.8
30.	HCA50	6.3	**76.**	RCA57	4.2
31.	HCA52	14.1	**77.**	RCA58	9.7
32.	HCA53	26.5	**78.**	RCA59	4.6
33.	HCA54	5.8	**79.**	RCA61	5.1
34.	HCA56	5.3	**80.**	RCA63	11.0
35.	HCA61	27.2	**81.**	RCA68	10.6
36.	HCA62	5.4	**82.**	RCA70	0.0
37.	HCA69	5.5	**83.**	RCA71	4.8
38.	HCA76	0.0	**84.**	RCA73	11.0
39.	HVA80	0.0	**85.**	RCA74	4.2
40.	HCA81	10.1	**86.**	RCA75	4.2
41.	HCA82	5.1	**87.**	RCA76	10.3
42.	HCA84	5.0	**88.**	RCA77	12.6
43.	HCA94	4.8	**89.**	RCA78	6.9
44.	HCA96	4.9	**90.**	RCA85	9.8
45.	HCA101	5.0	**91.**	RCA86	4.3
46.	HCA105	11.6	**92.**	SYB101	0.0

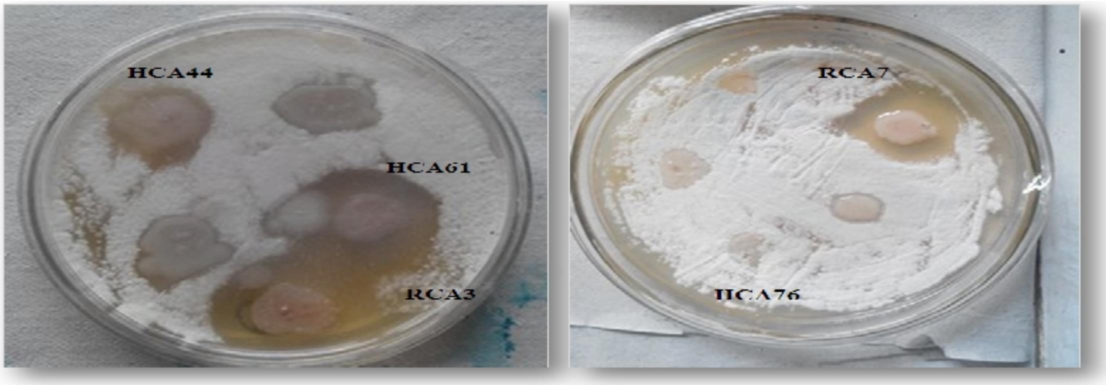


Fig. 5a. Growth inhibition of *F. oxysporum* by *Bacillus* isolates

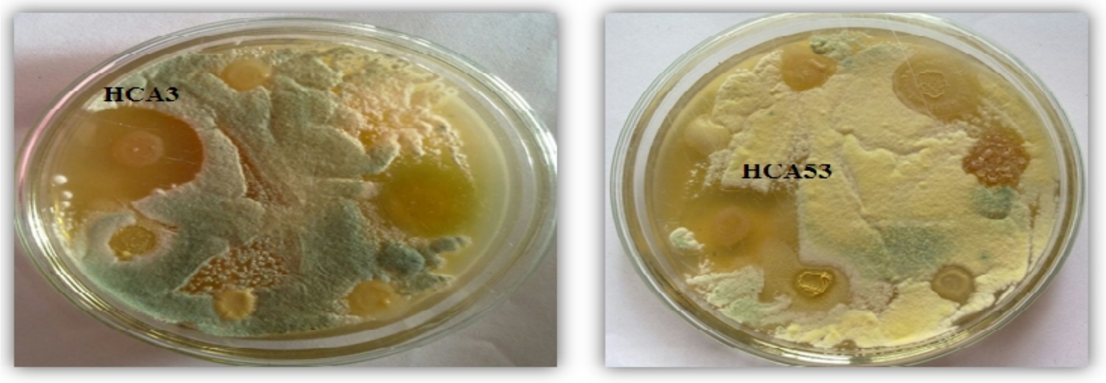


Fig. 5b. Antagonistic activity of *Bacillus* isolates *against F. oxysporum*

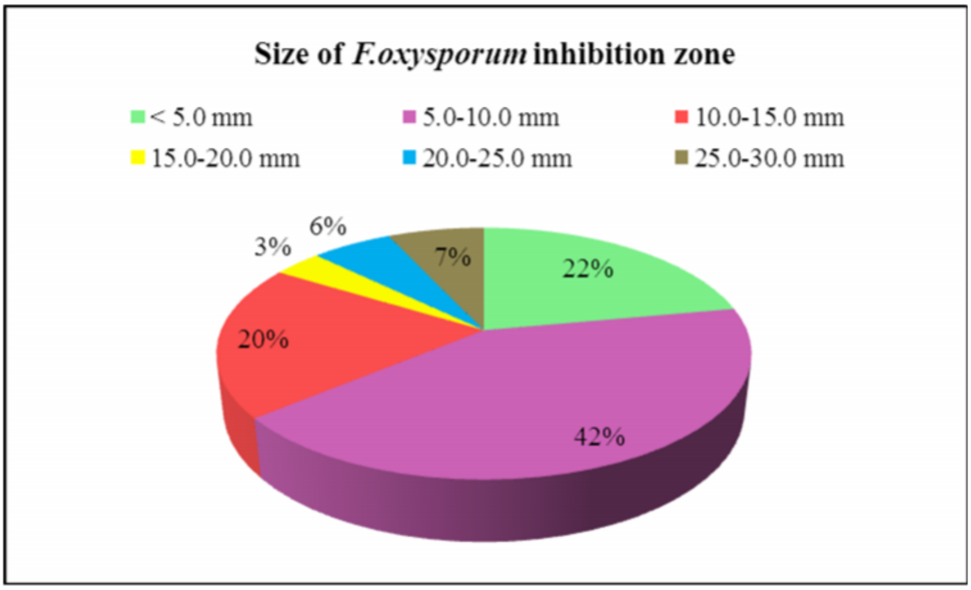


Fig. 6. Categorization of *Bacillus* isolates on the basis of size of *F. oxysporum* inhibition zone

Table 5. Selected antagonistic *Bacillus* isolates used for further studies

***Bacillus* isolates**	**Inhibition of *F.oxysporum* (Halo zone size) (mm)**	***Bacillus* isolates**	**Inhibition of *F. oxysporum* (Halo zone size)(mm)**
HCA1	13.3	HCA105	11.6
HCA3	25.4	RCA3	27.0
HCA10	20.6	RCA4	19.8
HCA11	13.2	RCA7	20.4
HCA26	25.1	RCA9	25.5
HCA41	20.2	RCA11	19.5
HCA44	21.3	RCA19	11.0
HCA53	26.5	RCA31	0.0
HCA61	27.2	RCA63	11.0
HCA76	0.0	RCA68	10.6
HCA80	0.0	RCA70	0.0
HCA81	10.1	SYB101	0.0

4.3. Growth inhibition of *Aspergillus niger, Neovossia indica* and *Rhizoctonia solani* by *Bacillus* isolates

Antifungal activity of *Bacillus* isolates was also tested against *Aspergillus niger, Neovossia indica* and *Rhizoctonia solani* on the basis of growth inhibition zone formed on modified LB medium plates. Out of twenty four *Bacillus* isolates tested, twelve isolates showed significant inhibition of *Aspergillus niger* on plates and three *Bacillus* isolates showed little inhibition of fungal growth (Table 6; Fig. 7). In case of *Neovossia indica*, *Bacillus*isolate RCA9 showed maximum antagonistic activity on fungal spore preseeded plates. Two *Bacillus* isolates RCA3 and HCA61 showed significant antagonistic activity and six isolates RCA63, HCA3, HCA26, HCA41, HCA53 and HCA105 caused moderate growth inhibition of fungi (Table 7; Fig. 8).

Table 6. Extent of growth inhibition of different bacterial isolates against *A. niger*

***Bacillus* isolates**	**Extent of inhibition**
RCA4, RCA7, RCA19, RCA31, RCA68, RCA70, HCA26, HCA76, HCA80	-
RCA9, HCA1, HCA81	+
RCA3, RCA63, HCA10, HCA11, HCA53, SYB101	++
RCA11, HCA3, HCA41, HCA44, HCA61, HCA105	+++

+++ : maximum inhibition, ++ : moderate inhibition, + : little inhibition, - : no inhibition

Table 7. Extent of growth inhibition of different bacterial isolates against *N. indica*

***Bacillus* isolates**	**Extent of inhibition**
RCA4, RCA9, RCA11, RCA31, RCA68, RCA70, HCA11, HCA76, HCA80, HCA81, SYB101	-
RCA7, HCA1, HCA10, HCA44	+
RCA63, HCA3, HCA26, HCA41, HCA53, HCA105	++
RCA3, HCA61	+++
RCA9	++++

++++ : maximum inhibition, +++ : moderate inhibition, ++ : little inhibition, + : very little inhibition, - : no inhibition

In case of *Rhizoctonia solani*, maximum inhibition of fungal growth was observed by *Bacillus* isolates HCA26 and RCA63, and isolates RCA9 and HCA41 also showed significant inhibition of fungal growth (Table 8; Fig. 9). Five *Bacillus* isolates RCA3, HCA53, HCA11, HCA61 and HCA105 showed comparatively less inhibition of fungal growth, whereas isolates RCA7, RCA11, RCA68, HCA1, HCA3, HCA10, HCA44, HCA80 and SYB101 showed little inhibition. Six *Bacillus* isolates did not inhibit the growth of *R. solani*.

Table 8. Extent of growth inhibition of different bacterial isolates against *R. solani*

***Bacillus* isolates**	**Extent of inhibition**
RCA4, RCA19, RCA31, RCA70, HCA76, HCA81	-
RCA7, RCA11, RCA68, HCA1, HCA3, HCA10, HCA44, HCA80, SYB101	+
RCA3, HCA53, HCA11, HCA61, HCA105	++
RCA9, HCA41	+++
RCA63	++++
HCA26	+++++

+++++ : maximum inhibition, ++++ : moderate inhibition, +++ : less inhibition, ++ : very less inhibition, + : very very less inhibition, - : no inhibition

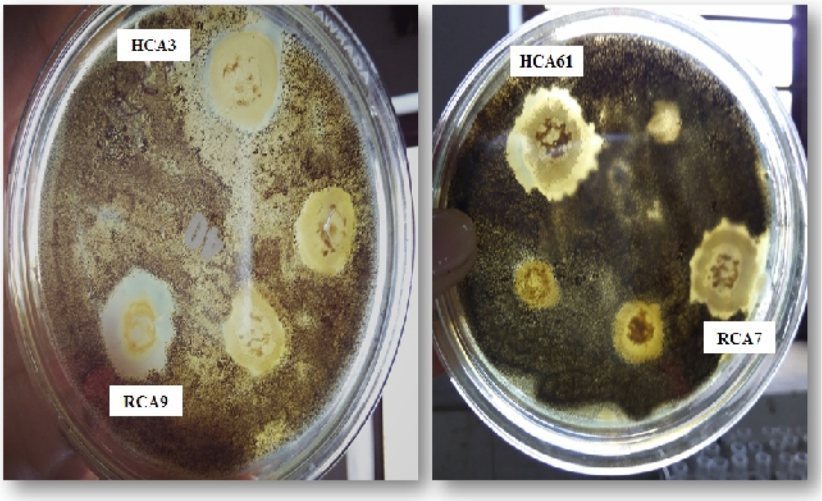


Fig. 7. Growth inhibition of *Aspergillus niger* by *Bacillus* isolates

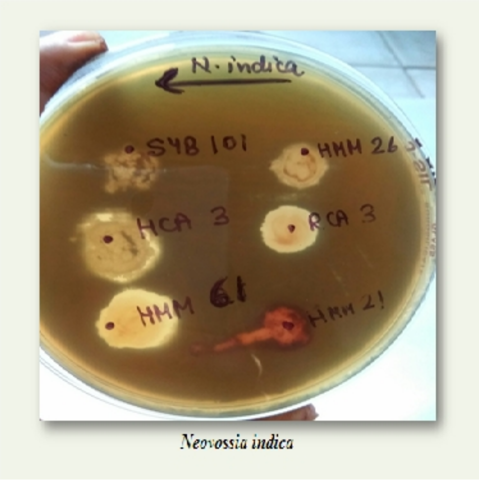


Fig. 8. Growth inhibition of *Neovossia indica* by *Bacillus* isolates

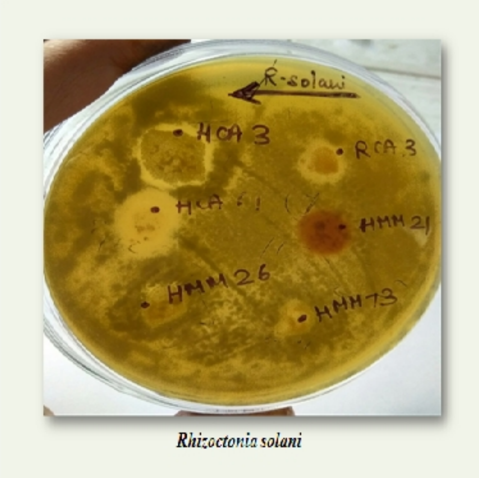


Fig. 9. Growth inhibition of *Rhizoctonia solani* by *Bacillus* isolates

Six *Bacillus* isolates HCA61, RCA3, HCA53, RCA9, HCA3 and HCA26 showed maximum inhibition (>25.0 mm) of *F. oxysporum*. RCA9 did not inhibit the growth of *N. indica* and very little inhibition of *A. niger* was observed. Isolate HCA61 showed significant growth inhibition of *F. oxysporum*, *A. niger* and *N. indica* but only moderate inhibition of *R. solani* was observed. Another isolate HCA26 which caused significant inhibition of *F. oxysporum* and *R. solani* did not inhibit the growth of *A. niger*. Isolate RCA63 caused significant inhibition of *R. solani*, whereas only moderate inhibition of other fungi was observed. The results showed the differential pattern of fungal growth inhibition due to antifungal metabolites production by different *Bacillus* isolates.

4.4. Characterization of beneficial activities of the selected antagonistic *Bacillus* cultures

Twenty four *Bacillus* isolates showing variable level of antagonistic activity against phytopathogenic fungus i.e., *F. oxysporum* were selected for study of different beneficial plant growth promoting activities.

4.4.1. Screening of *Bacillus* isolates for siderophore production

Siderophore production was tested by Chrome Azurol Sulfonate (CAS) assay method (Mayer and Abdallah, 1978). Siderophore producing isolates changed the blue color of the medium and formed yellow or orange halo zones around the colony. All 24 rhizobacterial isolates were spotted on the CAS medium plates and incubated at 28±2ºC in BOD incubator for ten days. Four isolates HCA1, HCA3, HCA53 and RCA11 showed large orange halo zones, whereas five isolates showed small halo zones around the bacterial growth (Table 9; Fig. 10a, b). The ability to produce siderophore was found only in 41% of the *Bacillus* isolates (Fig. 11).

Table 9. Siderophore production by selected antagonistic *Bacillus* isolates

***Bacillus* isolates**	**Siderophore production**
HCA1, HCA3, HCA53, RCA11	+++
HCA11, HCA26, HCA61, RCA3, RCA7	++
HAC1, HCA10, HCA41, HCA44, HCA76, HCA80, HCA81, HCA105, RCA4, RCA9, RCA31, RCA63, RCA68, RCA70, SYB101	-

+++ : maximum zone formation; ++ : moderate zone formation; - : no zone formation

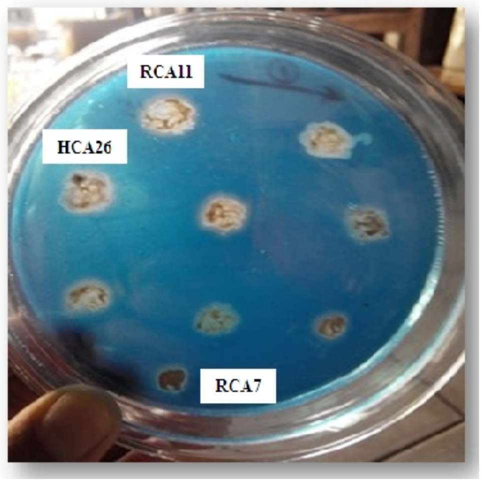


Fig. 10a. Zone formation by different *Bacillus* isolates on CAS medium

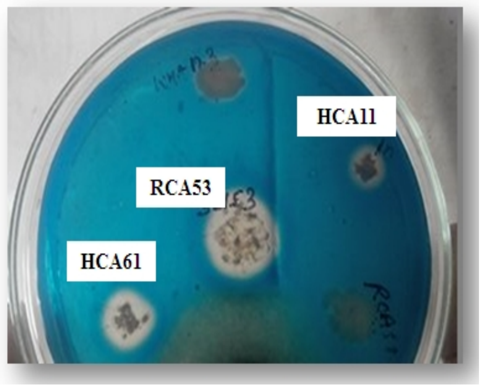


Fig. 10b. Siderophore production by *Bacillus* isolates on CAS medium

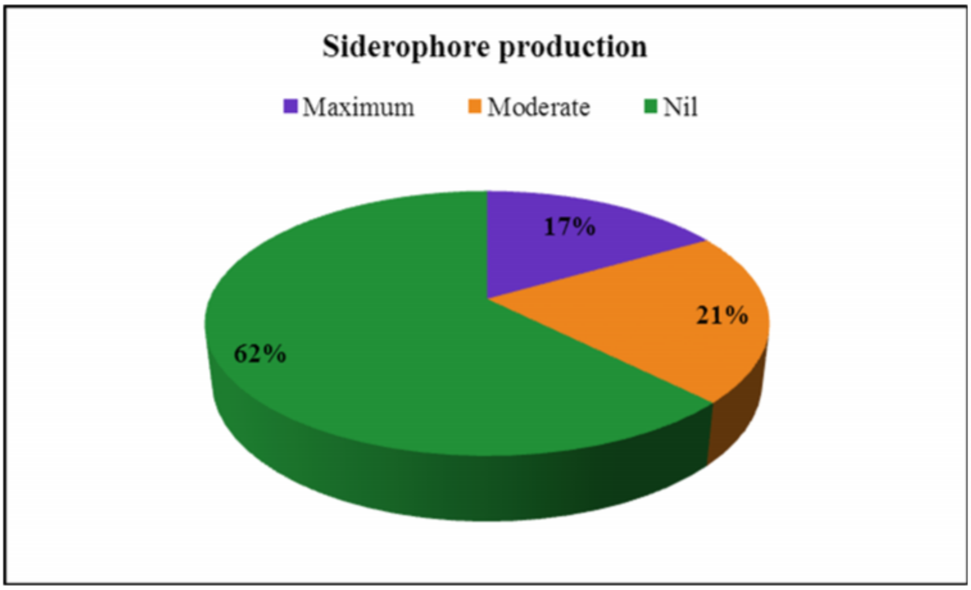


Fig. 11. Categorization of *Bacillus* isolates on the basis of siderophore production

4.4.2. Screening of *Bacillus* isolates for utilization of 1-aminocyclopropane-1- carboxylate (ACC)

Selected antagonistic *Bacillus* isolates were screened for utilization of ACC by spotting 2 days old bacterial growth on the minimal medium (Dworkin and Foster, 1958) plates supplemented with 3 mM ACC. Out of 24 isolates, seven isolates i.e., HCA3, HCA61, RCA3, RCA4, RCA7, RCA11 and RCA31 showed significant growth on ACC supplemented plates and sixteen isolates showed moderate growth (Table 10). RCA9 isolate showed little growth on ACC plates. Overall, 96% *Bacillus* isolates showed ACC utilization ability (Fig. 12). On ammonium sulphate containing plates, seven *Bacillus* isolates i.e., HCA11, RCA3, RCA4, RCA19, RCA31, RCA63 and RCA68 showed more growth, whereas eight isolates showed good growth (Table 11; Fig. 13). Seven isolates showed moderate growth and only two isolates i.e., RCA9 and HCA1 showed little growth on ammonium sulphate containing plates. Different isolates showed distinct and variable growth pattern on ACC and aluminum sulphate supplemented plates (Fig. 14).

Table 10. ACC utilization by *Bacillus* isolates on ACC supplemented medium plates

***Bacillus* isolates**	**Category of ACC utilizing isolates**
HCA3, HCA61, RCA3, RCA7, RCA4, RCA11, RCA31	+++
HCA1, HCA10, HCA11, HCA26, HCA41, HCA44, HCA61, HCA76, HCA80, HCA81, HCA105, RCA9, RCA63, RCA68, RCA70, SYB101	++
RCA9	+/-

Growth characteristics of *Bacillus* isolates on minimal medium: +++ : significant growth, ++ : moderate growth, + : less growth

Table 11. Growth of *Bacillus* isolates on ammonium sulphate containing medium

***Bacillus* isolates**	**Category of ammonium sulphate utilizing isolates**
HCA11, RCA3, RCA4, RCA19, RCA31, RCA63, RCA68	+++++
HCA3, HCA10, HCA41, HCA44, HCA61, HCA105, RCA7, RCA11	++++
HCA26, HCA53, HCA76, HCA80, HCA81, RCA70, SYB101	+++
RCA9, HCA1	++

Ammonium sulphate utilization characteristics of *Bacillus* isolates on minimal medium: +++++ : very good growth, ++++ : good growth, +++ : moderate growth, ++ : less growth

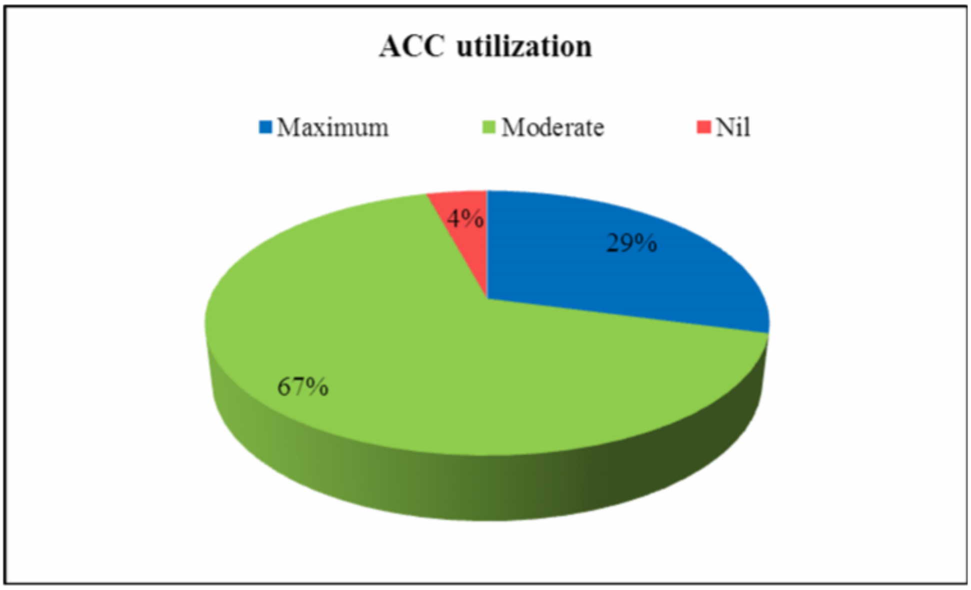


Fig. 12. Categorization of *Bacillus* isolates on the basis of ACC utilization on ACC supplemented medium

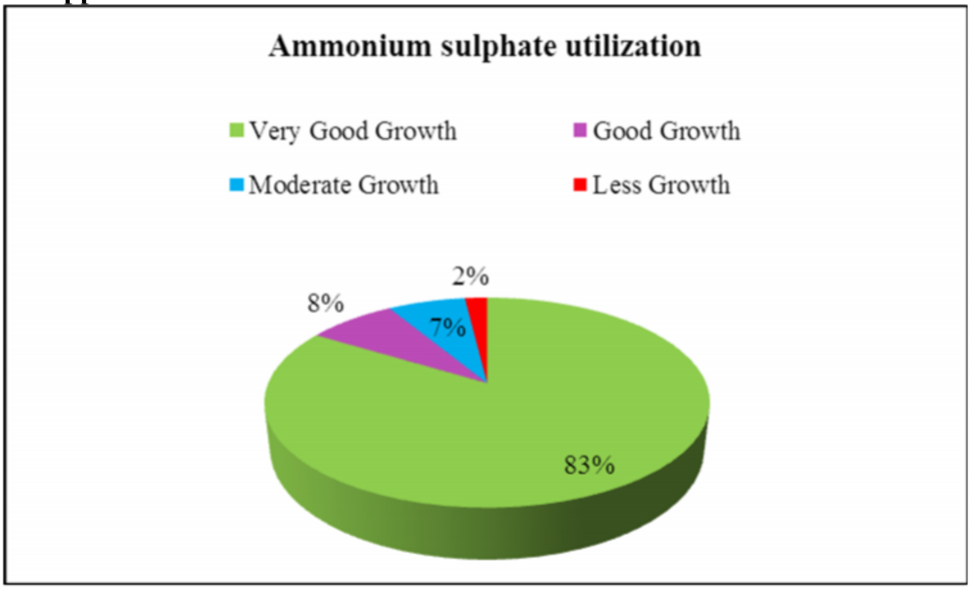
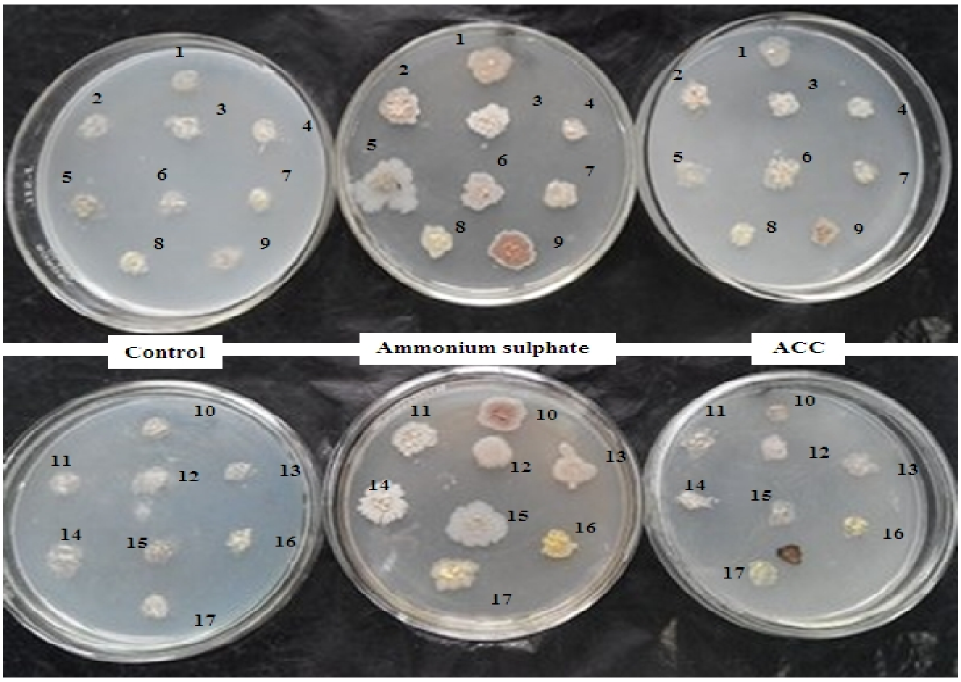


Fig. 13. Categorization of *Bacillus* isolates on the basis of ammonium sulphate utilization



1: HCA11; 2: RCA3; 3: RCA19; 4: HCA4; 5: HCA61; 6: RCA7; 7: RCA68; 8: HCA76; 9: HCA3; 10: RCA26; 11: HCA26; 12: HCA53; 13: HCA1; 14: RCA9; 15: RCA19; 16: HCA44; 17: HCA41

Fig. 14. Ammonium sulphate and ACC utilization by *Bacillus* isolates

4.4.3. Screening of *Bacillus* isolates for hydrogen cyanide production

All selected antagonistic *Bacillus* isolates were tested for the production of HCN on King's B medium supplemented with glycine (4.4 g/L). Six *Bacillus* isolates showed HCN production and HCN production varied from '+' to '4+' among different isolates (Table 12; Fig. 15). Three *Bacillus* isolates RCA3, RCA7 and RCA11 showed significant HCN production. Three isolates RCA4, HCA1 and HCA61 showed moderate HCN production. HCN production was not observed in eighteen isolates (Fig. 16).

Table 12. Qualitative estimation of HCN production by antagonistic *Bacillus* isolates

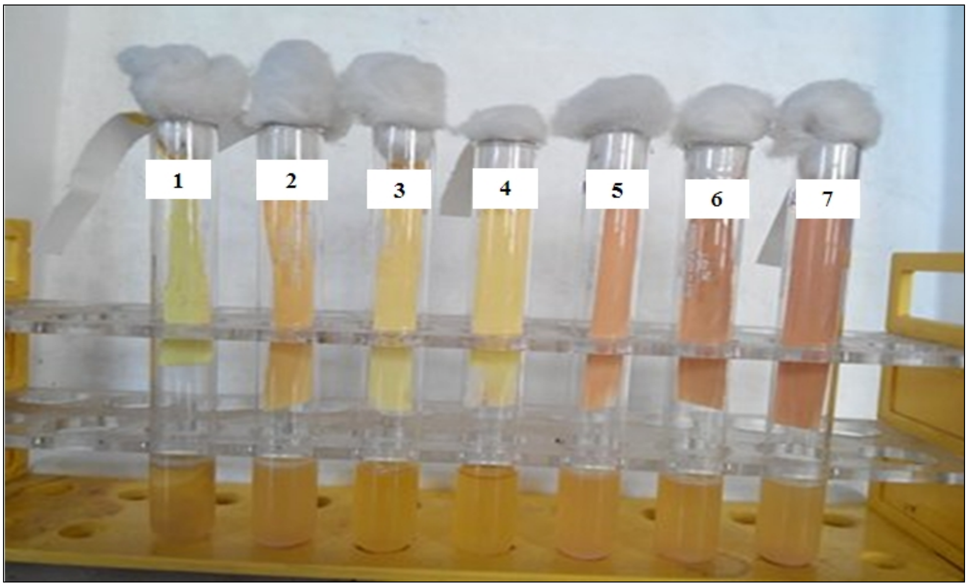
***Bacillus* isolates**	**Qualitative HCN production**
RCA3, RCA7	++++
RCA11	+++
RCA4	++
HCA1, HCA61	+
HCA3, HCA10, HCA11, HCA26, HCA41, HCA44, HCA53, HCA76, HCA80, HCA81, HCA105, RCA9, RCA19, RCA31, RCA63, RCA68, RCA70, SYB101	-

++++ : high; +++ : moderate; ++ : low: + : very low: - : no HCN production

In quantitative estimation, three *Bacillus* isolates RCA3, RCA7 and RCA11 showed significant HCN production and HCN production varied from 49.60 to 73.78 µl/ml (Table 13). Three isolates RCA4, HCA1 and HCA61 showed moderate HCN production. HCN production was not observed in eighteen isolates.

Table 13. Quantitative measurement of HCN production by antagonistic *Bacillus* isolates

***Bacillus* isolates**	**HCN production (µl/ml)**
RCA7	73.78
RCA3	57.04
RCA11	49.60
RCA4	30.38
HCA1	21.70
HCA61	15.99
HCA3, HCA10, HCA11, HCA26, HCA41, HCA44, HCA53, HCA76, HCA80, HCA81, HCA105, RCA9, RCA19, RCA31, RCA63, RCA68, RCA70, SYB101	-



1: Control; 2: HCA3; 3: HCA80; 4: HCA81; 5: RCA11; 6: RCA3; 7: RCA7

Fig. 15. Hydrogen cyanide production by *Bacillus* isolates

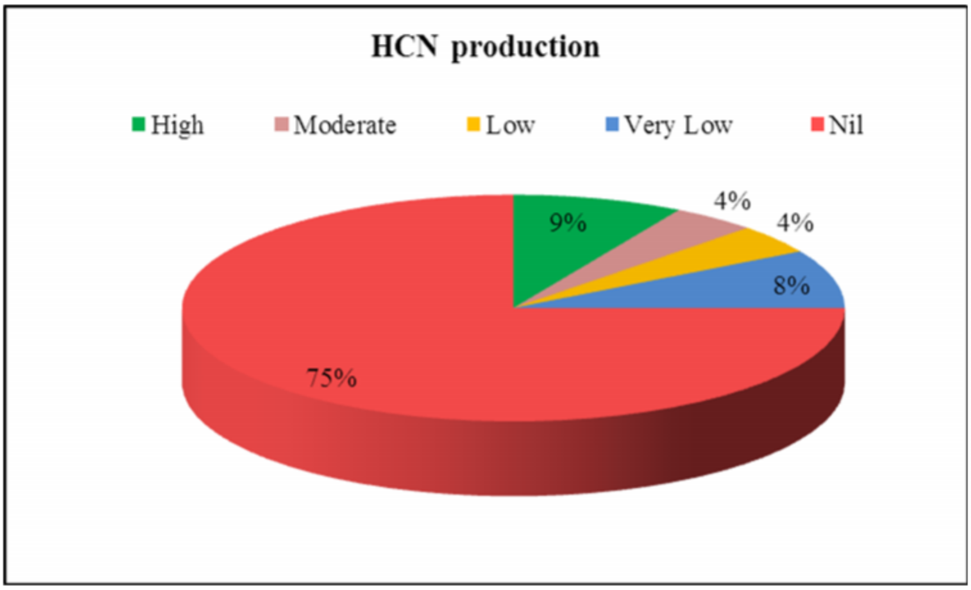


Fig. 16. Categorization of *Bacillus* isolates on the basis of HCN production

4.4.4. Screening of *Bacillus* isolates for δ-aminolevulinic acid (ALA) production

ALA has been reported to promote the growth and yield of agricultural crops at low concentration and also used as biodegradable herbicide, when produced at high concentration by rhizobacteria. All selected *Bacillus* isolates showed ALA production (Table 14; Fig 17). Significant ALA production (> 12 µg/ml) was observed in seven *Bacillus* isolates HCA61, HCA76, HCA80**,** RCA4, RCA9, RCA19 and RCA31. Seventeen isolates produced ALA ranging from 6.87 to 11.25 µg/ml (Fig. 18).

Table 14. Production of δ-aminolevulinic by *Bacillus* isolates

Sr. No.	*Bacillus* Isolates	ALA concentration (µg/ml)	Sr. No.	*Bacillus* isolates	ALA concentration (µg/ml)
1.	HCA1	10.54	**13.**	HCA105	11.25
2.	HCA3	11.08	**14.**	RCA3	10.42
3.	HCA10	6.87	**15.**	RCA4	12.01
4.	HCA11	11.08	**16.**	RCA7	9.59
5.	HCA26	10.75	**17.**	RCA9	12.54
6.	HCA41	9.12	**18.**	RCA11	10.49
7.	HCA44	9.58	**19.**	RCA19	12.83
8.	HCA53	10.55	**20.**	RCA31	12.21
9.	HCA61	14.21	**21.**	RCA63	8.87
10.	HCA76	12.02	**22.**	RCA68	10.56
11.	HCA80	12.09	**23.**	RCA70	10.42
12.	HCA81	11.21	**24.**	SYB101	9.14

Fig. 17. Production of δ-aminolevulinic acid by different *Bacillus* isolates

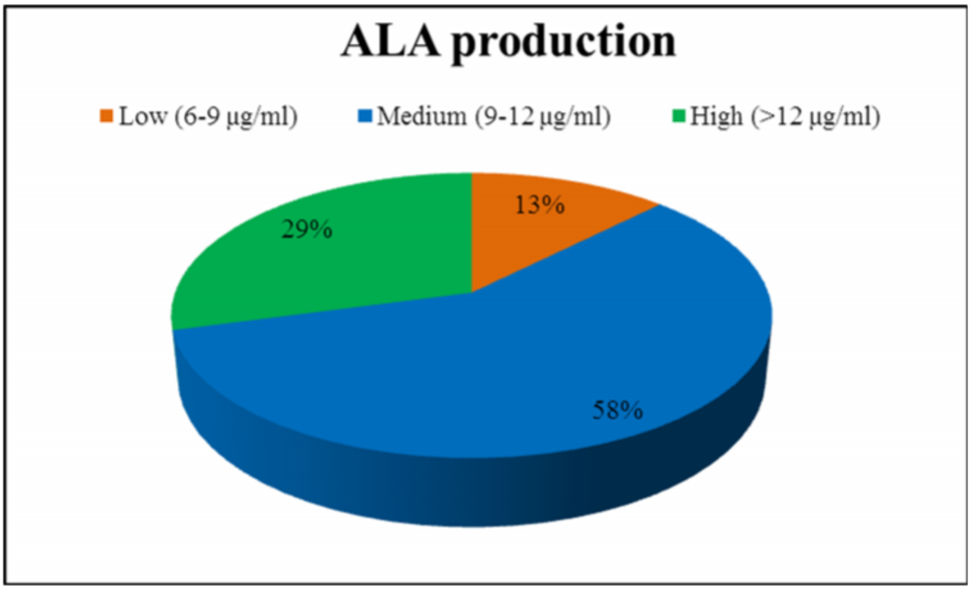


Fig. 18. Categorization of *Bacillus* isolates on the basis of δ-aminolevulinic acid production

4.4.5. Screening of *Bacillus*isolates for indole acetic acid (IAA) production

Production of phytohormones has been a dominant mechanism of plant growth promotion by *Pseudomonas* and *Bacillus* species. The IAA production was observed in all theisolates and it varied from 0.13 to 29.60 µg/ml (Table 15; Fig. 19). Seven isolates produced IAA ranging from 1.0 to 5.0 µg/ml. Five isolates i.e., HCA76, HCA80, RCA3, RCA7 and RCA11 produced IAA ranging from 6.0-9.0 µg/ml, while three isolates HCA61, RCA4 and RCA19 produced IAA ranging from 16.0-23.0 µg/ml. Maximum production of IAA (> than 25.0 µg/ml) was observed in isolate RCA9, whereas eight isolates showed very little (< 1.0 µg/ml) IAA production (Fig. 20).

Table 15. Production of indole acetic acid by antagonistic *Bacillus* isolates

Sr. No.	*Bacillus* isolates	IAA concentration (µg/ml)	Sr. No.	*Bacillus* isolates	IAA concentration (µg/ml)
1.	HCA1	0.72	**13.**	HCA105	0.84
2.	HCA3	1.33	**14.**	RCA3	8.11
3.	HCA10	1.30	**15.**	RCA4	19.54
4.	HCA11	1.18	**16.**	RCA7	7.90
5.	HCA26	4.55	**17.**	RCA9	29.60
6.	HCA41	0.18	**18.**	RCA11	6.54
7.	HCA44	0.13	**19.**	RCA19	22.54
8.	HCA53	4.37	**20.**	RCA31	0.45
9.	HCA61	16.4	**21.**	RCA63	0.21
10.	HCA76	7.99	**22.**	RCA68	0.42
11.	HCA80	8.08	**23.**	RCA70	1.06
12.	HCA81	0.51	**24.**	SYB101	2.80

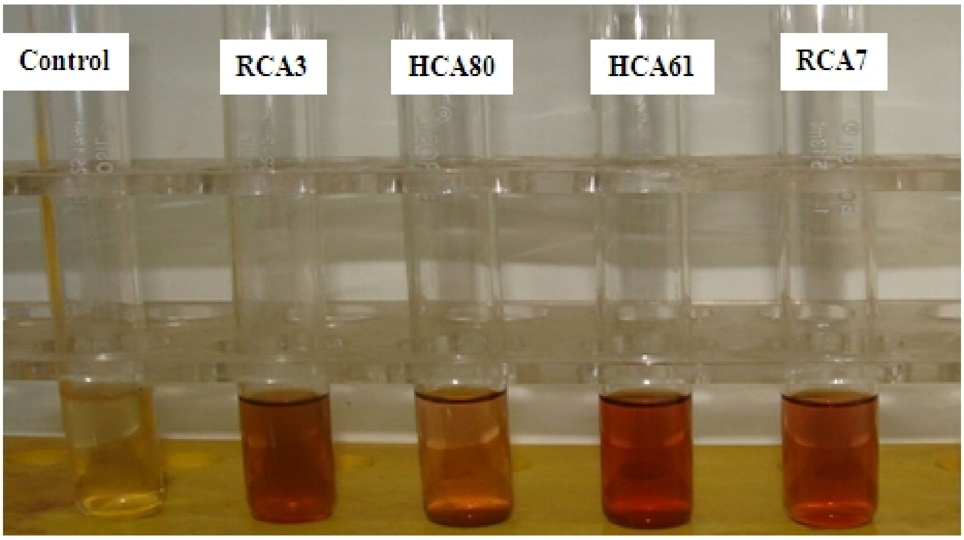


Fig. 19. Production of indole acetic acid by different *Bacillus* isolates

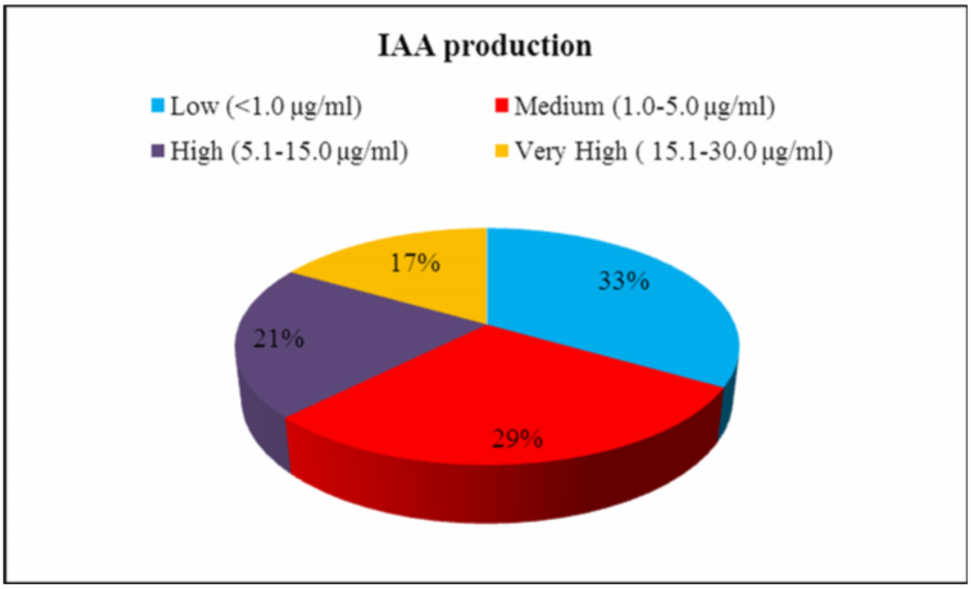


Fig. 20. Categorization of *Bacillus* isolates on the basis of indole acetic acid production

4.4.6. Phosphorous solubilization by *Bacillus* isolates

Bacillus isolates were scored depending on the ability of bacteria to form zone of solubilization on Pikovskaya medium plates with or without bromophenol blue dye. *Pseudomonas* sp. P-36 was used as reference strain. Out of 24 bacterial isolates, three isolates i.e. HCA61, RCA11 and RCA63 showed P solubilization zone varying between 4.1 to 5.0 mm and two isolates RCA3 and RCA4 showed P solubilization zone ranging from 3.1 to 4.0 mm (Table 16; Fig 21, 22). Three isolates HCA53, RCA7 and RCA19 showed moderate zone of phosphorus solubilization ranging 2.1-3.0 mm, whereas five isolates HCA44, HCA76, RCA70, HCA81 and SYB101 showed little zone of phosphorus solubilization ranging from 1.1-2.0 mm. Eleven *Bacillus* isolates (45.8%) did not solubilize phosphorus (Fig. 23).

Table 16. Phosphorous solubilization by *Bacillus* isolates

Size of P solubilization zone	*Bacillus* isolates
-	HCA1, HCA3, HCA10, HCA11, HCA26, HCA41, HCA80, HCA105, RCA9, RCA31, RCA68
1.1-2.0 mm	HCA44, HCA76, RCA70, HCA81, SYB101
2.1-3.0 mm	HCA53, RCA7, RCA19
3.1-4.0 mm	RCA3, RCA4
4.1-5.0 mm	HCA61, RCA11, RCA63
2.6 mm	P-36 (reference strain)

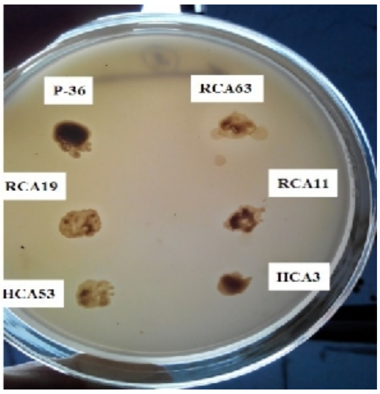


Fig. 21. Phosphorous solubilization on Pikovskaya medium by selected *Bacillus* isolates

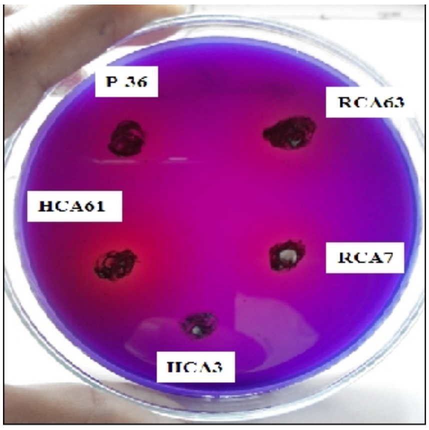


Fig. 22. Phosphorous solubilization by *Bacillus* isolates on Pikovskaya medium containing bromophenol blue dye

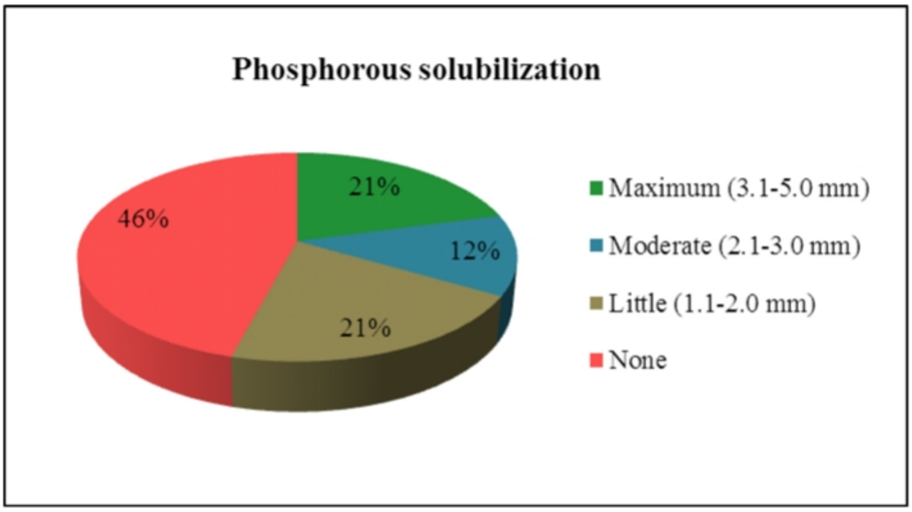


Fig. 23. Categorization of *Bacillus* isolates on the basis of phosphorous solubilization

4.4.7. Potassium solubilization by *Bacillus* isolates

Out of 24 *Bacillus* isolates tested, only nine isolates solubilized potassium and formed zone of K solubilization on mica powder supplemented medium plates. *Pseudomonas* isolate SMP54 was used as a reference strain. Three isolates i.e., HCA61, RCA3 and RCA63 showed potassium-solubilizing index (K-SI) more than 4.0 (Table 17; Fig. 24). Four *Bacillus* isolates RCA4, RCA7, RCA11 and RCA19 showed K-SI more than 3.0. Fifteen isolates did not cause K solubilization on mica containing plates. Only 37% *Bacillus* isolates solubilize the potassium on mica containing medium plates (Fig. 25).

Table 17. Potassium solubilization by *Bacillus* isolates

***Bacillus* isolates**	**K solubilizing index (K-SI)***	***Bacillus* isolates**	**K solubilizing index (K-SI)***
HCA1	-	RCA3	4.99
HCA3	-	RCA4	3.51
HCA10	-	RCA7	3.62
HCA11	2.97	RCA9	-
HCA26	-	RCA11	3.91
HCA41	-	RCA19	3.86
HCA44	-	RCA31	-
HCA53	-	RCA63	4.35
HCA61	5.78	RCA68	-
HCA76	-	RCA70	-
HCA80	-	SYB101	-
HCA81	2.88	SMP54	3.19
HCA105	-		

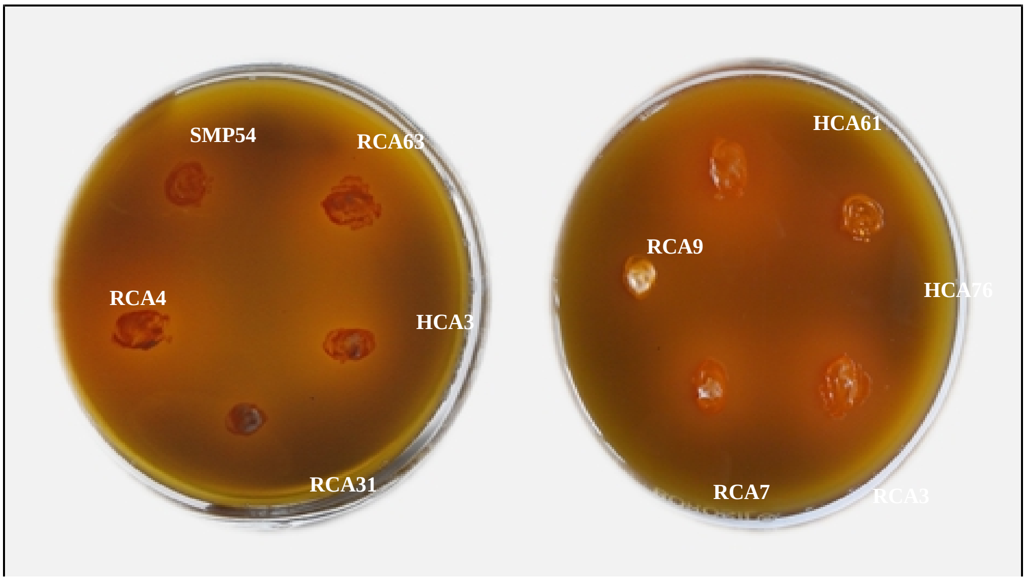


Fig. 24. Potassium solubilization by *Bacillus* isolates

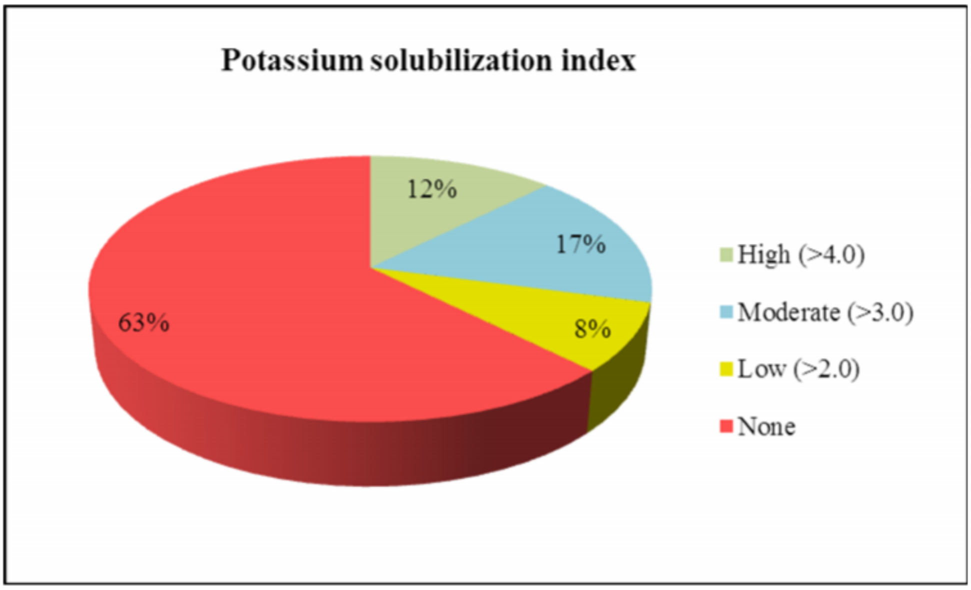


Fig. 25. Categorization of *Bacillus* isolates on the basis of potassium solubilization

4.5. Effect of *Bacillus* isolates on seed germination of chickpea

Selected antagonistic *Bacillus* isolates were studied for their effect on seed germination of chickpea. The observations for retardation/stimulation of root and shoot growth of chickpea seedlings were recorded at 5th and 10th day of seed germination. Out of 24 isolates tested, four *Bacillus* isolates i.e., RCA7, HCA10, HCA53, HCA61 and HCA81 showed significant stimulatory effect on seed germination of chickpea, while three isolates i.e., RCA4, RCA9 and RCA19 showed retarding effect on chickpea seedlings at both 5th and 10th day of inoculation (Table 18). Isolates RCA7, HCA10, HCA53, HCA61 and HCA81 showed increased growth, whereas isolates RCA4, RCA9 and RCA19 showed decreased growth of both root and shoot length as compared to control (Fig. 26, 27). Root length ranged from 1.1 to 8.33 cm and shoot length ranged from 1.37 to 9.7 cm at 10th day of seedling growth. Isolate HCA10 produced maximum root length (8.33 cm), whereas HCA81 produced shoot length (9.7 cm) at 10th day of inoculation. Other isolates also showed growth stimulatory effect over control on seedlings of chickpea at both 5th and 10th day of inoculation (Fig. 28, 29).

Table 18. Effect of *Bacillus* isolates on seedling growth of chickpea at 5th and 10th day of inoculation

Sr. no.	***Bacillus* isolates**	**Root length (cm)**		**Shoot length (cm)**	
		5th d	**10th d**	**5th d**	**10th d**
1.	Control	1.37	1.52	1.15	1.93
2.	HCA1	4.40	3.50	1.70	2.63
3.	HCA3	3.13	4.25	2.20	3.87
4.	HCA10	2.56	8.33	4.22	6.75
5.	HCA11	3.84	4.00	2.82	3.16
6.	HCA26	0.80	3.00	2.16	3.36
7.	HCA41	2.74	6.00	3.53	4.52
8.	HCA44	2.16	4.10	1.50	3.40
9.	HCA53	2.60	6.76	4.42	5.82
10.	HCA61	2.80	7.01	2.51	5.67
11.	HCA76	1.45	3.10	1.62	3.10
12.	HCA80	1.70	2.66	1.87	3.02
13.	HCA81	3.90	7.00	5.85	9.70
14.	HCA105	1.42	4.80	1.60	5.60
15.	RCA3	3.66	3.80	1.73	3.43
16.	RCA4	1.06	1.25	1.13	1.52
17.	RCA7	4.70	8.16	5.60	8.20
18.	RCA9	1.00	1.23	1.06	1.37
19.	RCA11	1.96	5.80	1.52	4.64
20.	RCA19	1.05	1.10	1.0	1.45
21.	RCA31	2.66	6.23	0.90	4.36
22.	RCA63	2.00	2.50	1.10	4.35
23.	RCA68	2.94	3.58	0.76	3.40
24.	RCA70	1.87	5.77	1.96	5.12
25.	SYB101	2.10	3.12	3.06	5.96

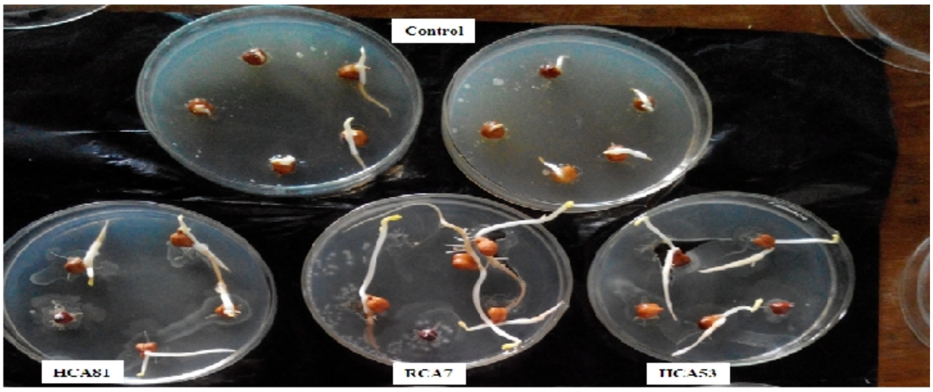


Fig. 26. Effect of *Bacillus* isolates on seedling growth of chickpea at 5th day of inoculation

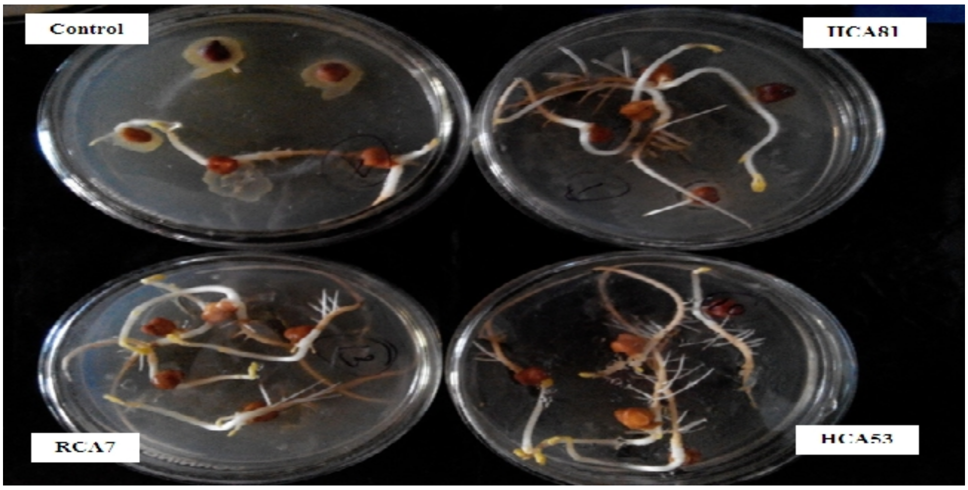


Fig. 27. Effect of *Bacillus* isolates on seedling growth of chickpea at 10th day of inoculation

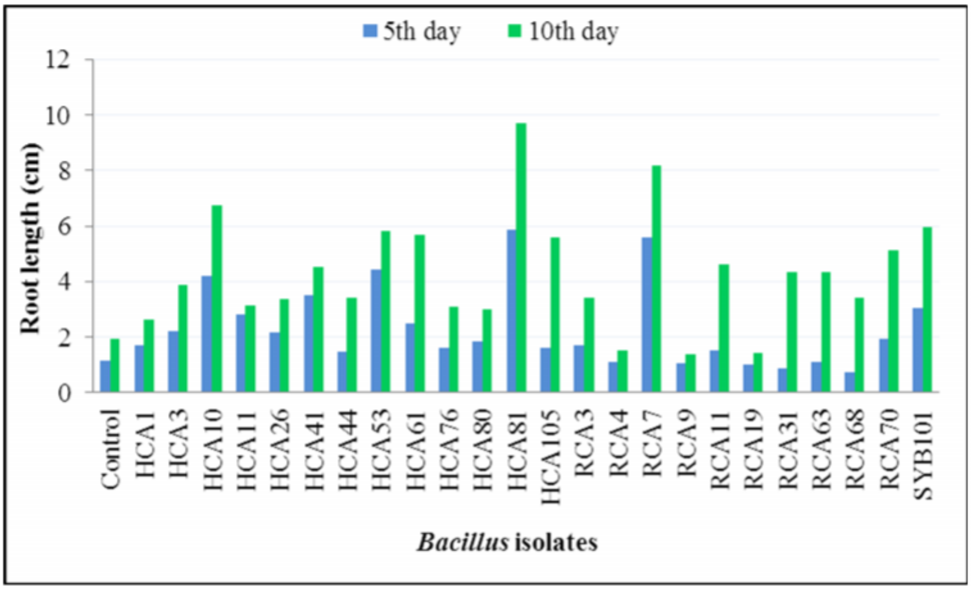


Fig. 28. Effect of *Bacillus* isolates on root growth of chickpea seedlings at 5th and 10th day of inoculation

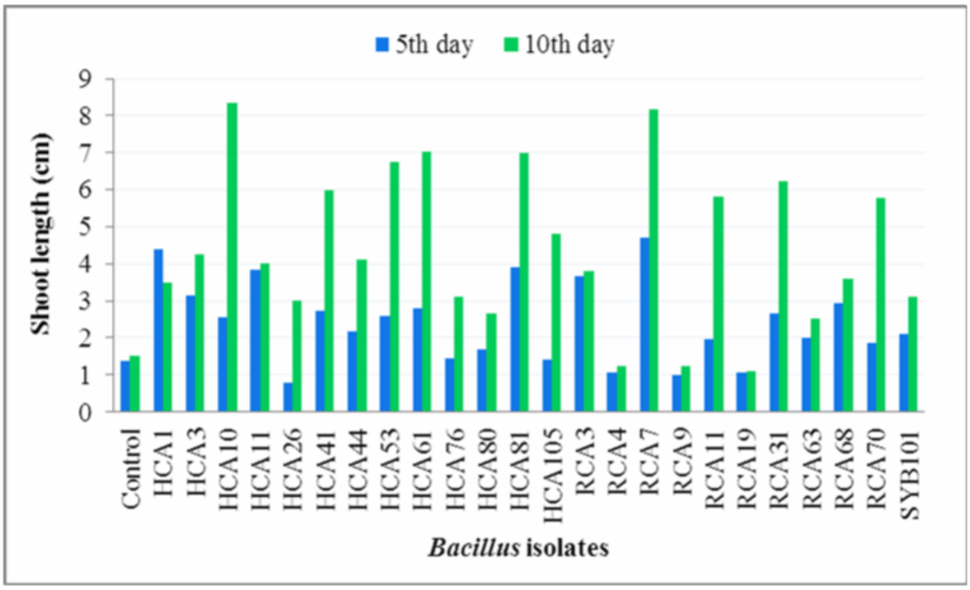


Fig. 29. Effect of *Bacillus* isolates on shoot growth of chickpea seedlings at 5th and 10th day of inoculation

4.6. Screening of *Bacillus* isolates for their ability to grow at different salt concentrations

Bacillus isolates were checked for their ability to grow at different concentrations of NaCl, i.e., 1, 2, 4, 6 and 8% (w/v), on LB medium plates. The susceptibility or tolerance towards NaCl was recorded by observing the growth and colony size at different salt concentrations. *Bacillus* isolates were categorized into six groups according to their colony size ranging from 0.5-2.5, 2.51-5.0, 5.1-10.0, 10.1-15.0 and 15.1-20.0 mm, and isolates which did not grow on salt containing plates. At 1% NaCl concentration, six *Bacillus* isolates formed larger colony size (15.1-20.0 mm; group 5) and fourteen isolates formed medium colony size (10.1-15.0 mm; group 4), whereas four isolates formed small colony size (5.1-10.0 mm; group 3) (Table 19; Fig. 30).

Table 19. Salt tolerance among *Bacillus* isolates at 1% NaCl concentration

Colony size	*Bacillus* isolates
Group 1 0.5-2.5 mm	-
Group 2 2.51-5.0 mm	-
Group 3 5.1-10.0 mm	HCA76, RCA4, RCA9, RCA70
Group 4 10.1-15.0 mm	HCA1, HCA3, HCA11, HCA26, HCA44, HCA61, HCA105, RCA3, RCA7, RCA11, RCA19, RCA31, RCA63, SYB101
Group 5 15.1-20.0 mm	HCA10, HCA53, HCA41, RCA68, HCA80, HCA81
Group 6 No growth	-

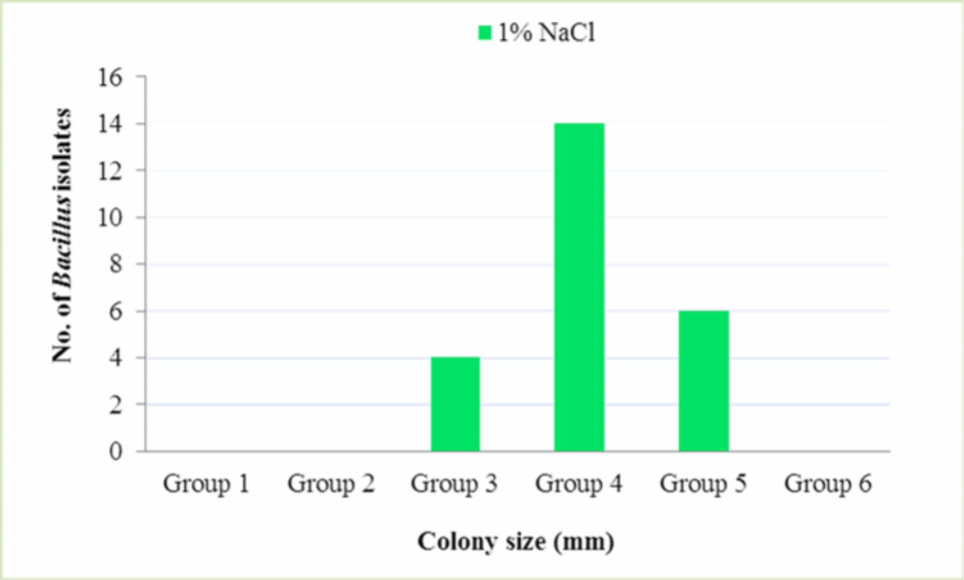


Fig. 30. **Categorization of *Bacillus* isolates on the basis of growth at 1% NaCl concentration**

At 2% NaCl concentration, six *Bacillus* isolates i.e., HCA10, HCA41, HCA53, HCA80, HCA81 and RCA68 formed colony size ranging from 15.1-20.0 mm and ten isolates formed colony size ranging from 10.1-15.0 mm, whereas eight isolates formed colony size ranging from 0.5-10.0 mm; group 1, 2, 3) (Table 20; Fig. 31). At 4% NaCl concentration, six *Bacillus* isolates i.e., HCA41, HCA53, HCA80, HCA81, RCA31 and RCA68 showed colony diameter ranging from 15.1-20.0 mm and eight isolates HCA1, HCA3, HCA10, HCA11, HCA61, HCA105, RCA7 and RCA11 showed colony diameter ranging from 10.1-15.0 mm (Table 21; Fig. 32). *Bacillus* isolates RCA3 and RCA63 showed colony diameter 7.56 mm and 9.5 mm, respectively. Isolates HCA26, HCA44 and SYB101 showed colony diameter ranging from 2.51-5.0 mm and HCA76, RCA4, RCA19 and RCA70 showed colony diameter ranging from 0.5-2.5 mm. *Bacillus* isolate RCA9 did not grow at 4% NaCl concentration.

Table 20. Salt tolerance among *Bacillus* isolates at 2% NaCl concentration

Colony size	***Bacillus* isolates**
Group 1 0.5-2.5 mm	HCA76, RCA9
Group 2 2.51-5.0 mm	RCA4, RCA70
Group 3 5.1-10.0 mm	HCA26, HCA44, RCA19, SYB101
Group 4 10.1-15.0 mm	HCA1, HCA3, HCA11, HCA61, HCA105, RCA3, RCA7, RCA11, RCA31 RCA63
Group 5 15.1-20.0 mm	HCA10, HCA41, HCA53, HCA80, HCA81, RCA68
Group 6 No growth	-

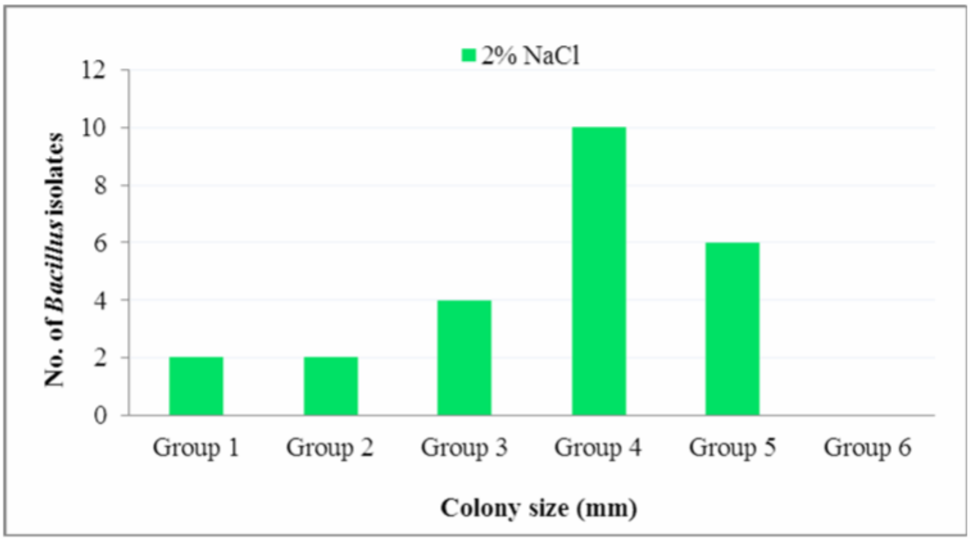


Fig. 31. Categorization of *Bacillus* isolates on the basis of growth at 2% NaCl concentration

Table 21. Salt tolerance among *Bacillus* isolates at 4% NaCl concentration

Colony size	***Bacillus* isolates**
Group 1 0.5-2.5 mm	HCA76, RCA4, RCA19, RCA70
Group 2 2.51-5.0 mm	HCA26, HCA44, SYB101
Group 3 5.1-10.0 mm	RCA3, RCA63
Group 4 10.1-15.0 mm	HCA1, HCA3, HCA10, HCA11, HCA61, HCA105, RCA7, RCA11
Group 5 15.1-20.0 mm	HCA41, HCA53, HCA80, HCA81, RCA31, RCA68
Group 6 No growth	RCA9

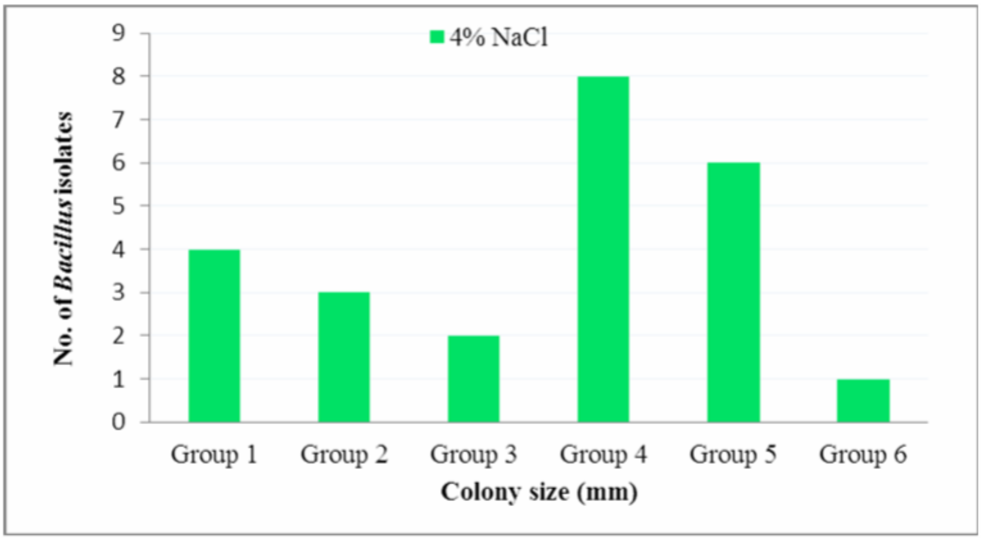


Fig. 32. Categorization of *Bacillus* isolates on the basis of growth at 4% NaCl concentration

At higher salt concentration (6%), only two *Bacillus* isolates i.e., HCA53 and HCA81 showed 15.1-20.0 mm colony size growth (group 5) (Table 22; Fig. 33). Six isolates HCA3, HCA10, RCA7, RCA11, RCA31 and RCA68 showed growth of 10.1 to 15.0 mm colony size (group 4) and seven isolates i.e., HCA1, HCA11, HCA41, HCA61, HCA80, HCA105 and RCA63 showed growth of 5.1-10.0 mm colony size (group 3). Isolates HCA26, HCA44, HCA76 and RCA19 showed growth in group 1 and three *Bacillus* isolates RCA4, RCA9 and RCA70 did not grow at 6% NaCl concentration.

Table 22. Salt tolerance among *Bacillus* isolates at 6% NaCl concentration

Colony size	***Bacillus* isolates**
Group 1 0.5-2.5 mm	HCA26, HCA44, HCA76, RCA19
Group 2 2.51-5.0 mm	RCA3, SYB101
Group 3 5.1-10.0 mm	HCA1, HCA11, HCA41, HCA61, HCA80, HCA105, RCA63
Group 4 10.1-15.0 mm	HCA3, HCA10, RCA7, RCA11, RCA31, RCA68
Group 5 15.1-20.0 mm	HCA53, HCA81
Group 6 No growth	RCA4, RCA9, RCA70

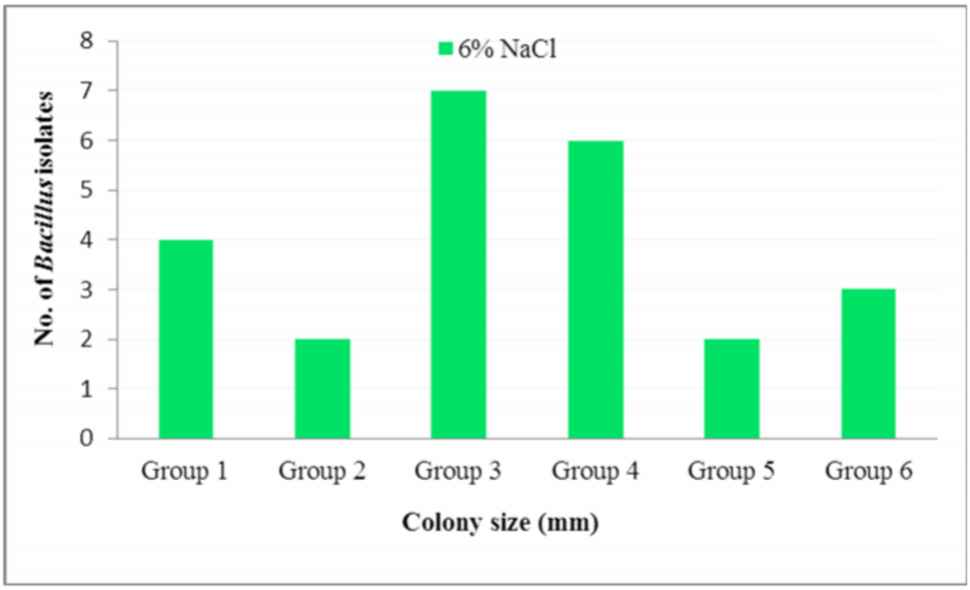


Fig. 33. Categorization of *Bacillus* isolates on the basis of growth at 6% NaCl concentration

At 8% NaCl concentration, five isolates were formed 5.1-10.0 mm colony size (group 3) and four isolates showed growth of 2.51-5.0 mm colony size (group 2) (Table 23; Fig. 34). Six *Bacillus* isolates i.e. HCA1, HCA41, HCA105, RCA7, RCA63 and RCA68 showed colony diameter ranging from 0.5 to 2.5 mm. Nine isolates i.e., HCA26, HCA44, HCA76, RCA3, RCA4, RCA9, RCA19, RCA70 and SYB101 did not grow on 8% salt containing plates. The comparative growth of different *Bacillus* isolates at higher salt concentration decreased and accordingly the colony size also reduced (Fig. 35).

Table 23.Salt tolerance among *Bacillus* isolates at 8% NaCl concentration

Colony size	***Bacillus* isolates**
Group 1 0.5-2.5 mm	HCA1, HCA41, HCA105, RCA7, RCA63, RCA68
Group 2 2.51-5.0 mm	HCA3, HCA10, HCA61, HCA80
Group 3 5.1-10.0 mm	HCA11, HCA81, HCA53, RCA11, RCA31
Group 4 10.1-15.0 mm	-
Group 5 15.1-20.0 mm	-
Group 6 No growth	HCA26, HCA44, HCA76, RCA3, RCA4, RCA9, RCA19, RCA70, SYB101

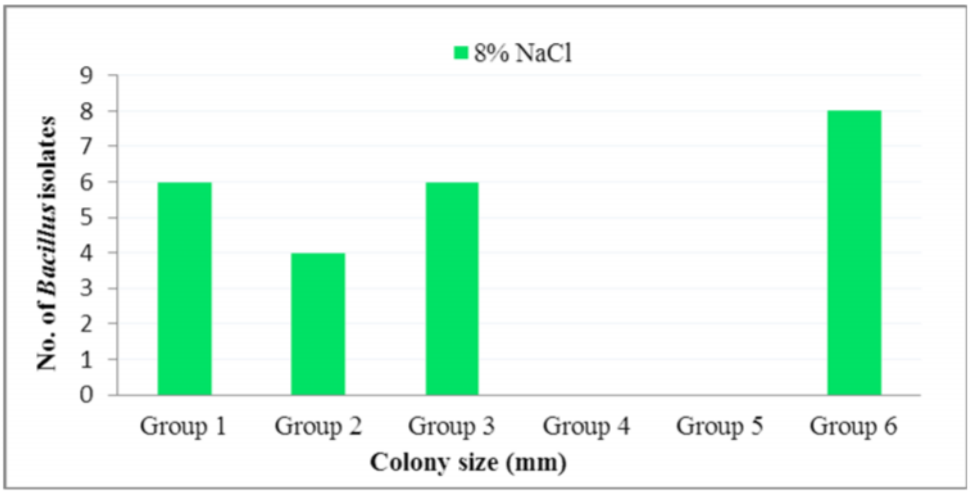


Fig. 34. Categorization of *Bacillus* isolates on the basis of growth at 8% NaCl concentration

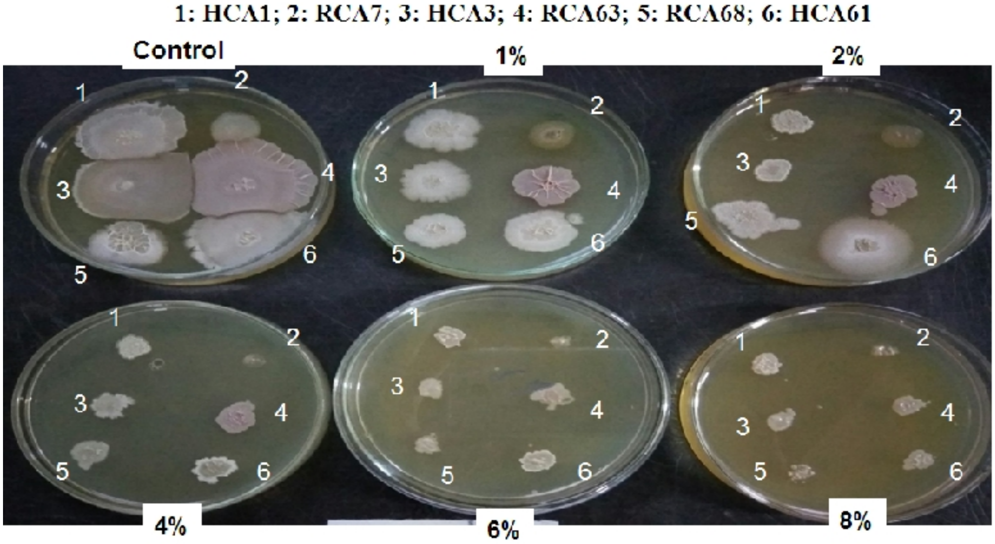


Fig. 35. Growth pattern of *Bacillus* isolates at different NaCl concentrations

4.7. Isolation of *Mesorhizobium* isolates from nodules of chickpea

Plant roots of chickpea along with nodules were collected from CCS H.A.U., Hisar farm at 80 days of plant growth. Twenty four *Mesorhizobium* isolates were obtained from the nodules of chickpea and used for further studies (Table 24; Fig. 36).

Table 24. Isolaion of *Mesorhizobium* isolates from chickpea nodules

Bacterial sp.	Location	Samples collected from chickpea field	Isolates obtained
Mesorhizobium isolates	CCS HAU, Hisar farm	Sample 1	7
		Sample 2	8
		Sample 3	5
		Sample 4	4

Fig. 36. Isolated colonies of *Mesorhizobium* species

4.8. Antagonistic effect of *Mesorhizobium* isolates against *Fusarium oxysporum*

Growth inhibition of pathogenic fungus *F. oxysporum* by *Mesorhizobium* isolates were studied by spot test method (Sindhu *et al.,* 1999) on modified YEMA-LB plates. Out of 24 *Mesorhizobium* isolates tested, only eight isolates inhibited the growth of *F. oxysporum* (Table 25, Fig. 37). Isolate MCA7 showed maximum inhibition of fungus. Four isolates i.e., MCA6, MCA14, MCA17 and MCA22 showed moderate inhibition of *F. oxysporum.* Sixteen isolates did not show antagonistic activity.

Table 25. Growth inhibition of *Fusarium oxysporum* by *Mesorhizobium* isolates

***Mesorhizobium* isolates**	**Extent of inhibition**
MCA1, MCA2, MCA3, MCA4, MCA5, MCA8, MCA10, MCA11, MCA12, MCA13, MCA15, MCA16, MCA18, MCA19, MCA21, MCA24	-
MCA9, MCA20, MCA23	+
MCA6, MCA14, MCA17, MCA22	++
MCA7	+++

+++ : maximum inhibition; ++ : moderate inhibition; + : minimum inhibition; - : no inhibition

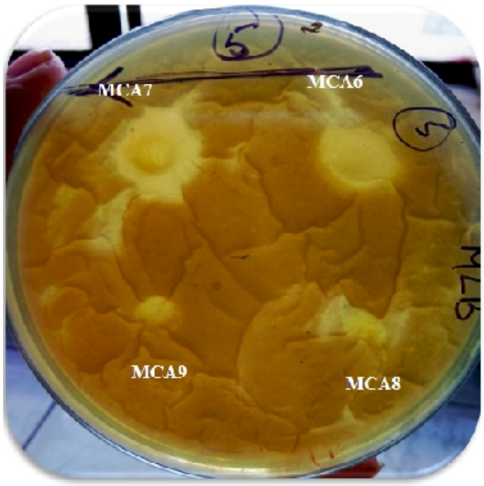


Fig. 37. Growth inhibition of *Fusarium oxysporum* by *Mesorhizobium* isolates

4.9. Screening of *Mesorhizobium* isolates for growth at different salt concentrations

All the 24 *Mesorhizobium* isolates were tested for their ability to grow at different concentrations of NaCl i.e., 1, 2, 4, 6 and 8% (w/v) on YEMA medium containing 20 mM HEPES (N-2-hydroxyethane-sulphonic acid) buffer. Four major groups were distinguished on the basis of different colony size on salt incorporated medium plates. Among the twenty four *Mesorhizobium* isolates, only two isolates i.e., MCA5 and MCA22 showed growth upto 8% salt concentration (Table 26; Fig. 38).All 24 *Mesorhizobium* isolates showed growth at 1 and 2% salt concentrations. At 1% salt concentration, thirteen isolates showed large colony size varying from 5.1 to 10.0 mm (3rd group). Six isolates showed medium colony size ranging from 2.1 to 5.0 mm (2nd group), whereas 5 isolates showed small colony size (1st group) at 1% salt concentration.

At 2% salt concentration, nine *Mesorhizobium* isolates showed colony diameter ranging from 5.1 to 10.0 mm, whereas 7 isolates showed 2.51-5.0 mm colony diameter. At 4% salt concentration, nine *Mesorhizobium* isolates showed colony growth of 5.1-10.0 mm (3rd group). Only 4 isolates i.e., MCA5, MCA7, MCA21 and MCA22 showed 2.51-5.0 mm colony size, whereas 8 isolates showed little growth (group 1). *Mesorhizobium* isolates MCA10, MCA16 and MCA17 did not grow at 4% salt concentration (Table 26). The growth of *Mesorhizobium* isolates further declined at high salt concentrations. Nine *Mesorhizobium* isolates did not grow at 6% salt concentration and only five isolates showed large colony size (3rd group). Seven *Mesorhizobium* isolates showed 2.51-5.0 mm colony diameter on these salt plates, whereas 4 isolates of *Mesorhizobium* showed small colony size (group 1). At 8% salt concentration, six *Mesorhizobium* isolates showed small colony having 0.5-2.5 mm size (1st group). *Mesorhizobium* isolates MCA6, MCA15, MCA18 and MCA19 showed 2.51-5.0 mm colony size, whereas only two isolates MCA5 and MCA22 were found salt-tolerant upto 8% of salt concentration (Fig. 39).

Table 26. Salt tolerance among various *Mesorhizobium* isolates

Colony size	*Mesorhizobium* isolates showing growth at different salt concentrations				
	1%	**2%**	**4%**	**6%**	**8%**
Group 1 0.5-2.5 mm	MCA1, MCA11, MCA14, MCA16, MCA24	MCA1, MCA2, MCA3, MCA10, MCA11, MCA14, MCA16, MCA24	MCA1, MCA2, MCA3, MCA11, MCA12, MCA13, MCA14, MCA24	MCA1, MCA3, MCA8, MCA9	MCA1, MCA4, MCA8, MCA9, MCA20, MCA23
Group 2 2.51-5.0 mm	MCA2, MCA3, MCA4, MCA10, MCA12, MCA13	MCA8, MCA9, MCA12, MCA13, MCA15, MCA17, MCA18	MCA8, MCA9, MCA15, MCA18	MCA4, MCA6, MCA15, MCA18, MCA19, MCA20, MCA23	MCA6, MCA15, MCA18, MCA19
Group 3 5.1-10.0 mm	MCA5, MCA6, MCA7, MCA8, MCA9, MCA15, MCA17, MCA18, MCA19, MCA20, MCA21, MCA22, MCA23	MCA4, MCA5, MCA6, MCA7, MCA19, MCA20, MCA21, MCA22, MCA23	MCA4, MCA5, MCA6, MCA7, MCA19, MCA20, MCA21, MCA22, MCA23	MCA5, MCA7, MCA21, MCA22	MCA5, MCA22
Group 4 No growth	-	-	MCA10, MCA16, MCA17	MCA2, MCA10, MCA11, MCA12, MCA13, MCA14, MCA16, MCA17, MCA24	MCA2, MCA3, MCA10, MCA11, MCA12, MCA13, MCA14, MCA16, MCA17, MCA24

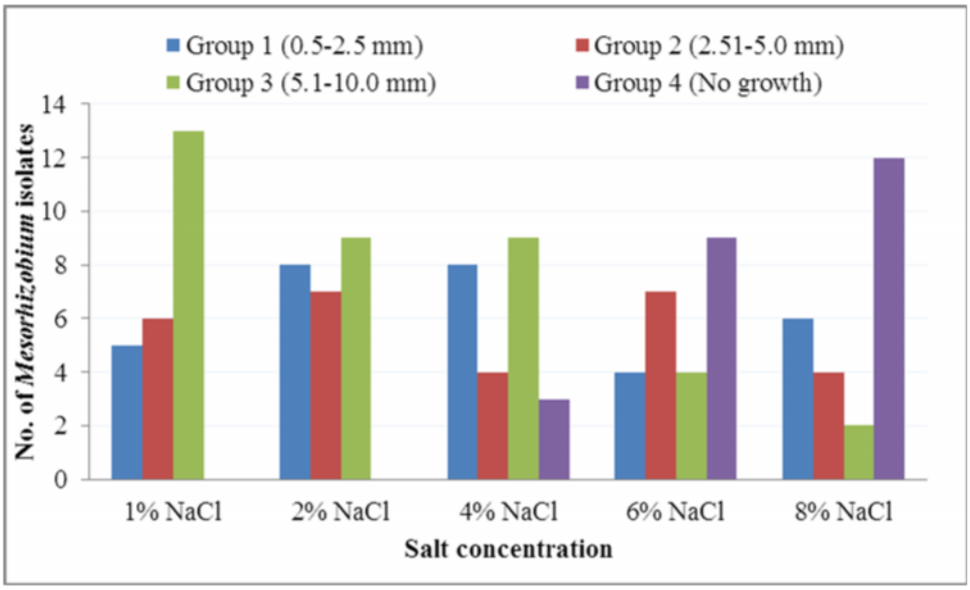
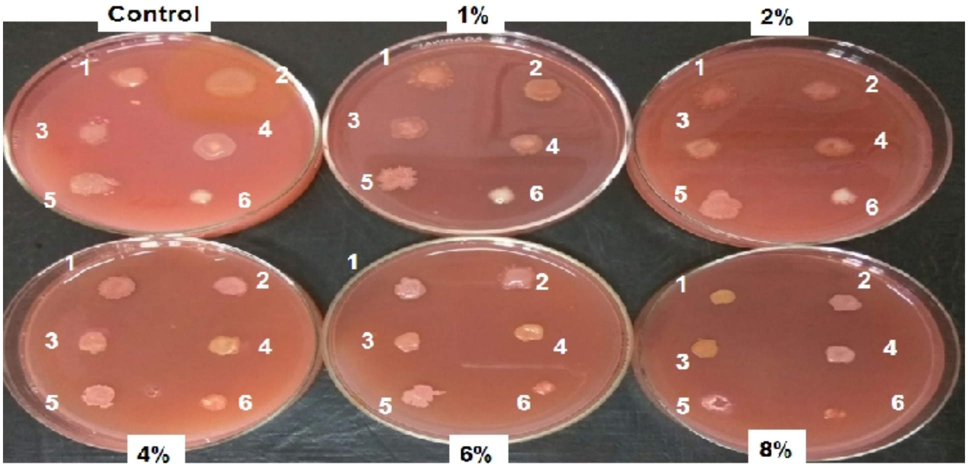


Fig. 38. **Categorization of *Mesorhizobium* isolates on the basis of growth at different NaCl concentrations**



1: MCA21; 2: MCA23; 3: MCA1; 4: MCA3; 5: MCA15; 6: MCA9

Fig. 39. Growth pattern of *Mesorhizobium* isolates at different NaCl concentrations

4.10. Inoculation effect of *Mesorhizobium* isolates on nodulation and plant growth of chickpea under chillum jar conditions

All the *Mesorhizobium* isolates obtained from chickpea nodules were studied for their effect on nodulation and plant growth of chickpea under chillum jar conditions. Maximum increase in all the plant growth parameters was observed by reference *Mesorhizobium* strains MBD26 and KR48. Maximum increase in all the symbiotic parameters i.e., nodule number (32 nodules/plant), nodule weight (325 mg/plant), shoot dry weight (SDW) (240 mg/plant) and root dry weight (RDW) (105 mg/plant) of chickpea was observed by inoculation of isolate MCA23 at 40 days of growth (Table 27). Likewise, *Mesorhizobium* isolates MCA4, MCA10 and MCA14 showed significant enhancement of nodulation and plant growth. Eighteen *Mesorhizobium* isolates showed stimulatory effect on growth of shoot and root of chickpea as compared to uninoculated control (Fig. 40,41). At 80 days of plant growth, maximum shoot dry weight (1336 mg/plant) was observed in MCA14 inoculated plants followed by inoculation of isolate MCA4 (1310 mg/plant), MCA10 (1302 mg/plant) and MCA23 (1303 mg/plant). On the other hand, significant increase in root dry weight was observed by inoculation of isolates MCA23 (241 mg/plant), MCA14 (238 mg/plant) and MCA4 (235 mg/plant) at 80 days of plant growth.

Table 27. Inoculation effect of *Mesorhizobium* isolates on chickpea growth at 40 and 80 days after sowing

Mesorhizobial isolates	Nodule no./plant		Nodule weight (mg/plant)		Shoot dry weight (mg/plant)		Root dry weight (mg/plant)	
	*	**	*	**	*	**	*	**
Control	-	-	-	-	167	600	77	300
MBD26	33	72	260	2040	249	1340	245	499
KR48	36	74	291	2059	285	1420	301	621
MCA1	10	41	149	1080	179	1181	110	401
MCA2	23	75	255	1370	188	1001	77	457
MCA3	-	-	-	-	210	501	170	243
MCA4	32	64	248	2010	235	1310	201	479
MCA5	-	7	-	180	208	400	101	280
MCA6	18	56	179	1480	217	1231	104	419
MCA7	13	22	162	390	209	603	118	435
MCA8	20	58	191	2030	153	1287	132	475
MCA9	25	55	210	1480	208	1031	120	405
MCA10	31	65	228	2010	210	1303	182	474
MCA11	-	-	-	-	155	514	121	310
MCA12	20	55	205	1570	239	1010	121	434
MCA13	-	23	-	380	179	511	112	372
MCA14	30	62	210	2028	238	1336	175	480
MCA15	-	4	-	190	212	330	180	281
MCA16	8	63	110	2006	235	1301	118	393
MCA17	-	29	-	510	230	641	165	333
MCA18	9	66	149	1790	203	1286	132	473
MCA19	11	58	156	1480	166	1093	78	477
MCA20	26	60	227	1810	243	1207	115	413
MCA21	18	72	185	1880	167	1217	130	476
MCA22	14	56	156	1550	228	1213	132	439
MCA23	32	65	263	2040	241	1303	205	485
MCA24	24	52	228	2008	231	1291	135	437
C.D. at 5% level of significance	-	-	-	-	8.183	10.630	9.403	8.605

* = 40 days of plant growth; ** = 80 days of plant growth
The values given are average value of 3 plants.

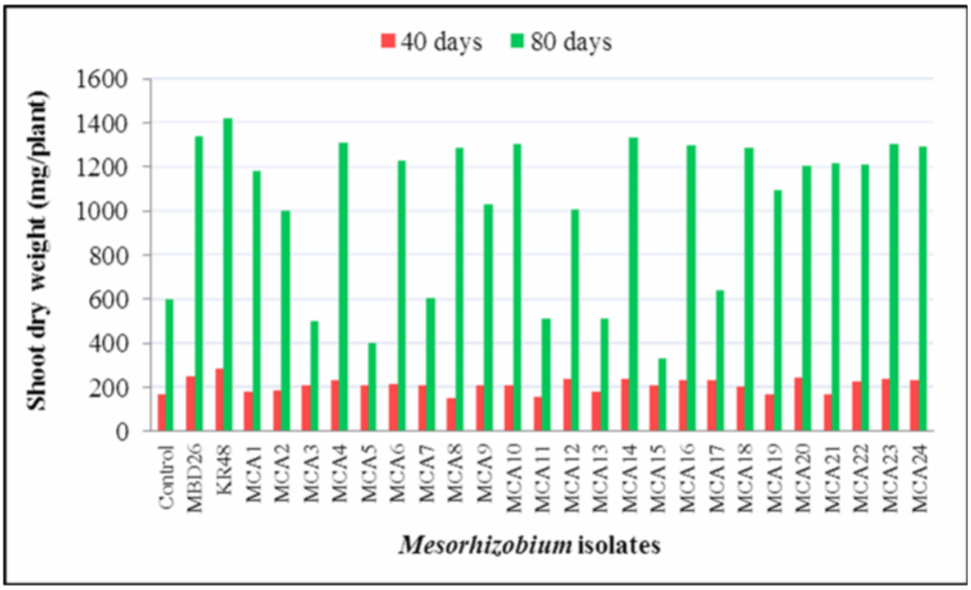


Fig. 40. Inoculation effect of *Mesorhizobium* isolates on shoot dry weight of chickpea

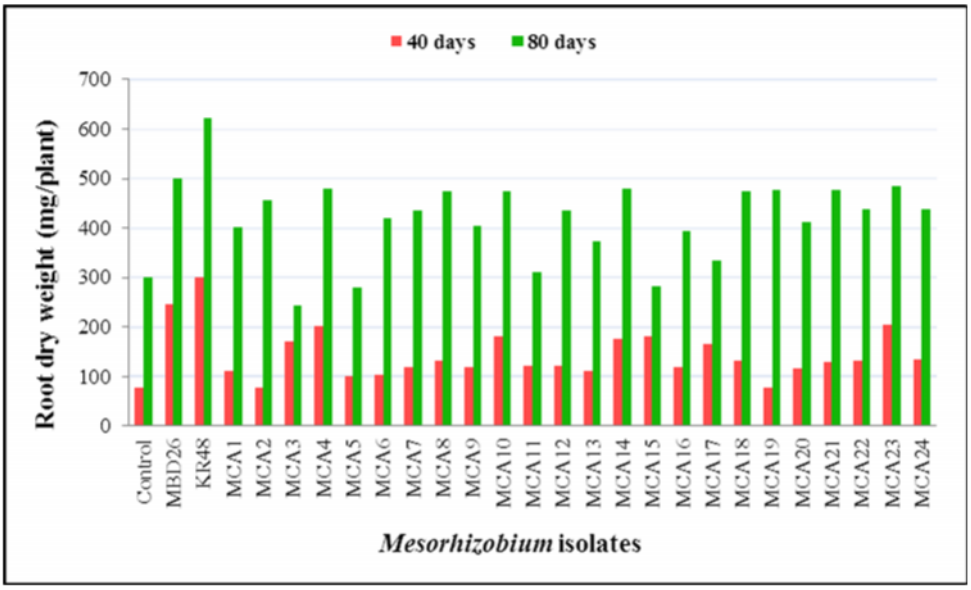


Fig. 41. Inoculation effect of *Mesorhizobium* isolates on root dry weight of chickpea

Maximum increase in SDW (45.5%)was observed by inoculation of isolate MCA20 followed by 44.3 and 43.1% increase in SDW by inoculation of isolates MCA23 and MCA12, respectively at 40 days of chickpea growth (Table 27, 28). It was followed by 42.5% increase in shoot dry weight by inoculation of isolate MCA14 and 38.3% increase in SDW by inoculation of MCA4 isolate (Fig. 42, 43). On the other hand, inoculation of chickpea with *Mesorhizobium* isolates MCA23 and MCA4 resulted in 166.2 and 161% increase in root dry weight, respectively. Inoculation of isolates MCA10 and MCA15 also increased RDW by 136.3 and 133.7% at 40th day of inoculation (Fig. 44, 45).

Similarly, significant increase in plant dry weight was observed on inoculation of mesorhizobial isolates at 80th day of observation. Maximum increase (122.6%) in SDW was observed on inoculation of MCA14 isolate followed by 118.3 and 117.2% increase in SDW on inoculation of MCA4 and MCA10 isolates, respectively (Table 27, 28). Inoculation of isolates MCA23 and MCA16 also increased SDW by 117.1 and 116.8% (Fig. 45). Likewise, 61.6% increase in RDW was observed by inoculation of isolate MCA23 followed by 60 and 59.6% increase in RDW by inoculation of MCA14 and MCA4 isolates, respectively at 80th day of observation (Fig. 46). Significant increase in root dry weight (58.3 and 57.9%) was observed on inoculation of MCA8 and MCA10, respectively. Reference *Mesorhizobium* strains MBD26 and KR48 caused maximum increase in all the symbiotic parameters including nodulation, SDW and RDW at both 40 and 80 days of plant growth (Table 27). Therefore, these two strains were used for coinoculation with *Bacillus* isolates to assess their symbiotic performance under chillum jar and pot house conditions.

Table 28. The per cent increase/decrease in shoot and root dry weight of chickpea plant inoculated with mesorhizobial isolates as compared to control

Mesorhizobial isolates	% Increase or decrease in shoot dry weight		% Increase or decrease in root dry weight	
	*	**	*	**
MBD26	49.1	123.3	218.1	66.3
KR48	70.6	136.6	290.9	107.0
MCA3	25.7	-16.5	120.7	-19.0
MCA4	40.7	118.3	161.0	59.6
MCA10	25.7	117.1	136.3	57.9
MCA14	42.5	122.6	127.2	60.0
MCA15	26.9	-45.0	133.7	-6.3
MCA16	40.7	116.8	53.2	31.0
MCA17	37.7	6.8	114.2	11.0
MCA20	45.5	101.1	49.3	37.6
MCA23	44.3	117.1	166.2	61.6
MCA24	38.3	115.1	75.3	45.6

* = 40 days of plant growth; ** = 80 days of plant growth, - = decrease in shoot/root dry weight

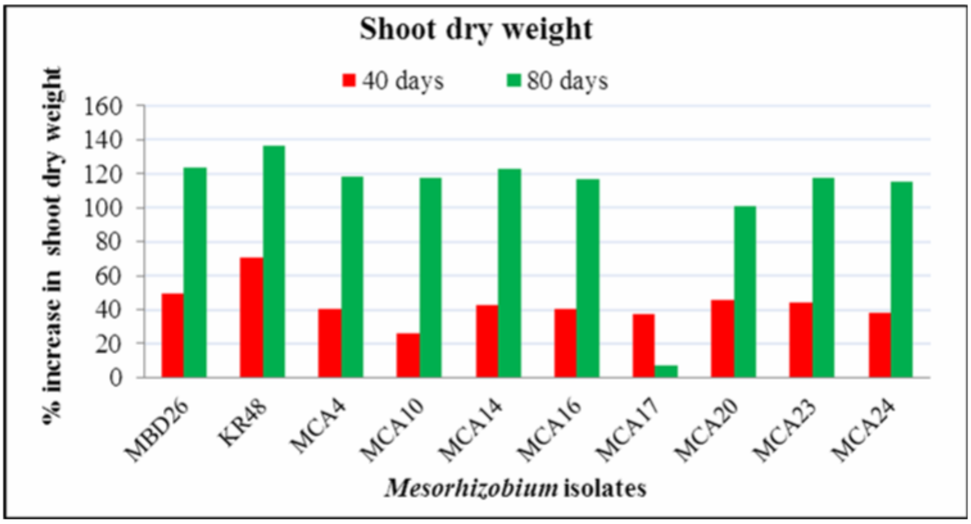


Fig. 42. The per cent increase in shoot dry weight of chickpea plants at 40 and 80 days

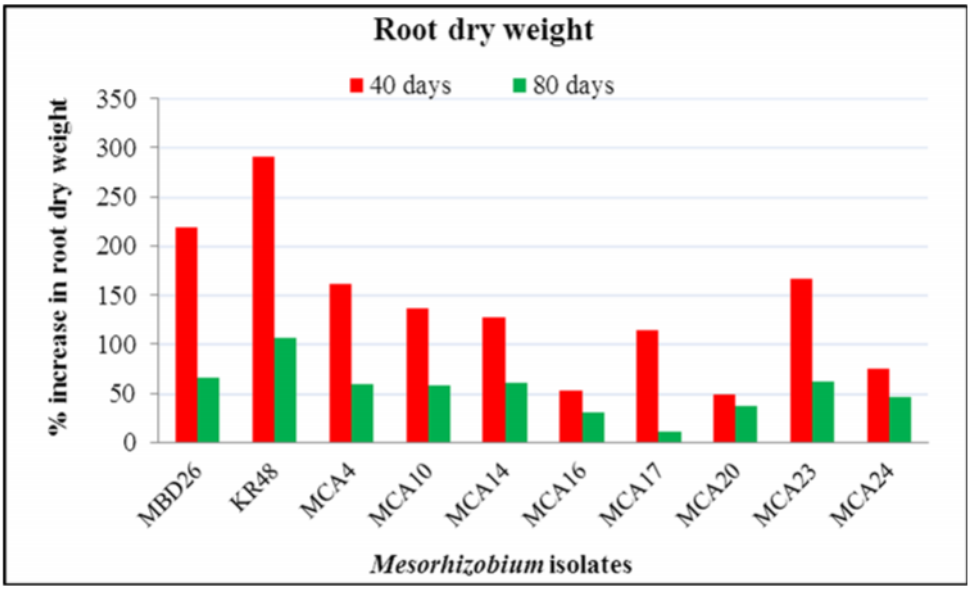


Fig. 43. The per cent increasein root dry weight of chickpea plant at 40 and 80 days

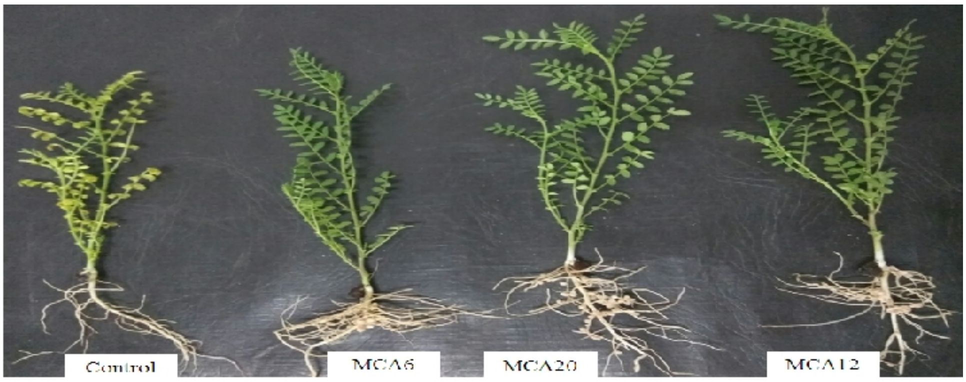


Fig. 44. Inoculation effect of *Mesorhizobium* isolates on nodulation of chickpea at 40 days of plant growth

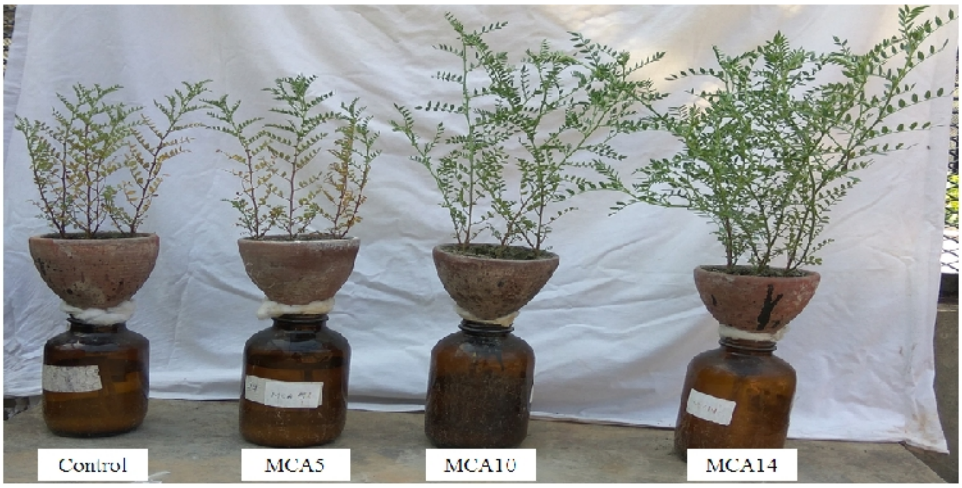


Fig. 45. Effect of *Mesorhizobium* isolates inoculation on shoot growth of chickpea at 40 day of plant growth

Fig. 46. Effect of *Mesorhizobium* isolates inoculation on shoot growth of chickpea growth at 80 day of plant growth

Fig. 47. Effect of *Mesorhizobium* isolates inoculation on nodulation of chickpea at 80 day of plant growth

4.11. Coinoculation studies of selected *Bacillus* isolates with *Mesorhizobium* strains for their effect on nodulation and plant growth of chickpea under chillum jar conditions

Based upon antagonistic and other plant growth promoting beneficial attributes, three antagonistic isolates i.e., HCA61, RCA3 and RCA7, and two non-antagonistic *Bacillus* isolates HCA76 and SYB101 were tested with *Mesorhizobium* strains i.e. MBD26 and KR48 on chickpea growth under chillum jar conditions (Table 29, 30). The observations for the nodulation and plant dry weight were recorded at 60, 75 and 90 days of plant growth. Coinoculation effect of selected *Bacillus* isolates with *Mesorhizobium* strains for their effect on nodulation and plant growth was compared to single inoculated *Mesorhizobium* strains.

Table 29. Characteristics of selected *Bacillus* isolates used for inoculation on chickpea

Characteristics		***Bacillus* isolates**				
		HCA61	**RCA3**	**RCA7**	**HCA76**	**SYB101**
Antagonistic activity against *F. oxysporum*		6+	5+	3+	-	-
A. niger		3+	++	-	-	-
N. indica		3+	3+	+	-	-
R. solani		++	++	+	-	+
Siderophore production		++	++	++	-	-
HCN production (µl/ml)		15.9	57.04	73.78	-	-
ACC utilization		+++	+++	+++	++	++
ALA production (µg/ml)		14.21	10.42	9.59	12.02	9.14
IAA production (µg/ml)		16.4	8.11	10.9	7.99	2.80
P-solubilizing activity (mm)		4.8	3.9	2.6	1.9	1.8
K-solubilizing index (K-SI)		5.78	4.99	3.62	-	-
Effect on seedling growth of chickpea at 10 days of germination	Shoot (cm)	5.67	3.43	8.2	3.1	5.96
	Root (cm)	7.01	3.8	8.16	3.1	3.12
Salt tolerance		8%	6%	8%	6%	6%

At 60 days of plant growth, *Mesorhizobium* strains MBD26 and KR48 formed 35 and 42 nodules/plant (Table 30). Maximum nodule number (65) was recorded in coinoculation treatment with KR48+SYB101 followed by KR48+RCA3, KR48+RCA7, KR48+HCA61 and MBD26+SYB101 (Fig. 48). *Bacillus* isolates SYB101 and RCA7 with *Mesorhizobium* strain KR48, and isolate RCA7 with strain MBD26 showed significant stimulatory effect on nodule weight. Coinoculation of MBD26+RCA3 showed maximum increase in shoot dry weight (SDW) (585 mg/plant) followed by coinoculation with KR48+SYB101 (478 mg/plant) and MBD26+RCA7 (473 mg/plant) at 60 days of plant growth (Fig. 49). Significant stimulatory effect on root dry weight of chickpea was recorded in KR48+RCA7 inoculated treatment (Fig. 51). Coinoculation with strains MBD26+RCA3 showed 87.7% increase in shoot dry weight and 24% increase in root dry weight of chickpea at 60 days of observation. Similarly, inoculation treatments KR48+SYB101 and KR48+HCA76 caused 23.5 and 10% increase in SDW, respectively. Coinoculation with KR48+RCA7 and KR48+RCA3 showed 15.2 and 8.7% increase in RDW as compared to *Mesorhizobium* strain KR48-inoculated plants, respectively.

Coinoculation of *Mesorhizobium* strain MBD26 with RCA3 and KR48 strain with *Bacillus* isolates HCA76, SYB101, RCA3, RCA7 and HCA61 showed significant increase in shoot dry weight of chickpea at 75 days after showing (DAS). Maximum nodule-promoting effects were observed with coinoculation of MBD26+RCA3, KR48+HCA76 and KR48+RCA3 (Table 31, Fig. 52). The symbiotic effectiveness of *Mesorhizobium* strain KR48 was improved on coinoculation with three *Bacillus* isolates i.e. HCA76, SYB101 and RCA3 at 75 days of plant growth. Significant increase in shoot biomass (30.6 to 38.4%) was observed on coinoculation of KR48+HCA76 (911 mg/plant) followed by KR48+SYB101 (870 mg/plant) and KR48+RCA3 (860 mg/plant). Inoculation of *Mesorhizobium* strain MBD26 with *Bacillus* isolate RCA3 also showed enhanced shoot growth as compared to uninoculated control. Improved root dry weight was observed on coinoculation of *Mesorhizobium* strains KR48 and MBD26 with *Bacillus* isolates RCA3, RCA7 and SYB101. Maximum root biomass was found in treatment KR48+RCA7 (567 mg/plant) followed by KR48+RCA3 (534 mg/plant). Inoculation of KR48+HCA76 caused 38.4% increase in SDW, whereas inoculation of KR48+HCA61 caused 13.5% and MBD26+RCA3 inoculation caused 49.3% increase in SDW. Increase in root biomass (6.0 to 17.3%) was observed in coinoculated treatments in comparision to *Mesorhizobium*-inoculated plants. At 75 DAS, maximum increase 13.3% in RDW was observed in KR48+RCA7 inoculated treatment followed by 7.6 and 5.8% increase with KR48+RCA3 and KR48+SYB101 inoculated treatments, respectively.

Table 30. Coinoculation effect of selected *Bacillus* isolates with *Mesorhizobium* strains KR48 and MBD26 on nodulation and plant growth of chickpea at 60 and 75 days after sowing

Sr. no.	**Treatments**	**Nodule no./plant**		**Nodule weight (mg/plant)**		**Shoot dry weight (mg/plant)**		**Root dry weight (mg/plant)**	
		*	**	*	**	*	**	*	**
1.	Control	-	3	-	19	227	386	206	327
2.	MBD26	35	58	276	1540	315	509	283	346
3.	KR48	42	54	284	1355	387	658	342	496
4.	HCA61	-	4	-	58	241	426	215	368
5.	RCA3	-	7	-	75	295	497	220	380
6.	RCA7	-	3	-	40	326	408	236	384
7.	SYB101	-	5	-	62	289	404	251	443
8.	HCA76	2	4	14	47	256	537	226	382
9.	MBD26+HCA61	-	3	-	39	241	430	266	406
10.	MBD26+RCA3	26	112	220	1808	585	760	351	405
11.	MBD26+RCA7	42	84	288	1767	473	604	267	367
12.	MBD26+SYB101	48	76	380	1650	442	678	295	382
13.	MBD26+HCA76	23	67	205	1476	372	456	306	394
14.	KR48+HCA61	48	89	355	1830	265	747	346	418
15.	KR48+RCA3	51	96	386	1608	385	860	372	534
16.	KR48+RCA7	48	57	341	1376	412	779	394	567
17.	KR48+SYB101	65	79	537	1584	478	870	355	525
18.	KR48+HCA76	45	106	326	2080	426	911	309	477
	C.D. at 5% level of significance	-	-	-	-	8.101	7.749	9.774	12.479

* = 60 days of plant growth; ** = 75 days of plant growth
The values given are average value of 3 plants.

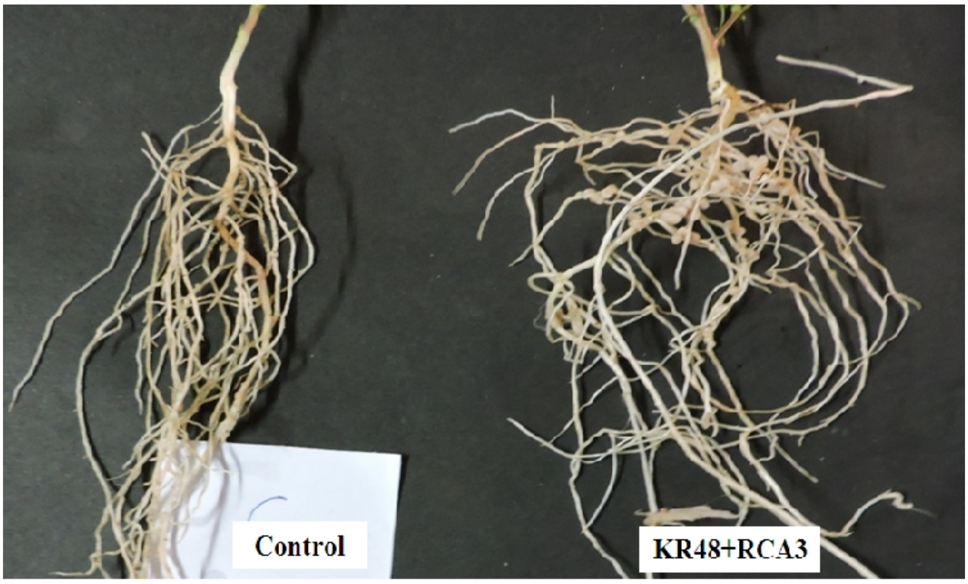


Fig. 48. **Inoculation effect of *Bacillus* isolate RCA3 with *Mesorhizobium* strain KR48 on nodulation of chickpea at 60 days**

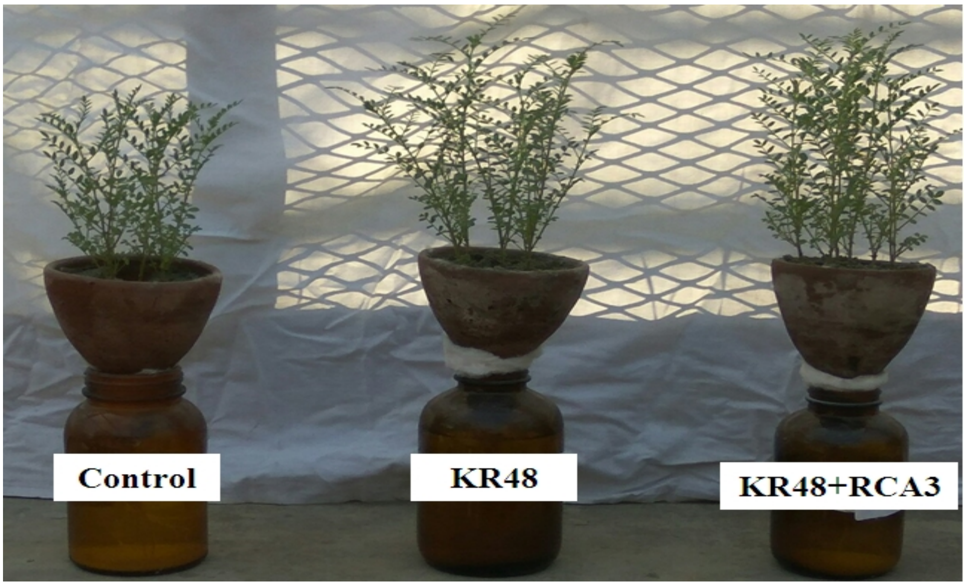


Fig. 49. **Inoculation effect of *Bacillus* isolate RCA3 with *Mesorhizobium* strain KR48 on plant growth of chickpea at 60 days**

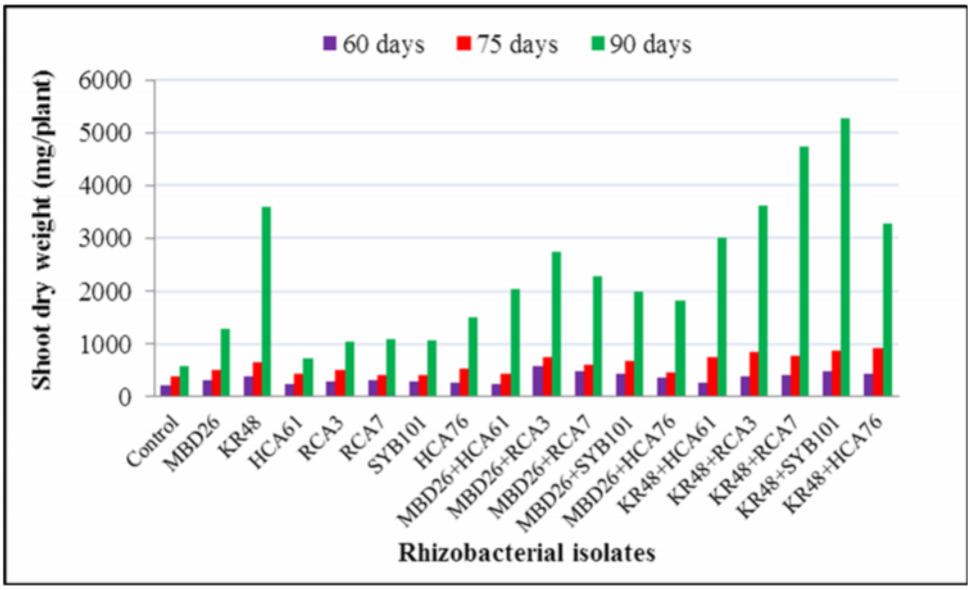


Fig. 50. **Inoculation effect of selected *Bacillus* isolates with *Mesorhizobium* strains on shoot dry weight of chickpea at 60 days of plant growth**

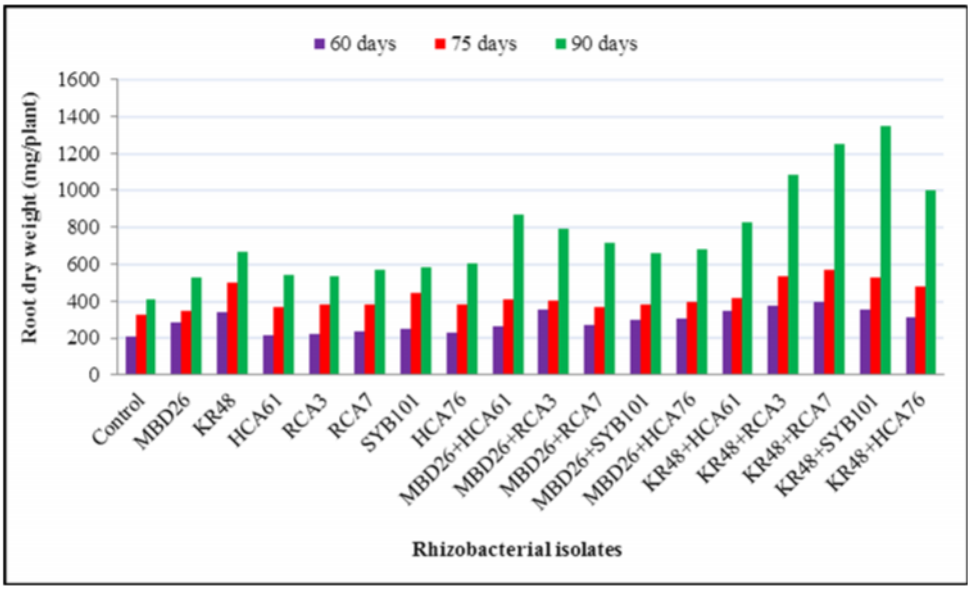


Fig. 51. **Inoculation effect of selected *Bacillus* isolates with *Mesorhizobium* strains on root dry weight of chickpea at 60 days of plant growth**

Fig. 52. Inoculation effect of *Bacillus* isolate RCA3 with *Mesorhizobium* strain MBD26 on plant growth of chickpea at 75 days

The symbiotic efficiency of chickpea was further improved by coinoculation of *Bacillus* isolates with *Mesorhizobium* sp. *Cicer* strains at 90 days of plant growth. Inoculation of *Mesorhizobium* strains KR48 and MBD26 showed significant gains on nodulation and root-shoot biomass of chickpea at 90 DAS (Table 31; Fig. 53, 54). Single inoculation of *Bacillus* isolates also showed stimulatory effect on plant growth of chickpea. Enhanced nodulation, root and shoot growth of chickpea was observed by coinoculation with MBD26+RCA3. Maximum nodulation and increased plant biomass was recorded on coinoculation of *Mesorhizobium* strain KR48 with *Bacillus* isolates SYB101 and RCA7 in comparison to uninoculated control. Coinoculation of KR48+SYB101 produced highest nodule weight (3894 mg/plant), shoot dry weight (5263 mg/plant), root dry weight (1346 mg/plant) and number of nodules per plant (172) followed by inoculation with KR48+RCA7 (3645, 4736 and 1251 mg/plant, 160) as compared to uninoculated control (369, 576 and 408 mg/plant, 17). Coinoculation of *Mesorhizobium* strain MBD26 and *Bacillus* isolate RCA3 showed a significant increase in nodulation (128 nodules/plant), nodule weight (2769 mg/plant), shoot dry weight (2738 mg/plant) and root dry weight (794 mg/plant). Prominent stimulatory effect of coinoculation of *Mesorhizobium* strain KR48 with *Bacillus* isolates was observed in comparison to inoculation with *Mesorhizobium* strain MBD26 at 90 days of chickpea growth at 90 days of observation and coinoculation of *Mesorhizobium* strain KR48 with *Bacillus* isolates i.e., SYB101, RCA7, HCA76, HCA61 and RCA3 showed 109.7, 95.1, 51.2, 34.1 and 26.8 % increase in nodule weight in comparison to *Mesorhizobium*-inoculated plants, respectively. Likewise, coinoculation of *Mesorhizobium* strain MBD26 with above mentioned *Bacillus* isolates showed 87.3, 75.3, 57.7, 17.6 and 25.4% increase in nodule weight. Significant gains were observed by coinoculation of chickpea with KR48+SYB101 in shoot dry weight (46.1%) and root dry weight (105.6%) followed by inoculation of KR48+RCA7 in SDW (31.4%)

and RDW (86.9%) at 90 days of observation. Similarly, coinoculation of *Mesorhizobium* strain MBD26 with *Bacillus* isolates i.e., RCA3, HCA61 and SYB101 showed significant gains (113.9, 79.2, 59.7 and 55.3%) in SDW, whereas coinoculation with these isolates caused 50.6, 35.6, 64.8 and 25.6% increase in RDW, respectively.

Table 31. Coinoculation effect of *Bacillus* isolates with *Mesorhizobium* strains KR48 and MBD26 on nodulation and plant growth of chickpea at 90 days after sowing

Sr. no.	Treatments	Nodule no./plant	Nodule weight (mg/plant)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)
1.	Control	17	369	576	408
2.	MBD26	84	2115	1280	527
3.	KR48	82	2079	3602	669
4.	HCA61	7	92	729	542
5.	RCA3	10	98	1054	535
6.	RCA7	8	87	1085	572
7.	SYB101	9	104	1078	585
8.	HCA76	11	118	1507	604
9.	MBD26+HCA61	52	1323	2045	869
10.	MBD26+RCA3	128	2769	2738	794
11.	MBD26+RCA7	91	2095	2295	715
12.	MBD26+SYB101	84	1978	1989	662
13.	MBD26+HCA76	70	1601	1827	678
14.	KR48+HCA61	110	2446	3013	825
15.	KR48+RCA3	104	2609	3622	1083
16.	KR48+RCA7	160	3645	4736	1251
17.	KR48+SYB101	172	3894	5263	1346
18.	KR48+HCA76	124	2635	3280	1003
	C.D. at 5% level of significance	-	-	28.233	10.704

The values given are average value of 3 plants.

Fig. 53. Inoculation effect of *Bacillus* isolate RCA7 with *Mesorhizobium* strain KR48 on plant growth of chickpea at 90 days

Fig. 54. **Inoculation effect of *Bacillus* isolate RCA7 with *Mesorhizobium* strain MBD26 on plant growth of chickpea at 90 days**

4.12. Coinoculation effect of *Mesorhizobium* strains with *Bacillus* isolates on Fusarium wilt incidence and disease control under pot house conditions

Effect of single and combined inoculation of *F. oxysporum* with *Mesorhizobium* strains as well as antagonistic and non-antagonistic *Bacillus* isolates was examined onFusarium wilt incidence and disease control at different periods of chickpea plant growth under pot house conditions. After, 60 days of *F. oxysporum* inoculation, 80% disease incidence was observed on chickpea plants and 20% plants also showed disease incidence in *Mesorhizobium* strain MBD26 incoulated treatment (Table 32; Fig. 55). Disease symptoms on shoot and root of chickpea were observed in treatments having non-antagonistic *Bacillus* isolates i.e., HCA76 and SYB101 (Fig. 56, 57). Disease symptoms were also observed in treatments having only *Mesorhizobium* strains. No Fusarium wilt symptoms were observed in mixed inoculated treatments containing antagonistic *Bacillus* isolates i.e. HCA61, RCA3 and RCA7 (Fig. 58). These antagonistic isolates caused 100% disease control either as single or on coinoculation.

At 75 days of growth, *Fusarium* treated chickpea plants showed 100% disease incidence. From 20 to 40% wilt disease symptoms were recorded in treatments having non-antagonistic *Bacillus* isolates (Fig. 59). The reduction in the percentage of wilted plants was observed from 60 to 100% in treatments inoculated with antagonistic isolates. The wilt disease free chickpea plants revealed the effectiveness of antagonistic isolates HCA61, RCA3 and RCA7 in disease control. Disease control was 100% in antagonistic *Bacillus* isolates and with fungicide (Bavistin) treatments at all the three stages of plant growth. Treatments of bacterial isolates KR48, KR48+SYB101 and KR48+HCA76 inoculated with *F. oxysporum* caused 100% disease control while single and mixed inoculation of SYB101 and HCA76 with

MBD26 strain caused only 60 to 80% disease control, respectively at 90 days of growth (Fig. 60-62).

Table 32. Coinoculation effect of *F. oxysporum* with *Mesorhizobium* strains and *Bacillus* isolates on chickpea growth at 60, 75 and 90 days

S. no.	Coinoculation of fungus with *Bacillus* isolates	% Disease incidence (% DI)	% Disease control (% DC)	% DI	% DC	% DI	% DC
		60 days		75 days		90 days	
T1	Fungus (*Fusarium oxysporum*)	80	0	100	0	100	0
T2	Fungus + MBD26	20	75	0	100	0	100
T3	Fungus + HCA61	0	100	0	100	0	100
T4	Fungus + RCA3	0	100	0	100	0	100
T5	Fungus + RCA7	0	100	0	100	0	100
T6	Fungus + SYB101	40	50	20	80	40	60
T7	Fungus + HCA76	60	25	40	60	20	80
T8	Fungus + MBD26 + HCA61	0	100	0	100	0	100
T9	Fungus + MBD26 + RCA3	0	100	0	100	0	100
T10	Fungus + MBD26 + RCA7	0	100	0	100	0	100
T11	Fungus + MBD26 + SYB101	60	25	20	80	40	60
T12	Fungus + MBD26 + HCA76	20	75	20	80	20	80
T13	Fungus + KR48	0	100	20	80	0	100
T14	Fungus + KR48 + HCA61	0	100	0	100	0	100
T15	Fungus + KR48 + RCA3	0	100	0	100	0	100
T16	Fungus + KR48 + RCA7	0	100	0	100	0	100
T17	Fungus + KR48 + SYB101	20	75	0	100	0	100
T18	Fungus + KR48 + HCA76	20	75	0	100	0	100
T19	Fungus + Bavistin	0	100	0	100	0	100

The values given are average value of 5 plants. Disease incidence is the % of plants infected and disease control is the % reduction of diseased plants after inoculation with bacteria.

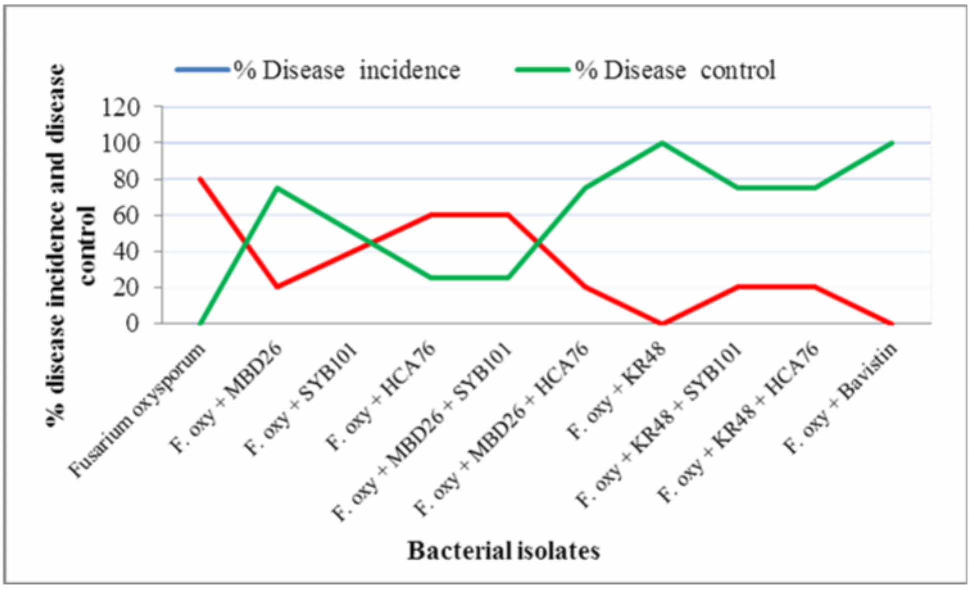


Fig. 55. Disease incidence by fungus and disease control (%) by antagonistic *Bacillus* isolates in chickpea at 60 days of plant growth

Fig. 56. **Disease symptoms caused by inoculation of *F. oxysporum* on chickpea at 60 days of plant growth**

Fungus infected roots Fungus infected nodules Stunting of plant root

Fig. 57. **Disease symptoms caused by inoculation of *F. oxysporum* on nodules and roots of chickpea plant at 60 days of plant growth**

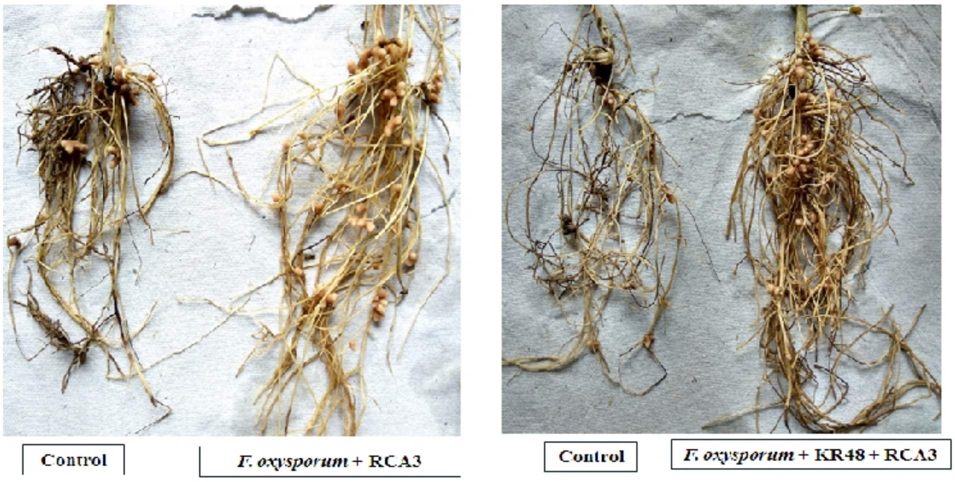


Fig. 58. **Effect of coinoculation of *Bacillus* isolate RCA3 alongwith *Mesorhizobium* strain KR48 and *Fusarium oxysporum* on nodulation of chickpea at 60 days of plant growth**

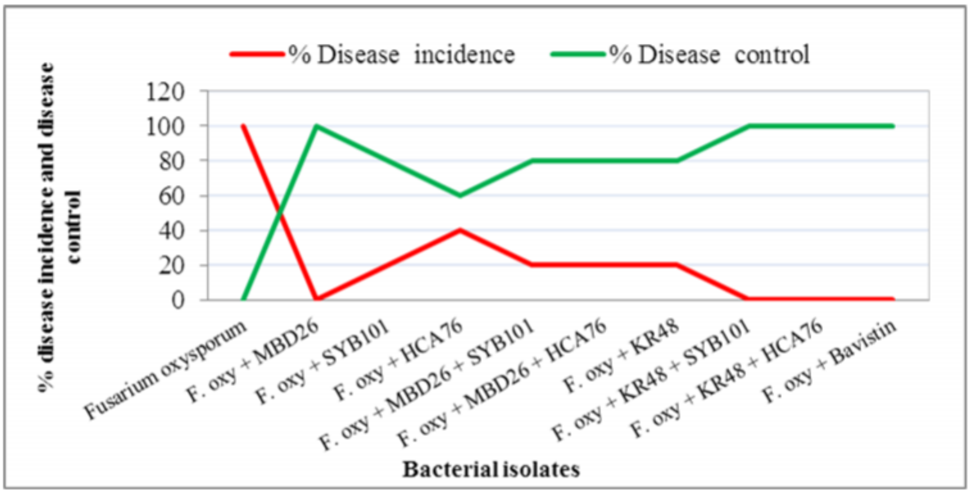


Fig. 59. **Disease incidence by fungus and disease control (%)by antagonistic *Bacillus* isolates in chickpea at 75 days of plant growth**

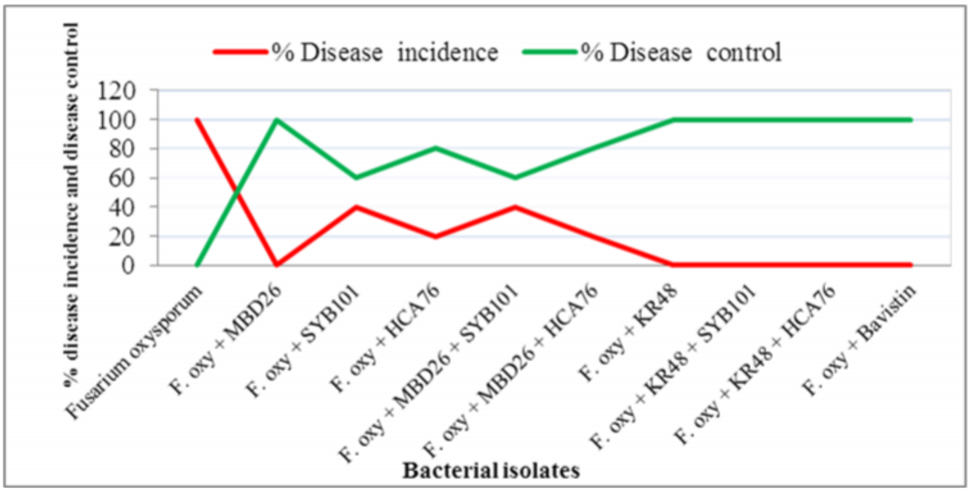


Fig. 60. **Disease incidence and disease control (%) by antagonistic *Bacillus* isolates in chickpea at 90 days of plant growth**

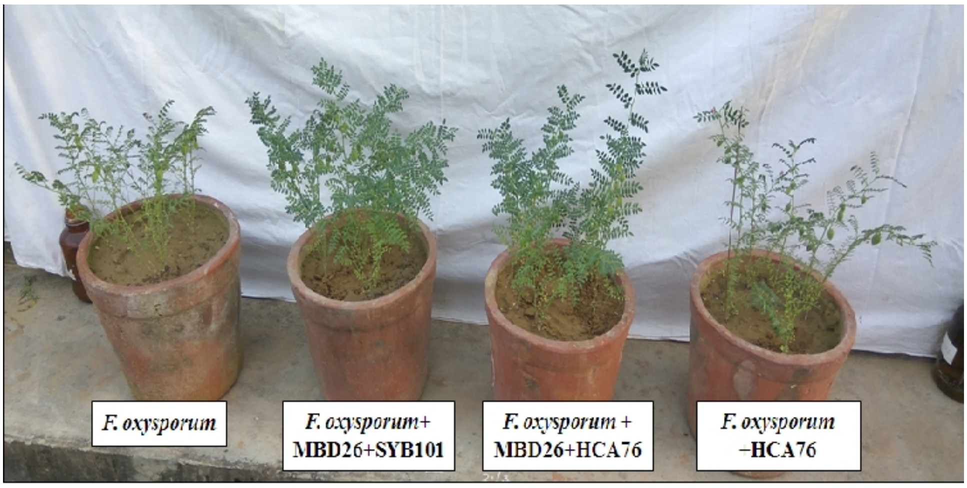


Fig. 61. **Effect of coinoculation of *Mesorhizobium* strain MBD26 and *F. oxysporum* along with antagonistic and non-antagonistic *Bacillus* isolates on plant growth of chickpea at 90 days of plant growth**

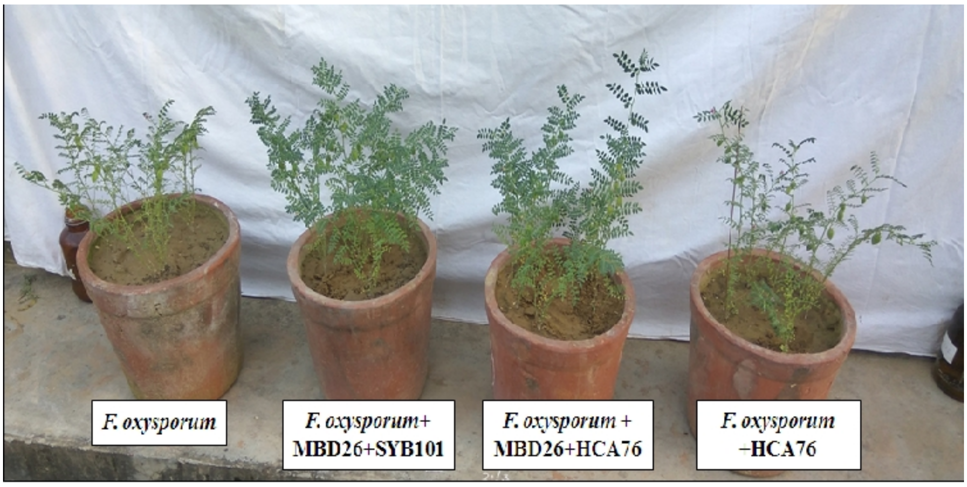


Fig. 62. **Control of Fusarium wilt disease by coinoculation of *Bacillus* isolates with *Mesorhizobium* strain MBD26 on chickpea plant at 90 days of plant growth**

4.13. Coinoculation effect of *Mesorhizobium* strains and *Bacillus* isolateson plant growth of chickpea under pot house conditions

Single inoculation of *Mesorhizobium* strains and *Bacillus* isolates RCA3, RCA7 and SYB101 showed significant increase in shoot and root dry weight compared to the untreated control under pot house conditions (Table 33). Reduced growth of chickpea was observed in *Fusarium oxysporum* inoculated treatment at different stages of plant growth. At 60 DAS, *Mesorhizobium* strains MBD26 and KR48 showed increase in nodule weight (88.7, 59.1%), SDW (27.7, 55.7%) and RDW (23.5, 52.5%) of chickpea plants, respectively. Coinoculation of these strains with *Bacillus* isolates i.e., MBD26+HCA61 and MBD26+RCA7 showed 140% increase in nodule number, whereas coinoculation of KR48+SYB101 and KR48+RCA7 showed 170 and 160% increase in nodule number, respectively. Maximum increase in SDW (658, 649 mg/plant) was observed on coinoculation with *Bacillus* isolates SYB101, HCA61 and fungus, respectively. Inoculation of *F. oxysporum*+KR48+SYB101 showed 155.4% increase in SDW followed by *F. oxysporum*+KR48+RCA7 (124%) and *F. oxysporum*+MBD26+RCA7 (121.1%) (Fig. 63).

At 75 DAS, coinoculation of *Bacillus* isolates SYB101 and HCA61 along with fungus showed 118.7 and 106.2% increase in nodule number and caused 128 and 68.5% increase in nodule weight, respectively. Likewise, 132% increase in nodule weight was observed in treatment with *F. oxysporum*+KR48+RCA3 followed by 98.9, 96.6% increase in nodule weight in *F. oxysporum*+MBD26+HCA61 and *F. oxysporum*+KR48+SYB101 treatments, respectively. At 75 days of growth, 173.3% increase in SDW was observed by inoculation of *F. oxysporum*+KR48+SYB101followed by 151.8 and 151.6% increase in the treatments with *F. oxysporum*+MBD26+SYB101 and *F. oxysporum*+KR48+RCA3 (Fig. 64). Similarly, inoculation of chickpea with the *Bacillus* isolates SYB101, RCA3 or RCA7 along with *Mesorhizobium* strain KR48 resulted in 118, 105.7 and 87.6% increase in RDW, respectively. Inoculation of *F. oxysporum*+MBD26+SYB101 and *F. oxysporum*+MBD26+RCA7 showed stimulatory effect on growth parameters of chickpea plant at 75 days. Inoculation of *F. oxysporum*+MBD26+RCA7 caused 128.3% increase in SDW and 60.4% gain in RDW, whereas *F. oxysporum*+MBD26+RCA3 showed 117.4% increase in SDW and 67.8% increase in RDW.

Table 33. Influence of *Mesorhizobium* strains and *Bacillus* isolateson symbiotic parameters in chickpea at 60 and 75 days after sowing

Treatments	**Nodule no./plant**		**Nodule weight (mg/plant)**		**Shoot dry weight (mg/plant)**		**Root dry weight (mg/plant)**	
	*	**	*	**	*	**	*	**
T1 : Control	10	16	98	181	350	540	310	420
T2 : *Mesorhizobium* strain MBD26	25	31	220	276	654	942	450	600
T3 : *Mesorhizobium* strain KR48	30	35	268	299	720	990	515	680
T4 : HCA61	14	20	120	202	472	870	399	542
T5 : RCA3	18	20	130	176	441	824	362	515
T6 : RCA7	16	27	108	290	450	1081	370	490
T7 : SYB101	15	18	114	194	552	860	411	516
T8 : HCA76	15	20	103	183	570	982	393	521
T9 : Fungus (*Fusarium oxysporum*)	10	16	95	183	384	670	300	412
T10 : Fungus + MBD26	21	29	185	296	447	988	383	476
T11 : Fungus + HCA61	19	33	180	305	649	956	491	505
T12 : Fungus + RCA3	17	24	169	274	584	1091	472	603
T13 : Fungus + RCA7	13	26	110	290	524	1104	448	578
T14 : Fungus + SYB101	10	35	145	413	658	1230	574	662
T15 : Fungus + HCA76	8	14	86	172	554	715	360	468
T16 : Fungus + MBD26 + HCA61	24	35	290	360	772	989	516	544
T17 : Fungus + MBD26 + RCA3	13	28	136	290	687	1174	584	705
T18 : Fungus + MBD26 + RCA7	24	30	254	310	774	1233	493	674
T19 : Fungus + MBD26 + SYB101	23	25	281	294	740	1360	634	744
T20 : Fungus + MBD26 + HCA76	19	26	170	290	602	755	462	541
T21 : Fungus + KR48	18	24	156	270	545	1170	473	584
T22 : Fungus + KR48 + HCA61	22	28	232	298	615	1246	564	632
T23 : Fungus + KR48 + RCA3	24	41	247	420	653	1359	576	788
T24 : Fungus + KR48 + RCA7	26	33	258	335	784	1247	635	864
T25 : Fungus + KR48 + SYB101	27	34	270	356	894	1476	760	916
T26 : Fungus + KR48 + HCA76	18	31	89	164	521	808	595	690
T27 : Fungus + Bavistin	16	22	158	262	565	915	387	534
C.D. at 5% level of significance	-	-	-	-	19.570	53.583	14.629	13.239

* = 60 days of plant growth; ** = 75 days of plant growth

Stimulatory effect of *Mesorhizobium* inoculation with different *Bacillus* isolates was observed on nodulation, shoot and root dry weight of chickpea at 90 days of plant growth. All single and coinoculated treatments (*Mesorhizobium* strains and *Bacillus* isolates) and fungal culture except treatment T9 (*Fusarium oxysporum*) showed significant growth promoting effect on root-shoot biomass of chickpea (Table 34; Fig. 65-68). *Mesorhizobium* strains MBD26 and KR48 showed increase in nodule number (114.9, 125%), nodule weight (148, 171.4%), SDW (64.1, 73.2%) and RDW (50.9, 52.5%), respectively as compared to uninoculated plants. Maximum increase in RDW (920 mg/plant) and SDW (1604 mg/plant) was observed by inoculation of *Bacillus* isolate RCA3 followed by RCA7 inoculated alongwith fungal culture (Table 33; Fig. 69). Coinoculation of *Bacillus* isolates SYB101 and HCA61 with fungal culture increased nodule number (90, 85%) and nodule weight (187.1, 176.1%), and coinoculation with RCA3 and RCA7 showed increase in SDW (113.5, 97.3%) and RDW (77.6, 65%), respectively.

Enhanced nodule number (135%), nodule weight (273.8%), RDW (123.1%) and SDW (86.2%) was observed on coinoculation of *F. oxysporum*+MBD26+RCA7 followed by 110, 177.6, 105.3 and 66.9% increase in nodule number, nodule weight, SDW and RDW, respectively on coinoculation with *F. oxysporum*+MBD26+HCA61. Similarly, enhanced nodulation and plant growth was recorded on coinoculation with *F. oxysporum*+KR48+SYB101, *F. oxysporum*+KR48+RCA7 and *F. oxysporum*+KR48+RCA3 (Fig. 65-68). Coinoculation of *F. oxysporum*+KR48+SYB101 caused 149 and 111% increase in SDW and RDW of chickpea, followed by *F. oxysporum*+KR48+RCA7 (129.5, 96.9%) and *F. oxysporum*+KR48+RCA3 (107.7, 77.2%), respectively (Fig. 69).

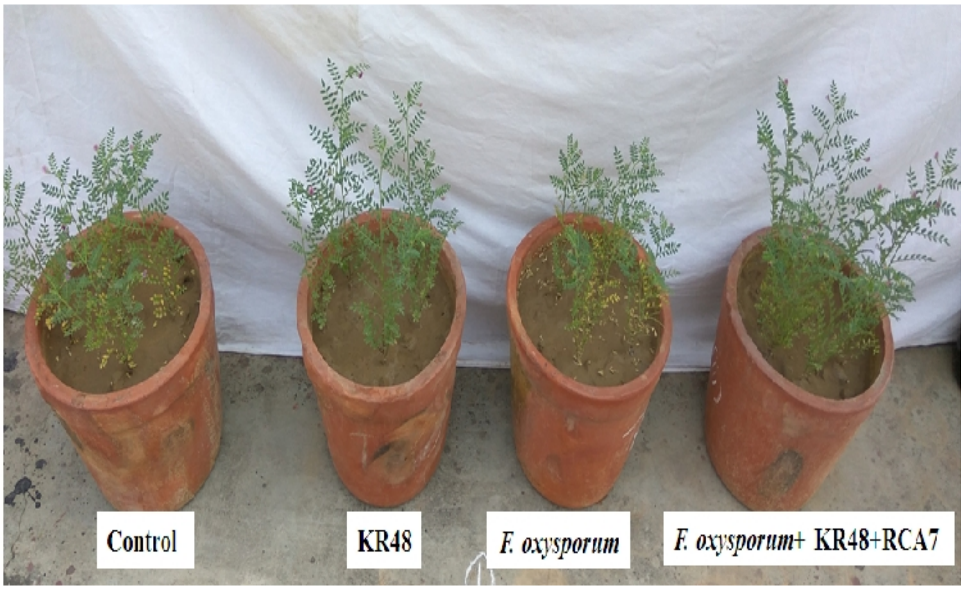


Fig. 63. Coinoculation effect of *Bacillus* isolate RCA7 with *Mesorhizobium* strain KR48 on plant growth of chickpea at 60 days of sowing

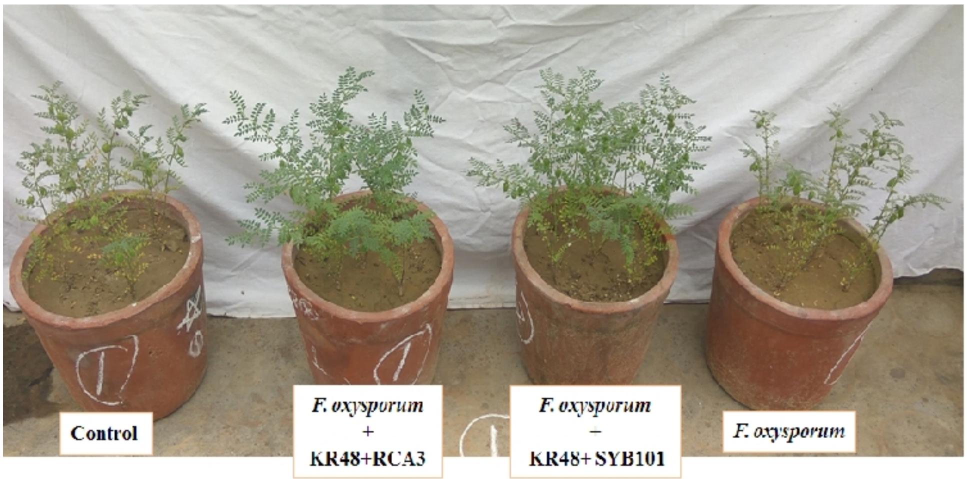


Fig. 64. Coinoculation effect of *Bacillus* isolates with *Mesorhizobium* strain KR48 on plant growth of chickpea at 75 days of sowing

Table 34. Influence of *Mesorhizobium* strains and *Bacillus* isolates on chickpea plant growth at 90 days after sowing

Treatments	**Nodule no./plant**	**Nodule weight (mg/plant)**	**Shoot dry weight (mg/plant)**	**Root dry weight (mg/plant)**
T1 : Control	20	210	751	518
T2 : *Mesorhizobium* strain MBD26	43	521	1233	782
T3 : *Mesorhizobium* strain KR48	45	570	1301	790
T4 : HCA61	26	300	1142	690
T5 : RCA3	28	315	1031	701
T6 : RCA7	31	351	1151	770
T7 : SYB101	35	383	1080	758
T8 : HCA76	25	274	920	615
T9 : Fungus (*Fusarium oxysporum*)	23	232	713	370
T10 : Fungus + MBD26	32	489	1061	736
T11 : Fungus + HCA61	37	580	1433	698
T12 : Fungus + RCA3	36	564	1604	920
T13 : Fungus + RCA7	30	376	1482	855
T14 : Fungus + SYB101	38	603	1395	778
T15 : Fungus + HCA76	22	250	851	493
T16 : Fungus + MBD26 + HCA61	42	583	1542	865
T17 : Fungus + MBD26 + RCA3	34	570	1452	958
T18 : Fungus + MBD26 + RCA7	47	785	1676	965
T19 : Fungus + MBD26 + SYB101	28	491	1573	876
T20 : Fungus + MBD26 + HCA76	30	480	1012	745
T21 : Fungus + KR48	29	415	1481	960
T22 : Fungus + KR48 + HCA61	39	524	1530	870
T23 : Fungus + KR48 + RCA3	44	654	1560	918
T24 : Fungus + KR48 + RCA7	42	570	1724	1020
T25 : Fungus + KR48 + SYB101	45	660	1870	1093
T26 : Fungus + KR48 + HCA76	39	320	1291	890
T27 : Fungus + Bavistin	29	432	1401	832
C.D. at 5% level of significance	-	-	30.279	11.746

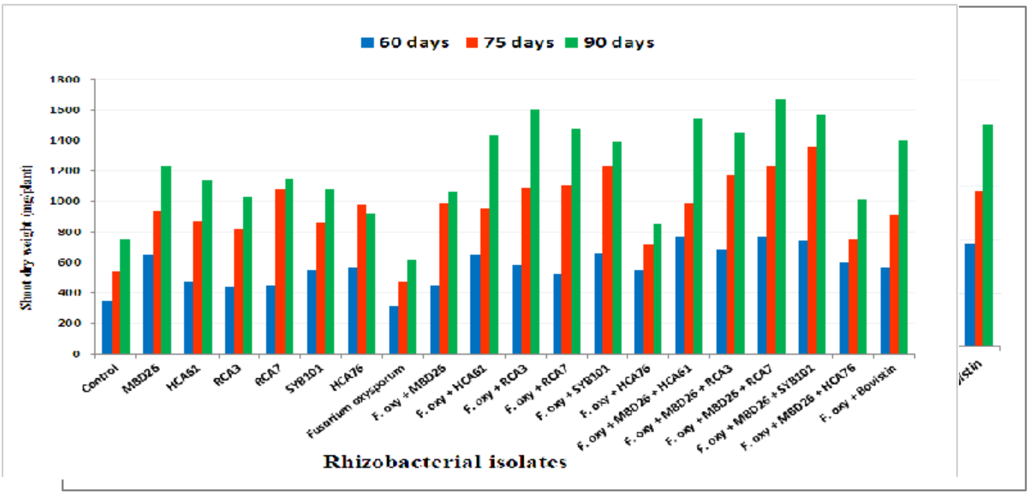


Fig. 65. **Coinoculation effect of *Mesorhizobium* strain MBD26 and *Bacillus* isolates on shoot growth of chickpea plant at 60, 75 and 90 days**

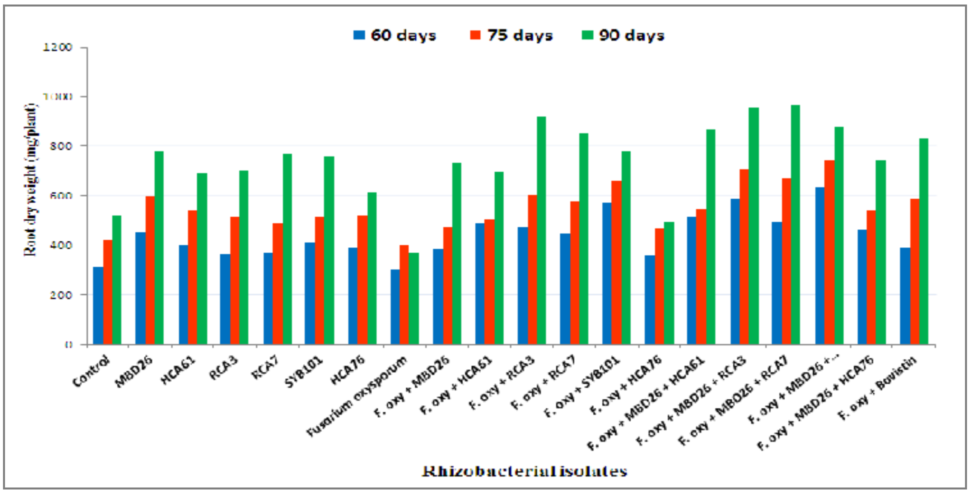


Fig. 66. **Coinoculation effect of *Mesorhizobium* strain MBD26 and *Bacillus* isolates on root growth of chickpea plant at 60, 75 and 90 days**

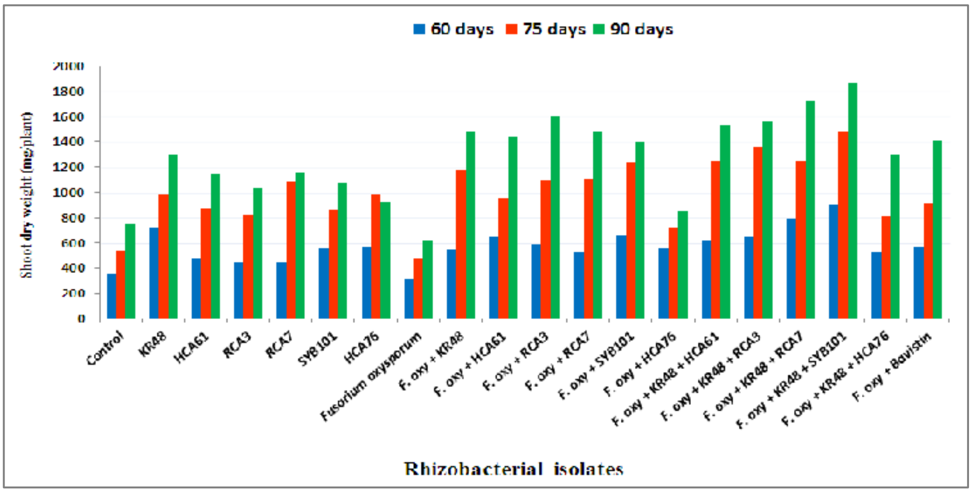


Fig. 67. **Coinoculation effect of *Mesorhizobium* strain KR48 and *Bacillus* isolates on shoot growth of chickpea plant at 60, 75 and 90 days**

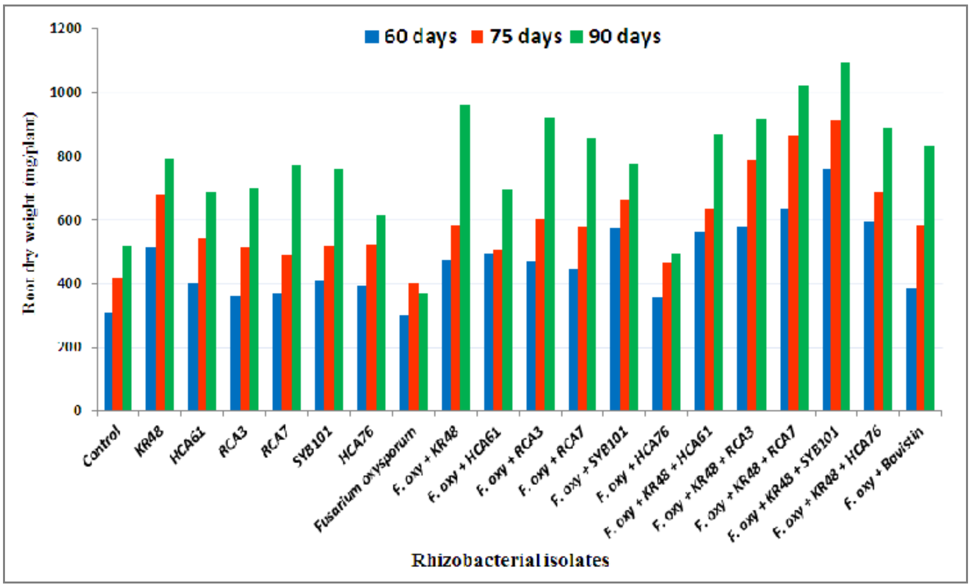


Fig. 68. **Coinoculation effect of *Mesorhizobium* strain KR48 and *Bacillus* isolates on root growth of chickpea plant at 60, 75 and 90 days**

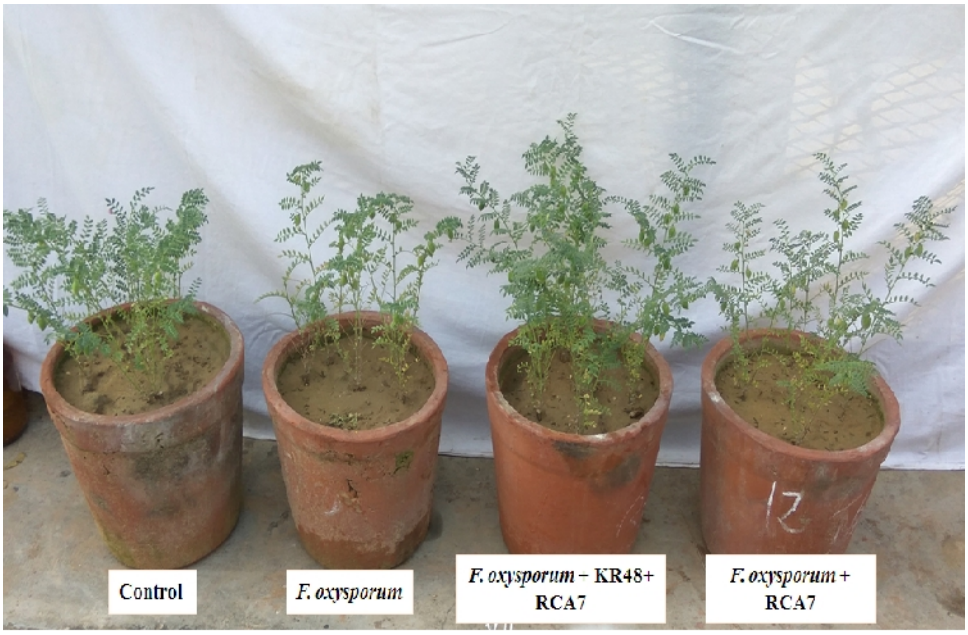


Fig. 69. **Effect of single and coinoculation of *Mesorhizobium* strain KR48 and *F. oxysporum* with *Bacillus* isolate RCA7 on plant growth of chickpea at 90 days of sowing**

CHAPTER-V

DISCUSSION

Plant rhizosphere is an important ecological environment in the soil for plant-microbe interactions (Pii *et al.*, 2015). The underground root and soil microbe interactions are extremely complex, but vitally important for aboveground plant growth, health and disease control (Zhang *et al.*, 2017b). The pathogenic, saprophytic and plant-growth promoting bacteria colonize the same ecological niche and possess similar mechanisms of plant colonization (Miller *et al.*, 1989). Antagonistic microorganisms, by their interactions with various soil-borne plant pathogens, play a major role in microbial equilibrium and serve as powerful agents for biological disease control (Anal *et al.*, 2009). Finding the microorganism very close to epidermis, plants secrete signal molecules for protection against invasion of the heterogenous microbes in the root zone and this is the stage where differentiation takes place between symbiotic, associative, neutralistic or pathogenic adaptation of microbes with the plant. The interactions between biocontrol agents and plant pathogens have been studied extensively (Sindhu *et al.*, 2016) and the applications of biocontrol agents in the protection of some commercially important crops are increasingly capturing the attention of many plant pathologists and microbiologists. Currently, biocontrol is gaining stature as a possible practical agricultural practice for control of soil-borne pathogens (Weller, 2007). Biocontrol of plant pathogens provides an alternative means of reducing the incidence of plant diseases without the negative aspects of chemical pesticides.

Chickpea is a major food legume grown mostly by the poor and subsistence farmers in the semi-arid tropics of Asia and Africa. It also has advantages in improving soil fertility, particularly in dry lands and the semiarid tropics (Saxena *et al.*, 1996). Low yield of chickpea has been attributed to its susceptibility to several biotic and abiotic stresses. Among the biotic stress, chickpea is frequently attacked by wilt disease caused by *Fusarium oxysporum* f.sp. *ciceris*, which is worldwide in distribution and most prevalent in the Mediterranean Basin and the Indian subcontinent (Khan *et al.*, 2002; Patra and Biswas, 2017). The fungus is soil as well as seed borne pathogen and causes wilting of crop plants (Pande *et al.*, 2007). In India, annual yield loss due to *Fusarium* wilt was estimated at 10% (Dubey *et al.*, 2007). Under favourable conditions for fungal growth, the wilt infection may damage the crop completely and cause 100% yield loss (Navas-Cortes *et al.*, 2000; Sharma *et al.*, 2005). Thus, plant pathogenic microorganisms are a major threat to food production, and traditional methods seem to be insufficient to control root diseases of important crop plants (Johri *et al.*, 2003).

The rich microbial diversity in plant rhizosphere possesses a resource which could replace the synthetic agrochemicals to control phytopathogens (Saraf *et al.*, 2014). Because of their multilayered cell wall, secretion of peptide antibiotics, peptide signal molecules, extracellular enzymes and stress resistant endospore formation, members of the genus *Bacillus* survive under extremes of environment and can be exploited as biocontrol agents. Several *Bacillus* species have been reported as efficient producers of antimetabolites and allelochemicals, and are used in plant disease control leading to plant growth promotion (Zaim *et al*., 2013). The principal mechanisms of growth promotion and control of phytopathogens include production of growth stimulating phytohormones (Glick, 2012), solubilization and mobilization of phosphate (Taurian *et al*., 2010), siderophore production (Haas and Défago, 2005), antibiosis through production of antibiotics, inhibition of plant ethylene synthesis, production of cell wall degrading enzymes and induction of plant systemic resistance to pathogens, and cyanide production (Ribeiro and Cardoso, 2012).

Being an important legume grain crop, chickpea has been widely cultivated throughout the world and the nodule bacteria of this plant have been intensively studied in the last decades, especially in Mediterranean countries. *Mesorhizobium ciceri*, *M. mediterraneum* and *M. muleiense* were reported as dominant groups but several *Mesorhizobium* sp. strains were also isolated from chickpea nodules in Europe, India and China (Zhang *et al.*, 2017a). Some of nodule forming mesorhizobia prevent the deleterious effects of plant pathogens mostly through the synthesis of antibiotics and fungicidal compounds (Dobbelaere *et al*., 2003), competition for nutrients (siderophore production) or by the induction of induced systemic resistance (ISR) against pathogens (Gururani *et al*., 2013).

The present study was planned with the objectives to evaluate antagonistic effect of *Bacillus* species against pathogenic fungi under cultural conditions. Coinoculation effect of *Bacillus* species with *Mesorhizobium* strains was studied for their effect on nodulation and plant growth promotion of chickpea under sterilized/chillum jar conditions as well as for disease control of wilt caused by *Fusarium oxysporum* under pot house conditions.

5.1. Isolation of *Bacillus* species from chickpea rhizosphere

One hundred ninety three rhizobacteria were obtained from the rhizosphere soil of chickpea (Table 2, 3). Bacterial colonies were isolated by dilution plate method and picked from LB medium plates spreaded with 10^{-4} and 10^{-5} dilutions of chickpea rhizosphere samples. The bacteria isolated were mainly selected on the basis of morphological characteristics to those resembling to *Bacillus* species (Fig. 4). One reference strain was obtained from Department of Microbiology, CCS H.A.U., Hisar. Karimi *et al*. (2012) isolated two hundred and thirty two bacteria from the rhizosphere and root of chickpea showing substantial inhibition zones against *Fusarium oxysporum* f. sp *ciceris* under *in vitro* condition. Twelve bacterial strains showed high antifungal activity against pathogenic fungi. Based on biochemical, physiological and morphological tests, selected isolates were identified as

Bacillus subtilis (B1, B6, B28, B40, B99 and B108), *Pseudomonas putida* (P9 and P10) and *P. aeuroginosa* (P11, P12 P66 and P112).

In the present study, rhizobacteria were isolated from different fields of Alwar (Rajasthan) and Hisar (Haryana) to identify the organisms which have adapted to various environmental conditions. Zaim *et al*. (2013) isolated 131 rhizobacterial isolates from the chickpea rhizosphere from nine locations in Algeria: Mascara, Sidi Bel-Abbes, Tlemcen, Tiaret, Relizane, Mostaganem, Chlef, Constantine and Guelma, and isolates having efficient fungal antagonism were identified. Kumari and Khanna (2014) reported antagonistic potential of 28 rhizobacteria isolated from chickpea rhizosphere, among which 11 isolates belonged to *Bacillus*, 2 isolates to *Serratia* and 15 isolates were representatives of pseudomonads.

The use of rhizobacteria for plant disease control has been found more effective when rhizobacteria is isolated from rhizosphere of the same host plant (Weller, 1988). Therefore, all the *Bacillus* isolates were selected from rhizosphere of healthy chickpea plants in the present study. The predominance of *Pseudomonas* and *Bacillus* sp. in legume rhizosphere has been reported by many workers. Parmar and Dadarwal (2001) reported that fluorescent pseudomonads and spore forming *Bacillus* species predominantly colonize the rhizosphere of healthy chickpea plants. Yadav *et al*. (2010) reported that population of *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia* and *Serratia* dominated the chickpea rhizosphere. Similarly, populations of *Pseudomonas*, *Bacillus* and *Azotobacter* predominantly colonized the rhizosphere and rhizoplane of healthy chickpea plants (Joseph *et al*., 2007; Ahmad *et al*., 2008). They found that bacterial population ranged from $0.5\text{-}2.1\times10^6$ of *Bacillus* sp., $1.1\text{-}2.1\times10^6$ of *Pseudomonas* sp. and $0.3\text{-}1.7\times10^6$ of *Azotobacter* sp. Hynes *et al*. (2008) reported that plant growth promoting bacteria isolated from the roots of pea, lentil and chickpea belonged to the families *Pseudomonadaceae* and 36-42% of the *Enterobacteriaceae*. Similarly, Kundu *et al*. (2009) found that isolates from chickpea rhizosphere mostly belonged to genera *Pseudomonas*, *Aeromonas* and *Enterobacter*. Ninety eight rhizobacterial isolates were isolated from thirty-six chickpea rhizospheric soil samples and were subjected to antagonism tests against *F. oxysporum* (Kumari and Khanna, 2016). These were further examined for morphological, biochemical, molecular characters and identified as *Bacillus* sp. Out of 14 isolates obtained from rhizosphere soils along the vicinity of Rusizi river at Cibitoke province (North Western of Burundi), eight strains (57%) belonged to *Bacillus* (Nihorimbere and Ongena, 2017). Further, identification of these isolates characterized these isolates as *Bacillus subtilis*, *B.amyloliquefaciens*, *B. atrophaeus* and *B. mojavensis* sp.

5.2. Antagonistic interactions of *Bacillus* isolates with *Fusarium oxysporum* under *in vitro* conditions

In plant disease management programmes, the use of a rapid method for screening efficient biocontrol agents is a prerequisite (Anith *et al*., 2003). Microorganisms that grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the frontline defense for roots against attack by pathogens (Nautiyal, 1997). In present study, antagonistic activity of selected *Bacillus* isolates was determined on the ability of bacteria to inhibit fungal growth under culture conditions. Out of 193 *Bacillus* isolates tested, only 87 isolates inhibited the growth of *F. oxysporum* (Table 4). The fungal growth inhibition zone varied from 4.2 to 27.2 mm in different isolates tested (Fig. 5a, b). Thirty one isolates showed more than 10 mm inhibition zone, whereas eleven isolates showed large inhibition zones more than 20 mm. Most of the isolates exhibited weak antagonistic activity against the pathogen (Fig. 6). Similar mycoparasitic potential of *Bacillus* sp. has previously been reported (Saharan and Nehra, 2011; Suneeta *et al*., 2016). *Bacillus* sp. has been reported to modify the fungal mycelium appearance, due to antifungal secondary metabolite production. Generally, biocontrol capacity through antagonistic bacteria involves either competition or bacterial metabolite production, such as siderophores, hydrogen cyanide, antibiotics or extracellular enzymes for antagonism towards plant pathogens (Kamilova *et al*., 2005).

Kaur *et al*. (2007) reported that 14 out of 96 *Pseudomonas* isolates from chickpea rhizosphere were highly antagonistic to *F. oxysporum* sp. Different *Pseudomonas* isolates showed inhibition zone ranging from 5-7 mm under *in vitro* growth conditions. Growth inhibition of *F. oxysporum* may be due to fungistatic effect or might be attributed to the secretion of antibiotics by the fungi or other inhibitory substances produced by the antagonists. Karimi *et al*. (2012) studied antagonistic effects of *Pseudomonas* and *Bacillus* species isolated from rhizosphere of chickpea and evaluated against *Fusarium oxysporum* f. sp. *ciceris* as potential biocontrol agents *in vitro* and *in vivo*. In dual culture test, *B. subtilis* strains were shown to have more growth inhibition of pathogen on the PDA medium than *Pseudomona*s isolates. *B. subtilis* B28 isolate showed the highest inhibition percentages (51.16%) than other isolates. Among 131 rhizobacterial isolates tested, only 29 isolates were found potentially antagonistic strains. Five antagonistic *Bacillus* spp. Rb29, Rb6, Rb12, Rb4 and Rb15 showed the most inhibitory effect against FOC1 (from 25.63 to 71.11%) mycelial growth and FOC2 (from 28.43 to 60.65%) *in vitro*. Karuppiah and Rajaram (2011) reported that eight *Bacillus* sp., out of 63 different *Bacillus* isolates tested, exhibited plant growth promoting activities and six of these *Bacillus* isolates also inhibited the growth of *Penicillium* sp., *Cercospora* sp. and *F. oxysporum*. Kumari *et al*. (2013) reported that rhizobacterial isolates Ps-17, Ps-14 (*Pseudomonas*) and S-10 (*Serratia*) isolated from chickpea rhizosphere inhibited growth of *F. oxysporum* sp. *ciceris* under *in vitro* dual plate assay by 42.8, 31.4 and 28.6%, respectively.

5.3. Growth inhibition of *Aspergillus niger*, *Neovossia indica* and *Rhizoctonia solani* by *Bacillus*isolates

Many pathogenic fungal species viz., *Alternaria porri*, *Aspergillus nidulans*, *A. niger*, *A. wentii*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Penicillium notatum*, *Rhizoctonia* sp. and *Rhizopus arrhizus* been reported in chickpea fields (Ahmad *et al*., 1993). Out of twenty four *Bacillus* isolates tested in this study, twelve isolates showed significant inhibition of *Aspergillus niger* on plates and three *Bacillus* isolates showed little inhibition of fungal growth (Table 6; Fig. 7). In similar study, a rhizobacterial strain of *Bacillus subtilis* AF1 grown for 6 h was coinoculated with *Aspergillus niger* (Podile and Prakash, 1996). Growth of AF1 resulted in damage to the cell wall, followed by lysis. AF1 inoculation into media containing *A. niger* at 0, 6, and 12 h suppressed > 90% fungal growth, while in 18- and 24-h cultures fungal growth inhibition was 70 and 56%, respectively, in terms of dry weight. Bacteria associated with 6 habitats of groundnut were evaluated for suppression of collar rot (*A. niger*) of groundnut (Kishore *et al*., 2005). Three hundred and ninety-three strains were tested against 8 fungal pathogens of groundnut including 5 necrotrophic fungi, *Aspergillus flavus*, *A. niger*, *Rhizoctonia bataticola*, *Rhizoctonia solani* and *Sclerotium rolfsii*, and 3 biotrophic fungi, *Cercospora arachidicola*, *Phaeoisariopsis personata* and *Puccinia arachidis*. *Pseudomonas* sp. GRS175, *Pseudomonasaeruginosa* GPS21, GSE18, GSE19 and GSE30, and their cell-free culture filtrates were found highly antagonistic to all the test fungi. *P. aeruginosa* GSE18 emerged as an effective biocontrol agent of collar rot closely followed by *P. aeruginosa* GSE19. Biocontrol of *R. solani* damping-off disease in cucumber with inoculation of *Bacillus pumilus* SQR-N43 was described (Huang *et al*., 2012).

Growth inhibition of *A. niger* (66.5%) and *F. oxysporum* (64.4%) has also been reported by Parani and Saha (2009) when inoculated with potential antagonist *Serratia marcescens*. *Bacillus* species were isolated from the rhizosphere of *Calendula officinalis* (Kaki *et al*., 2013). Fifteen per cent of the isolated bacteria were screened for their important antifungal activity against *F. oxysporum*, *Botrytis cinerea*, *A. niger*, *Cladosporium cucumerinium* and *Alternaria alternata*. *Bacillus* isolates showed significant growth inhibition percentages i.e., 39–83% against *F. oxysporium* and *B. cinerea*.

Bacillus isolate RCA9 showed maximum antagonistic activity against *Neovossia indica*. Two *Bacillus* isolates RCA3 and HCA61 showed significant antagonistic activity and six isolates also caused growth inhibition of fungi (Table 7; Fig. 8). The potentiality of fungal, bacterial and actinomycete biocontrol agents isolated from soil was investigated against *Neovossia indica* (Amer *et al*., 1998). Culture filtrates of *Trichoderma pseudokoningii*, *T. lignorum*, *T. koningii*, *Gliocladium roseum*, *G. deliquescens*, *G. virens*, fluorescent pseudomonad isolate (FP-VII), bacterial isolate Bact-II and an actinomycete isolate act-V inhibited germination of teliospore and secondary sporidia of Karnal bunt fungus.

Maximum inhibition of *Rhizoctonia solani* was observed by *Bacillus* isolates HCA26 and RCA63, and isolates RCA9 and HCA41 also showed significant inhibition of fungal growth (Table 8; Fig. 9). Five *Bacillus* isolates showed comparatively less inhibition of fungal growth, whereas nine isolates showed little inhibition. Khot *et al*. (1996) isolated 36 rhizobacteria from rhizosphere of chickpea and five bacteria were found to inhibit the growth of *F. oxysporum* and *R. bataticola*. Siddiqui *et al*. (2001) showed that *P. aeruginosa* and *B. subtilis* strains produced inhibition zones by inhibiting the radial growth of *Macrophomina phaseolina, F. oxysporum* and *R. solani*. Lemessa and Zeller (2007) found that six strains of rhizobacteria i.e., RP87, B2G, APF1, APF2, APF3 and APF4 showed good inhibitory activity against *Rhizoctonia solanacearum* out of 118 strains tested. Ahmad *et al*. (2008) reported that siderophore production and antifungal activity was exhibited by 10 to 12.77% of *Azotobacter* and *Pseudomonas* isolates. *Pseudomonas* Ps5 and *Bacillus* B1 isolates showed broad-spectrum antifungal activity on Muller-Hinton medium against *Aspergillus*, *Fusarium* and *Rhizoctonia bataticola*. Josic *et al.* (2015) reported that PGP isolates, Q16 and B25, showed the best antifungal activity against *Trichoderma viride* and good antifungal effect against *Aspergillus fumigatus* and *Aspergillus niger*. *Pseudomonas* sp. B25 exhibited higher antifungal potential than Q16 isolate. To characterize antagonistic bacteria, one hundred and thirty bacterial isolates were screened against *R. solani* under cultural conditions (Dua and Sindhu, 2012). Sixteen bacterial isolates inhibited the growth of *R. solani* and growth inhibition zone varied from 6-15 mm by different rhizobacterial isolates. Two isolates WPS3 and WPS90 caused maximum growth inhibition of the fungi. Growth inhibiton of the pathogenic fungi was also observed using culture filtrates of antagonistic rhizobacterial isolates.

5.4. Characterization of beneficial activities of the selected antagonistic *Bacillus* cultures

Twenty four *Bacillus* isolates showing variable level of antagonistic activity against phytopathogenic fungus i.e., *F. oxysporum* were selected for study of different beneficial plant growth promoting activities like sideophore production, 1-aminocyclopropane-1-carboxylate utilization and production of hydrogen cyanide, δ-aminolevulinic acid and indole acetic acid (IAA). *Bacillus* isolates were also studied for phosphorous and potassium solubilization.

5.4.1. Screening of *Bacillus* isolates for siderophore production

Siderophore producing bacteria may play a pivotal role in controlling several fungal pathogens. *Fusarium oxysporium* f. sp. *ciceri* (FOC) is major phytopathogen amongst the several constraints affecting the productivity of chickpea in India and other countries in Asia. Siderophore producing *Bacillus* isolates changed the blue color of the medium and formed yellow or orange halo zones around the colony in CAS assay method. The color change from blue to orange was the result of siderophoral removal of Fe from the dye, which is also an indicative of a hydroxymate type of siderophore (Milagres *et al*., 1999). Siderophore production by *B. subtilis* CTS-G24 was confirmed by developing yellow to orange colored zone on blue coloured CAS agar plate indicating siderophore production (Patil *et al*., 2015).

In this study, all 24 rhizobacterial isolates were spotted on the CAS medium plates. Four isolates HCA1, HCA3, HCA53 and RCA11 formed large orange halo zone, whereas five isolates formed small halo zones around the bacterial growth (Table 9; Fig. 10a, b). The ability to produce siderophore was found only in 41% of the *Bacillus* isolates (Fig. 11). Gupta *et al*. (2002) reported similar results on CAS agar plates where diameter of halo ranged from 0.72-2.6 cm after 24-72 h of incubation. Akhtar and Siddiqui (2010) reported siderophores production by *Pseudomonas* sp. isolated from chickpea rhizosphere and the halos formed on CAS medium ranged from 1.6-1.7 cm. Buyer *et al*. (1993) using monoclonal antibodies, confirmed the production of siderophore by PGPR in rhizosphere under iron-limiting conditions. Purified siderophores showed disease-suppressive effect similar to the producer strain (Neilands and Leong, 1986). A siderophore over-producing mutant of *Pseudomonas putida* was found more effective than the wilt type in control of Fusarium wilt of tomato (Vandenburgh and Gonzalez, 1984), while a siderophore-deficient mutant of *P. aeruginosa* lost its biocontrol ability (Buysens *et al*., 1994). The production of siderophores by microorganisms is beneficial to plants, because it can inhibit the growth of plant pathogens (Sharma and Johri, 2003). In a dual culture assay for fungicidal activity of *Bacillus subtilis* CTS-G24 against FOC and *M. phaseolina*, strain CTS-G24 vigorously inhibited the growth of the phytopathogenic fungi (Patil *et al*., 2015).

Sindhu *et al.* (1999) reported that some *Pseudomonas* isolates inhibited the growth of phytopathogenic fungi (*Aspergillus* sp., *Curvularia* sp., *Fusarium oxysporum* and *Rhizoctonia solani*). The rhizobacterial isolates produced siderophores in Fe-deficient succinate medium. Yeole *et al.* (2001) showed that twelve fluorescent pseudomonads isolates obtained from chilli (CHRB2, CHRB3 and CHRB4), cotton (CORB1, CORB2, CORB3 and CORB4), groundnut (GNRB1, GNRB2 and GNRB3) and soybean (SBRB4 and SBRB5) inhibited the growth of twelve soil borne plant pathogens (*Aspergillus* sp., *A. niger, Fusarium* sp., *Fusarium oxysporum, F. solani, Mucor* sp., *Penicillum* sp., *Pythium* sp., *Rhizopus oryzae, Rhizoctonia solani* and *Sclerotium rolfsii*) in Fe deficient Kings'B medium. Yu *et al.* (2011) screened bacteria from the rhizospheric soil of pepper in Hainan, China. *Bacillus subtilis* CAS15, which produced a large orange halo on CAS plates, strongly antagonized the growth of 15 plant fungal pathogens, with rates of inhibition ranging from 19.26 to 94.07%. Nineteen of the antagonistic rhizobacteria showed a distinct orange halo on CAS plates indicating siderophore production. Out of these eleven isolates belonged to *Pseudomonas* spp., seven belonged to *Bacillus* spp. and one isolate belonged to *Serratia* spp. Siderophores can directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria, which suppresses the growth of phytopathogenic fungi and function as stress factors in inducing host resistance (Ahmad *et al*., 2008; Zhou *et al.*, 2012).

5.4.2. Screening of *Bacillus* isolates for utilization of 1-aminocyclopropane-1- carboxylate

Some of the plant growth-promoting bacteria contain the enzyme ACC deaminase and their colonisation in the vicinity of developing seedlings may decrease the ethylene level and thus, it may prevent the inhibition of the root growth by stress hormone ethylene (Kumari *et al*., 2017). Several bacterial strains which can utilize ACC as a sole source of nitrogen have been isolated from rhizosphere soil samples and have been subsequently used for inoculation and coinoculation purposes (Glick *et al*., 2007a, b).

Out of 24 isolates tested in this study, seven isolates i.e., HCA3, HCA61, RCA3, RCA4, RCA7, RCA11 and RCA31 showed significant growth on ACC supplemented plates and sixteen isolates showed moderate growth (Table 10; Fig. 12, 14). On ammonium sulphate containing plates, seven *Bacillus* isolates i.e., HCA11, RCA3, RCA4, RCA19, RCA31, RCA63 and RCA68 showed very good growth, whereas eight isolates showed good growth (Table 11; Fig. 13). Seven isolates showed moderate growth and only two isolates i.e., RCA9 and HCA1 showed little growth on ammonium sulphate containing plates (Fig. 14). Overall, 96% *Bacillus* isolates showed ACC utilization ability.

Ghosh *et al*. (2003) recorded ACC deaminase activity in three *Bacillus* species namely, *Bacillus circulans* DUC1, *Bacillus firmus* DUC2 and *Bacillus globisporus* DUC3 that stimulated root elongation in *Brassica campestris*. Ma *et al.* (2003) reported that only a small fraction of rhizobial strains contain ACC deaminase enzyme. In the screening of 13 different known rhizobial strains, they observed that only five strains possessed ACC deaminase activity. Zafar *et al.* (2007) obtained twenty seven isolates of rhizobacteria containing ACC deaminase from the lentil rhizosphere and all the rhizobacterial isolates showed the potential to modify the growth of lentil seedlings under axenic conditions. Onofre-Lemus *et al*. (2009) investigated the presence of ACC deaminase activity and the *acd*S gene in 45 strains, which were nonpathogenic, plant associated and rhizospheric or endophytic bacteria. In the genus *Burkholderia*, eighteen species exhibited ACC deaminase activities in the range from 2 to 15 mol of ketobutyrate/h/mg protein.

Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Ralstonia, Serratia* and *Rhizobium* etc. (Kang *et al*., 2010). Khandelwal and Sindhu (2012) found that 46.3% *Bradyrhizobium* and *Rhizobium* isolates obtained from clusterbean rhizosphere showed good growth on ACC supplemented plates. ACC deaminase containing rhizobacterial isolates GSA6, GSA11 and GSA110 formed 42-48 nodules per plant and caused 80.7 to 205.3% gain in plant dry weight in comparison to uninoculated plants at 60 days of plant growth under chillum jar assembly conditions. Ninety six rhizobacterial isolates were screened for ACC utilization (Khandelwal, 2015) and sixty three per cent of rhizobacterial isolates showed growth on ACC supplemented plates.

5.4.3. Screening of *Bacillus* isolates for hydrogen cyanide production

Isolates obtained from rhizosphere of rice, mangrove, chickpea and effluent contaminated soil showed HCN production (Joseph *et al.,* 2007). The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules (Ahmad *et al*., 2008; Ahemad and Kibret, 2014). In this study, six *Bacillus* isolates showed HCN production and HCN production varied from '+' to '4+' among different isolates (Table 12; Fig. 15). Three *Bacillus* isolates RCA3, RCA7 and RCA11 showed significant HCN production. Three isolates RCA4, HCA1 and HCA61 showed moderate HCN production. HCN production was not observed in eighteen isolates (Fig. 16). In quantitative estimation, HCN production varied from 49.60 to 73.78 µl/ml. *Bacillus* isolates RCA3, RCA7 and RCA11, whereas other three isolates RCA4, HCA1 and HCA61 showed moderate HCN production (Table 13). Only 33.3% *Bacillus* isolates showed HCN production (Fig. 16).

Many bacterial species including *Pseudomonas*, *Chromobacterium*, *Rhizobium* and several cyanobacterial species have been reported to produce cyanide as a secondary metabolite (Blumer and Haas, 2000). Wani *et al.* (2007b) tested rhizosphere bacterial isolates for HCN producing ability *in vitro*. *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium produced significant amount of hydrogen cyanide (Selvakumar *et al*., 2009b). Saraf *et al*. (2008) isolated 10 strains of *Pseudomonas* from chickpea rhizosphere, out of which three produced HCN, the strongest producer being *Pseudomonas* M1P3. Siddiqui and Shakeel (2009) reported HCN production by twenty-one *Pseudomonas* isolates from pigeonpea rhizosphere, out of which three were potent producers of HCN, whereas eleven were moderate producers. Ghodsalavi *et al*. (2013) isolated 40 bacteria from the rhizosphere of valerian in which most of the isolated bacteria produced siderophore, lipase, HCN and protease, and these bacterial isolates also increased shoot length of plant. Reetha *et al*. (2014b) obtained two strains of *B. subtilis* and two strains of *P. fluorescens*. Qualitative analysis of HCN in these strains indicated a strong production of HCN in Pf1 and CPf5. Out of 28 antagonistic bacterial isolates, only twelve isolates were found positive for HCN production, of which seven belonged to *Pseudomonas* spp. and five belonged to *Bacillus* sp. (Kumari and Khanna, 2014). Isolates 3B, 9B, 12B, 20P, 27P, 29P, 34P, 38P and 39P were found to be strong producer of HCN, causing colour change from yellow to reddish brown, whereas 4B, 6B and 25P were moderate HCN producers indicated by orange brown colour.

Lakshmi *et al*. (2015) reported that bacterial isolate KC1 isolated from the rhizosphere of castor plants (*Ricinus communis*) produced cyanide (4.78 nmol L^{-1}) over a period of 36 h. Khandelwal (2015) showed that 29 bacterial isolates showed HCN production and HCN production varied from + to 4+ among different isolates. Two bacterial isolates i.e., WHA123 and WHA125 showed maximum HCN production and three isolates i.e., MSA50, MSA56 and WHA87 showed moderate HCN production. Overall, 30% bacterial isolates showed hydrogen cyanide production. Similar results were obtained by Phour (2016), in which eight bacterial isolates showed HCN production out of 18 isolates tested and it varied from + to 3+ among different isolates. Two bacterial isolates i.e., JMM19 and JMM24 showed maximum HCN production and other two isolates JMM22 and JMM35 showed moderate HCN production. Four bacterial isolates HMM37, HMM92, HMM97 and JMM15 showed very little HCN production.

5.4.4. Production of δ-aminolevulinic acid by *Bacillus* isolates

Tanaka *et al.* (1992) found that low concentration of ALA application increased the chlorophyll content and accelerated the growth of plant tissue and rice seedlings. ALA could also be used in agricultural applications as a herbicide, insecticide and growth promoting factor including salt and cold temperature tolerance in plants (Watanabe *et al.*, 2000). In this study, all the antagonistic *Bacillus* isolates showed ALA production (Table 14; Fig. 17, 18). Significant ALA production (> 12 µg/ml) was observed in seven *Bacillus* isolates. Seventeen isolates produced ALA ranging from 6.87 to 11.25 µg/ml. Liu *et al.* (2005) selected seven stains of *Rhodopseudomonas* sp. from 36 photosynthetic bacterial strains. *Rhodopseudomonas* sp. strain 99-28 showed highest 5-aminolevulinic acid (ALA) production ability among these seven strains. ALA production was 2.819, 1.531, 2.166 and 2.424 mg/L in monosodium glutamate waste water, succotash waste water, brewage waste water and citric acid waste water, respectively. When levulinic acid (LA), glycine and succinate were supplied, ALA production was dramatically increased. One hundred sixty two bacterial isolates were obtained from mustard rhizosphere soil and significant ALA production (> 15 µg ml^{-1}) was observed in 10 bacterial isolates (Phour, 2016). Other eighty four isolates produced ALA ranging from 10.1 to 15.0 µg ml^{-1}. Similarly, two hundred fifty rhizosphere bacteria were isolated from the rhizosphere of wheat and mustard (Khandelwal, 2016). Rhizobacterial isolates WSA38, MSA57, WSA68, WSA56, MSA42, MSA39, WHA98 and MSA11 showed >11.0 µg ml^{-1} δ-aminolevulinic acid production.

5.4.5. Screening of *Bacillus* isolates for indole acetic acid production

Diverse bacterial species possess the ability to produce IAA (Ashrafuzzaman *et al*., 2009; Saharan and Nehra, 2011). It functions as an important signal molecule in the regulation of plant development and indirectly influence bacterial ACC deaminase activity (Wahyudi *et al.,* 2011). Production of IAA has been observed in species of *Bacillus, Pseudomonas, Azotobacter, Azospirillium, Phosphobacteria, Glucanoacetobacter, Aspergillus niger* and *Penicillium.* Bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defense mechanisms (Patel *et al*., 2012). It has been reported that *B. megaterium* from tea rhizosphere produced IAA and helped in plant growth promotion (Chakraborty *et al.*, 2006).

The IAA production was observed in all the 24 isolates and it varied from 0.13 to 29.60 µg/ml (Table 15; Fig. 19). Seven isolates produced IAA ranging from 1.0 to 5.0 µg/ml. Five isolates produced IAA ranging from 6.0-9.0 µg/ml while three isolates HCA61, RCA4 and RCA19 produced IAA ranging from 16.0-23.0 µg/ml. Maximum production of IAA (> than 25.0 µg/ml) was observed in isolate RCA9, whereas eight isolates showed very little (< 1.0 µg/ml) IAA production (Fig. 20).

Seven isolates of *B. megaterium* JUMB1, JUMB2, JUMB3, JUMB4, JUMB5, JUMB6 and JUMB7 were screened *in vitro* for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia, HCN, phosphate, siderophores and evaluated for the ability to suppress fusarial growth (Shobha and Kumudini, 2012). All the isolates produced IAA, ammonia, HCN and siderophores. Ten bacterial isolates were found as IAA producer from rhizosphere soil (Mohite, 2013). These isolates were coded as br1, br2, br3 (from banana rhizosphere), wr2 (from wheat rhizosphere) and mr2 (from maize rhizosphere). The isolates were identified as *B. megaterium, Lactobacillus casei*, *B. subtilis*, *B. cereus* and *Lactobacillus acidophilus*, respectively for isolates coded as br1, br2, br3, wr2 and mr2. Reetha *et al*. (2014b) isolated *P. fluorescens* and *Bacillus subtilis*from the rhizosphere of onion plant and screened for their plant growth promoting activities and indole acetic acid production. In the quantitative measurements, the highest value of IAA production was obtained by *P. fluorescens* followed by *B. subtilis* and these strains produced 15.38±0.537 and 12.67±0.325 IAA, respectively.

5.4.6. Phosphorous solubilization by *Bacillus* isolates

Phosphorus is the second most important key element in the nutrition of plants, next to nitrogen. Plants absorb phosphate only in two soluble forms, the monobasic (H_2PO_4) and the diabasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). Strains from genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers, while tricalcium phosphate and hydroxyl apatite seem to be more degradable substrates than rock phosphate (Banerjee *et al*., 2006). PGPR together with PSB can reduce P fertilizer application by 50% without any significant reduction in crop yield (Jilani *et al*., 2007). Inoculation of seeds with PSB is a promising technique which alleviates the deficiency of P (Qureshi *et al*., 2012). Phosphate solubilizing microorganisms largely include bacteria and fungi viz. species of *Bacillus*, *Pseudomonas*, *Penicillium* and *Aspergillus* (Tilak *et al*., 2005).

Out of 24 bacterial isolates tested, three isolates i.e., HCA61, RCA11 and RCA63 showed P-solubilization zone varying between 4.1 to 5.0 mm and two isolates RCA3 and RCA4 showed P-solubilization zone ranging from 3.1 to 4.0 mm (Table 16). Three isolates

HCA53, RCA7 and RCA19 showed moderate zone of phosphorus solubilization ranging 2.1-3.0 mm, whereas five isolates HCA44, HCA76, RCA70, HCA81 and SYB101 showed little zone of phosphorus solubilization ranging from 1.1-2.0 mm (Fig 21, 22). Maximum zone of P-solubilization was formed by HCA61, RCA11 and RCA63. Eleven *Bacillus* isolates (45.8%) did not solubilize phosphorus (Fig. 23). Ashrafuzzaman *et al*. (2009) showed that seven *Bacillus* isolates isolated from rice rhizosphere were not able to solubilize phosphate. Wahyudi *et al*. (2011) showed that eleven out of twelve isolates of *Bacillus* from soybean rhizosphere possess the ability to solubilize the phosphorus.

Vazquez *et al*. (2000) isolated bacteria belonging to genera *Bacillus*, *Paenibacillus*, *Vibrio, Enterobacter*, *Kluyvera* and *Pseudomonas* etc. from mangroves rhizosphere and found that the most common mechanism of phosphorous solubilization was by secretion of organic acids. Gupta *et al*. (2002) described *Pseudomonas* species as a potent phosphate solubilizer obtained heavy metal resistant mutants of phosphate solubilizing *Pseudomonas* NBRI 4014. Gull *et al*. (2004) isolated phosphate solubilizing bacterial strains from rhizosphere, roots and nodules of chickpea. The potential of isolated bacterial strains to solubilize phosphorous was evaluated qualitatively by measurement of clear zone around the colonies and the diameter of clear zone ranged from 21 to 83 mm. Phosphate solubilization was also quantified by spectophotomertry and was found to range from 65 to 130.5 µg/ml. Phosphate solubilizing bacterial strains CPS-2, CPS-3 and Ca-18 had the maximum positive effect on shoot length, shoot dry weight and nodulation of chickpea plants. Anu and Kundu (2005) observed that among 76 *Pseudomonas* strains isolated from chickpea rhizosphere, phosphate solubilization efficiency varied from 22-248 µg/ml in liquid medium. The isolates belonging to *Bacillus* sp. (67%) and *Burkholderia* sp. (58.5%) obtained from maize rhizosphere showed solubilization of aluminium, phosphate and tricalcium phosphate (Oliveira *et al*., 2009).

5.4.7. Potassium solubilization by *Bacillus* isolates

Only nine *Bacillus* isolates formed zone of K-solubilization on mica powder supplemented medium plates out of 24 *Bacillus* isolates tested. Three isolates i.e., HCA61, RCA3 and RCA63 showed potassium-solubilizing index (K-SI) more than 4.0 (Table 17; Fig. 24). Four *Bacillus* isolates RCA4, RCA7, RCA11 and RCA19 showed K-SI more than 3.0. Two isolates showed K-SI more than 2.0. Fifteen isolates did not cause K-solubilization on mica containing plates. Only 37% *Bacillus* isolates solubilized the potassium on mica containing medium plates (Fig. 25).

In earlier studies, out of 55 rhizobacterial isolates tested, only 14 isolates formed significant zone of K-solubilization on mica containing medium (Choudhary, 2013). Nine isolates i.e., HCS2, HCS7, HCS36, HCS41, HCS42, HCS44, HFS6, HFS11 and HFS12 showed large solubilization zone and five isolates HCS16, HCS21, HFS5, HFS9 and HFS12 showed moderate solubilization zone. Parmar and Sindhu (2013) reported that out of 137 rhizobacterial isolates/strains tested, only 27.7% isolates/strains formed large zone of K-solubilization on mica containing medium plates and the amount of potassium released by different strains varied from 15 to 48 mg/L.

Hu *et al.* (2006) isolated two phosphate and potassium solubilizing *Bacillus* sp. from the soils using the modified medium containing phosphorite and potassium minerals like kaolinite and potassium feldspar. Sugumaran and Janarthanam (2007) showed that K solubilizing activity of the five slime producing bacterial isolates varied from 1.90 mg/L to 2.26 mg/L from acid leached soil. MCRCp1 was found to have maximum K-solubilization activity (2.26 mg/L) to dissolve the silicate than other bacterial isolates. Zhang *et al*. (2013) reported that strain CX-7 caused 71.60 mg/L and 5.18 mg/L release of water-soluble phosphorus and 3.44 mg/L potassium release from phosphate powder, lecithin and potassium feldspar powder, respectively. Meena *et al*. (2014) showed release of K from waste mica (muscovite and biotite) with four K solubilizing isolates after 7, 14 and 21 days of incubation. K-solubilization by different bacterial isolates showed significant change on muscovite and biotite powder supplemented plates and the release of K varied from 1.28-46.75 µg/ml.

5.5. Effect of *Bacillus* isolates on seed germination of chickpea

The observations for retardation/stimulation of root and shoot growth of chickpea seedlings were recorded at 5th and 10th day of seed germination. Out of 24 isolates tested, isolates HCA10, HCA53, HCA61 and HCA81 showed increased seedling growth, whereas three isolates RCA4, RCA9 and RCA19 showed decreased growth of both root and shoot length as compared to control at both 5th and 10th day of inoculation (Table 18; Fig. 26, 27). In the present investigation, root length ranged from 1.1 to 8.33 cm and shoot length ranged from 1.37 to 9.7 cm at 10th day of seedling growth. Isolate HCA10 produced maximum root length (8.33 cm), whereas HCA81 produced shoot length (9.7 cm) at 10th day of inoculation. Other isolates also showed growth stimulatory effect over control on seedlings of chickpea at both 5th and 10th day of inoculation (Fig. 28, 29).

Nelson (2004) observed that PGPR strains were able to exert beneficial effects on plant growth and increased the germination rate. Sivaramaiah *et al*. (2007) studied ten *Bacillus* strains for their antifungal activity, effect on seedling emergence and plant growth promotion. Seed inoculation with different *Bacillus* strains showed stimulatory effect on root and shoot growth at 10 d of observation in comparison to control, whereas four *Bacillus* strains CBS24, CBS127, CBS129 and CBS155 caused retardation of shoot growth at 10 d. Five *Bacillus* strains i.e. CBS17, CBS106, CBS127, CBS153 and CBS155 caused inhibition of root growth of the seedlings at 5 d of observation. *Bacillus* strain CBS20 showed significant stimulation of root and shoot growth at both 5 and 10 d of seedling growth. Inoculation with *Bacillus* strain CBS155 also stimulated lateral root formation. The inhibition of seedling growth in some of the treatments may be due to synthesis or secretion of some toxic metabolites by rhizosphere bacteria in the growth media as well as in root exudates of chickpea (Astom *et al*., 1993)

The effect of inoculation of five PGPR isolates designated as *P. aeruginosa* strain BHUPSB02, *P. putida* strain BHUPSB04, *Bacillus subtilis* strain BHUPSB13, *Paenibacillus polymyxa* strain BHUPSB17 and *Bacillus boronophilus* strain BHUPSB19 was studied on the growth of chickpea plant (Yadav *et al*., 2010). PGPR isolates produced plant hormone (IAA), solubilized phosphate and produced ammonia to enhance plant growth. Most of isolates resulted in a significant increase in shoot length, root length of chickpea seedlings. The highest shoot length 15.6 cm plant^{-1} was recorded in treatment of *P. putida* BHUPSB04 isolate followed by statistically at par values due to inoculation of isolate *P. aeruginosa* BHUPSB02 (14.5 cm plant^{-1}). *B. subtilis* BHUPSB13, *P. polymyxa* BHUPSB17 and *B. boronophillus* BHUPSB19 showed significantly higher shoot length over control. Root length ranged from 3.87 to 9.2 cm plant^{-1}. The isolate BHUPSB02 produced the highest root length (9.2 cm plant^{-1}) followed by BHUPSB04 and BHUPSB17 in comparison to control and other isolates. BHUPSB13 and BHUPSB19 isolates also showed significant increase in root length over control. Seven strains of bacteria (*Pseudomonas plecoglossicida* SRI-156, *Brevibacterium antiquum* SRI-158, *Bacillus altitudinis* SRI-178, *Enterobacter ludwigii* SRI-211, *E. ludwigii* SRI-229, *Acinetobacter tandoii* SRI-305 and *Pseudomonas monteilii* SRI-360 did not show any influence on germination as 100% germination was found in both bacteria-treated as well as untreated control plants in both chickpea as well as pigeonpea (Gopalakrishanan *et al*., 2016). However, the shoot height and root lengths of seedlings were increased significantly up to 17 and 30%, respectively for chickpea and up to 29 and 22%, respectively for pigeonpea. Among the bacterial treatments, SRI-229 and SRI-305 in chickpea and SRI-158 and SRI-211 in pigeonpea were found to increase both shoot height and root length significantly over the uninoculated control.

5.6. Screening of *Bacillus* isolates for growth at different salt concentrations

The susceptibility or tolerance of *Bacillus* isolates towards NaCl was recorded by observing the growth and colony size at different salt concentrations i.e., 1, 2, 4, 6 and 8% (w/v), on LB medium plates. At 1% NaCl concentration, six *Bacillus* isolates formed larger colony size (15.1-20.0 mm; group 5) and fourteen isolates formed medium colony size (10.1-15.0 mm; group 4), whereas four isolates formed small colony size (5.1-10.0 mm; group 3) (Table 19; Fig. 30). At 2% NaCl concentration, six *Bacillus* isolates formed colony size ranging from 15.1-20.0 mm and ten isolates formed colony size ranging from (10.1-15.0 mm), and eight isolates formed colony size ranging from 0.5-10.0 mm) (Table 20; Fig. 31). At 4% NaCl concentration, six *Bacillus* isolates i.e., HCA41, HCA53, HCA80, HCA81, RCA31 and RCA68 showed colony diameter ranging from 15.1-20.0 mm and eight isolates showed colony diameter ranging from 10.1-15.0 mm (Table 21; Fig. 32). *Bacillus* isolates RCA3 and RCA63 showed colony diameter 7.56 mm and 9.5 mm, respectively. Isolates HCA26, HCA44 and SYB101 showed colony diameter ranging from 2.51-5.0 mm and HCA76, RCA4, RCA19 and RCA70 showed colony diameter ranging from 0.5-2.5 mm. Only two *Bacillus* isolates i.e., HCA53 and HCA81 showed 15.1-20.0 mm colony size growth at 6% NaCl concentration (Table 22; Fig. 33). Six isolates HCA3, HCA10, RCA7, RCA11, RCA31 and RCA68 showed growth of 10.1 mm to 15.0 mm colony size and three *Bacillus* isolates RCA4, RCA9 and RCA70 did not grow at 6% NaCl concentration. At 8% NaCl concentration, five isolates formed 5.1-10.0 mm colony size and four isolates showed growth of 2.51-5.0 mm colony size (group 2) (Table 23; Fig. 34). Nine isolates did not show growth on 8% salt containing plates. *Bacillus* isolates showed different growth pattern at different salt concentrations (Fig. 35). Among the 55 rhizobacterial isolates obtained from the chickpea rhizosphere, 28 isolates showed growth up to 4% salt concentration with different colony size. Twenty two rhizobacterial isolates showed 0-5 mm colony size (Chaudhary and Sindhu, 2017). Phour (2016) reported that ninety three bacterial isolates showed larger colony size ranging from 10.1-20 mm at 1% NaCl concncentration. At 4% NaCl concentration, only 56 rhizobacterial isolates showed large colony size, whereas only 17 bacterial isolates showed larger colony size at 6% NaCl concentration. Eleven bacterial isolates showed larger colony size (ranging from 10.1-20.0 mm) even at 8% NaCl concentration.

Plant-microbe interactions are influenced by increased biotic and abiotic stress tolerance, including plant disease resistance and salt and drought tolerance (del Rosario *et al*., 2013). Medeiros *et al*. (2011) found that transcriptional profiling in cotton was linked with *Bacillus subtilis* (UFLA285) which promoted biotic stress tolerance. *B. subtilis* strain GB03 has also been shown to confer growth promotion and abiotic stress tolerance in *Arabidopsis thaliana* (Han *et al*., 2014). Two PGPR strains, *Pseudomonas putida* NBRIRA (RA) and *Bacillus amyloliquefaciens* NBRISN13 (SN13) were identified. Strain RA displayed multiple PGP activities like auxin production, phosphate solubilisation and tolerance to drought and salt stresses (Kumar *et al*., 2016). Other PGPR strain SN13 showed salt stress amelioration under hydroponic and soil conditions, and it showed enhanced colonization with increased ACC deaminase activity (Nautiyal *et al*., 2013). Studies revealed that plants inoculated with PGPR containing ACC deaminase were able to thrive better by lowering the level of salinity induced ethylene (Ahmad *et al*., 2011). Sapsirisopa *et al*. (2009) studied the effects of three groups of salt-tolerant *Bacillus* inoculums namely: single inoculum of *Bacillus megaterium* A12ag, a phosphate solubilizing bacterium (PSB); single inoculum of *Bacillus licheniformis* B2r, an ACC deaminaseproducing bacterium; and coinoculum of *B. megaterium* A12ag and *B. licheniformis* B2r at mixture ratio of 1:1, on germination of rice under laboratory saline conditions (0, 5, 10, 15 and 20 dS/m). The single inoculum of salt-tolerant PSB aand *B. megaterium* A12ag was found as the most efficient inoculum to enhance germination of rice.

Upadhyay *et al*. (2012) reported that 33% of bacterial isolates survived up to 8% NaCl (w/v) and only 19% showed PGP attributes at higher NaCl concentration. Proline content was increased with NaCl stress and maximum production was recorded with isolate SU8 i.e., 2.73 and 11.95 g mg protein at 0 and 10% NaCl (w/v), respectively. The reducing sugars (RS) and total soluble sugars (TSS) production in rhizobacterial isolates were reversely proportional to the salt (NaCl) concentration which could mitigate salinity levels and improved agriculture crops growth under saline condition. A total 85 isolates were obtained from the rhizosphere of a halotolerant plant *Suaeda fruticosa* from saline desert of Little Rann of Kutch, Gujarat (India) having 4.33% salinity (Goswami *et al*., 2014). Out of 84 screened isolates, *Bacillus licheniformis* strain A2 showed most prominent PGP traits *in vitro* and it was tested *in vivo* under saline soil condition. In presence of soil supplemented with 50 mM NaCl, *B. licheniformis* treated groundnut (*Arachis hypogaea*) plants showed increase in fresh biomass, total length and root length by 28, 24 and 17% and in absence of NaCl, it was 43, 31 and 39%, respectively.

5.7. Isolation of *Mesorhizobium* isolates from nodules of chickpea

Twenty four *Mesorhizobium/Rhizobium* isolates were obtained from the nodules of chickpea by streaking crushed nodule suspension on YEMA medium plates (Table 24; Fig. 36). Shahzad *et al*. (2012) tested fifty samples of root nodules from alfalfa (*Medicago sativa*) and 25 out of 50 sampleswere found positive for the presence of *Sinorhizobium meliloti*. A total of 20 *Mesorhizobium* sp. were isolated from soil and nodule samples of chickpea rhizosphere collected from Punjab (Verma *et al*., 2012).

As an important legume grain crop, chickpea has been widely cultivated throughout the world. *Mesorhizobium ciceri* and *M. mediterraneum* were reported as dominant groups but several *Mesorhizobium* sp. strains were also isolated from chickpea nodules in Europe and India. Moreover, *M. muleiense* was recently described as the dominant chickpea rhizobia in Xinjiang, China (Zhang *et al*., 2014; Zhang *et al*., 2017a), indicating that chickpea rhizobia may follow biogeographic patterns. Similarly, 11 Caragana-nodulating *Mesorhizobium* genomic species were identified, among fourteen strains of different *Mesorhizobium* species, representing *Mesorhizobium amorphae, Mesorhizobium huakuii, Mesorhizobium septentrionale* and groups related to *Mesorhizobium plurifarium, Mesorhizobium temperatum, Mesorhizobium tianshanense* and *Mesorhizobium mediterraneum*. All these *Mesorhizobium* strains possessed similar symbiotic genes (probably acquired by a phenomenon of lateral gene transfer) and common host range was observed (Chen *et al.*, 2008). Laranjo *et al*. (2008) analysed 21 Portuguese chickpea rhizobial isolates to derive the evolutionary relationships between these different species with the ability of nodulating the same host. The comparison of 16S rRNA gene-based phylogeny with the phylogenies based on symbiosis genes revealed evidence of lateral transfer of symbiosis genes across different species. Results suggested that sequencing of *nif*H or *nod*C genes could be used for rapid detection of chickpea nodulating mesorhizobia.

5.8. Antagonistic effect of *Mesorhizobium* isolates against *Fusarium oxysporum*

Out of 24 *Mesorhizobium* isolates tested, eight isolates inhibited the growth of *F. oxysporum* (Table 25, Fig. 37). Isolate MCA7 showed maximum inhibition of fungus. Four isolates i.e., MCA6, MCA14, MCA17 and MCA22 showed moderate inhibition of *F. oxysporum.* Sixteen isolates did not show antagonistic activity. Reduction of fungal growth *in vitro* by certain rhizobia and formation of inhibition zones were presumably due to the metabolites released by the bacteria into the culture medium. These metabolites may include antibiotics and/or cell-wall degrading enzymes. Different studies have implicated antifungal secondary metabolites produced by *Rhizobium* sp. in the control of plant diseases caused by pathogenic fungi (Siddiqui *et al*., 2000a; Arfaoui *et al*., 2006). *Mesorhizobium* sp. promote the growth of plants either directly through N_2 fixation, supply of nutrients, synthesis of phytohormones and solubilization of minerals or indirectly as a biocontrol agent by inhibiting the growth of pathogens particularly Fusarium wilt of chickpea. Sixteen *Mesorhizobium* sp. isolates along with reference *Mesorhizobium* sp. (LGR 33) were tested as biocontrol agent *in vitro* against the causative agent of Fusarium wilt of chickpea (Bhagat *et al*., 2014). All *Mesorhizobium* sp. inhibited the growth of *Fusarium oxysporum* f. sp. *ciceris*, with growth inhibition varied from 34.2 (LGR 1)-59.3% (LGR 16), whereas variation in production of volatile antifungal compounds was from 20 (LGR 9)-46.3% (LGR 16). Out of 17 *Mesorhizobium* sp., 88% isolates produced siderophores and protease, and 64% produced HCN. Three *Mesorhizobium* isolates LGR 14, LGR 15 and LGR 16 produced maximum HCN, siderophore, cell wall degradation enzymes, antagonistic effect and volatile antifungal compounds.

Among the 21 *Rhizobium* isolates tested in dual culture, 19 isolates inhibited the growth of Foc race 0. Fourteen isolates gave more than 30% inhibition (Arfaoui *et al*., 2006). Isolates Rh8, Rh16, PchSOM and Rh11 were the most effective *in vitro* and caused more than 50% growth inhibition. Jayamani (2006) reported the antagonistic effect of *P. fluorescens* and *B. amyloliquefaciens* against soil borne *Phytophthora* sp. by dual culture technique. Lamessa and Zeller (2007) secreened 118 rhizobacteria against *R. solanacearum* strain and on the basis of *in vitro* secreening, six strains (RP87, B2G, AFP1, AFP2 and AFP4) were selected with good inhibitory effects. Kucuk (2013) proposed that *Mesorhizobium* strains can be used as potential biocontrol agents against *Fusarium* sp. Reduction of fungal growth *in vitro* by certain rhizobia and formation of inhibition zones were presumably due to the metabolites released by the bacteria into the culture medium. *Mesorhizobium* sp. produced HCN in response to contact with pathogenic fungi, which results in killing of fungi. A total of 207 isolates of *M. ciceri* obtained from root nodules of chickpea were screened for antagonistic effect against *Fusarium oxysporum*, out of which seven isolates viz; MC69, MC84, MC96, MC99, MC180, MC183 and MC190 specifically inhibited the growth of *F. oxysporum* and growth inhibition zone varied from 5 mm to 15 mm (Suman and Yadav, 2015). The *M. ciceri* isolate MC99 revealed the maximum inhibition zone (15 mm), while MC96 showed the minimum inhibition zone (5 mm).

5.9. Screening of *Mesorhizobium* isolates for growth at different salt concentrations

Salinity is one of the major factors restricting symbiotic nitrogen fixation. Rhizobia may grow at higher salt levels than their host plant species. The *Rhizobiumleguminosarum* nodulating common beans (*Phaseolus vulgaris*) can tolerate up to 350 mM NaCl concentration in broth culture while rhizobia nodulating *Vigna unguiculata* can tolerate up to 450 mM NaCl concentration (Zahran, 2001). Some rhizobia species can tolerate moderate salinity soils and fix nitrogen effectively (Hafeez *et al*., 1988). In order to establish a successful rhizobia-legume symbiosis for saline environments, efficient salt tolerant native rhizobial strains should be isolated from saline soils (Bouhmouch *et al*., 2005).

All the 24 *Mesorhizobium* isolates obtained in this study were tested for their ability to tolerate different concentrations of NaCl i.e., 1, 2, 4, 6 and 8% (w/v) on YEMA medium. Among 24 *Mesorhizobium* chickpea isolates, only two isolates i.e., MCA5 and MCA22 showed growth upto 8% salt concentration(Table 26; Fig. 38, 39).At 1% salt concentration, thirteen isolates showed large colony size varying from 5.1 to 10.0 mm and six isolates showed medium colony size ranging from 2.1 to 5.0 mm. At 2% salt concentration, nine *Mesorhizobium* isolates showed colony diameter ranging from 5.1 to 10.0 mm, whereas 7 isolates showed 2.51-5.0 mm colony diameter. At 4% salt concentration, nine *Mesorhizobium* isolates showed colony growth of 5.1-10.0 mm and only 4 isolates i.e., MCA5, MCA7, MCA21 and MCA22 showed 2.51-5.0 mm colony size. The growth of *Mesorhizobium* isolates further declined at high salt concentrations (Fig. 39). Nine *Mesorhizobium* isolates did not grow at 6% salt concentration and only five isolates showed large colony size. Seven *Mesorhizobium* isolates showed 2.51-5.0 mm colony diameter on these salt plates. At 8% salt concentration, *Mesorhizobium* isolates MCA6, MCA15, MCA18 and MCA19 isolates showed 2.51-5.0 mm colony size, whereas only two isolates MCA5 and MCA22 were found salt-tolerant upto 8% of salt concentration.

Saraf and Dhandhukia (2005) reported that *Sinorhizobium meliloti* growth was not completely inhibited by 4% of NaCl concentration. Jida and Assefa (2012) reported that 75% of the tested rhizobia could grow well with 1% NaCl. However, at higher concentrations the percentage of tolerant isolates decreased with increasing salt concentration as only 11.1% of the isolates tolerated 5% NaCl. Berrada *et al*. (2012) observed the growth responses of *Rhizobium* strains to different NaCl concentrations (0.5, 1, 1.5, and 2%) and found 31% of the strains were tolerant to 2% NaCl. Similar results were reported by Chaudhary and Sindhu

(2015). Among fifty *Mesorhizobium* chickpea isolates, only four i.e., MHD2, MSD41, MHD12 and MHD14 isolates showed growth up to 4% salt concentration and only 8% *Mesorhizobium* isolates showed growth upto 4% NaCl incorporated medium plates. Similarly, Cevheri *et al*. (2011) found that 100% *Rhizobium* isolates grew at 1% and 2% NaCl, 65% isolates grew at 3% and 40% isolates grew at 4% NaCl. High concentration of NaCl solution could give high competitive value in the rhizosphere to survive and nodulate the host plants in harsh environmental conditions particularly at high concentrations of salt in the soil. Gauri *et al*. (2012) screened 40 mesorhizobial isolates for different (2%, 3% and up to 8%) NaCl concentrations and found that all isolates grew up to 4% NaCl concentration and salt tolerance ability was reduced with increasing salt concentration.

5.10. Inoculation effect of *Mesorhizobium* isolates on nodulation and plant growth of chickpea under chillum jar conditions

Nodulation and plant growth of chickpea was studied by inoculation of *Mesorhizobium* isolates alongwith reference *Mesorhizobium* strains MBD26 and KR48 under chillum jar conditions. Reference *Mesorhizobium* strains MBD26 and KR48 caused maximum increase in all the symbiotic parameters including nodulation, SDW and RDW at both 40 and 80 days of plant growth (Table 27). Maximum increase in SDW (45.5%) was observed by inoculation of isolate MCA20 followed by 43.7 and 43.1% increase in SDW by inoculation of isolates MCA23 and MCA12, respectively at 40 days of growth (Table 27, 28). At 40 days of growth, 42.5% increase in shoot dry weight was observed by inoculation of isolate MCA14 followed by 38.3% increase in SDW by inoculation of MCA4 isolate. On the other hand, inoculation of chickpea with *Mesorhizobium* isolates MCA23 and MCA4 resulted in 166.2 and 161% increase in root dry weight, respectively. Inoculation of isolates MCA10 and MCA15 also increased RDW by 136.3 and 133.7% at 40th day of inoculation (Table 28; Fig. 42-45). At 80th day of observation, maximum increase (122.6%) in SDW was observed on inoculation of MCA14 isolate followed by 118.3 and 117.2% increase in SDW on inoculation of MCA4 and MCA10 isolates, respectively (Table 28; Fig. 46, 47). Inoculation of isolates MCA23 and MCA16 also increased SDW by 117.1 and 116.8%. Likewise, 61.6% increase in RDW was observed by inoculation of isolate MCA23 followed by 60 and 59.6% increase in RDW by inoculation of MCA14 and MCA4 isolates, respectively at 80th day of observation.

Nodules are positive outcome of host legume plant with bacteria (rhizobia) during symbiosis between microbes and host plants. Romdhane *et al.* (2007) found that nodule number was increased significantly in chickpea plants treated with *Mesorhizobium ciceri* isolates over control. Similarly, significant increase in nodulation of various legumes was observed with inoculation of different rhizobial strains (Akthar and Siddiqui, 2009). Sindhu *et al*. (2002) also reported that plants inoculated with *Rhizobium* isolates produced the highest weight of effective and total nodules in chickpea. The increase in weight of nodules in chickpea may be ascribed to enhanced nodulation due to effective N_2 fixation rate and a general improvement of root development. Otieno *et al*. (2007) reported that rhizobia inoculation increased the number and weight of nodules. Similarly, increase in dry weight of shoot was observed by Ahmad *et al*. (2011) and Romdhane *et al*. (2007) in chickpea seedlings after inoculation with *Mesorhizobium ciceri* isolates. These results are also well corroborated with Singh *et al*. (2005) who found significant increase (29.09%) in straw dry weight by rhizobial inoculation over control. Phosphate solubilization and plant growth regulators production were proposed as the key mechanisms. Solaiman *et al*. (2005) also reported that inoculation in chickpea with *Mesorhizobium ciceri* significantly improved the fresh and dry root weight and length over the control. Similarly, 42% increase in straw dry weight was attributed by Chi *et al.* (2005) due to auxin and gibberellin production by inoculated strains of *Mesorhizobium*.

Malik and Sindhu (2011) reported that inoculation of chickpea with *Mesorhizobium* sp. alone increased the plant dry weight that varied from 1.14 to 1.80 times in comparison to uninoculated control. Increase in dry weight of shoot compared to uninoculated control was also reported by Akthar and Siddiqui (2009). Ahmad *et al*. (2011) reported enhancement in root weight by *Mesorhizobium* isolates which ranged from 25 to 65%. Enhanced plant growth by inoculation of *Rhizobium* was attributed through the changes in root physiology and morphology along with nitrogen fixation (Biswas *et al*., 2000). Kunal and Sharma (2012) reported 28.3% increase in the nodule weight in the *Mesorhizobium* treatment over the uninoculated control at 90 DAS. Four isolates of *Mesorhizobium* sp. (LGR46, LGR50, LGR52 and LGR54) significantly enhanced dry weight of root and highest dry weight of root was recorded with LGR46 (0.52 g) over uninoculated control (0.37 g) (Kaur, 2014).

5.11. Coinoculation studies of selected *Bacillus* isolates with *Mesorhizobium* strains for their effect on nodulation and plant growth of chickpea under chillum jar conditions

Based upon antagonistic activity against *Fusarium oxysporum* and other plant growth promoting beneficial attributes like phytohormones production (IAA, ALA), siderophore production, ACC utilization, P and K solubilization and salt tolerance, three antagonistic i.e. HCA61, RCA3 and RCA7, and two non-antagonistic *Bacillus* isolates; HCA76 and SYB101were selected (Table 29) for coinoculation studies with *Mesorhizobium* strains i.e. MBD26 and KR48 under chillum jar conditions. Coinoculation effect of selected *Bacillus* isolates with *Mesorhizobium* strains for their effect on nodulation and plant growth was compared to single inoculated *Mesorhizobium* strains.

At 60 days of plant growth, *Mesorhizobium* isolates MBD26 and KR48 formed 35 and 42 nodules/plant (Table 30). Maximum nodule number (65) was recorded in treatment

KR48+SYB101 followed by KR48+RCA3, KR48+RCA7, KR48+HCA61 and MBD26+SYB101 (Fig. 48). Coinoculation of MBD26+RCA3 showed 87.7% increase in shoot dry weight and 24% increase in root dry weight of chickpea at 60 days of observation. Similarly, inoculation of treatments KR48+SYB101 and KR48+HCA76 caused 110.5 and 87.6% increase in SDW, respectively. Coinoculation with strains KR48+RCA7 and KR48+RCA3 isolates showed 23.5 and 10% increase in RDW as compared to *Mesorhizobium* strain KR48-inoculated plants, respectively (Fig. 49-51). Coinoculation of MBD26+RCA3 and KR48 strain with all the five *Bacillus* isolates showed significant increase in shoot dry weight of chickpea at 75 days after showing (DAS). Maximum nodule-promoting effects were observed with coinoculation of MBD26+RCA3 and KR48+HCA76 (Fig. 52). Significant increase in shoot biomass (30.6 to 38.4%) was observed on coinoculation of KR48+HCA76 followed by KR48+SYB101 and KR48+RCA3. Improved root weight was observed in coinoculated treatments of *Mesorhizobium* strains KR48 and MBD26 with *Bacillus* isolates RCA3, RCA7 and SYB101. Inoculation of treatments KR48+HCA76 caused 38.4% increase in SDW, whereas inoculation of treatments KR48+HCA61 caused 13.5% and MBD26+RCA3 caused 49.3% increase in SDW in comparison to *Mesorhizobium*–inoculated plants. At 75 DAS, maximum increase in RDW (13.3%) was observed in KR48+RCA7 inoculated treatment followed by 7.6 and 5.8% increase with KR48+RCA3and KR48+SYB101 inoculated treatments, respectively.

At 90 days of plant growth, inoculation of *Mesorhizobium* strains MBD26 and KR48 with *Bacillus* isolates showed significant effect on nodulation and root-shoot biomass of chickpea (Table 31; Fig. 53, 54). Coinoculation of *Mesorhizobium* strain KR48 with *Bacillus* isolates i.e., SYB101, RCA7, HCA76, HCA61 and RCA3 showed significant increase in nodule weight, respectively. Likewise, coinoculation of *Mesorhizobium* strain MBD26 with above mentioned *Bacillus* isolates showed 87.3, 75.3, 57.7, 17.6 and 25.4% increase in nodule weight. Significant gain was observed by coinoculation of chickpea with KR48+SYB101 in shoot dry weight (46.1%) and root dry weight (105.6%) at 90 days of observation followed by inoculation of KR48+RCA7 in SDW (31.4%)and RDW (86.9%), respectively. On the other hand, coinoculation of *Mesorhizobium* strain MBD26 with *Bacillus* isolates i.e., RCA3, RCA7, HCA61 and SYB101 showed significant gain (113.9, 79.2, 59.7 and 55.3%) in SDW, whereas these treatments caused 50.6, 35.6, 64.8 and 25.6% increase in RDW, respectively.

Different bacterial strains have the ability to cause growth promotion on diverse crops (Nieto-Jacobo *et al*., 2017) either through the induction of nitrogen fixation or the production of varying level of plant hormones. In earlier studies, mostly synergistic effects have been observed on nodulation and plant growth of legumes by dual inoculation of *B. japonicum* and *P. fluorescens* in soybean (Chebotar *et al.*, 2001), *R. leguminosarum* with an antibiotic-producing *P. fluorescens* strain F113 in pea (Leij, 1998) and

Bradyrhizobium/Mesorhizobium strains with *Pseudomonas* sp. in green gram and chickpea (Goel *et al.*, 2000; Sindhu *et al.*, 2002). Some PGPR strains enhanced legume growth, nodulation and nitrogen fixation when coinoculated with rhizobia (Petersen *et al*., 1996). Coinoculation studies with PGPR and *Rhizobium* sp. have been found to increase root and shoot biomass, nodule dry matter in chickpea, and common bean, green gram and soybean. In the present study, the *Mesorhizobium* strain KR48 and MBD26coinoculated with different *Bacillus* isolatesstimulated nodulation and growth of chickpea. Valverde *et al*. (2006) have reported that the coinoculation of *Pseudomonas jessenii* PS06 and *Mesorhizobium ciceri* C-2/2 strain showed significant increase in nodulation, growth and seed yield of chickpea under greenhouse and field experiments.

Wani *et al.* (2007a) reported that dual bacterial inoculation of *Mesorhizobium* and *Pseudomonas* significantly increased the plant growth of chickpea plant. Similarly, *Pseudomonas* and other PGPR strains exhibited enhanced nodulation, nodule dry weight and plant growth in chickpea (Verma *et al.*, 2014). Hameeda *et al*. (2010) reported that the dual inoculation of *Rhizobium* with *Pseudomonas* significantly increased the nodule number, nodule weight and nitrogenase activity for chickpea under greenhouse conditions. Stefan *et al.* (2013) reported that coinoculation of two rhizobacterial strains significantly increased nodule number, nodule weight, total biomass and yield of runner bean compared to single strain application. Malik and Sindhu (2011) reported that coinoculation of *Pseudomonas* with *Mesorhizobium* sp. that significantly improved chickpea growth and its yield components as compared with the sole application. Coinoculation increased growth and yield of *Phaseolus vulgaris,* compared to single inoculation provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus and mineral nutrients (Sánchez *et al.*, 2014).

Similarly, Verma *et al*. (2013) demonstrated that the root dry weight was significantly increased by 44, 15 and 10%, and shoot dry weight was increased by 26, 20 and 11% after combined inoculation of seeds with *Mesorhizobium* sp. and *P. aeruginosa*, *B. megaterium* and *A. chroococcum*, respectively, compared to *Mesorhizobium* sp. inoculation alone at 70 DAS in pot. Seed treatment of chickpea with combined inoculation of *Mesorhizobium* sp. and *P. aeruginosa* was found to be most effective with highest increase in nodules number (86%); dry weight of nodules (90%), root (57%) and shoot (45%) plant^{-1} followed by coinoculations of *Mesorhizobium* sp. with *B. megaterium* and *A. chroococcum* over uninoculated control at 70 DAS in field condition. Combined inoculation of *Mesorhizobium* sp. with *P. aeruginosa* showed significant increase in nodulation, root and shoot dry weight followed by *Mesorhizobium* sp. with *B. megaterium*, *A. chroococcum* as compared to individual seed inoculation and uninoculated control.

Strains of *Bacillus* have been reported to produce plant growth regulators (like IAA), antifungal peptides and phosphorus solublization (Nautiyal *et al*., 2013). Solubilization of phosphorus correlates with the production of organic acid in rhizosphere. *Bacillus* and *Pseudomonas* sp. are major contributors of solubilization of phosphorus in soil that ultimately help in the promotion of plant growth (Rajkumar *et al*., 2009). Siderophore production is another important character of *Bacillus* sp. that increases the uptake of iron under saline stress. The role of ACC deaminase in decreasing ethylene level by the enzymatic hydrolysis of ACC into α-ketobutyrate and ammonia has been presented as one of the critical mechanism of *Bacillus* sp. in promoting plant growth (Islam *et al*., 2016). The ACC utilizing bacteria lowered the stress ethylene level in plants (Glick *et al*., 2007b). The adverse effects of salinity on root, shoot dry and fresh weights could overcome by inoculation with the bacterium *B. cereus* (Chakraborty *et al*., 2011). Shukla *et al*. (2012) observed similar responses of growth in *Arachis hypogaea* inoculated with different bacterial isolates. It has been suggested that apart from bacterial ACC deaminase activity, elevated phosphorous availability (Upadhyaya *et al*., 2011) potassium uptake and phytohormones secretion might play a critical role in plant growth under saline stress (Mayak *et al*., 2004). *Mesorhizobium ciceri, M. mediterraneum* and *Sinorhizobium medicae* strains showed different symbiotic performances when inoculated to chickpea at unstressed conditions and under salt stress (Mhadhbi *et al*., 2004).

5.12. Coinoculation effect of *Mesorhizobium* strains with *Bacillus* isolateson Fusarium wilt incidence and disease control under pot house conditions

Fusarium oxysporum f. sp. *ciceri* caused wilt disease has been considered as most devastating for the production of chickpea (Khan *et al*., 2002). The present investigation was undertaken to isolate antagonistic *Bacillus* isolates against *F. oxysporum*. Single and combined inoculation effect of *Fusarium oxysporum* with *Mesorhizobium* strains as well as antagonistic and non-antagonistic *Bacillus* isolates was examined on chickpea at 60, 75 and 90 days. At 60 days of *F. oxysporum* inoculation, 80% disease incidence was observed on chickpea plant and 20% plants also showed disease incidence in MBD26 incoulated treatment (Table 32; Fig. 55). Disease symptoms on shoot and root of chickpea were observed in treatments having non-antagonistic *Bacillus* isolates i.e. HCA76 and SYB101 (Fig. 56, 57). Disease symptoms such as yellowing and wilting of leaves were visible in treatments having only *Mesorhizobium* strains. No *Fusarium* wilt symptoms were observed in single and mixed inoculated treatments containing antagonistic *Bacillus* isolates i.e. HCA61, RCA3 and RCA7 (Fig. 58). 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.

At 75 days of growth, *Fusarium* treated chickpea plants showed 100% disease incidence and 20 to 40% wilt disease symptoms were recorded in treatments having non-antagonistic *Bacillus* isolates (Fig. 59). The reduction in the percentage of wilted plants was observed from 60 to 100% in treatments inoculated with antagonistic isolates. The wilt disease free chickpea plants revealed the effectiveness of antagonistic isolates HCA61, RCA3 and RCA7. Disease control was 100% in antagonistic *Bacillus* isolates and fungicide (Bavistin) treatments at all the three stages of plant growth. Treatments of bacterial isolates KR48, KR48+SYB101 and KR48+HCA76 inoculated with *F. oxysporum* caused 100% disease control while single and mixed inoculation of SYB101 and HCA76 with MBD26 strain caused only 60 to 80% disease control, respectively at 90 days of growth (Fig. 60-62).

*Pseudomo*nas and *Bacillus* strains are the major root colonizers (Joseph *et al*., 2007; Manikandan *et al*., 2010). Different mechanisms have been reported for their performance as biocontrol agents such as production of antibiotics, siderophore hydrogen cyanide, competition for nutrition and space, inducing resistance, inactivation of pathogen's enzymes and enhancement of root and plant development (Intana *et al*., 2008). Thus, *Pseudomonas* and *Bacillus* strains have great potential in control of Fusarium wilt disease of chickpea (Anjajah *et al*., 2003). *Bacillus* spp. is recognized as a powerful biocontrol agent because species of *Bacillus* are able to synthesize more than 60 different types of antifungal metabolites, which also act as plant growth promoters (Zongzheng *et al*., 2009). *Bacillus* sp. have showed significant inhibitory activity against many plant pathogens including *Fusarium moniliforme* (Agarry *et al*., 2005), *Phytophthora capsici* (Jo, 2005), *Botrytis cinerea* and *Sclerotium rolfsii* (Prapagdee *et al*., 2008) and *Fusarium oxysporum* (Nikam *et al*., 2011) etc.

Species of *Bacillus* have also been known to produce compounds which inhibit fungal growth directly or indirectly *viz*., hydrogen cyanide (HCN), siderophores and antifungal activity (Saharan and Nehra, 2011). Three antagonistic *Bacillus* isolates i.e., HCA61, RCA3 and RCA7 used in this study possess the capability to produce siderophores and HCN alongwith ACC deaminase activity (Table 29), which resulted in 100% disease control on coinoculation of *Mesorhizobium* isolates with these antagonistic *Bacillus* isolates (Table 32, Fig. 58). Hydrogen cyanide has been reported as effective in control of cucumber wilt caused by *Pythium ultimum* (Keel *et al*., 1996). Chickpea *Fusarium* wilt severity caused by FOC1 was reduced from 60 to 99% in the susceptible cultivar ILC 482 treated with antagonistic *Bacillus* spp. (Rb29, Rb6, Rb12, Rb4, and Rb15) in pot assays and by 98, 81, 68, 64, 57.20%, respectively, in the field trials (Zaim *et al*., 2013). Biocontrol of incidence of *Fusarium* wilt disease in chickpea was studied by Inam ul Haq *et al*. (2003) and among 36 rhizosphere bacteria, isolate no. 3, 6, 12, 16, 18, 21, 22, 23, 27, 29 and 33 inhibited the growth of *Fusarium oxysporum* under *in vitro* studies. Various individual *Bacillus* strains applied as seed treatment have been reported to suppress plant pathogens (Das *et al*., 2008).

5.13. Coinoculation effect of *Mesorhizobium* strains and *Bacillus* isolateson plant growth of chickpea under pot house conditions

In the present study, nitrogen fixing bacteria *Mesorhizobium* sp. strains MBD26 and KR48 and *Bacillus* isolates i.e., HCA61, RCA3, RCA7, HCA76 and SYB101 were used to enhance crop yield under unsterilized soil conditions. Single inoculation of *Mesorhizobium* strains and *Bacillus* isolates RCA3, RCA7 and SYB101 also showed significant increase in shoot and root dry weight compared to the untreated control (Table 33). Reduced growth of chickpea was observed in *Fusarium oxysporum* inoculated treatment at different stages of plant growth. At 60 DAS, *Mesorhizobium* strains MBD26 and KR48 showed increase in nodule weight (88.7, 59.1%), SDW (27.7, 55.7%) and RDW (23.5, 52.5%) of chickpea plant, respectively. Coinoculation of these strains with *Bacillus* isolates i.e., MBD26+HCA61 and MBD26+RCA7 showed 140% increase in nodule number, whereas KR48+SYB101 and KR48+RCA7 showed 170 and 160% increase in nodule number, respectively. Inoculation of *F.oxysporum*+KR48+SYB101 showed 155.4% increase in SDW followed by *F. oxysporum*+KR48+RCA7 (124%) and *F. oxysporum*+MBD26+RCA7 (121.1%) (Fig. 63). At 75 DAS, inoculation of *Bacillus* isolates SYB101 and HCA61 along with fungus showed 118.7 and 106.2% increase in nodule number, respectively. At 75 days of chickpea growth, 173.3% increase in SDW was observed followed by 151.8 and 151.6% increase in the treatments with *F. oxysporum*+KR48+SYB101, *F. oxysporum*+MBD26+SYB101 and *F. oxysporum*+KR48+RCA3 (Fig. 64). Inoculation of chickpea with *Bacillus* isolates SYB101, RCA3 and RCA7 with *Mesorhizobium* strain KR48 resulted in 118, 105.7 and 87.6% increase in RDW, respectively. Inoculation of treatment *F. oxysporum*+MBD26+RCA7 showed 128.3% in SDW and 60.4% RDW, whereas *F. oxysporum*+MBD26+RCA3 showed 117.4% increase in SDW and 67.8% increase in RDW at 75 DAS.

At 90 days of plant growth, all the coinoculated treatments (*Mesorhizobium* strains and *Bacillus* isolates) alongwith fungal culture except treatment T9 (*Fusarium oxysporum*) showed significant growth promoting effect on root-shoot biomass of chickpea (Table 34; Fig. 65-68). Single inoculation with *Mesorhizobium* strains MBD26 and KR48 also showed increase in nodule number (114.9, 125%), nodule weight (148, 171.4%), SDW (64.1, 73.2%) and RDW (50.9, 52.5%), respectively. Maximum increase in RDW (920 mg/plant) and SDW (1604 mg/plant) was observed by coinoculation of isolate RCA3 followed by RCA7 inoculated with fungal culture (Table 34; Fig. 69). Coinoculation of *Bacillus* isolates SYB101 and HCA61 with fungal culture increased the nodule number (90, 85%) and nodule weight (187.1, 176.1%), and coinoculation with RCA3 and RCA7 showed increase in SDW (113.5, 97.3%) and RDW (77.6, 65%), respectively. Enhanced nodule number (135%), nodule weight (273.8%), RDW (123.1%) and SDW (86.2%) was observed on coinoculation of *F. oxysporum*+MBD26+RCA7 followed by 110, 177.6, 105.3 and 66.9% increase on coinoculation with *F. oxysporum*+MBD26+HCA61. Similarly, enhanced nodulation and plant growth was observed on inoculation with *F. oxysporum*+KR48+SYB101, *F. oxysporum*+KR48+RCA7 and *F. oxysporum*+KR48+RCA3. Inoculation of *F. oxysporum*+KR48+SYB101 caused 149 and 111% increase in SDW and RDW of chickpea, followed by *F. oxysporum*+KR48+RCA7 (129.5, 96.9%) and *F. oxysporum*+KR48+RCA3 (107.7, 77.2%), respectively (Fig. 69).

Rhizobacteria promote plant growth through phosphate solubilization, indole and siderophore production and therefore, have a potential for use as inoculants to improve crops. IAA like compounds are the most common growth regulators produced by PGPR and is known to enhance plant growth (Glick, 2012). Varying levels of IAA production was recorded in *Paenibacillus* sp., *Bacillus* sp. and *Klebsiella* sp. (Ji *et al*., 2014). The solubilization of insoluble and fixed forms of phosphorus by bacterial inoculants also play an important role in increasing soil phosphorus availability and simultaneously enhancing plant growth and crop yield (Hameeda *et al.*, 2008). Bacteria belonging to genera *Paenibacillus*, *Bacillus*, *Pseudomonas* etc. are reported to solubilize the insoluble phosphate compounds and aid in plant growth promotion (Ahmad *et al.*, 2008). Siderophores suppresses the growth of phytopathogenic fungi and function as stress factors in inducing host resistance (Ahmad *et al*., 2008; Zhou *et al.*, 2012). Lowering of plant ethylene by deamination of its immediate precursor 1-aminocyclopropane-1-carboxylate (ACC) is a key trait found in many rhizobacteria (Duan *et. al.*, 2009). The inoculation effects of ACC deaminase containing bacteria were found to promote plant growth cultivated under axenic conditions and in drying soil, flooded soil and under normal soil (Belimov *et al.*, 2009; Shahzad *et al.*, 2010). Many other studies have also shown positive effects of inoculation with PGPR strains, especially *Pseudomonas* and *Bacillus* sp. on growth of chickpea under greenhouse and field conditions (Malik and Sindhu, 2011; Yadav and Verma, 2014) which could promote plant growth through different mechanisms such as production of IAA, phosphates solubilization, siderophore production and other plant growth promoting activities (Glick, 2012).

Bacillus species have been reported to promote plant growth through phosphate solubilization, indole acetic acid and siderophore production. In this study, three *Bacillus* isolates i.e., HCA61, RCA3 and RCA7 possessing plant growth promoting attributes like production of phytohormone IAA, utilization of ACC (precursor of stress hormone) and solubilization of macronutrients (P and K) showed significant growth promoting effect on chickpea plants either on single inoculation or coinoculation alongwith *Mesorhizobium* strains MBD26 and KR48 as compared to uninoculated control (Table 29). Salt tolerance ability of these *Bacillus* isolates and their stimulatory effect on seed germination of chickpea seedlings acted as supplementary factor in chickpea plant growth promotion. IAA like compounds are the most common growth regulators produced by PGPR andare known to enhance plant growth (Glick, 2012; Ji *et al.*, 2014). The bacterial IAA together with endogenous plant IAA may induce the synthesis of the plant enzyme ACC synthase that converts the compound S-adenosyl methionine to ACC, the immediate precursor of ethylene in all higher plants (Patten and Glick, 1996). Seedor root inoculation with ACC deaminase-producing bacteria have been found to enhance root elongation and shoot growth promotion of maize (Shaharoona *et al.*, 2006). Solubilization of insoluble and fixed forms of phosphorus by bacterial inoculants belonging to genera *Paenibacillus, Bacillus* and *Pseudomonas* etc. increase soil phosphorus availability and simultaneously enhance plant growth and crop yield (Ahmad *et al*., 2008; Hameeda *et al.*, 2008; Pastor *et al.*, 2014). Sapsirisopa *et al*. (2009) studied the effects of three groups of salt-tolerant *Bacillus* inoculums namely: *Bacillus megaterium* A12ag, a phosphate solubilizing bacterium *Bacillus licheniformis* B2r, an ACC deaminase producing bacterium and co-inoculum of *B. megaterium* A12ag and *B. licheniformis* B2r at mixture ratio of 1:1, on germination of rice under laboratory saline conditions. The single inoculum of salt-tolerant PSB and *B. megaterium* A12ag was found as the most efficient inoculum to enhance germination of rice.

The positive effect of inoculation with PGPR strains, especially *Pseudomonas* sp. on growth of chickpea was reported by many workers (Valverde *et al*., 2006). The synergistic effect of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on chickpea was demonstrated by Wani *et al*. (2007b). The yield, dry matter, N and P uptake and nodulation was maximum in the treatment that received combination of *Mesorhizobium ciceri* RC4 + *Azotobacter chroococcum* A10 + *Bacillus* sp. PSB9 as seed inoculation than in the treatment that received single strain or 100% RDF. Combined inoculation of *Rhizobium* with *Pseudomonas striata* or/and with *Bacillus megaterium* increased dry matter, grain yield and P uptake significantly over the uninoculated control in legumes (Elkoca *et al.,* 2008; Selvakumar *et al.*, 2009a). A total of 74 bacteria isolated from compost and rhizosphere soils were screened for their antagonistic potential against soil-borne fungal pathogens of chickpea (Sreevidyaand Gopalakrishnan, 2017). Of which, four bacterial isolates (VBI-4, VBI-19, VBI-23 and SBI-23) were identified as *Bacillus* species by 16S ribosomal DNA (rDNA) sequence analysis. Allthe isolates increased growth parameters including nodule number, plant growth, and yield parameters when compared to uninoculated controlunder greenhouse and field conditions on chickpea.

Verma *et al*. (2009) found a positive influence of coinoculation with PGPR and *Mesorhizobium* sp. strain BHURC03 on nodulation, plant biomass and yield of chickpea in field experiment. Maximum increase in nodule number, dry weight of nodule, root and shoot were recorded on coinoculation of *Mesorhizobium* sp. and *P. fluorescens* followed by coinoculation of *Mesorhizobium* sp., *Azotobacter chroococcum* and *Bacillus megaterium* over uninoculated control in field study. The *Mesorhizobium* sp. and *P. fluorescens* showed significant increase in all parameters due to higher nitrogen fixation by *Mesorhizobium* sp. and strong phosphate solubilization, higher production of hormones like indole acetic acid by *P. fluorescens*. Mohan (2010) showed that coinoculation of *Bacillus* strains with *Mesorhizobium* sp. strain Ca181 in chickpea cv. C235 caused significant increase in nodule number, nodule fresh weight and plant dry weight.

Further advantages of mixed cultures over single strains inoculation have been well documented and *in vitro* studies have shown that rhizobacteriacan produce more phytohormones when grown in mixed culture (Spaepen *et al.*, 2007). Moreover, mixed cultures may provide conditions more suitable for nitrogen fixation than pure cultures (Remans *et al.,* 2008). In addition, mixed inoculation of biocontrol microorganisms is more efficient in controlling pathogens than the use of single strain inoculants, e.g., combinations of *Pseudomonas* with *Serratia* (Hameeda *et al*., 2010; Verma *et al*., 2014). Five rhizobacteria i.e., *Paenibacillus durus* (PNF16) alone and in combination with other rhizobacteria (*Azotobacter* (AZT3), *Achromobacter* (PNF11), *Bacillus* (Bc1), *Pseudomonas* (Ps5) and *Mesorhizobium* (IARI) were tested for plant growth promoting effect under pot conditions (Ahmad *et al*., 2016). PNF16 was found to produce 21.7 µg ml^{-1} of indole acetic acid like substances, hydroxymate type of siderophores (salicylate type 11 µg ml^{-1} and benzoate type 6.5 µg ml^{-1}) and solubilized phosphate (405.33 µg ml^{-1}). PNF16-*Mesorhizobium* combination was found significantly better as compared to other combinations for growth parameters, nodulation and yield of chickpea over control. Significant increase in plant growth (32%) and nodulation (43%) was observed as compared to untreated control. The results indicated the potential usefulness of PNF16 alone and in combinations for enhancement of nodulation and stimulation of plant growth in chickpea. Tena *et al*. (2016) reported that chickpea inoculation with *Mesorhizobium* strains gave higher nodule number, nodule dry weight and biological yield compared to uninoculated plants in Shoa-Robit, Ethiopia.

The ability of several isolates belonging to rhizobacteria (*Pseudomonas* and *Bacillus*) collected from several chickpea growing areas in Algeria was studied to control the mycelium growth of *Fusarium oxysporum* f. sp. *ciceris* (Abed *et al*., 2016). Six *Bacillus* species (*B. subtilis, B. circulans, B. lentus, B. aneurinilyticus, B. firmus, B. licheniformis* and three *Pseudomonas* species (*P. aeruginosa, P. luteola, P. fluorescens*) were identified. The ability of bacterial isolates varied in production of protease, gelatinase, amylase, cellulase, indole acetic acid, lipase, catalase and hydrogen cyanide. Different rates of inhibition growth were observed due to various extracellular compounds, where B61 (*Bacillus aneurinilyticus)* and P39 (*Pseudomonas luteola*) and P70 (*Pseudomonas fluorescens)* were the most efficient with 77 and 55.5% respectively, while B39 (*Bacillus firmus*) and P41 (*Pseudomonas luteola*) were the most efficient by volatile compounds with 70.5 and 77.5%, respectively.

5.14. Approaches to enhance plant growth and disease control

Genetic variability is essential to develop disease resistant plants and novel tolerant lines were identified that may be used for a breeding program as donors of Fusarium resistance and combined with high-yield genotypes (De Lucchi *et al.*, 2017). The interaction between *F. oxysporum* and host plants has been well studied in several crops and resistance genes have been identified (Oumouloud *et al.*, 2008). Moreover, the genes involved in Fusarium resistance of tomato and melon are dominant (Joobeur *et al.*, 2004). One dominant and one recessive gene for Fusarium resistance were also identified in pigeon pea (Saxena *et al.*, 2012). Currently, the use of resistant cultivars appears to be the most practical and economically efficient control measure for management of chickpea Fusarium wilt. Resistant chickpea cultivars represent a key component in integrated disease management (IDM) programs that involve the use of additive or synergistic combinations of biotic, cultural and chemical control measures (Jimenez-Diaz *et al.*, 2011b). Resistance to Foc races had been identified mainly in Desi germplasm and to a lesser extent in Kabuli chickpeas, as well as in wild *Cicer* sp. (Jimenez-Diaz *et al.*, 2015). The deployment of resistant cultivars has not been extensive because of undesirable agronomic characteristics in some developed materials. Furthermore, the high pathogenic variability in Foc populations may limit the effectiveness and extensive use of available resistance (Bayraktar *et al.*, 2012).

The major diseases affecting chickpea are *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*. Resistant cultivars, tillage practices, crop rotation, inter cropping and soil solarization are effective measures for control of diseases in chickpea. The suppressive ability of neem organic amendment in inhibiting growth of soil borne pathogens has been demonstrated to be through competition, antibiosis or due to increase of soil microbial populations (Zakaria and Lockwood, 1980). Baily and Lazarovits (2003) reported that organic amendments, manures and composts with high nitrogen contents may suppress soil borne diseases by releasing allelochemicals during microbial decomposition. Continuous application of compost extract reduces disease incidence due to induced natural defense mechanism against *Fusarium oxysporum* in tomato (Al-Dhamani *et al.*, 2003). Chattopadhyay *et al.* (1999) reported that soil application of green manure, mustard cake and farm yard manure lead to significant reduction of population of *Fusarium oxysporum* f. sp. *ciceri* under field condition.

Bacteria could parasitize disease-causing fungi by the production of glycanases, proteases, cellulases and chitinase enzymes. Production of extracellular cell wall degrading enzymes has been associated with biocontrol abilities of the producing bacteria (Singh *et al.*, 1999). Tn5 mutants of *Enterobacter* which were deficient in chitinolytic activity were unable to protect plants against the disease (Chernin *et al.*, 1995). Addition of *Pseudomonas* pyoverdine to soil reduced chlamydospore germination of pathogenic *F. oxysporum* (Elad and Baker, 1985). In addition, some siderophores like pyocyanin and pyoveridin are essential for the induction of systemic resistance (Ahmad *et al.*, 2008). *P. fluorescens* CHA0 enhanced root growth and could suppress black root rot of tobacco caused by *Thielaviopsis basicola* by excreting several metabolites with antifungal properties including pyoverdine, DAPG, pyoluteorin and HCN (Maurhofer *et al*., 1995). HCN might constitute a stress in the plants, provoking an enhancement of their resistance to fungal diseases (De´fago *et al*., 1990). Suppressive effect on black root rot was found to be related to hydrogen cyanide production as demonstrated by the less protective effect of *hcn* mutant defective in HCN biosynthesis and effective disease suppression when *hcn*+ genes were reintroduced into the mutant genome or inserted into the genome of an initially nonactive strain (Voisard *et al*., 1989).

Bioengineering of synthetic microbial communities for plant/crop growth promotion, disease resistance, and stress tolerance/regulation presents a unique opportunity. Many microbial genera are known that colonize the rhizosphere, have publically available genome sequences and are amenable to genetic engineering efforts. These genera include *Pseudomonas*, *Streptomyces*, *Rhizobium*, *Paenibacillus* and *Bacillus*. While *Streptomyces* sp. offer great examples of PGPRs with tractable genetic systems and many available complete genome sequences, *Streptomyces* sp. have some drawbacks in that they have very large genomes (on average ~7 MB) (Köberl *et al*., 2015), and have many mobile elements, which make them difficult to engineer and/or grow in cooperative synthetic microbial communities. The base of a synthetic community would be *Bacillus* sp., as it is relatively easy to genetically engineer has a large depth of complete genome sequences (Sharma and Satyanarayana, 2013), contains many isolates that have plant growth proprieties (Köberl *et al*., 2013; Köberl *et al*., 2015), and/or are currently used in bio-control applications. For example, a *Bacillus* sp. could be engineered to contain a nitrogen-fixation machinery (e.g., *nif*H from *Paenibacillus* (Kim and Timmusk, 2013), produce high concentrations of plant hormones (Arkhipova *et al*., 2005), or add pathways from other *Bacillus* sp. to control pathogens (Köberl *et al*., 2013). A simple three-strain member consortium, including an engineered *Bacillus* sp. with two natural or engineered nitrogen fixers like *Pseudomonas, Rhizobium* and/or *Bradyrhizobium*, could provide many of the benefits of a more complex natural rhizosphere community. The potential ecological functional interactions increase with the number of strains added; a three strain consortium would potentially contain >729 predicted interactions, and a four member consortium 531,441 predicted interactions (Grosskopf and Soyer, 2014). Efforts should be taken to limit the number of strains within a synthetic microbial community to three strains in order to exert control of potential interactions.

Microbes that are motile and chemotactically driven to roots by the host's plant root exudates and with strong attachment to the host plant allow for stronger association and possibly better beneficial effects to the plant (Bashan *et al*., 2014). Density of growth is an important factor for whether or not the microbe will have a beneficial effect. For example, *Pseudomonas* sp. needs a minimal growth density (i.e., 10^5-10^6 CFU g^{-1} of root) in order to protect plants from pathogens (*G. tritici* and *Pythium* sp.) (Hass and Defago, 2005). If standard agronomic farming practices are used, then the microbial consortia must be able to tolerate herbicides, fertilizers and pesticides without losing any of the beneficial effects.

As our understanding of the mechanisms used by PGPB advances, it becomes feasible to enhance their capacity to stimulate plant growth by modifying promising traits. The activity and utility of a biocontrol-PGPB may be enhanced by integration of genes responsible for the biosynthesis of antibiotics, extending the range of pathogens against which a single biocontrol-PGPB can be used, or by genetically manipulating the bacterium to increase production of the antibiotic. Because many of the enzymes that hydrolyze fungal cell walls are encoded by a single gene, it would be relatively easy to isolate these genes, transfer them to other biocontrol-PGPB, and thus construct new biocontrol-PGPB armed with antibiotics, siderophores and hydrolytic enzymes. It is possible to isolate bacterial genes for ACC deaminase and transfer them to biocontrol-PGPB strains that employ other plant growth-promoting mechanisms, allowing them to modulate ethylene levels in the host plant and reduce disease severity (Glick, 2014, 2015). Potential growth-promoting traits can be transferred from any bacteria to a PGPB (Bashan and de Bashan, 2005). For example, the transfer of the gene for acid phosphatase from the saprophytic soil bacterium *Morganella morganii* to *B. cepacia* and *Azospirillum* strains would create a biocontrol-PGPB and a nitrogen-fixing bacterium with phosphate solubilization activity and therefore enhanced phosphate uptake. Regardless of the type of genetic insertion aimed at improving the PGPB, as a general rule, wild strains are likely to persist in the environment longer than their transformed relatives (Bashan and de Bashan, 2005). However, a transformed PGPB with a short survival capacity (but long enough to last a growing season) is a bonus for commercial suppliers, who can then provide fresh inoculant to the grower on a regular basis.

CHAPTER-VI

SUMMARY AND CONCLUSIONS

Chickpea is the third world's most important food legume crop after dry beans and peas, grown throughout tropical, subtropical and temperate regions in South and West Asia, East and North Africa, southern Europe, North and South America, and Australia (FAOSTAT, 2014). Approximately 13.5×10^6 hectare of chickpea are cultivated in more than 50 countries worldwide that yield nearly 13.1×10^6 tons (FAOSTAT, 2014). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is the major soil-borne fungus affecting chickpea globally and has become a major factor limiting for chickpea production in the Mediterranean Basin (Jimenez-Diaz *et al*., 2015). Fusarium wilt epidemics can devastate crops and cause up to 100% loss in highly infested fields under favorable conditions (Halila and Strange, 1997). Since soil-borne pathogens are difficult to control using conventional strategies, such as the use of resistant cultivars and synthetic fungicides, alternative methods of environmentally benign disease control are urgently required (Bailey and Lazarovits, 2003). Biological control using microorganisms such as *Bacillus* sp. (Cao *et al*., 2011), *Pseudomonas* sp. (Manikandan *et al*., 2010) and *Rhizobium* sp. (Das *et al*., 2017) have been proposed to control diseases caused by soil-borne pathogens. Quite apart from the antagonistic mechanism of *Bacillus* species, these microbes also have an important role in plant growth promotion by enhancing the biosynthesis of plant hormones (gibberellic acid and indole-3-acetic acid that have a close relation with plant nutrient availability (Nain *et al*., 2012). Inoculation of crop plants with certain strains of *Bacillus* improves biomass production through direct effect on root and shoot growth. High yielding varieties of chickpea along with use of effective rhizobial strains can enhance the yield. The use of biofertilizer and biopesticide is advocated to get the maximum benefits due to additive and synergistic effect.

During this study, one hundred ninety three bacterial isolates were obtained from chickpea rhizosphere soil and *Bacillus* isolates were selected based on morphological characterstics with one reference strain of *Bacillus subtilis*. All *Bacillus* isolates were screened for their antagonistic interactions against *Fusarium oxysporum* and only 87 isolates inhibited the growth of *F. oxysporum* on modified LB medium plates. Eleven *Bacillus* isolates i.e., HCA3, HCA10, HCA26, HCA29, HCA41, HCA44, HCA53, HCA61, RCA3, RCA7 and RCA9 showed large inhibition zones measuring more than 20 mm. Antifungal activity of selected *Bacillus* isolates was also tested against *Aspergillus niger*, *Neovossia indica* and *Rhizoctonia solani.* Out of twenty four *Bacillus* isolates tested, twelve isolates showed significant inhibition of *A. niger***,** RCA9 showed maximum antagonistic activity against *N.*

indica, whereas in case of *R. solani*, maximum inhibition of fungal growth was observed by *Bacillus* isolates HCA26 and RCA63.

Selected *Bacillus* isolates having variable level of antagonistic activity were further screened for different beneficial plant growth promoting activities. The ability to produce siderophore was found only in 41% of the *Bacillus* isolates. Four isolates HCA1, HCA3, HCA53 and RCA11 showed large orange halo zones around the bacterial growth. Overall, 96% *Bacillus* isolates showed ACC utilization ability, whereas seven *Bacillus* isolates i.e., HCA11, RCA3, RCA4, RCA19, RCA31, RCA63 and RCA68 showed more growth on ammonium sulphate containing plates as compared to control. Among 24, three *Bacillus* isolates RCA3, RCA7 and RCA11 showed significant HCN production in qualitative and quantitative estimation of HCN and it varied from 49.60 to 73.78 µl/ml. All the selected *Bacillus* isolates showed ALA production. Significant ALA production (> 12 µg/ml) was observed in seven *Bacillus* isolates HCA61, HCA76, HCA80**,** RCA4, RCA9, RCA19 and RCA31. The IAA production was observed in all the isolates and it varied from 0.13 to 26.6 µg/ml. Thirteen *Bacillus* isolates HCA44, HCA53, HCA61, HCA76, HCA81, SYB101, RCA3, RCA4, RCA7, RCA11, RCA19, RCA63 and RCA70 were found to possess phosphorus solubilizing activity. Three isolates i.e., HCA61, RCA3 and RCA63 showed potassium-solubilizing index (K-SI) more than 4.0. Fifteen isolates did not cause K-solubilization on mica containing plates.

Out of 24 isolates tested, four *Bacillus* isolates i.e., RCA7, HCA10, HCA53, HCA61 and HCA81 showed significant stimulatory effect on seed germination of chickpea, while three isolates i.e., RCA4, RCA9 and RCA19 showed retarding effect on chickpea seedlings at both 5th and 10th day of inoculation. *Bacillus* isolates were checked for their ability to grow at different concentrations of NaCl, i.e., 1, 2, 4, 6 and 8% (w/v), on LB medium plates. Twenty *Bacillus* isolates showed larger colony size ranging from 10.1-20 mm at 1% NaCl concentration. At 4% NaCl concentration, fourteen isolates showed large colony size whereas only eight *Bacillus* isolates showed larger colony size at 6% NaCl concentration. Only five *Bacillus* isolates HCA11, HCA81, HCA53, RCA11 and RCA31 showed colony size (ranging from 5.1-10.1 mm) even at 8% NaCl concentration. Out of 24 *Mesorhizobium* isolates obtained from the nodules of chickpea, 8 isolates inhibited the growth of *F. oxysporum* and isolate MCA7 showed maximum inhibition of fungus. On screening for salt tolerance all 24 *Mesorhizobium* isolates showed growth at 1 and 2% salt concentrations. Nine *Mesorhizobium* isolates at 4% and only five isolates at 6% salt concentration showed colony growth of 5.1-10.0 mm, whereas only two isolates MCA5 and MCA22 were found salt-tolerant upto 8% of salt concentration.

All the *Mesorhizobium* isolates obtained from chickpea nodules were studied for nodulation and plant growth of chickpea under chillum jar conditions. Eighteen

Mesorhizobium isolates showed stimulatory effect on growth parameters shoot and root dry weight of chickpea as compared to uninoculated control. At 40 days of growth, Maximum increase in shoot dry weight (45.5%) was observed by inoculation of *Mesorhizobium* isolate MCA20 and 166.2% increase in root dry weight by inoculation of MCA23. Maximum increase (122.6%) in SDW was observed on inoculation of MCA14 isolate followed by 118.3% increase in SDW on inoculation of isolate MCA4, whereas 61.6% increase in RDW was observed by inoculation of isolate MCA23 followed by 60% increase in RDW by inoculation of isolate MCA14 at 80th day of observation. Based upon antagonistic and other plant growth promoting beneficial attributes, three antagonistic isolates i.e., HCA61, RCA3 and RCA7, and two non-antagonistic *Bacillus* isolates; HCA76 and SYB101 were tested with *Mesorhizobium* strains i.e. MBD26 and KR48 on chickpea growth under chillum jar conditions. Coinoculation effect of selected *Bacillus* isolates with *Mesorhizobium* strains for their effect on nodulation and plant growth was compared to single inoculated *Mesorhizobium* strains. Maximum nodule-promoting effect was observed with treatments MBD26+RCA3 and KR48+HCA76. At 75 DAS, significant increase in shoot biomass (30.6 to 38.4%) was observed on coinoculation of KR48+HCA76 followed by KR48+SYB101 and KR48+RCA3. Inoculation of *Mesorhizobium* strain MBD26 with *Bacillus* isolates also showed enhanced plant biomass as compared to uninoculated control. Inoculation of treatments KR48+HCA76 caused 38.4% increase in SDW, whereas 49.3% increase was observed in MBD26+RCA3 inoculated treatment as compared to *Mesorhizobum*-inoculated plants. At 90 days of observation, coinoculation of *Mesorhizobium* strain KR48 with *Bacillus* isolates i.e., SYB101, RCA7, HCA76, HCA61, and RCA3 showed 109.7, 95.1, 51.2, 34.1 and 26.8% increase in nodule weightin comparison to *Mesorhizobum*-inoculated plants, respectively. Likewise, coinoculation of *Mesorhizobium* strain MBD26 with above mentioned *Bacillus* isolates showed 87.3, 75.3, 57.7, 17.6 and 25.4% increase in nodule weight. Significant gains were observed by coinoculation of chickpea with treatment KR48+SYB101 in shoot dry weight (46.1%) and root dry weight (105.6%) at 90 days of observation. On the other hand, coinoculation of *Mesorhizobium* strain MBD26 with *Bacillus* isolates i.e., RCA3, RCA7, HCA61 and SYB101 showed significant gains (113.9, 79.2, 59.7 and 55.3%) in SDW, whereas coinoculation with these isolates caused 50.6, 35.6, 64.8 and 25.6% increase in RDW, respectively.

Coinoculation effect of *Mesorhizobium* strains with five selected *Bacillus* isolates was studied on chickpea for biocontrol and plant growth promotion under pot house conditions. Reduction in the percentage of wilted plants was found from 60 to 100% in treatments inoculated with antagonistic isolates at 70 DAS. The wilt disease free chickpea plants revealed the effectiveness of antagonistic isolates HCA61, RCA3 and RCA7 and fungicide (Bavistin) in disease control at all the three stages of plant growth. Treatments of *Bacillus* isolate KR48, KR48+SYB101 and KR48+HCA76 inoculated with *F. oxysporum* caused 100% disease control while single and mixed inoculation of SYB101 and HCA76 with MBD26 strain caused only 60 to 80% disease control, respectively at 90 days of growth.

At 75 DAS, coinoculation of *Bacillus* isolates SYB101 and HCA61 along with fungus showed 118.7 and 106.2% increase in nodule number and caused 128 and 68.5% increase in nodule weight, respectively. Coinoculation of *Bacillus* isolates SYB101 and HCA61 with fungal culture increased nodule number (90, 85%) and nodule weight (187.1, 176.1%), whereas coinoculation with RCA3 and RCA7 showed increase in SDW (113.5, 97.3%) and RDW (77.6, 65%), respectively at 90 days of growth. Inoculation of treatment *F. oxysporum*+KR48+SYB101 caused 149 and 111% increase in SDW and RDW of chickpea, followed by *F. oxysporum*+KR48+RCA7 (129.5, 96.9%) and *F. oxysporum*+KR48+RCA3 (107.7, 77.2%), respectively. *Mesorhizobium* strain MBD26 with *Bacillus* isolates showed significant stimulatory effect on plant growth parameters at different stages of plant growth.

This study is providing an initial assessment of the potential of *Bacillus* bacteria associated with chickpea in Haryana and Rajasthan to control Fusarium wilt disease of chickpea and promote plant growth individually, and in combination with *Mesorhizobium* under chillum jar and pot house conditions. Three *Bacillus* isolates, RCA3, RCA7 and HCA61 appeared to be the most promising biological control agents against chickpea wilt while both antagonistic and non-antagonistic *Bacillus* isolates showed significant effect on chickpea growth. Study of these antagonists under field condition is needed for the more evident confirmation of their activity against Fusarium wilt and plant growth promoting traits for chickpea growth.

REFERENCES

Abbasdokht, H. and Gholami, A. (2010) The effect of seed inoculation (*Pseudomonas putida* + *Bacillus lentus*) and different levels of fertilizers on yield and yield components of wheat (*Triticum aestivum* L.) cultivars. *World Acad. Sci. Eng. Technol.* **68**: 979-983.

Abed, H., Rouag, N., Mouatassem, D. and Rouabhi, A. (2016) Screening for *Pseudomonas* and *Bacillus* antagonistic rhizobacteria strains for the biocontrol of Fusarium wilt of chickpea. *Eurasian J. Soil Sci.* **5(3):** 182-191.

Agarry, O.O., Akinyosoye, F.A. and Adetuyi, F.C. (2005) Antagonistic properties of microogranisms associated with cassava (*Manihot esculenta* Crantz) products. *Afri. J. Biotech.* **4(7):** 627-632.

Agbodjato, N.A., Noumavo, P.A., Baba-Moussa, F., Salami, H.A., Sina, H., Sèzan, A., Bankolé, H., Adjanohoun, A. and Baba-Moussa, L. (2015) Characterization of potential plant growth promoting rhizobacteria isolated from maize (*Zea mays* L.) in central and northern Benin (West Africa). *Appl. Environ. Soil Sci.* **2015:** 1-9.

Ahemad, M. and Khan, M.S. (2009) Toxicity assessment of herbicides quizalafop-p-ethyl and clodinafop towards *Rhizobium*-pea symbiosis. *Bull. Environ. Contam. Toxicol.***82:** 761–766.

Ahemad, M. and Khan, M.S. (2011) Plant growth promoting fungicide-tolerant *Rhizobium* improves growth and symbiotic characteristics of lentil (*Lens esculentus*) in fungicide-applied soil. *J. Plant Growth Regul.***30:** 334–342.

Ahemad, M. and Kibret, M. (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J. King Saud University Sci.* **26(1):** 1-20.

Ahmad, E., Zaidi, A. and Khan, M.S. (2016) Effects of plant growth promoting rhizobacteria on the performance of green gram under field conditions. *Jordan J. Biol. Sci.* **9(2):** 79-88.

Ahmad, F., Ahmad, I. and Khan, M.S. (2008) Screening of free-living rhizospheric bacteria for their multiple growth promoting activities. *Microbiol. Res.* **163:** 173-181.

Ahmad, I., Iftikhar, S. and Bhutta, A.R. (1993) Seed borne microorganism in Pakistan. A checklist 1991.Pakistan Agricultural Research Council, Islamabad, Pakistan.p. 32.

Ahmad, M., Zahir Z.A., Khalid M., Nazli F. and Arshad M. (2013) Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiol. Biochem.* **63:** 170-176.

Ahmad, M., Zahir, Z.A., Asghar, H.N. and Asghar, M. (2011a) Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. *Can. J. Microbiol.* **57(7):** 578-589.

Ahmad, M.J., Khalid, M. and Jami, A. (2011b) Screening of *Mesorhizobium ciceri* isolates from rainfed area for improvement in growth and nodulation of chickpea seedlings under controlled conditions. *Soil Environ.* **30(2):** 160-165.

Ahmadzadeh, M., Afsharmanesh, H., Javan-Nikkhah, M. and Sharifi-Tehrani, A. (2006) Identification of some molecular traits in fluorescent pseudomonads with antifungal activity. *Iranian J. Biotechnol.* **4(4):** 245-253.

Ahmed, A. and Hasnain, S. (2010) Auxin-producing *Bacillus* sp.: Auxin quantification and effect on the growth of *Solanum tuberosum*. *Pure Appl. Chem.* **82(1):** 313-319.

Ahmed, T.H., Elsheikh, E.A.E. and Mahadi, A.A. (2009) The *in vitro* compatibility of some *Rhizobium* and *Bradyrhizobium* strains with fungicides. *Afr. Crop. Sci. Conf. Proc. B* **8:** 1171-1178.

Akhtar, M.S. and Siddiqui, Z.A. (2010) Role of plant growth promoting rhizobacteria in biocontrol of plant diseases and sustainable agriculture. In: *Plant growth and health promoting bacteria* (eds. Microbiology Monograohs). Springer Berlin Heidelberg. pp. 157-195.

Alamri, S.A. (2015) Enhancing the efficiency of the bioagent *Bacillus subtilis* JF419701 against soil-borne phytopathogens by increasing the productivity of fungal cell wall degrading enzymes. *Archi.Phytopathol. Plant Protect.* **48(2):** 159-170.

Aleksandrov, V.G., Blagodyr, R.N. and Ilev, I.P. (1967) Liberation of phosphoric acid from apatite by silicate bacteria. *Microbiol. J.***29:** 111–114.

Alstrom, S. and Burns, R.G. (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. *Biol. Fértil. Soils* **7:** 232–238.

Alves-Santos, F.M., Cordeiro-Rodrigues, L., Sayagués, J.M., Martín-Domínguez, R., García-Benavides, P., Crespo, M.C., Díaz-Mínguez, J.M. and Eslava, A.P. (2002) Pathogenicity and race characterization of *Fusarium oxysporum* f. sp. *phaseoli* isolates from Spain and Greece. *Plant Pathol.* **51(5):** 605-611.

Amer, G.A. and Utkhede, R.S. (2007) Development of formulations of biological agents for management of root rot of lettuce and cucumber. *Can. J. Microbiol.* **46(9):** 809-816.

Amer, G.A.M., Singh, D.V., Aggarwal, R. and Dureja, P. (1998) Microbial antagonism to *Neovossia indica*, causing Karnal bunt of wheat. *Curr. Sci.***21:** 1393-1396.

Anal, E.P., Moreno, V.M., Cordo, C., Cecilia, M. and Siman, A. (2009) Biological control of *Septoria tritici* blotch on wheat by *Trichoderma* sp. under field conditions in Argentina. *Biocontrol* **54:** 113-122.

Anith, K.N., Radhakrishnan, N.V. and Manomohandas, T.P. (2003) Screening of antagonistic bacteria for biological control of nursery wilt of black pepper (*Piper nigrum*). *Microbiol. Res.* **158(2):** 91-97.

Anjajah, V., Cornelis, P. and Koedam, N. (2003) Effect of genotype and root colonization in biological control of Fusarium wilt in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. *Can. J. Microbiol.* **49:** 85-91.

Anu and Kundu, B.S. (2005) Development and characterization of *lac*Z marked strains of phosphate solubilizing bacteria. *Indian J. Microbiol.* **45(4):** 261-263.

Anusuya, V. and Manimekalai, G. (2016) Potential of *Bacillus* isolates as biocontrol agent against Fusarium wilt of banana. *World J. Pharm. Pharmaceutical Sci.* **5(5):** 1679-1694.

Arfaoui, A., Sifi, B., Boudabous, A., Hadrami, I. E. and Chérif, M. (2006) Identification of *Rhizobium* isolates possessing antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris*, the causal agent of Fusarium wilt of chickpea. *J. Plant Pathol.* **88(1):** 67-75.

Arkhipova, T.N., Veselov, S.U., Melentiev, A.I., Martynenko, E.V. and Kudoyarova, G.R. (2005) Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. *Plant Soil* **272:** 201-209.

Arnon, I. (1972) Crop production in dry regions, Vol. I. London: Leonard Hill. pp. 460–462.

Arora, N.K., Kang, S.C. and Maheshwari, D.K. (2001) Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Curr. Sci.***81:** 673-677.

Arshad, M. and Frankenberger, W.T. Jr. (1992) Microbial production of plant growth regulators. In: Soil microbial ecology, applications in agricultural and environmental management. Metting FB Jr (ed). Dekker, New York, pp. 27–32.

Asari, S., Matzén, S., Petersen, M.A., Bejai, S. and Meijer, J. (2016) Multiple effects of *Bacillus amyloliquefaciens* volatile compounds: Plant growth promotion and growth inhibition of phytopathogens. *FEMS Microbiol. Ecol.* **92(6):** 70-81.

Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, M.A., Islam, M.Z., Shahidullah, S.M. and Meon, S. (2009) Efficiency of plant growth promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.* **8(7):** 1247-1252.

Bailey, K.L. and Lazarovits, G. (2003) Suppressing soil borne diseases with residue management and organic amendments.*Soil Till. Res.***72:** 169-180.

Banerjee, M.R., Yesmin, L. and Vessey, J.K. (2006) Plant growth promoting rhizobacteria as biofertilizers and biopesticides. Handbook of Microbial Biofertilizers. Food Products Press, New York, 137-181.

Basak, B.B. and Biswas, D.R. (2010) Coinoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. *Biol. Fertil. Soils* **46(6):** 641-648.

Bashan, Y. and De-Bashan, L.E. (2005) Plant growth promoting bacteria. *Encyclopedia Soils Environ.* **1:** 103-115.

Bashan, Y., de-Bashan, L.E., Prabhu, S.R. and Hernandez, J.P. (2014) Advances in plant growth promoting bacterial inoculant technology: Formulations and practical perspectives. *Plant Soil* **378:** 1-33.

Bayaa, B., Kumari, S.G., Akkaya, A., Erskine, W., Makkouk, K.M., Turk, Z. and Özberk, I. (1998) Survey of major biotic stresses of lentil in South-East Anatolia, Turkey. *Phytopathol.Mediter.* **37(2):** 88-95.

Bayraktar, H. and Dolar, F.S. (2012) Pathogenic variability of *Fusarium oxysporum* f. sp. *ciceris* isolates from chickpea in Turkey. *Pak. J. Botany* **44:** 821–823.

Baysal, Ö., Lai, D., Xu, H.H., Siragusa, M., Çalışkan, M., Carimi, F., Da Silva, J.A.T. and Tör, M. (2013) A proteomic approach provides new insights into the control of soil-borne plant pathogens by *Bacillus* species. *PloS One* **8(1):** e53182.

Beattie, G.A. (2006) Plant-associated bacteria: Survey, molecular phylogeny, genomics and recent advances. In: Plant-associated bacteria. Gnanamanickam, S.S. (ed.). Dordrecht, the Netherlands: Springer. pp. 1–56.

Belete, E., Amare, A. and Seid, A. (2015) Antagonistic effect of native *Bacillus* isolates against black root rot of faba bean. *Afri. Crop Sci. J.* **23(3):** 249-259.

Belimov, A.A., Dodd, I.C., Hontzeas, N., Theobald, J.C., Safronova, V.I. and Davies, W.J. (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytol.* **181(2):** 413-423.

Berrada, H., Nouioui, I., Houssaini, M.I., Gtari, M. and Benbrahim, K.F. (2012) Phenotypic and genotypic characterizations of rhizobia isolated from root nodules of multiple legume species native of Fez, Morocco. *Afri. J. Microbiol. Res.* **6(25):** 5314-5324.

Bertrand, H., Nalin, R., Bally, R. and Cleyet-Marel, J.C. (2001) Isolation and identification of the most efficient plant growth promoting bacteria associated with canola (*Brassica napus*). *Biol. Fertil. Soils* **33:** 152–156.

Bhagat, D., Sharma, P., Sirari, A. and Kumawat, K.C. (2014) Screening of *Mesorhizobium* spp. for control of Fusarium wilt in chickpea *in vitro* conditions. *Int. J. Curr. Microbiol. Appl. Sci.* **3:**923-930.

Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A. and Samiyappan, R. (2004) Rhizobacteria-based bioformulations for the management of fruit rot infection in chillies. *Crop Protect.* **23(9):** 835-843.

Bhattacharya, S., Das, A., Samadder, S. and Rajan, S.S. (2016) Biosynthesis and characterization of a thermostable, alkali-tolerant chitinase from *Bacillus pumilus* JUBCH08 displaying antagonism against phytopathogenic *Fusarium oxysporum*. *3 Biotech.* **6(1):** 1-8.

Bhattacharyya, P.N. and Jha, D.K. (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbiol.Biotechnol.* **28(4):** 1327-1350.

Biswas, J.C., Ladha, J.K. and Dazzo, F.B. (2000) Rhizobia inoculation improves nutrient uptake and growth of lowland rice. *Soil Sci. Soc. Am. J.***64:** 1644–1650.

Blumer, C. and Haas, D. (2000) Mechanism, regulation and ecological role of bacterial cyanide biosynthesis. *Archi.Microbiol.* **173(3):** 170-177.

Bottini, R., Cassán, F. and Piccoli, P. (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Appl. Microbiol. Biotechnol.* **65(5):** 497-503.

Bouhmouch, I., Souad-Mouhsine, B., Brhada, F. and Aurag, J. (2005) Influence of host cultivars and *Rhizobium* species on the growth and symbiotic performance of *Phaseolus vulgaris* under salt stress. *J. Plant Physiol.* **162(10):** 1103-1113.

Buyer, J.S., Kratzke, M.G. and Sikora, L.J. (1993) A method for detection of pseudobactin, the siderophore produced by a plant-growth-promoting *Pseudomonas* strain, in the barley rhizosphere. *Appl. Environ. Microbiol.* **59(3):** 677-681.

Buysens, S., Poppe, J. and Höfte, M. (1994) Role of siderophores in plant growth stimulation and antagonism by *Pseudomonas aeruginosa* 7NSK2. In: Improving plant productivity with rhizosphere bacteria (Ryder, M.H., Stephens, P.M. and Bowen, G.D. eds.). Proceedings of the third international workshop on plant growth promoting rhizobacteria Adelaide, Australia, March 7-11, 1994. pp. 139-141.

Cao, Y., Zhang, Z., Ling, N., Yuan, Y., Zheng, X., Shen, B. and Shen, Q. (2011) *Bacillus subtilis* SQR9 can control Fusarium wilt in cucumber by colonizing plant roots. *Biol. Fertil. Soils* **47:** 495–506.

Cevheri, C., Küçük, C. and Çetin, E. (2011) Fungicide, antibiotic, heavy metal resistance and salt tolerance of root nodule isolates from *Vicia palaestina*. *Afri. J. Biotech.* **10(13):** 2423-2429.

Chakraborty, A.P., Dey, P., Chakraborty, B., Chakraborty, U. and Roy, S. (2011) Plant growth promotion and amelioration of salinity stress in crop plants by a salt-tolerant bacterium. *Recent Res. Sci. Technol.* **3:** 61–70.

Chakraborty, U., Chakraborty, B. and Basnet, M. (2006) Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. *J. Basic Microbiol.* **46(3):** 186-195.

Charest, M.H., Beauchamp, C.J. and Antoun, H. (2005) Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and *Pythium ultimum*. *FEMS Microbiol. Ecol.* **52(2):** 219-227.

Chattopadhyay, N., Kaiser, S.A.K.M. and Sengupta, P.K. (1999) Effect of organic amendment of soil on the population of three soil-borne fungal pathogen of chickpea. *Annals Plant Protect. Sci.* **7:** 243-245.

Chaudhary, D. and Sindhu, S.S. (2015) Inducing salinity tolerance in chickpea (*Cicer arietinum* L.) by inoculation of 1-aminocyclopropane-1-carboxylic acid deaminase-containing *Mesorhizobium* strains. *Afri. J. Microbiol. Res.* **9(2):** 117-124.

Chaudhary, D. and Sindhu, S.S. (2017) Amelioration of salt stress in chickpea (*Cicer arietinum* L.) by coinculation of ACC deaminase-containing rhizospheric bacteria with *Mesorhizobium* strains. *Legume Res.* **40(1):** 80-86.

Chauhan, Y.S., Nene, Y.L., Johansen, C., Haware, M.P., Saxena, N.P., Sardar, S., Sharma, S.B., Sahrawat, K.L., Burford, J.R., Rupela, O.P., Kumar Rao, J.V.D.K. and Sithanantham, S. (1988) Effects of soil solarization on pigeon pea and chickpea. Research Bulletin no 11. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India.

Chebotar, V.K., Asis, C.A. and Akao, S. (2001) Production of growth promoting substances and high colonization ability of rhizobacteria enhance the nitrogen fixation of soybean when coinoculated with *Bradyrhizobium japonicum*. *Biol. Fertil. Soils* **34:** 427-432.

Chehri, K., Abbasi, S., Reddy, K.R.N. and Salleh, B. (2010) Occurrence and pathogenicity of various pathogenic fungi on cucurbits from Kermanshah province, Iran. *Afri. J. Microbiol.Res.* **4:** 1215-1222.

Chen, L., Dodd, I.C., Theobald, J.C., Belimov, A.A. and Davies, W.J. (2013) The rhizobacterium *Variovorax paradoxus* 5C-2, containing ACC deaminase, promotes growth and development of *Arabidopsis thaliana* via an ethylene-dependent pathway. *J. Expt. Botany* **64(6):** 1565-1573.

Chen, W.F., Guan, S.H., Zhao, C.T., Yan, X.R., Man, C.X., Wang, E.T. and Chen, W.X. (2008) Different *Mesorhizobium* species associated with Caragana carry similar symbiotic genes and have common host ranges. *FEMS Microbiol. Lett.* **283(2):** 203-209.

Chen, Y., Cao, S., Chai, Y., Clardy, J., Kolter, R., Guo, J.H. and Losick, R. (2012) A *Bacillus subtilis* sensor kinase involved in triggering biofilm formation on the roots of tomato plants. *Mol. Microbiol.* **85(3):** 418-430.

Chernin, L., Ismailov, Z., Haran, S. and Chet, I. (1995) Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. *Appl. Environ. Microbiol.* **61:** 1720–1726.

Chi, F., Shen, S.H., Cheng, H.P., Jing, Y.X., Yanni, Y.G. and Dazzo, F.B. (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Appl. Environ. Microbiol.***71:** 7271-7278.

Choudhary, D.K. and Johri, B.N. (2009) Interactions of *Bacillus* spp. and plants with special reference to induced systemic resistance (ISR). *Microbiol. Res.* **164(5):** 493-513.

Choudhary, D.K., Sharma, K.P. and Gaur, R.K. (2011) Biotechnological perspectives of microbes in agro-ecosystems. *Biotechnol. Lett.* **33:** 1905–1910.

Choudhary, S.R. (2013) Biological control of root rot disease in clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) by rhizospherebacteria. M. Sc. thesis. Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Chowdhury, S.P., Hartmann, A., Gao, X. and Borriss, R. (2015) Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42–A review. *Front. Microbiol.* **6:** 1-11.

Compant, S., Duffy, B., Nowak, J., Clément, C. and Barka, E.A. (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. *Appl. Environ. Microbiol.* **71(9):** 4951-4959.

Cother, E.J. (1977) Identification and control of root-rot fungi in *Cicer arietinum* (chickpea). *Plant Dis. Reporter* **61(9):** 736-740.

Dary, M., Chamber-Perez, M.A., Palomares, A.J. and Pajuelo, E. (2010) 'In situ'' phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant growth promoting rhizobacteria. *J. Hazard. Mater.* **177:** 323–330.

Das, I.K., Indira, S., Annapurna, A. and Seetharama, N. (2008) Biocontrol of charcoal rot in sorghum by fluorescent pseudomonads associated with the rhizosphere. *Crop Protect.* **27(11):** 1407-1414.

Das, K., Prasanna, R. and Saxena, A.K. (2017) Rhizobia: A potential biocontrol agent for soilborne fungal pathogens. *Folia Microbiol.***5:** 1-11.

Davies, P. (2013) Plant hormones: Physiology, biochemistry and molecular biology. Springer Science & Business Media.

De Lucchi, C., Stevanato, P., Hanson, L., McGrath, M., Panella, L., De Biaggi, M., Broccanello, C., Bertaggia, M., Sella, L. and Concheri, G. (2017) Molecular markers for improving control of soil-borne pathogen *Fusarium oxysporum* in sugar beet. *Euphytica* **213(3):** 71.

del Rosario, C.L., Santoro, M.V., Nievas, F., Giordano, W. and Banchio, E. (2013) Increase of secondary metabolite content in marigold by inoculation with plant growth-promoting rhizobacteria. *Appl. Soil Ecol.* **70:** 16-22.

Demir, S. and Akkopru, A. (2007) Using of arbuscular mycorrhizal fungi (AMF) for biocontrol of soil-borne fungal plant pathogens. *Biol. Control Plant Dis.* Haworth Press, USA. pp. 17-37.

Deshwal, V.K., Pandey, P., Kang, S.C. and Maheshwari, D K. (2003) Rhizobia as a biological control agent against soilborne plant pathogenic fungi. *Indian J. Expt. Biol.***41:** 1160-1164.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan, S.M. (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. *Microbiol. Res.* **159:** 371-394.

Dileep Kumar, B.S. (1999) Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chickpea growing in soils infested with *Fusarium oxysporum* f. sp. *ciceris*. *Biol. Fertil. Soils***29:** 87–91.

Dobbelaere, S., Vanderleyden, J. and Okon, Y. (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Rev. Plant Sci.* **22(2):** 107-149.

Dua, S. and Sindhu, S.S. (2012) Effectiveness of rhizosphere bacteria for control of root rot disease and improving plant growth of wheat (*Triticum aestivum* L.). *J. Microbiol. Res*. **2(2):** 26-35.

Duan, J., Müller, K.M., Charles, T.C., Vesely, S. and Glick, B.R. (2009) 1- aminocyclopropane-1-carboxylate (ACC) deaminase genes in rhizobia from southern Saskatchewan. *Microbial Ecol.* **57(3):** 423-36.

Dubey, S.C., Singh, S.R. and Singh, B. (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Archi. Phytopathol. Plant Protect.* **43(2):** 174-190.

Dubey, S.C., Suresh, M. and Singh, B. (2007) Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. *Biol. Control* **40(1):** 118-127.

Duke, J.A. (1981) *Cicer arietinum* L. Handbook of legumes of world economic importance. pp. 52-57.

Dworkin, M. and Foster, J. (1958) Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol*. **75:** 592–601.

Effmert U., Kalderás J., Warnke R. and Piechulla B. (2012) Volatile mediated interactions between bacteria and fungi in the soil. *J. Chem. Ecol.* **38:** 665–703.

Ehsan, M., Ahmed, I., Hayat, R., Iqbal, M., Bibi, N. and Khalid, N. (2016) Molecular identification and characterization of phosphate solubilizing *Pseudomonas* sp. isolated from rhizosphere of mash bean (*Vigna mungo* L.) for growth promotion in wheat. *J. Agri. Sci. Technol.* **18(3):** 775-788.

Ekundayo, E.A., Adetuyi, F.C. and Ekundayo, F.O. (2011) *In vitro* antifungal activities of bacteria associated with maize husks and cobs. *Res. J. Microbiol.* **6(4):** 418-424.

Elad, Y. and Baker, R. (1985) The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. *Phytopathology* **75:** 1053-1059.

Elbadry, M.R.M., Taha, Eldougdoug, A. and Gamal-Eldin, H. (2006) Induction of systemic resistance in faba bean (*Vicia faba* L.) to bean yellow mosaic potyvirus (BYMV) via seed bacterization with plant growth promoting rhizobacteria. *J. Plant Dis. Protect.* **113(6):** 247-251.

Elkoca, E., Kantar, F. and Sahin, F. (2008) Influence of nitrogen-fixing and phosphorus-solubilizing bacteria on the nodulation, plant growth and yield of chickpea. *J. Plant Nutri.* **31:** 157-171.

Emmert, E.A. and Handelsman, J. (1999) Biocontrol of plant disease: A (Gram-) positive perspective. *FEMS Microbiol. Lett.* **171(1):** 1-9.

Erdogan, O. and Benlioglu, K. (2010) Biological control of Verticillium wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. *Biol. Control* **53(1):** 39-45.

Essalmani, H. and Lahlou, H. (2003) Bioprotection mechanisms of the lentil plant by *Rhizobium leguminosarum* against *Fusarium oxysporum* f. sp. *lentis*. *Comptes Rendus Biologies* **326(12):** 1163-1173.

FAO, I. (2014) The state of food insecurity in the world.p. 12.

FAOSTAT (Food and Agriculture Organization of the United Nations Statistical Database) (2014). FAOSTAT Production Statistics of Crops.

FAOSTAT, F.A.O. (2009) Statistical databases. Food and Agriculture Organization of the United Nations, Rome.

Farmer, E.E. (2001) Surface-to-air signals. *Nature* **411:** 854-856.

Fernando, W.G.D., Nakkeeran, S. and Zhang, Y. (2006) Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In: PGPR: Biocontrol and Biofertilization. Siddiqui, Z.A. (ed). Springer, Netherlands. pp. 67-109.

Fiddaman, P.J. and Rossall, S. (1994) Effect of substrate on the production of antifungal volatiles from *Bacillus subtilis*. *J. Appl. Bacteriol.* **76(4):** 395-405.

Figueiredo, M.V.B., Martinez, C.R., Burity, H.A. and Chanway, C.P. (2008) Plant growth promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.).*World J. Microbiol. Biotechnol.* **24:** 1187–1193.

Garcia, J.A.L., Probanza, A., Ramos, B., Palomino, M. and Mañero, F.J.G. (2004) Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. *Agronomy* **24(4):** 169-176.

Gauri, A., Singh, A.K. and Bamania, M. (2012) Characterization of *Mesorhizobium* sp. isolated from root nodules of *Cicer arietinum*. *Int. J. Agric. Sci. Res.* **2:** 142-154.

Ghodsalavi, B., Ahmadzadeh, M., Soleimani, M., Madloo, P.B. and Taghizad-Farid, R. (2013) Isolation and characterization of rhizobacteria and their effects on root extracts of *Valeriana officinalis*. *Austr. J. Crop Sci.* **7(3):** 338-344.

Ghosh, S., Penterman, J.N., Little, R.D., Chavez, R. and Glick, B.R. (2003) Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica campestris*. *Plant Physiol. Biochem.* **41:** 277–281.

Glick, B.R. (1995) The enhancement of plant growth by free-living bacteria. *Can J. Microbiol.* **41(2):** 109-117.

Glick, B.R. (2004) Bacterial ACC deaminase and the alleviation of plant stress. *Adv. Appl.Microbiol*. **56:** 291–312.

Glick, B.R. (2012) Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* **2012:** 1-15.

Glick, B.R. (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol. Res.* **169(1):** 30-39.

Glick, B.R. (2015) Beneficial plant-bacterial interactions. Springer.

Glick, B.R., Cheng, Z., Czarny, J. and Duan, J. (2007a). Promotion of plant growth by ACC deaminase-producing soil bacteria. *European J. Plant Pathol.* **119(3):** 329-339.

Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J. and McConkey, B. (2007b) Promotion of plant growth by bacterial ACC deaminase. *Critical Rev. Plant Sci.* **26(5-6):** 227-242.

Goel, A., Sindhu, S. and Dadarwal, K. (2002) Stimulation of nodulation and plant growth of chickpea (*Cicer arietinum* L.) by *Pseudomonas* spp. antagonistic to fungal pathogens. *Biol. Fertil. Soils* **36(6):** 391-396.

Goel, A.K., Sindhu, S.S. and Dadarwal, K.R. (2000) Pigment diverse mutants of *Pseudomonas* sp.: Inhibition of fungal growth and stimulation of growth of *Cicer arietinum*. *Biol. Plant* **43:** 563-569.

Goel, A.K., Sindhu, S.S. and Dadarwal, K.R. (2001) Seed bacterization with fluorescent *Pseudomonas* enhances the synthesis of flavonoid-like compounds in chickpea (*Cicer arietinum* L.). *Physiol. Mol. Biol. Plants* **6:** 195–198.

Gopalakrishnan, S., Vadlamudi, S., Samineni, S. and Kumar, C.S. (2016) Plant growth-promotion and biofortification of chickpea and pigeonpea through inoculation of biocontrol potential bacteria, isolated from organic soils. *Springer Plus* **5(1):**1882-1893.

Gordon, S.A. and Weber, R.P. (1951) Colorimetric estimation of indole acetic acid. *Plant Physiol.* **26(1):**192-195.

Goswami, B.K., Pandey, R.K., Goswami, J. and Tewari, D.D. (2007) Management of disease complex caused by root knot nematode and root wilt fungus on pigeonpea through soil organically enriched with vesicular arbuscular mycorrhiza, karanj (*Pongamia pinnata*) oilseed cake and farmyard manure. *J. Environ. Sci. Health Part B* **42(8):** 899-904.

Goswami, D., Dhandhukia, P., Patel, P. and Thakker, J.N. (2014) Screening of PGPR from saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. *Microbiol. Res.* **169(1):** 66-75.

Gotor-Vila, A., Teixidó, N., Di Francesco, A., Usall, J., Ugolini, L., Torres, R. and Mari, M. (2017) Antifungal effect of volatile organic compounds produced by *Bacillus amyloliquefaciens* CPA-8 against fruit pathogen decays of cherry. *Food Microbiol.* **64:** 219-225.

Gowda, S.J.M., Radhika, P., Kadoo, N.Y., Mhase, L.B. and Gupta, V.S. (2009) Molecular mapping of wilt resistance genes in chickpea. *Mol. Breeding* **24(2):** 177-183.

Graham, P.H. and Vance, C.P. (2003) Legumes: Importance and constraints to greater use. *Plant Physiol*. **131(3):** 872-877.

Grobelak, A., Napora, A. and Kacprzak, M. (2015) Using plant growth promoting rhizobacteria (PGPR) to improve plant growth. *Ecol. Engg.* **84:** 22-28.

Groppa, M.D., Zawoznik, M.S. and Tomaro, M.L. (1998) Effect of coinoculation with *B. japonicum* and *Azospirillum brasilense* on soybean plants. *Euro. J. Soil Biol.* **34(2):** 75-80.

Grosskopf, T. and Soyer, O.S. (2014) Synthetic microbial communities.*Curr. Opinion Microbiol.* **18:** 72-77.

Gull, M. and Hafeez, F.Y. (2012) Characterization of siderophore producing bacterial strain *Pseudomonas fluorescens* Mst 8.2 as plant growth promoting and biocontrol agent in wheat. *Afri. J. Microbiol. Res.* **6(33):** 6308-6318.

Gull, M., Hafeez, F.Y., Saleem, M. and Malik, K.A. (2004) Phosphorus uptake and growth promotion of chickpea by coinoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. *Aus. J. Expt. Agri.* **44:** 623–628.

Gupta, A., Meyer, J. M. and Goel, R. (2002) Development of heavy metal-resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI4014 and their characterization. *Curr.Microbial.* **45(5):** 323-327.

Gururani, M.A., Upadhyaya, C.P., Baskar, V., Venkatesh, J., Nookaraju, A. and Park, S.W. (2013) Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *J. Plant Growth Regul.* **32(2):** 245-258.

Haas, D. and Défago, G. (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Rev. Microbiol.* **3(4):** 307-319.

Hafeez, F.Y., Aslam, Z. and Malik, K.A. (1988) Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* (L.) Wilczek. *Plant Soil* **106(1):** 3-8.

Hafeez, F.Y., Yasmin, S., Ariani, D., Zafar, Y. and Malik, K.A. (2006) Plant growth promoting bacteria as biofertilizer. *Agro. Sustainable Dev.* **26(2):** 143-150.

Hahm, M.S., Sumayo, M., Hwang, Y.J., Jeon, S.A., Park, S.J., Lee, J.Y., Ahn, J.H., Kim, B.S., Ryu, C.M. and Ghim, S.Y. (2012) Biological control and plant growth promoting capacity of rhizobacteria on pepper under greenhouse and field conditions. *J. Microbiol.* **50(3):** 380-385.

Halila, M.H. and Strange, R.N. (1996) Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f. sp *ciceris* race 0. *Phytopathol. Mediter.* **35(2):** 67-74.

Halila, M.H. and Strange, R.N. (1997) Screening of Kabuli chickpea germplasm for resistance to Fusarium wilt. *Euphytica* **96(2):** 273-279.

Halverson, L.J. and Handelsman, J. (1991) Enhancement of soybean nodulation by *Bacillus cereus* UW85 in the field and in a growth chamber. *Appl. Environ. Microbiol.* **57(9):** 2767-2770.

Hameeda, B., Harini, G., Rupela, O.P., Rao, J.K. and Reddy, G. (2010) Biological control of chickpea collar rot by coinoculation of antagonistic bacteria and compatible rhizobia. *Ind. J. Microbiol.* **50(4):** 419-424.

Hameeda, B., Harini, G., Rupela, O.P., Wani, S.P. and Reddy, G. (2008) Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. *Microbiol. Res.* **163(2):** 234-242.

Han, Q.Q., Lü, X.P., Bai, J.P., Qiao, Y., Paré, P.W., Wang, S.M., Zhang, J.L., Wu, Y.N., Pang, X.P., Xu, W.B. and Wang, Z.L. (2014) Beneficial soil bacterium *Bacillus subtilis* (GB03) augments salt tolerance of white clover. *Front. Plant Sci.* **5:** 525.

Harding, D.P. and Raizada, M.N. (2015) Controlling weeds with fungi, bacteria and viruses: A review. *Front. Plant Sci.* **6:** 659.

Hartman, A., Singh, M. and Klingmuler, W. (1983) Isolation and characterization of *Azospirillum* mutants excreting high amounts of indole acetic acid. *Can. J. Microbiol.* **29:** 916–923.

Haware, M.P. (1998) Diseases of chickpea. In: The plant pathology of food and pasture legumes. Allen, D.J. and Lenne, J.M. (eds). CAB International, Wallingford, UK. pp. 473–516.

Haware, M.P. and Nene, Y.L. (1982) Races of *Fusarium oxysporum* f. sp. c*iceris*. *Plant Dis*. **66:** 809-810.

Haware, M.P., Nene, Y.L. and Mathur, S.B. (1986) Seedborne diseases of chickpea.Technical Bulletin No. 1. ISP. Copenhagen. ICRISAT, India.

Haware, M.P., Nene, Y.L. and Natarajan, M. (1996) The survival of *Fusarium oxysporum* f. sp. *ciceri* in the soil in the absence of chickpea. *Phytopathol. Mediter.* **35(1):** 9-12.

Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. (2010) Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals Microbiol.* **60(4):** 579-598.

Heinen, W. (1960) Silicon metabolism in micorganisms. *Arkiv. Mikrobiol*. **37:** 199-210.

Hemissi, I., Mabrouk, Y., Neila, A.B.D.I., Bouraoui, M., Saidi, M. and Bouaziz, S.I.F.I. (2011) Effects of some *Rhizobium* strains on chickpea growth and biological control of *Rhizoctonia solani*. *Afri. J. Microbiol. Res.* **5(24):** 4080-4090.

Henry, M.B., Lynch, J.M. and Fermor, T.R. (2008) Role of siderophores in the biocontrol of *Pseudomonas tolaasii* by fluorescent pseudomonad antagonists. *J. Appl. Microbiol.* **70(2):** 104–108.

Hervas, A., Trapero-Casas, J.L. and Jimenez-Dıaz, R.M. (1995) Induced resistance against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f. sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. *Plant Dis.***79:** 1110–1116.

Holliday, P. (1980) Fungus diseases of tropical crops. Courier Corporation.

Hotta, Y., Tanaka, H., Takaoka, Y., Takeuchi, Y. and Konnai, M. (1997) Promotive effects of 5-aminolevulinic acid on the yield of several crops.*Plant Growth Regul.***22:** 109–114.

Hu, X.F., Chen, J. and Guo, J.F. (2006) Two phosphate and potassium solubilizing bacteria isolated from Tiannu mountain, Zhejiang, China. *World J. Microbiol.Biotechnol.***22:** 983–990.

Huang, H.C. and Erickson, R.S. (2007) Effect of seed treatment with *Rhizobium leguminosarum* bv. *viceae* on *Pythium* damping-off, seedling height, root nodulation, root biomass, shoot biomass and seed yield of pea and lentil. *J. Phytopathol*. **155:** 31–37.

Huang, X., Zhang, N., Yong, X., Yang, X. and Shen, Q. (2012) Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. *Microbiol. Res.* **167(3):** 135-143.

Husen, E. (2003) Screening of soil bacteria for plant growth promotion activities *in vitro*. *Indo. J. Agri. Sci.* **4(1):** 27-31.

Hussain, S. and Ghaffar, A. (1990) Biological control of *Macrophomina phaseolina* charcoal rot of sunflower and mung bean. *J. Phytopathol*. **130:** 157-160.

Hymowitz, T. (1990) Grain legumes. In: *Advances in new crops*. Proceedings of the first national symposium New crops: Research, development, economics', Indianapolis, Indiana, USA, 23-26 October 1988. Timber Press. pp. 154-158.

Hynes, R.K., Leung, G.C., Hirkala, D.L. and Nelson, L.M. (2008) Isolation, selection and characterization of beneficial rhizobacteria from pea, lentil and chickpea grown in western Canada. *Can. J. Microbiol.* **54(4):** 248-258.

Inam-ul-Haq, M., Javed, N., Ahmad, R. and Rehman, A. (2003) Evaluation of different strains of *Pseudomonas fluorescens* for the bioconrol of Fusarium wilt of chickpea. *Pak. J. Phytopathol.* **2(1):** 65-74.

Intana, W., Yenjit, P., Suwanno, T., Sattasakulchai, S., Suwanno, M. and Chamswarng, C. (2008) Efficacy of antifungal metabolites *Bacillus* spp. for controlling tomato damping off caused by *Pythium aphanidermatum*. *Walailak J. Sci. Technol.* **5(1):** 29-38.

Islam, F., Yasmeen, T., Arif, M.S., Ali, S., Ali, B., Hameed, S. and Zhou, W. (2016) Plant growth promoting bacteria confer salt tolerance in *Vigna radiata* by up-regulating antioxidant defense and biological soil fertility. *Plant Growth Regul.* **80(1):** 23-36.

Jacobson, C.B., Pasternak, J.J. and Glick, B.R. (1994) Partial purification and characterization of ACC deaminase from the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Can. J. Microbiol.* **46:** 898-907.

Jamali, F., Sharifi-Tehrani, A., Okhovvat, M., Zakeri, Z and Saberi-Riseh, R. (2004) Biological control of chickpea Fusarium wilt by antagonistic bacteria under greenhouse condition**.** *Commun. Agric. Appl. Biol. Sci.***69**: 649-51.

Jayantibhai, M.S. (2014) Plant growth promotion, translocation of iron and zinc, prevention of Fusarium wilt disease by native *Rhizobium* isolates in chickpea varieties. Ph. D. thesis, Anand Agricultural University.

Ji, S.H., Gururani, M.A. and Chun, S.C. (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiol. Res.* **169(1):** 83-98.

Jida, M. and Assefa, F. (2012) Phenotypic diversity and plant growth promoting characteristics of *Mesorhizobium* species isolated from chickpea (*Cicer arietinum* L.) growing areas of Ethiopia. *Afri. J. Biotechnol.* **11(29):** 7483-7493.

Jilani, G., Akram, A., Ali, R.M., Hafeez, F.Y., Shamsi, I. H., Chaudhry, A.N. and Chaudhry, A.G. (2007) Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. *Annals Microbiol.* **57(2):** 177-184.

Jimenez Diaz, R.M., Jimenez Gasco, M.M., Landa, B.B., Castillo, P. and Navas-Cortes, J.A.(2011a) Fusarium wilt of chickpea. In: Compendium of Chikpea and Lentil Diseases and Pests, Chen, W., Sharma, H.C. and Muehlbauer, F.J. (eds). The American Phytopathological Society: St. Paul, MN, USA.

Jimenez-Díaz, R.M., Alcala-Jimenez, A.R., Hervas, A. and Trapero-Casas, J.L. (1993) Pathogenic variability and hosts resistance in the *Fusarium oxysporum* f. sp. *ciceris*/*Cicer arietinum* pathosystem. In: Proc. 3rd European Seminar on Fusarium mycotoxins, taxonomy, pathogenicity and host resistance. Hodowsla Roslin Aklimatyazacjai Nasiennictwo. Plant Breeding and Acclimatization Institute, Radzikov, Poland, pp. 87-94.

Jiménez-Díaz, R.M., Castillo, P., del Mar Jiménez-Gasco, M., Landa, B.B. and Navas-Cortés, J.A. (2015) Fusarium wilt of chickpea: Biology, ecology and management. *Crop Prot.* **73:** 16–27.

Jiménez-Fernández, D., Montes-Borrego, M., Jiménez-Díaz, R.M., Navas-Cortés, J.A. and Landa, B.B. (2011b) *In planta* and soil quantification of *Fusarium oxysporum* f. sp. *ciceris* and evaluation of Fusarium wilt resistance in chickpea with a newly developed quantitative polymerase chain reaction assay. *Phytopathology* **101(2):** 250-262.

Jimenez-Gasco, M.M. and Jimenez-Diaz, R.M. (2003) Development of a specific polymerase chain reaction-based assay for the identification of *Fusarium oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 5 and 6. *Phytopathology* **93:** 200-209.

Jimenez-Gasco, M.M., Navas-Cortes, J.A. and Jimenez-Díaz, R.M. (2004) The *Fusarium oxysporum* f. sp. *ciceris*/*Cicer arietinum* pathosystem: A case study of the evolution of plant pathogenic fungi into races and pathotypes. *Inter. Microbiol.* **7:** 95-104.

Jimenez-Gasco, M.M., Perez-Artes, E. and Jimenez-Díaz, R.M. (2001) Identification of pathogenic races 0, 1B/C, 5 and 6 of *Fusarium oxysporum* f. sp. *ciceris* with random amplified polymorphic DNA (RAPD). *Eur. J. Plant Pathol.***107:** 237-248.

Jo, G.J. (2005) Production of antifungal substance for biological control of *Phytophthora capsici* causing phytophthora blight in red peppers by *Streptomyces halstedii*. *Biotech. Lett.* **27:** 201-205.

Johri, B., Sharma, A. and Virdi, J. (2003) Rhizobacterial diversity in India and its influence on soil and plant health. *Biotechnol. India.* **1:**49-89.

Jones, L.H.P. and Handrecht, K.A. (1967) Silica in soils, plants and animals. *Adv. Agron.* **19:** 107-149.

Joobeur, T., King, J.J., Nolin, S.J., Thomas, C.E. and Dean, R.A. (2004) The Fusarium wilt resistance locus Fom-2 of melon contains a single resistance gene with complex features. *Plant J.* **39:** 283–297.

Jorge, I., De La Rosa, O., Navas-Cortes, J.A., Jiménez-Díaz, R.M. and Tena, M. (2005) Extracellular xylanases from two pathogenic races of *Fusarium oxysporum* f. sp. *ciceris*: enzyme production in culture and purification and characterization of a major isoform as an alkaline endo-β-(1, 4)-xylanase of low molecular weight. *Anton. van Leeuwenh.* **88(1):** 48-59.

Joseph, B., Patra, R.R. and Lawrence, R. (2007) Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int. J. Plant Prod.* **1:** 141-152.

Josic, D., Ćirić, A., Soković, M., Stanojković-Sebić, A., Pivić, R., Lepšanović, Z. and Glamočlija, J. (2015) Antifungal activities of indigenous plant growth promoting *Pseudomonas* spp. from alfalfa and clover rhizosphere. *Front. Life Sci.* **8(2):** 131-138.

Jung, H.W., Kim, W. and Hwang, B.K. (2003) Three pathogen inducible genes encoding lipid transfer protein from pepper are differentially activated by pathogens, abiotic and environmental stress. *Plant Cell Environ.* **26:** 915–928.

Kai, M., Effmert, U., Berg, G. and Piechulla, B. (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. *Archi. Microbiol.* **187(5):** 351-360.

Kaiser, W.J., Alcala-Jimenez, A.R., Hervas-Vargas, A. Trapero-Casas, J.L. and Jimenez-Diaz, R.M. (1994) Screening of wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*. *Plant Dis.***78:** 962–967.

Kaki, A.A., Chaouche, N.K., Dehimat, L., Milet, A., Youcef-Ali, M., Ongena, M. and Thonart, P. (2013) Biocontrol and plant growth promotion characterization of *Bacillus* species isolated from *Calendula officinalis* rhizosphere. *Indian J. Microbiol.* **53(4):** 447-452.

Kamilova, F., Validov, S., Azarova, T., Mulders, I. and Lugtenberg, B. (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environ. Microbiol.* **7(11):** 1809-1817.

Kang, B.G., Kim, W.T., Yun, H.S. and Chang, S.C. (2010) Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. *Plant Biotech. Reports* **4(3):** 179-183.

Kang, Z., Zhang, J., Zhou, J., Qi, Q., Du, G. and Chen, J. (2012) Recent advances in microbial production of δ-aminolevulinic acid and vitamin B12. *Biotechnol. Advan.* **30:** 1533–1542.

Kannaiyan, J. and Nene, Y.L. (1975) Note on the effect of sowing dates on the reaction of twelve lentil varieties to wilt disease. *Madras Agri. J.* **62(4):** 240-242.

Karimi, K., Amini, J., Harighi, B. and Bahramnejad, B. (2012) Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against Fusarium wilt of chickpea. *Aust. J. Crop Sci.* **6(4):** 695-703.

Karlidag, H., Esitken, A., Turan, M. and Sahin, F. (2007) Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. *Sci. Hort.* **114(1):** 16–20.

Karuppiah, P. and Rajaram, S. (2011) Exploring the potential of chromium reducing *Bacillus* sp. and there plant growth promoting activities. *J. Microbiol. Res.* **1(1):** 17-23.

Kaur, G. (2014) Phenotypic and molecular characterization of chickpea (*Cicer arietinum* L.) mesorhizobia.Ph. D. thesis, Punjab Agricultural University, Ludhiana, Punjab.

Kaur, N.P. and Mukhopadhyay, A.N. (1992) Integrated control of chickpea wilt-complex by *Trichoderma* and chemical methods in India. *Trop. Pest Manag.* **38:** 20-23.

Kaur, R., Singh, R.S. and Alabouvette, C. (2007) Antagonistic activity of selected isolates of fluorescent *Pseudomonas* against *Fusarium oxysporum* f. sp. *ciceri*. *Asian J. Plant Sci.* **6(3):** 446-454.

Keel, C.D., Weller, M., Natsch, A., Defago, G., Cook, R.G. and Thomashow, L.S. (1996) Conservation of the 2, 4 diacetylphloroglucinol biosynthesis locus among *Pseudomonas fluorescens* strains from diverse geographic locations. *Appl. Environ. Microbiol.* **62:** 552-563.

Khalequzzaman, K.M. and Hossain, I. (2008) Efficacy of *Rhizobium* strains and biofertilizers for controlling foot and root rot and increasing green pod yield of bush bean. *Bangladesh J. Agri. Res.* **33(4):** 617-622.

Khan, M.S., Zaidi, A. and Aamil, M. (2002) Biocontrol of fungal pathogens by the use of plant growth promoting rhizobacteria and nitrogen fixing microorganisms. *Indian J. Bot. Soc.* **81:** 255–263.

Khan, M.S., Zaidi, A. and Rizvi, P.Q. (2006) Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity and seed production in chickpeas. *Commun. Soil Sci. Plant* **37:** 1783-1793.

Khan, M.S., Zaidi, A., Ahemad, M., Oves, M. and Wani, P.A. (2010) Plant growth promotion by phosphate solubilizing fungi: Current perspective. *Arch. Agron. Soil Sci.* **56:** 73-98.

Khandelwal, A. (2015) Evaluation of herbicidal potential of rhizospheric bacteria against *Chenopodium album* (Bathu) and *Asphodelous tenuifolius* (Piazi) weeds. Ph. D. thesis. Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Khandelwal, A. and Sindhu S.S. (2012) Expression of 1-aminocyclopropane-1-carboxylate deaminase in rhizobia promotes nodulation and plant growth of clusterbean (*Cyamopsis tetragonoloba* L.). *Res. J. Microbiol.* **7:** 158-170.

Khandelwal, A. and Sindhu S.S. (2013) ACC deaminase containing rhizobacteria enhance nodulation and plant growth in clusterbean (*Cyamopsis tetragonoloba* L.). *J. Microbiol. Res.* **3:** 117-123.

Khandelwal, A. and Sindhu, S.S. (2012) Expression of 1-aminocyclopropane-1-carboxylate deaminase in rhizobia promotes nodulation and plant growth of clusterbean (*Cyamopsis tetragonoloba* L.). *Res. J. Microbiol.* **7:** 158–170.

Khanzada, K.A., Rajput, M.A., Shah, G.S., Lodhi, A.M. and Mehboob, F. (2002) Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat. *Asian J. Plant Sci.* **1:** 441-444.

Khot, G.G., Tauro, P. and Dadarwal, K.R. (1996) Rhizobacteria from chickpea (*Cicer arietinum* L) rhizosphere effective in wilt control and promote nodulation. *Indian J. Microbiol.* **36:** 217-222.

Kilani, O., Culioli, G., Ortalo-Magné, A., Zouari, N., Blache, Y. and Jaoua, S. (2011) Environmental *Burkholderia cepacia* strain Cs5 acting by two analogous alkyl-quinolones and a didecyl-phthalate against a broad spectrum of phytopathogens fungi. *Curr. Microbiol.* **62(5):** 1490-1495.

Kim, B.Y., Lee, S.Y., Ahn, J.H., Song, J., Kim, W.G. and Weon, H.Y. (2015) Complete genome sequence of *Bacillus amyloliquefaciens* subsp. *plantarum* CC178, a phyllosphere bacterium antagonistic to plant pathogenic fungi. *Genome Announ.* **3(1):** e01368-14.

Kim, H.S., Park, J.Y., Choi, S.W., Choi, K.H., Lee, G.P., Ban, S.J., Lee, C.H. and Kim, C.S. (2003) Isolation and characterization of *Bacillus* strains for biological control. *J. Microbiol.* **41(3):** 196-201.

Kim, K.J., Sung, W.S., Suh, B.K., Moon, S.K., Choi, J.S., Kim, J.G. and Lee, D.G. (2009) Antifungal activity and mode of action of silver nanoparticles on *Candida albicans*. *Biometals* **22(2):** 235-242.

Kim, P. and Chung, K.C. (2004) Production of an antifungal protein for control of *Colletotrichum lagenarium* by *Bacillus amyloliquefaciens* MET0908. *FEMS Microbiol. Lett.* **234(1):** 177-183.

Kim, S.B. and Timmusk, S. (2013) A simplified method for gene knockout and direct screening of recombinant clones for application in *Paenibacillus polymyxa*. *PloS One* 8.

King, E.O., Ward, M.K. and Raney, D.E. (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.* **44:** 301-307.

Kishore, G.K., Pande, S. and Podile, A.R. (2005) Biological control of collar rot disease with broad-spectrum antifungal bacteria associated with groundnut. *Can. J. Microbiol.* **51(2):** 123-132.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286:** 883-884.

Kloepper, J.W., Ryu, C.M. and Zhang, S. (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* **94(11):** 1259-1266.

Koberl, M., Ramadan, E.M., Adam, M., Cardinale, M., Hallmann, J., Heuer, H., Smalla, K. and Berg, G. (2013) *Bacillus* and *Streptomyces* were selected as broad-spectrum antagonists against soilborne pathogens from arid areas in Egypt. *FEMS Microbiol. Lett.* **342:** 168-178.

Köberl, M., White, R.A., Erschen, S., El-Arabi, T.F., Jansson, J.K. and Berg, G. (2015) Draft genome sequence of *Streptomyces* sp. strain Wb2n-11, a desert isolate with broad-spectrum antagonism against soilborne phytopathogens. *Genome Announ.* **3(4):** 860-815.

Koh, R.H. and Song, H.G. (2007) Effects of application of *Rhodopseudomonas* sp. on seed germination and growth of tomato under axenic conditions. *J. Microbiol. Biotechnol.* **17(11):** 1805-1810.

Kucuk, C. (2013) *In vitro* antagonism of *Rhizobium* strains isolated from various legumes. *J. Appl. Biol. Sci.* **7:** 24-30.

Kumar Rao, J.V.D, Sen, A. and Shinde, S.T. (1974) Inhibition of groundnut *Rhizobium* in India soils. *Proc. Indian Natl. Sci. Acad. Part B.* **40**: 535-539.

Kumar, A., Prakash, A. and Johri, B.N. (2011) *Bacillus* as PGPR in crop ecosystem. In: Bacteriain Agrobiology*:* Crop Ecosystems. Springer Berlin Heidelberg. pp. 37-59.

Kumar, H., Bajpai, V.K., Dubey, R.C., Maheshwari, D.K. and Kang, S.C. (2010) Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L) var. Manak by bacterial combinations amended with chemical fertilizer. *Crop Protect.* **29(6):** 591-598.

Kumar, M., Mishra, S., Dixit, V., Kumar, M., Agarwal, L., Chauhan, P.S. and Nautiyal, C.S. (2016) Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). *Plant Signaling Behav.* **11(1):** e1071004.

Kumar, P., Dubey, R.C. and Maheshwari, D.K. (2012) *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiol. Res.* **167(8):** 493-499.

Kumari, P. and Khanna, V. (2016) Allelopathic effects of native *Bacillus* sp. against *Fusarium oxysporum* causing chickpea wilt. *Allelopathy J.* **38(1):** 77-90.

Kumari, P., Khanna, V., Kaur, L. and Mukhija, B. (2013) Characterization of functionality traits of plant growth promoting rhizobacteria antagonistic to *Fusarium oxysporum* f. sp. *ciceris*. *Plant Dis. Res.* **28(1):** 11-15.

Kumari, S. and Khanna, V. (2014) Effect of antagonistic rhizobacteria coinoculated with *Mesorhizobium ciceris* on control of Fusarium wilt in chickpea (*Cicer arietinum* L.). *Afri. J. Microbiol. Res.* **8(12):** 1255-1265.

Kumari, S., Varma, A., Tuteja, N. and Choudhary, D.K. (2016) Bacterial ACC-deaminase: An eco-friendly strategy to cope abiotic stresses for sustainable agriculture. In: Plant-microbe interaction: An approach to sustainable agriculture. Springer Singapore. pp. 165-185.

Kunal and Sharma, P. (2012) Compatibility of *Mesorhizobium* sp. *Cicer* with seed treatment of fungicide and insecticide in chickpea. *Archi. Agro. Soil Sci.* **58(1):** 115-123.

Kundu, B.S., Nehra, K., Yadav, R. and Tomar, M. (2009) Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian J. Microbiol.* **49(2):** 120-127.

Lakshmi, V., Kumari, S., Singh, A. and Prabha, C. (2015) Isolation and characterization of deleterious *Pseudomonas aeruginosa* KC1 from rhizospheric soils and its interaction with weed seedlings. *J. King Saud Univ. Sci.* **27(2):** 113-119.

Lange, R. and McLaren, D. (2002) Fusarium wilt-A new disease of canola.http://www.umanitoba.ca/afs/agronomists conf/2002/pdf/lange.pdf.

Laranjo, M., Alexandre, A., Rivas, R., Velázquez, E., Young, J.P.W. and Oliveira, S. (2008) Chickpea rhizobia symbiosis genes are highly conserved across multiple *Mesorhizobium* species. *FEMS Microbiol. Ecol.* **66(2):** 391-400.

Lee, J.P., Lee, S.W., Kim, C.S., Son, J.H., Song, J.H., Lee, K.Y. and Moon, B.J. (2006) Evaluation of formulations of *Bacillus licheniformis* for the biological control of tomato gray mold caused by *Botrytis cinerea*. *Biol. Control* **37(3)**: 329-337.

Lee, K.D., Bai, Y., Smith, D. and Han, H.S. (2005) Isolation of plant growth promoting endophytic bacteria from bean nodules. *Res. J. Agri. Biol. Sci.* **1(3):** 232-236.

Lee, K.H., Koh, R.H. and Song, H.G. (2008) Enhancement of growth and yield of tomato by *Rhodopseudomonas* sp. under greenhouse conditions. *J. Microbiol.* **46(6):** 641-646.

Leelasuphakul, W., Hemmanee, P. and Chuenchitt, S. (2008) Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit. *Postharvest Biol. Technol.* **48(1):**113-121.

Leij, D. (1998) Plant mediated interactions between *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizae on pea. *Lett. Appl. Microbiol.* **26(4):** 311-316.

Lemessa, F. and Zeller, W. (2007) Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Biol. Control* **42(3):** 336-344.

Leslie, J.F. and Summerell, B.A. (2006) *Fusarium* laboratory workshops - A recent history. *Mycotoxin Res.* **22(2):** 73-74.

Li, Y., Dong, F., Liu, X., Xu, J., Li, J., Kong, Z., Chen, X. and Zheng, Y. (2012) Environmental behavior of the chiral triazole fungicide fenbuconazole and its chiral metabolites: Enantioselective transformation and degradation in soils. *Environ. Sci. Technol.* **46(5):** 2675-2683.

Liu, D., Lian, B. and Dong, H. (2012) Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. *Geomicrobiol. J.* **29:** 413-421.

Liu, F., Xing, S., Ma, H., Du, Z. and Ma, B. (2013) Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in *Platycladusorientalis* container seedlings. *Appl. Microbiol. Biotechnol.* **97(20):** 9155-9164.

Liu, S., Zhang, G., Li, X. and Zhang, J. (2014) Microbial production and applications of 5-aminolevulinic acid. *Appl. Microbiol. Biotechnol.* **98(17):** 7349-7357.

Liu, X.Y., Xu, X.Y., Ma, Q.L. and Wu, W.H. (2005) Biological formation of 5-aminolevulinic acid by photosynthetic bacteria.*J. Environ. Sci.* **17:** 152–155.

Loper, J.E. and Gross, H. (2007) Genomic analysis of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5. *Euro. J. Plant Pathol.* **119(3):** 265-278.

Lugtenberg, B. and Kamilova, F. (2009) Plant-growth promoting rhizobacteria. *Annu. Rev. Microbiol.* **63:** 541–556.

Ma, W., Guinel, F.C. and Glick, B.R. (2003) *Rhizobium leguminosarum* biovar *viciae* 1-aminocyclopropane-1-carboxylate deaminase promotes nodulation of pea plants. *Appl. Environ. Microbiol.* **69:** 4396–4402.

Mahdi, A.A. and Mustafa, E.M. (2005) Response of guar to *Bradyrhizobium* inoculation and to nitrogen and phosphate fertilization. *University Khartoum J. Agri. Sci.* (Sudan).

Maheshwari, D.K. (2010) Plant growth and health promoting bacteria (Vol. 18).Springer Science & Business Media.

Malik, D.K. and Sindhu, S.S. (2011) Production of indole acetic acid by *Pseudomonas* sp.: Effect of coinoculation with *Mesorhizobium* sp. *Cicer* on nodulation and plant growth of chickpea (*Cicer arietinum*). *Physiol. Mol. Biol. Plants* **17(1):** 25-32.

Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T. and Samiyappan, R. (2010) Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. *Biol. Control* **54(2):** 83-89.

Mantelin, S. and Touraine, B. (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *J. Expt. Botany* **55:** 27–34.

Marsudi, N.D.S., Glenn, A.R. and Dilworth, M.J. (1999) Identification and characterization of fast and slow growing root nodule bacteria from South Western Australian soils able to nodulate *Acacia saligna*. *Soil Biol. Biochem*. **31:** 1229–1238.

Marulanda, A., Azcón, R., Chaumont, F., Ruiz-Lozano, J.M. and Aroca, R. (2010) Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays* L.) plants under unstressed and salt-stressed conditions. *Planta* **232:** 533–543.

Matthijs, S., Tehrani, K.A., Laus, G., Jackson, R.W., Cooper, R.M. and Cornelis, P. (2007) Thioquinolobactin, a *Pseudomonas* siderophore with antifungal and anti-Pythium activity. *Environ. Microbial.* **9(2):** 425-434.

Maurhofer, M., Keel, C., Haas, D. and Defago, G. (1995) Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. *Plant Pathol.* **44:** 40–50.

Mauzerall, D. and Granick, S. (1955) The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. *J. Biol. Chem.* **219:** 435–446.

Mayak, S., Tirosh, T. and Glick, B. (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sci.* **166:** 525–530.

Mayer, J.M. and Abdallah, M.A. (1978) The fluorescent pigment of *Pseudomonas fluorescens* biosynthesis, purification and physicochemical properties. *J. Gen. Microbiol.***107**: 319–332.

Mazzola, M. (2002) Mechanisms of natural soil suppressiveness to soilborne diseases. *Anton. van Leeuwenh.* **81(1):** 557-564.

McKerral, A. (1923) A note on Fusarium wilt of gram in Burma and measures taken to combat it. *Agri. J. India* **18:** 608-613.

McRae, W. (1932) Export of the Imperial Mycologist. *Sclent. Repts. Irnper. Inst. Agri. Res.* pp.73-86.

McTaggart, A. (2007) Fusarium wilt of chickpea *Fusarium oxysporum* f. sp. *ciceris*. Updated on 2/9/2009 1:35:23 PM Available online: PaDIL- http://www.padil.gov.au

Medeiros, F.H., Souza, R.M., Medeiros, F.C., Zhang, H., Wheeler, T., Payton, P., Ferro, H.M. and Paré, P.W. (2011) Transcriptional profiling in cotton associated with *Bacillus subtilis* (UFLA285) induced biotic-stress tolerance. *Plant Soil* **347(1-2):** 327-337.

Meena, M.D. and Biswas, D.R. (2014) Changes in biological properties in soil amended with rock phosphate and waste mica enriched compost using biological amendments and chemical fertilizers under wheat-soybean rotation. *J. Plant Nutri.* **37(12):** 2050-2073.

Meena, V.S., Maurya, B.R., Meena, S.K., Meena, R.K., Kumar, A., Verma, J.P. and Singh, N.P. (2016) Can *Bacillus* species enhance nutrient availability in agricultural Soils? In: *Bacilli Agrobiotechnol.* Springer International Publishing. pp. 367-395.

Memenza, M., Mostacero, E., Camarena, F. and Zúñiga, D. (2016) Disease control and plant growth promotion (PGP) of selected bacterial strains in *Phaseolus vulgaris*. In: *Biological Nitrogen Fix. Beneficial Plant-Microbe Interact*. Springer International Publishing. pp. 237-245.

Meyer, S.L. and Roberts, D.P. (2002) Combinations of biocontrol agents for management of plant-parasitic nematodes and soilborne plant-pathogenic fungi. *J. Nematol.* **34(1):**1-8.

Mhadhbi, H., Jebara, M., Limam, F. and Aouani, M.E. (2004) Rhizobial strain involvement in plant growth, nodule protein composition and antioxidant enzyme activities of chickpea-rhizobia symbioses: modulation by salt stress. *Plant Physiol. Biochem.* **42(9):** 717-722.

Milagres, A.M., Machuca, A. and Napoleao, D. (1999) Detection of siderophore production from several fungi and bacteria by modification of chrome azurol S (CAS) agar plate assay. *J. Microbiol. Methods* **37(1):** 1-6.

Miller, H.J., Henken, G. and van Veen, J.A. (1989) Variation and composition of bacterial population in the rhizospheres of maize, wheat and grass cultivars. *Can. J. Microbiol.* **35:** 656-660.

Miransari, M. and Smith, D.L. (2014) Plant hormones and seed germination. *Environ. Expt. Botany* **99:** 110-121.

Mishra, B., Haack, E.A., Maurice, P.A. and Bunker, B.A. (2009) Effects of the microbial siderophore DFO-B on Pb and Cd speciation in aqueous solution. *Environ. Sci. Technol.* **43:** 94-100.

Mishra, M., Kumar, U., Mishra, P.K. and Prakash, V. (2010) Efficiency of plant growth promoting rhizobacteria for the enhancement of *Cicer arietinum* L. growth and germination under salinity. *Adv. Biol. Res.* **4(2):** 92-96.

Mohan, J. (2010) Assessment of the potential of plant growth promoting rhizobacteria *Bacillus* sp. in enhancing symbiotic efficiency in chickpea with *Mesorhizobium*. *Biochem. Cell. Archi.* **10(1):** 57-62.

Mohite, B. (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J. Soil Sci. Plant Nutr.* **13(3):** 638-649.

Moores, J.C., Magazin, M., Ditta, G.S. and Leong, J. (1984) Cloning of genes involved in the biosynthesis of pseudobactin, a high-affinity iron transport agent of a plant growth-promoting *Pseudomonas* strain. *J. Bacteriol.* **157:** 53-58.

Mora, I., Cabrefiga, J. and Montesinos, E. (2015) Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. *PloS One* **10(5):** e0127738.

Moradi, H., Bahramnejad, B., Amini, J., Siosemardeh, A. and Allahverdipoor, K.H. (2012) Suppression of chickpea (*Cicer arietinum* L.) *Fusarium* wilt by *Bacillus subtillis* and *Trichoderma harzianum*. *Plant Omics J.* **5(2):** 68-74.

Motamedi, H., Zahedi, E. and Zarei-Mahmoud Abadi, A. (2017) Optimizing conditions for the production of antifungal agents using the native *Bacillus cereus* SB15. *Feyz J. Kashan University Medical Sci.* 21-31.

Mouloud, G., Samir, M., Hani, B. and Daoud, H. (2015) Biocontrol of wheat Fusarium head blight (FHB) by *Streptomyces* spp. isolated from the rhizosphere of *Astragalus combo* Coss. & Dur. and *Ononis angustissima* Lam. *American-Eurasian J. Agric. Environ. Sci.* **15(12):** 2499-2511.

Munimbazi, C. and Bullerman, L.B. (1998) Isolation and partial characterization of antifungal metabolites of *Bacillus pumilus*. *J. Appl. Microbiol.* **84(6):** 959-968.

Munoz, N., Soria-Diaz, M.E., Manyani, M., Sanchez-Matamoros, R.C., Serrano, A.G., Megias, M. and Lascano, R. (2014) Structure and biological activities of lipochitooligosaccharide nodulation signals produced by *Bradyrhizobium japonicum* USDA138 under saline and osmotic stress. *Biol. Fertil. Soils* **50:** 207–215.

Nadeem, S.M., Zahir, Z.A., Naveed, M. and Ashraf, M. (2010) Microbial ACC-deaminase; prospects and applications for inducing salt tolerance in plants. *Crit. Rev. Plant Sci.* **29:** 360-393.

Nagórska, K., Bikowski, M. and Obuchowski, M. (2007) Multicellular behaviour and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. *Acta Biochimica Polonica* **54(3):** 495-508.

Nain, L., Yadav, R. and Saxena, J. (2012) Characterization of multifaceted *Bacillus* sp. RM-2 for its use as plant growth promoting bioinoculant for crops grown in semiarid deserts. *Appl. Soil Ecol.* **59:** 124–135.

Nautiyal, C.S. (1997) Selection of chickpea-rhizosphere-competent *Pseudomonas fluorescens* NBRI1303 antagonistic to *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Pythium* sp. *Curr. Microbiol.* **35(1):** 52-58.

Nautiyal, C.S., Srivastava, S., Chauhan, P.S., Seema, K., Mishra, A. and Sopory, S.K. (2013) Plant growth promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. *Plant Physiol. Biochem*. **66:** 1–9.

Navas-Cortes, J.A., Hau, B. and Jimenez-Diaz, R. M. (2000) Yield loss in chickpea in relation to development to Fusarium wilt epidemics. *Phytopathology* **90:** 1269-1278.

Neilands, J.B. and Leong, S.A. (1986) Siderophores in relation to plant growth and disease. *Ann. Rev. Plant Physiol.* **37(1):** 187-208.

Nelson, L.M. (2004) Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. *Crop Manag.* OkanaganUniversity,Kelowna.

Nene, Y.L. and Reddy, M.V. (1987) Chickpea diseases and their control. In: The Chickpea. Saxena, M.C. and Singh, K.B. (eds.), CAB Int., Oxon, UK, pp. 233-270.

Nene, Y.L., Kannaiyan, J., Haware, M.P. and Reddy, M.V. (1979) Review of the work done at ICRISAT on soil-borne diseases of pigeonpea and chickpea. ICRISAT pp. 1-78.

Nene, Y.L., Reddy, M.V., Haware, M.P., Ghanekar, A.M. and Amin, K.S. (1991) Field diagnosis of chickpea diseases and their control. In: Information Bulletin no. 28. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Nene, Y.L., Sheila, V.K. and Sharma, S.B.A, (1996) A world list of chickpea and pigeonpea pathogens, ICRISAT, Patencheru, 1996, 5th Edition, p. 27.

Nieto-Jacobo, M.F., Steyaert, J.M., Salazar-Badillo, F.B., Nguyen, D.V., Rostás, M., Braithwaite, M., De Souza, J.T., Jimenez-Bremont, J.F., Ohkura, M., Stewart, A. and Mendoza-Mendoza, A. (2017) Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds and plant growth promotion. *Front. Plant Sci.* **8:** 1-18.

Nihorimbere, V. and Ongena, M. (2017) Isolation of plant growth-promoting *Bacillus* strains with biocontrol activity *in vitro*. *Merit Res. J. Microbiol. Biol. Sci.* **5(2):** 13-21.

Nikam, P.S., Jagtap, G.P. and Sontakke, P.L. (2011) Survey, surveillance and cultural characteristics of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri*. *Afri. J. Agri. Res.* **6(7):** 1913-1917.

Noel, T.C., Sheng, C., Yost, C.K., Pharis, R.P. and Hynes, M.F. (1996) *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: Direct growth promotion of canola and lettuce. *Can. J. Microbiol.* **42:** 279–283.

Norkina, S.P. and Pumpyansakya, L.V. (1956) Certain properties of silicate bacteria. *Crop Sci. Soc. Japan.* **28:** 35-40.

Nunkaew, T., Kantachote, D., Kanzaki, H., Nitoda, T. and Ritchie, R.J. (2014) Effects of 5 aminolevulinic acid (ALA)-containing supernatants from selected *Rhodopseudomonas palustris* strains on rice growth under NaCl stress, with mediating effects on chlorophyll, photosynthetic electron transport and antioxidative enzymes. *Electr. J. Biotechnol.* **17:** 19–26.

Obagwu, J. and Korsten, L. (2003) Integrated control of citrus green and blue molds using *Bacillu*s *subtilis* in combination with sodium bicarbonate or hot water. *Postharvest Biol. Technol.* **28(1):** 187-194.

Okigbo, R.N. (2004) A review of biological control methods for post harvest yams *Dioscorea* spp. in storage in South Eastern Nigeria. *KMITL Sci. J.* **4(1):** 207-215.

Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P. and Sa, N.M.H. (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biol. Biochem.* **41(9):** 1782-1787.

On, A., Wong, F., Ko, Q., Tweddell, R.T., Antoun, H. and Avis, T.J. (2015) Antifungal effect of compost tea microorganism on tomato pathogens. *Biol. Control***80:** 63-69.

Ongena, M. and Jacques, P. (2008) *Bacillus* lipopeptides: Versatile weapons for plant disease biocontrol. *Trends Microbiol.* **16(3):** 115-125.

Onofre-Lemus, J., Hernández-Lucas, I., Girard, L. and Caballero-Mellado, J. (2009) ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species and its growth-promoting effect on tomato plants. *Appl. Environ. Microbiol.* **75(20):** 6581-6590.

Otieno, P.E., Muthomi, J.W., Chemininngwa, G.N. and Nderitu, J.H. (2007) Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. In *8th African Crop Science Society Conference, El-Minia, Egypt, 27-31 October 2007* African Crop Science Society. pp. 305-312.

Oumouloud, A., Arnedo-Andres, M.S., Gonzalez-Torres, R. and Alvarez, J.M. (2008) Development of molecular markers linked to the Fom-1 locus for resistance to Fusarium race 2 in melon. *Euphytica* **164:** 347–356.

Pal, K. K. and Gardener, B.M. (2006) Biological control of plant pathogens. The Plant Health Instructor **2:** 1117-1142.

Pande, S., Rao, J.N. and Sharma, M. (2007) Establishment of the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceris* in the soil through seed transmission. *Plant Pathol. J.* **23(1):** 3-6.

Pandey, P. and Maheshwari, D.K. (2007) Two-species microbial consortium for growth promotion of *Cajanus cajan*. *Curr. Sci.* **92(8):** 1137-1142.

Pandey, V.C., Singh, J.S., Kumar, A. and Tewari, D.D. (2010) Accumulation of heavy metals by chickpea grown in fly ash treated soil: Effect on antioxidants. *CLEAN–Soil, Air, Water* **38(12)**: 1116-1123.

Pandya, M., Rajput, M. and Rajkumar, S. (2015) Exploring plant growth promoting potential of non rhizobial root nodules endophytes of *Vigna radiata*. *Microbiology* **84(1):** 110-119.

Pane, C., Villecco, D., Campanile, F. and Zaccardelli, M. (2012) Novel strains of *Bacillus*, isolated from compost and compost-amended soils, as biological control agents against soil-borne phytopathogenic fungi. *Biocontrol Sci. Technol.* **22(12):** 1373-1388.

Parani, K. and Saha, B.K. (2009) Studies on interaction of *Serratia marcescens* strain (SR1) with fungal pathogens. *American-Eurasian J. Agri. Environ. Sci.* **5(2):** 215-218.

Parmar, P. and Sindhu, S.S. (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. *J. Microbiol. Res. 3*(1): 25-31.

Parmar, N. and Dadarwal, K.R. (2001) Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacteria. *J. Appl. Microbiol.* **86(1):** 36-44.

Pastor, N., Rosas, S., Luna, V. and Rovera, M. (2014) Inoculation with *Pseudomonas putida* PCI2, a phosphate solubilizing rhizobacterium, stimulates the growth of tomato plants. *Symbiosis* **62:**157–167.

Patel, D., Jha, C.K., Tank, N. and Saraf, M. (2012) Growth enhancement of chickpea in saline soils using plant growth-promoting rhizobacteria. *J. Plant Growth Regul.* **31(1):** 53-62.

Pathma, J., Kennedy, R.K. and Sakthivel, N. (2011) Mechanisms of fluorescent pseudomonads that mediate biological control of phytopathogens and plant growth promotion of crop plants. In *Bacteria in Agrobiology: Plant growth responses*. Springer Berlin Heidelberg. pp. 77-105.

Patil, S., Shivannavar, C.T., Bheemaraddi, M.C. and Gaddad, S.M. (2015) Antiphytopathogenic and plant growth promoting attributes of *Bacillus* strains isolated from rhizospheric soil of chickpea. *J. Agri. Sci. Technol.* **17(5):** 1365-1377.

Patra, S., Biswas, M.K. and Mahato, A. (2017) Sustainable management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *Ciceri*. *Int. J. Pure Appl. Biosci*. **5(1):** 526-529.

Patten, C.L. and Glick, B.R. (1996) Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol.* **42:** 207–220.

Paulucci, N.S.,. Gallarato, L.A., Reguera, Y.B., Vicario, J.C. Cesari, A.B., Garciade-Lema, M.B. and Dardanelli, M.S. (2015) *Arachis hypogaea* PGPR isolated from Argentina soil modifies its lipids components in response to temperature and salinity. *Microbiol. Res.* **173:** 1-9.

Penrose, D.M. and Glick, B.R. (2003) Methods for isolating and characterizing ACC deaminase containing plant growth-promoting rhizobacteria. *Physiol. Plant* **118:** 10–15.

Petersen, D.J., Srinivasan, M. and Chanway, C.P. (1996) *Bacillus polymyxa* stimulates increased *Rhizobium etli* populations and nodulation when coresident in the rhizosphere of *Phaseolus vulgaris*. *FEMS Microbiol. Lett.* **142:** 271–276.

Phour, M. (2016) Aminolevulinic acid production by rhizobacteria: its role in salt tolerance and weed control in mustard [*Brassica juncea* (L.)]. Ph. D. thesis. Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S. and Crecchio, C. (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process: A review. *Biol. Fertil. Soils* **51(4):** 403-415.

Pikovskaya, R.I. (1948) Mobilization of phosphates in soil in connection with the vital activities of some microbial species. *Microbiology* **17:** 362–370.

Pishchik, V.N., Vorobyev, N.J., Chernyaeva, L.I., Timofeeva, S.V., Kazhemyakov, A.P. and Alexeev, Y.V. (2002) Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interaction under cadmium stress. *Plant Soil* **243:**173–186.

Podile, A.R. and Prakash, A.P. (1996) Lysis and biological control of *Aspergillus niger* by *Bacillus subtilis* AF1. *Can. J. Microbiol.* **42(6):** 533-538.

Prapagdee, B., Kuekulvong, C. and Mongkolsuk, S. (2008) Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. *Int. J. Biol. Sci.* **4(5):** 330-337.

Prashar, P., Kapoor, N. and Sachdeva, S. (2013) Isolation and characterization of *Bacillus* sp with *in-vitro* antagonistic activity against *Fusarium oxysporum* from rhizosphere of tomato. *J. Agri. Sci. Technol.* **15:** 1501-1512.

Pretorius, D., Van Rooyen, J. and Clarke, K.G. (2015) Enhanced production of antifungal lipopeptides by *Bacillus amyloliquefaciens* for biocontrol of postharvest disease. *New Biotechnol.* **32(2):** 243-252.

Qureshi, M.A., Ahmad, Z.A., Akhtar, N., Iqbal, A., Mujeeb, F. and Shakir, M.A. (2012) Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. *J. Anim. Plant Sci*. **22(1):** 204-210.

Qureshi, M.A., Shakir, M.A., Naveed, M. and Ahmad, M.J. (2009) Growth and yield response of chickpea to coinoculation with *Mesorhizobium ciceri* and *Bacillus megaterium*. *J. Anim. Plant Sci*. **19(4):** 205-211.

Raj, S.A., Kannaiyan, S., Kumar, K. and Govindarajan, K. (2004) Solubilization of silicate and concurrent release of phosphorus and potassium in rice ecosystem. *Biofertil. Technol. Rice Based Cropping Sys*. **1:** 372-378.

Rajkumar, M., Ae, N. and Freitas, H. (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. *Chemosphere* **77:** 153–160.

Rajkumar, M., Ae, N., Prasad, M.N.V. and Freitas, H. (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.* **28(3):** 142-149.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. and Samiyappan, R. (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protect.* **20(1):** 1-11.

Rayavarapu, V.B. and Padmavathi, T. (2016) *Bacillus* sp as potential plant growth promoting rhizobacteria. *Int. J. Adv. Lif. Sci.* **9(1):** 29-36.

Reetha, A.K., Pavani, S.L. and Mohan, S. (2014a) Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on *Macrophomina phaseolina* (Tassi.) Goid. *Int. J. Curr. Microbiol. Appl. Sci.* **3(5):** 172-178.

Reetha, S., Bhuvaneswari, G., Thamizhiniyan, P. and Mycin, T.R. (2014b) Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allim cepa*. L). *Int. J. Curr. Microbiol. Appl. Sci.* **3(2):** 568-574.

Remans, R., Bebee, S., Manrique, M.B., Tovar, E., Rao, I., Croonenborghs, A., Torres-Gutierrez, R., El-Howeity, M., Michiels, J. and Vanderleyden, J. (2008a) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth promoting bacteria in common bean (*Phaseolus vulgaris* L.). *Plant Soil* **302:** 149–161.

Remans, R., Ramaekers, L., Schelkens, S., Hernandez, G., Garcia, A., Reyes, J.L., Mendez, N., Toscano, V., Mulling, M., Galvez, L. and Vanderleyden, J. (2008b) Effect of *Rhizobium–Azospirillum* coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in Cuba. *Plant Soil* **312(1-2):** 25-37.

Ribeiro, C.M. and Cardoso, E.J.B.N. (2012) Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (*Araucaria angustifolia*). *Microbiol. Res.* **167(2):** 69-78.

Robison, M.M., Shah, S., Tamot, B., Pauls, K.P., Moffatt, B.A. and Glick, B.R. (2001) Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase. *Mol. Plant Pathol.* **2*:*** 135–145.

Romdhane, S.B., Tajini, F., Trabelsi, M., Aouani, M E. and Mhamdi, R. (2007) Competition for nodule formation between introduced strains of *Mesorhizobium ciceri* and the native populations of rhizobia nodulating chickpea (*Cicer arietinum*) in Tunisia. *World J. Microbiol. Biotech.* **23(9):** 1195-1201.

Rondon, M.A., Lehmann, J., Ramírez, J. and Hurtado, M. (2007) Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with biochar additions. *Biol. Fertil. Soils* **43(6):** 699-708.

Ryall, B., Mitchell, H., Mossialos, D. and Williams, H.D. (2009) Cyanogenesis by the entomopathogenic bacterium *Pseudomonas entomophila*. *Lett. Appl. Microbiol.* **49(1):** 131-135.

Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Kloepper, J.W. and Paré, P.W. (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol.* **134(3):** 1017-1026.

Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Wei, H.X., Paré, P.W. and Kloepper, J.W. (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci.***100:** 4927–4932.

Sadasivam, S. and Manickam, A. (1992) Biochemical methods for agricultural sciences. Wiley Eastern Ltd, New Delhi, p. 246.

Saharan, B.S. and Nehra, V. (2011) Plant growth promoting rhizobacteria: A critical review. *Life Sci. Med. Res.* **21(1):** 1-30.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sánchez, A.C., Gutiérrez, R.T., Santana, R.C., Urrutia, A.R., Fauvart, M., Michiels, J. and Vanderleyden, J. (2014) Effects of coinoculation of native *Rhizobium* and *Pseudomonas* strains on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. *Euro. J. Soil Biol.* **62:** 105-112.

Sansinenea, E., Almaraz, M., Ramírez, M.D. and Ortiz, A. (2016) Cellular damage of plant pathogenic fungi by antifungal compounds produced by *Bacillus* spp. isolates. *Chem. Ecol.* **32(8):** 722-732.

Sapsirisopa, S., Chookietwattana, K., Maneewan, K. and Khaengkhan, P. (2009) Effect of salt-tolerant *Bacillus* inoculum on rice KDML105 cultivated in saline soil. *Asian J. Food Agro-Ind.* S69-S74.

Saraf, M. and Dhandhukia, P. (2005) Response of *Sinorhizobium meliloti* to high salt concentration and effect of added osmotica. *J. Microbiol. World* **7:** 250-257.

Saraf, M., Pandya, U. and Thakkar, A. (2014) Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. *Microbiol. Res.* **169(1):** 18-29.

Saraf, M., Thakker, A. and Patel, B.V. (2008) Biocontrol activity of different species of *Pseudomonas* against phytopathogenic fungi *in vitro* and *in vivo* condition. *Int. J. Biotechnol. Biochem.* **4:** 223-232.

Sarti, G.C. and Miyazaki, S.S. (2013) *Bacillus subtilis* crude extracts with antifungal activity against soybean (*Glycinemax*) phytopathogens and *Bradyrhizobium japonicum* coinoculation effect. *Agrociencia (Montecillo)* **47(4):** 373-383.

Sasaki, K., Watanabe, M. and Tanaka, T. (2002) Biosynthesis, biotechnological production and applications of 5-aminolevulinic acid. *Appl. Microbiol. Biotechnol.* **58:** 23–29.

Saxena, A., Singh, D.V. and Joshi, N.L. (1996) Autotoxic effects of pearl millet aqueous extracts on seed germination and seedling growth. *J. Arid Environ.* **33(2):** 255-260.

Saxena, K.B., Kumar, R.V., Saxena, R.K., Sharma, M., Srivastava, R.K., Sultana, R., Varshney, R.K., Vales, M.I. and Pande, S. (2012) Identification of dominant and recessive genes for resistance to Fusarium wilt in pigeonpea and their implication in breeding hybrids. *Euphytica* **188:** 221–227.

Schwyn, B. and Neilands, J.B. (1987) Universal chemical assay for the detection and determination of siderophores. *Anal.Biochem.* **160(1):** 47-56.

Selvakumar, G., Joshi, P., Nazim, S., Mishra, P., Bisht, J. and Gupta, H. (2009a) Phosphate solubilization and growth promotion by *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium isolated from a high altitude Himalayan rhizosphere. *Biologia* **64(2):** 239-245.

Selvakumar, G., Lenin, M., Thamizhiniyan, P. and Ravimycin, T. (2009b) Response of biofertilizers, on the growth and yield of blackgram (*Vigna mungo* L.). *Recent Res. Sci. Technol.* **1(4):** 169-175.

Sen, A., Dadarwal, K.R. and Subba Rao, N.S. (1972) Soak seed in water to avoid seed coat toxins and produce more pulses. *Indian Farming* **22**: 25.

Senthilkumar, M., Paulraj, S., Alagupalamuthirsolai, M., Singh, M. and Singh, J. (2016) Synergistic effect of *Mesorhizobium ciceri* and 1-amino cyclopropane 1-carboxylate (ACC) deaminase producing rhizobacteria on chickpea growth and yield. *Food Leg.* **29(1):** 37-42.

Senthilkumar, M., Swarnalakshmi, K., Govindasamy, V., Lee, Y.K. and Annapurna, K. (2009) Biocontrol potential of soybean bacterial endophytes against charcoal rot fungus, *Rhizoctonia bataticola*. *Curr.Microbial.* **58(4):** 288-293.

Shaharoona, B., Arshad, M. and Zahir, Z.A. (2006) Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett. Appl. Microbiol.* **42:**155-159.

Shahzad, F., Shafee, M., Abbas, F., Babar, S., Tariq, M. M. and Ahmad, Z. (2012) Isolation and biochemical characterization of *Rhizobium meliloti* from root nodules of Alfalfa (*Medicago sativa*). *J. Ani. Plant Sci*. **22(2):** 522-524.

Shahzad, S.M., Khalid, A., Arsha, M. and Rehman, K. (2010) Screening rhizobacteria containing ACC deaminase for growth promotion of chickpea seedlings under axenic conditions**.** *Soil Environ.* **29:** 38-46.

Sharma KD, Chen W. and Muehlbauer, F.J. (2005) Genetics of chickpea resistance to five races of Fusarium wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceris*. *Plant Dis.* **89:** 385–390.

Sharma, A. and Johri, B.N. (2003) Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiol. Res.* **158(3):** 243-248.

Sharma, A. and Satyanarayana, T. (2013) Comparative genomics of *Bacillus* species and its 34 relevance in industrial microbiology.*Genomics Insights* **6:** 25-36.

Sharma, P. and Gill, R.K. (2010) Grain legumes (pulses) role in revitalizing soil health and human nutrition. *Ecol. Environ. Conserv*. **16:** 359-364.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. and Gobi, T.A. (2013) Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus* **2(1):** 587-601.

Sheng, X.F. and He, L.Y. (2006) Solubilization of potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Can. J.Microbiol.***52:** 66-72.

Shilev, S. (2013) Soil rhizobacteria regulating the uptake of nutrients and undesirable elements by plants. In *Plant Microbe Symbiosis: Fundamentals and Advances.* Springer India. pp. 147-167.

Shobha, G. and Kumudini, B.S. (2012) Antagonistic effect of the newly isolated PGPR *Bacillus* spp. on *Fusarium oxysporum*. *Inter. J. Appl. Sci. Engin. Res.* **1(3):** 463-474.

Shukla, P.S., Agarwal, P.K. and Jha, B. (2012) Improved salinity tolerance of *Arachis hypogaea* (L.) by the interaction of halotolerant plant growth promoting rhizobacteria. *J. Plant Growth Regul.* **31(2):** 195-206.

Siddiqui, I., Ehetshamul-Haque, S. and Shaukat, S. (2001) Use of rhizobacteria in the control of root rot–root knot disease complex of mungbean. *J. Phytopathol.* **149(6):** 337-346.

Siddiqui, I.A., Ehteshamul-Haque, S., Zaki, M.J. and Ghaffar, A. (2000a). Greenhouse evaluation of rhizobia as biocontrol agent of root infecting fungi in Okra. *Acta Botanica* **53:** 13-22.

Siddiqui, I.A., Qureshi, S.A., Sultana, V., Ehteshamul-Haque, S. and Ghaffar, A. (2000b) Biological control of root rot and root knot disease complex of tomato. *Plant Soil* **227:** 163-169.

Siddiqui, Z.A. and Singh L.P. (2004) Effects of soil inoculants on the growth, transpiration and wilt disease of chickpea. *J. Plant Dis. Protect.* **111:** 151-157.

Siddiqui, Z.A. and Shakeel, U. (2009) Biocontrol of wilt disease complex of pigeonpea (*Cajanus cajan* (L.) by isolates of *Pseudomonas* spp. *Afr. J. Pl. Sci*. **3(1):** 1-12.

Sindhu, S.S. and Dadarwal, K.R. (2000) Competition for nodulation among rhizobia in *Rhizobium*-legume symbiosis. *Indian J. Microbiol.* **40**: 211-246.

Sindhu, S.S. and Dadarwal, K.R. (2001) Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. *Cicer* in chickpea. *Microbiol. Res.* **156(4):** 353-358.

Sindhu, S.S., Gupta, S.K. and Dadarwal, K.R. (1999) Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of plant growth in green gram (*Vigna radiata*). *Biol. Fertil. Soils* **29:** 62-68.

Sindhu, S.S., Gupta, S.K., Suneja, S. and Dadarwal, K.R. (2002a) Enhancement of green gram nodulation and plant growth by *Bacillus* species. *Biol. Plant.* **45:** 117–120.

Sindhu, S.S., Rakshiya, Y.S. and Sahu, G. (2009) Biological control of soilborne plant pathogens with rhizosphere bacteria. *Pest Technol.* **3:** 10-21.

Sindhu, S.S., Sehrawat, A., Sharma, R. and Dahiya, A. (2016) Biopesticides: use of rhizosphere bacteria for biological control of plant pathogens. *Defence Life Sci. J.* **1:** 135-148.

Sindhu, S.S., Suneja, S., Goel, A.K., Parmar, N. and Dadarwal, K.R. (2002b) Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions. *Appl. Soil Ecol*. **19:** 57-64.

Sindhu, S.S., Malik, D.K. and Dadarwal, K.R. (2003) Enhancing the potential of biological nitrogen fixation by genetic manipulations of diazotrophic bacteria for sustainable agriculture. In: *Plant Genetic Engineering: Volume 1*. Applications and Limitations (eds. Singh, R.P. and Jaiwal, P.K.). *Sci. Tech. Publ.* LCC Houston, USA. pp. 199-228.

Singh G., Biswas, D.R. and Marwah, T.S. (2010) Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (*Zea mays*) and wheat (*Triticum aestivum* L.). *J. Plant Nutr.* **33:** 1236-1251.

Singh, A.K., Tomar, R.K.S., Kasana, B.S., Rikhari, Y.C. and Kushwaha, P. (2016) Integrated wilt management in chickpea (*Cicer arietinum* L.) in Bundelkhand region. *Indian Res. J. Exten. Edu.* **16(2):** 65-69.

Singh, N., Raina, S., Singh, D., Ghosh, M. and Helfish, A.I.A.I. (2017) Exploitation of promising native strains of *Bacillus subtilis* with antagonistic properties against fungal pathogens and their PGPR characteristic. *J. Plant Pathol.* **99(1):** 27-35.

Singh, N.P. and Sewak, S. (2013) Global perspective of chickpea research. *AICRP on chickpea*, pp. 8-13.

Singh, P.P., Shin, Y.C., Park, C.S. and Chung, Y.R. (1999) Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. *Phytopathology* **89(1):** 92-99.

Singh, R.K., Mishra, R.P.N. and Jaiswal, H.K. (2005) Role of rhizobial endophytes as nitrogen fixer in promoting plant growth and productivity of Indian cultivated upland rice (*Oryza sativa* L.) plants. In: *Biological Nitrogen Fixation, Sustainable Agriculture and the Environment.* Springer Netherlands. pp. 289-291.

Sivaramaiah, N., Malik, D.K. and Sindhu, S.S. (2007) Improvement in symbiotic efficiency of chickpea (*Cicer arietinum*) by coinoculation of *Bacillus* strains with *Mesorhizobium* sp. *Cicer*. *Indian J. Microbiol.* **47(1):** 51-56.

Slattery, J.F., Pearce, D.J. and Slatlery, W.J. (2004) Effects of resident rhizobial communities and soil types on the effective nodulation of pulse legumes. *Soil Biol. Biochem*. **36**: 1339-1346.

Slimene, I.B., Tabbene, O., Gharbi, D., Mnasri, B., Schmitter, J.M., Urdaci, M.C. and Limam, F. (2015) Isolation of a chitinolytic *Bacillus licheniformis* S213 strain exerting a biological control against *Phoma medicaginis* infection. *Appl. Biochem. Biotechnol.* **175(7):** 3494-3506.

Sloger, C. (1969) Symbiotic effectiveness and nitrogen fixation in nodulated soybean. *Plant Physiol.***44:** 1666-1668.

Smith, I.M., Dunez, J., Phillips, D.H., Lelliott, R.A. and Archer, S.A. (1988) European handbook of plant diseases. Blackwell Scientific Publications: Oxford. 583.

Smitha, K.P., Mohan, R., Devadason, A. and Raguchander, T. (2015) Exploiting novel rhizosphere *Bacillus* species to suppress the root rot and wilt pathogens of chickpea. *Afr. J. Microbiol. Res.* **9(15):** 1098–1104.

Solaiman, A.R.M., Rabbani, M. G. and Molla, M.N. (2005) Effects of inoculation of *Rhizobium* and arbuscular mycorrhiza, poultry litter, nitrogen and phosphorus on growth and yield in chickpea. *Korean J. Crop Sci.* **50(4):** 256-261.

Somers, E., Vanderleyden, J. and Srinivasan, M. (2004) Rhizosphere bacterial signaling: A love parade beneath our feet. *Crit. Rev. Microbiol.* **30:** 205-240.

Spaepen, S., Vanderleyden, J. and Remans, R. (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev.* **31:** 425-448.

Sreevidya, M. and Gopalakrishnan, S. (2017) Direct and indirect plant growth-promoting abilities of *Bacillus* species on chickpea isolated from compost and rhizosphere soils. *Organic Agri.* **7(1):** 31-40.

Srinivasan, M., Holl, F.B. and Petersen, D.J. (1996) Influence of indole-acetic acid producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gnotobiotic conditions. *Can. J. Microbiol.***42:** 1006-1014.

Srinivasan, M., Holl, F.B. and Petersen, D.J. (1997) Nodulation of *Phaseolus vulgaris* by *Rhizobium etli* is enhanced by the presence of *Bacillus*. *Can. J. Microbiol.* **43(1):** 1-8.

Stefan, M., Munteanu, N., Stoleru, V., Mihasan, M. and Hritcu, L. (2013) Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.). *Sci. Horti.* **151:** 22-29.

Stein, T. (2005) *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol. Microbiol.* **56(4):** 845-857.

Stobart, A.K. and Ameen-Bukhari, J. (1984) Regulation of δ-aminolevulinic acid synthesis and protochlorophyllide regeneration in the leaves of dark-grown barley (*Hordeum vulgare*) seedlings. *Biochem. J.* **222:** 419–426.

Sturz, A.V. and Nowak, J. (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. *Appl. Soil Ecol.* **15:** 183–190.

Subhani, M.N., Sahi, S.T., Ali, L., Hussain, S., Iqbal, J. and Hussain, N. (2013) Management of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceris* through antagonistic microorganisms. *Can. J. Plant Protect.* **1(1):** 1-6.

Subhani, M.N., Sahi, S.T., Hussain, S., Ali, A., Iqbal, J. and Hameed, K. (2011) Evaluation of various fungicides for the control of gram wilt caused by *Fusarium oxysporium* f. sp. *ciceris*. *Afri. J. Agri. Res.* **6(19):** 4555-4559.

Sugumaran, P. and Janarthanam, B. (2007) Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World J. Agri. Sci.* **3(3):** 350-355.

Suman and Yadav, A.S. (2015) Antagonistic effect of *Mesorhizobium ciceri* isolates on *Fusarium oxysporum* f. sp. *ciceri*. *Legume Res.* **38(1):** 115-120.

Suneeta, P., Aiyanathan, K. and Nakkeeran, S. (2016) Efficacy of *Bacillus* spp. in the management of collar rot of Gerbera under protected cultivation. *Res. Crops* **17(4):** 745-752.

Supanjani, Han, H.S., Jung, S.J. and Lee, K.D. (2006) Rock phosphate potassium and rock solubilizing bacteria as alternative sustainable fertilizers. *Agro. Sustain. Develop.* **26:** 233-240.

Syamala, M. and Sivaji, M. (2017) Functional characterization of various plant growth promoting activity of *Pseudomonas fluorescens* and *Bacillus subtilis* from *Aloe vera* rhizosphere. *J. Pharmac. Phytochem.* **6(3):** 120-122.

Sziderics, A.H., Rasche, F., Trognitz, F., Sessitsch, A. and Wilhelm, E. (2007) Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). *Can. J. Microbiol.* **53(11):** 1195-1202.

Tanaka, T., Takahashi, K., Hotta, T., Takeuchi, Y., Konnai, M. (1992) Promotive effects of 5-aminolevulinic acid on yield of several crops. In: Proceedings of the 19th annual meeting of plant growth regulator Society of America, San Francisco. Plant Growth Regulator Society of America, Washington DC: pp. 237-241.

Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Ludueña, L., Pena, D., Ibáñez, F. and Fabra, A. (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. *Plant Soil* **329:** 421–431.

Tchebotar, V.K., Kang, U.G., Asis Jr, C.A. and Akao, S. (1998) The use of GUS-reporter gene to study the effect of *Azospirillum-Rhizobium* coinoculation on nodulation of white clover. *Biol. Fertil. Soils* **27(4):** 349-352.

Tena, W., Wolde-Meskel, E. and Walley, F. (2016) Response of chickpea (*Cicer arietinum* L.) to inoculation with native and exotic *Mesorhizobium* strains in Southern Ethiopia. *Afri. J. Biotechnol.* **15(35):** 1920-1929.

Tilak, K.V.B.R., Ranganayaki, N., Pal, K.K., De, R., Saxena, A.K., Nautiyal, C.S. and Johri, B.N. (2005) Diversity of plant growth and soil health supporting bacteria. *Curr. Sci.* **89(1):** 136-150.

Tiwari, S., Singh, P., Tiwari, R., Meena, K.K., Yandigeri, M., Singh, D.P. and Arora, D.K. (2011) Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. *Biol. Fertil. Soils***47:** 907–916.

Toro, M., Azcon, R. and Barea, J. (1998) The use of isotopic dilution techniques to evaluate the interactive effects of *Rhizobium* genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. *New Phytol.* **138:** 265-273.

Torres, M.J., Brandan, C.P., Sabaté, D.C., Petroselli, G., Erra-Balsells, R. and Audisio, M.C. (2017) Biological activity of the lipopeptide-producing *Bacillus amyloliquefaciens* PGPBacCA1 on common bean *Phaseolus vulgaris* L. pathogens. *Biol. Control* **105:** 93-99.

Trivedi, L. and Rathi, Y.P.S. (2016) Integrated management of seedborne *Fusarium oxysporum* f. sp. *ciceri* in chickpea wilt complex. *World J. Pharm. Pharmac. Sci.* **5(6):** 2392-2402.

Trivedi, P. and Pandey, A. (2008) Plant growth promotion abilities and formulation of *Bacillusmegaterium* strain B 388 (MTCC6521) isolated from a temperate Himalayan location. *Indian J. Microbiol.* **48(3):** 342-347.

Upadhyay, S.K., Singh, J.S., Saxena, A.K. and Singh, D.P. (2012) Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biol.* **14(4):** 605-611.

Upadhyaya, C.P., Akula, N., Kim, H.S., Jeon, J.H., Ho, O.M., Chun, S.C. and Park, S.W. (2011) Biochemical analysis of enhanced tolerance in transgenic potato plants overexpressing δ-galacturonic acid reductase gene in response to various abiotic stresses. *Mol. Breeding* **28(1):** 105-115.

Upadhyaya, H.D., Dwivedi, S.L., Baum, M., Varshney, R.K., Udupa, S.M., Gowda, C.L., Hoisington, D. and Singh, S. (2008) Genetic structure, diversity and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biol.* **8(1):** 106.

Usha rani, C., Priyanka and Srinivasa Rao, A. (2016) Antifungal properties exhibited by bacteria isolated from agriculturally cultivable soils and their antagonistic nature towards fungal phytopathogen suppression. *Agri. Sci. Digest.* **36(1):** 17-23.

Valverde, A., Burgos, A., Fiscella, T., Rivas, R., Velazquez, E., Rodríguez-Barrueco, C. and Igual, J.M. (2006) Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. In: *First International Meeting on Microbial Phosphate Solubilization.* Springer Netherlands. pp. 43-50.

Van der Maesen, L.J.G. (1987) Origin, history and taxonomy of chickpea. In: The chickpea. Saxena, M.C. and Singh, K.B. (eds). CAB International Publication, UK. pp. 11–34.

Vazquez, P., Holguin, G., Puente, M. E., Lopez-Cortes, A. and Bashan, Y. (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol. Fertil. Soils* **30(5):** 460-468.

Verma, J.P., Yadav, J. and Tiwari, K.N. (2009) Effect of *Mesorhizobium* and plant growth promoting rhizobacteria on nodulation and yields of chickpea. *Biol. Forum* **1:** 11-14.

Verma, J.P., Yadav, J., Tiwari, K.N. and Jaiswal, D.K. (2014) Evaluation of plant growth promoting activities of microbial strains and their effect on growth and yield of chickpea (*Cicer arietinum* L.) in India. *Soil Biol. Biochem.* **70:** 33-37.

Verma, J.P., Yadav, J., Tiwari, K.N. and Kumar, A. (2013) Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. *Ecol. Engg.* **51:** 282-286.

Voisard, C., Keel, C., Haas, D. and Dèfago, G. (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *The EMBO J.* **8(2):** 351-358.

Vyas, P., Joshi, R., Sharma, K.C., Rahi, P., Gulati, A. and Gulati, A. (2010) Cold-adapted and rhizosphere-competent strain of *Rahnella* sp. with broad-spectrum plant growth promotion potential. *J. Microbiol. Biotechnol.* **20(12):** 1724-1734.

Wahyudi, A.T., Astuti, R.P., Widyawati, A., Mery, A. and Nawangsih, A.A. (2011) Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting rhizobacteria. *J. Microbiol. Antimicrob.* **3(2):** 34-40.

Wang, C., Knill, E., Glick, B.R. and Défago, G. (2000) Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its *gacA* derivative CHA96 on their growth promoting and disease-suppressive capacities. *Can. J. Microbiol.* **46:** 898–907.

Wani, P.A., Khan, M.S. and Zaidi, A. (2007a) Coinoculation of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. *Acta Agron. Hungarica.* **55(3):** 315-323.

Wani, P.A., Khan, M.S. and Zaidi, A. (2007b) Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. *J. Plant Nutr. Soil Sci.* **170(2):** 283-287.

Wani, P.A., Khan, M.S. and Zaidi, A. (2007c) Synergistic effects of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on the performance of field grown chickpea.*J. Plant Nutr. Soil Sci.* 170: 283–287.

Wani, P.A., Khan, M.S., Zaidi, A. (2008) Chromium-reducing and plant growth promoting *Mesorhizobium* improves chickpea growth in chromium-amended soil. *Biotechnol. Lett.* **30:** 159–163.

Watanabe, K., Tanaka, T., Hotta, Y., Kuramochi, H. and Takeuchi, Y. (2000) Improving salt tolerance of cotton seedling with 5-aminolevulinic acid.*Plant Growth Regul.* **32:** 99-103.

Weller, D.M. (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* **26:** 379–407.

Weller, D.M. (2007) *Pseudomonas* biocontrol agents of soilborne pathogens: Looking back over 30 years. *Phytopathology* **97:** 250-256.

Wensing, A., Braun, S.D., Büttner, P., Expert, D., Völksch, B., Ullrich, M.S. and Weingart, H. (2010) Impact of siderophore production by *Pseudomonas syringae* pv. *syringae* 22d/93 on epiphytic fitness and biocontrol activity against *Pseudomonas syringae* pv. *glycinea* 1a/96. *Appl. Environ. Microbiol.* **76(9):** 2704-2711.

Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. and Wong, M.H. (2005) Effect of biofertilizer containing N-fixer, P and K soluiblizers and AM-fungi on maize growth. *Geoderma* **125:** 155-166.

Xu, S.J., Park, D.H., Kim, J.Y. and Kim, B.S. (2016) Biological control of gray mold and growth promotion of tomato using *Bacillus* spp. isolated from soil. *Tropical Plant Pathol.* **41(3):** 169-176.

Yadav, J. and Verma, J. P. (2014) Effect of seed inoculation with indigenous *Rhizobium* and plant growth promoting rhizobacteria on nutrients uptake and yields of chickpea (*Cicer arietinum* L.). *Euro. J. Soil Biol.* **63:** 70-77.

Yadav, J., Verma, J.P. and Tiwari, K.N. (2010) Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicer arietinum* L.) under *in vitro* conditions. *Biol. Forum An. Int. J.* **2(2):** 15- 18.

Yadav, J., Verma, J.P. and Tiwari, K.N. (2011) Plant growth promoting activities of fungi and their effect on chickpea plant growth. *Asian J. Biol. Sci.* **4(3):** 291-299.

Yadegari, M., Rahmani, H.A., Noormohammadi, G. and Ayneband, A. (2008) Evaluation of bean (*Phaseolus vulgaris*) seeds inoculation with *Rhizobium phaseoli* and plant growth promoting rhizobacteria on yield and yield components. *Pak. J. Biol. Sci.* **11:** 1935-1939.

Yahalom, E., Okon, Y. and Dovrat, A. (1987) *Azospirillum* effects on susceptibility to *Rhizobium* nodulation and on nitrogen fixation of several forage legumes. *Can. J. Microbiol.* **33(6):** 510-514.

Yeole, R.D., Dave, B.P. and Dube, H.C. (2001) Siderophore production by fluorescent pseudomonads colonizing roots of certain crop plants. *Indian J. Expt. Biol.* **36:** 464-468.

Yoshida, M., Maeda, T., Okazaki, K., Nagamune, H., Kunikata, K., Tsuchiya, H. and Kourai, H. (2001) Synthesis of 4, 4'-(tetramethylenedicarbonyldiamino)-bis (1-decylpyridinium bromide) and its antimicrobial and deodorant characteristics. *Biocontrol Sci.* **6(2):** 75-80.

Yu, X., Ai, C., Xin, L. and Zhou, G. (2011) The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. *Euro. J. Soil Biol.* **47(2):** 138-145.

Yuan, J., Raza, W., Shen, Q. and Huang, Q. (2012) Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f.sp. *cubense*. *Appl. Environ. Microbiol.* **78:** 5942–5944.

Yuming, B., Xiaomin, Z. and Smith. D.L. (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. *Crop Sci.* **43:** 1774-1778.

Zafar, M., Zahir, Z.A., Shahzad, S.M., Naveed, M., Arshad, M. and Khalid, M. (2007) Preliminary screening of rhizobacteria containing ACC-deaminase for promoting growth of lentil seedlings under axenic condition. *Pak. J. Botany* **39(5):** 1725-1738.

Zahir, Z.A., Munir, A., Asghar, H.N., Arshad, M. and Shaharoona, B. (2008) Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J. Microbiol. Biotechnol.* **18:** 958–963.

Zahir, Z.A., Shah, M.K., Naveed, M. and Akhter, M.J. (2010) Substrate-dependent auxin production by *Rhizobium phaseoli* improves the growth and yield of *Vigna radiata* L. under salt stress conditions. *J. Microbiol. Biotechnol.* **20:** 1288–1294.

Zahran, H.H. (2001) Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *J. Biotechnol.* **91(2):** 143-153.

Zaidi, A., Khan, M.S. and Amil, M. (2003) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *Euro. J. Agron.* **19:** 15–21.

Zaim, S., Belabid, L. and Bellahcene, M. (2013) Biocontrol of chickpea Fusarium wilt by *Bacillus* spp. rhizobacteria. *J. Plant Prot. Res.* **53(2):** 177-183.

Zaim, S., Belabid, L., Bayaa, B. and Bekkar, A.A. (2016) Biological control of chickpea Fusarium wilts using rhizobacteria “PGPR”. In: *Microbial-mediated Induced Systemic Resistance in Plants.* Springer Singapore. pp. 147-162.

Zakaria, M.A. and Lockwood, J.T. (1980) Reduction of Fusarium population in soil by oil seed meal amendments. *Phytopathology* **70:** 240-243.

Zeller, S.L., Brandl, H. and Schmid, B. (2007) Host-plant selectivity of rhizobacteria in a crop/weed model system. *PLoS One* **2(9):** e846.

Zhang, A.M., Zhao, G.Y., Gao, T.G., Wang, W., Li, J., Zhang, S.F. and Zhu, B.C. (2013) Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: a soil microorganism with biological control potential. *Afri. J. Microbiol. Res.* **7(1):** 41-47.

Zhang, C.J., Tu, G.Q. and Cheng, C.J. (2004) Study on potassium dissolving ability of silicate bacteria. *Shaguan College J.* **26:** 1209-1216.

Zhang, F., Dashti, N., Hynes, R.K. and Smith, D.L. (1996) Plant growth promoting rhizobacteria and soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. *Ann. Bot.* **77:** 453–459.

Zhang, J., Yang, X., Guo, C., de Lajudie, P., Singh, R.P., Wang, E. and Chen, W. (2017a) *Mesorhizobium muleiense* and *Mesorhizobium* gsp. *nov*. are symbionts of *Cicer arietinum* L. in alkaline soils of Gansu, Northwest China. *Plant Soil* **410(1-2):** 103-112.

Zhang, R., Vivanco, J.M. and Shen, Q. (2017b) The unseen rhizosphere root–soil–microbe interactions for crop production. *Curr. Opinion Microbiol.* **37:** 8-14.

Zhang, W.F., Zhang, F., Raziuddin, R., Gong, H.J., Yang, Z.M., Lu, L., Ye, Q.F. and Zhou, W.J. (2008) Effects of 5-aminolevulinic acid on oilseed rape seedling growth under herbicide toxicity stress. *J. Plant Growth Regul.* **27:** 159–169.

Zhang, Z., Li, H., Zhou, W., Takeuchi, Y. and Yoneyama, K. (2006) Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers in vitro. *Plant Growth Regul.* **49:** 27–34.

Zhou, T., Chen, D., Li, C., Sun, Q., Li, L., Liu, F., Shen, Q. and Shen, B. (2012) Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. *Microbiol. Res.* **167(7):** 388-394.

Zongzheng, Y., Xin, L., Zhong, L., Jinzhao, P., Jin, Q. and Wenyan, Y. (2009) Effect of *Bacillus subtilis* SY1 on antifungal activity and plant growth. *Inter. J. Agri. Biol. Engg.* **2(4):** 55-56.

ABSTRACT

Title of the thesis	:	**Fusarium wilt management and growth stimulation of chickpea (*Cicer arietinum* L.) by inoculation with antagonistic *Bacillus* species**
Full name of the degree holder	:	**Anju Sehrawat (2013BS14D)**
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Key words: *Bacillus* isolates, *F. oxysporum*, plamt growth promotion, seed germination, *Mesorhizobium*, chickpea

In the present study, one hundred ninety three *Bacillus* isolates were obtained from chickpea rhizosphere soil and screened for their antagonistic interactions against *Fusarium oxysporum* under *in vitro* conditions. Eighty eight isolates showed antagonistic activity against *F. oxysporum* and 24 isolates were selected. *Bacillus* isolates HCA3, HCA26, HCA53, HCA61, RCA3 and RCA9 showed maximum inhibition of *F. oxysporum* growth. Nine *Bacillus* isolates also inhibited the growth of *Aspergillus niger*, *Neovossia indica* and *Rhizoctonia solani.* Siderophore production was observed in four isolates HCA1, HCA3, HCA53 and RCA11. ACC utilization was observed in all selected isolates except RCA9. Three *Bacillus* isolates RCA3, RCA7 and RCA11 showed significant HCN production. Significant ALA production (> 12 µg/ml) was observed in seven *Bacillus* isolates. The IAA production varied from 0.13 to 29.60 µg/ml in different isolates. Thirteen *Bacillus* isolates showed phosphorus solubilizing activity and nine isolates caused potassium solubilization. Three *Bacillus* isolates i.e. RCA4, RCA9 and RCA19 retarded the growth of chickpea seedlings on 0.8% soil extract agar plates, whereas other isolates showed growth stimulation effect on seedling growth in comparison to uninoculated control at both 5th and 10th day of growth. Out of 24 *Mesorhizobium* isolates obtained from chickpea nodules, eight isolates showed antagonistic effect against *F. oxysporum*. *Bacillus* and *Mesorhizobium* isolates were tested for their salt tolerance capacity at 1, 2, 4, 6 and 8% NaCl concentrations. Six isolates showed growth of 10.1 mm to 15.0 mm colony size at 6% NaCl concentration and five *Bacillus* isolates i.e. HCA11, HCA81, HCA53, RCA11, RCA31 showed larger colony size ranging from 5.1-10.0 mm even at 8% NaCl concentration. Only two *Mesorhizobium* isolates MCA5 and MCA22 were found salt-tolerant upto 8% of salt concentration. Maximum increase in SDW (45.5%) was observed by inoculation of isolate MCA20 followed by 44.3% increase in SDW by inoculation of isolate MCA23 at 40 days of chickpea growth under chillum jar conditions, whereas isolates MCA23 and MCA4 resulted in 166.2 and 161% increase in RDW, respectively. Likewise, 112.6% increase in SDW was observed on inoculation of MCA14 isolate followed by 118.3 and 117.2% increase in SDW on inoculation of MCA4 and MCA10 isolates, respectively at 80th day of observation.

The inoculation effect of three antagonistic i.e., HCA61, RCA3 and RCA7, and two non-antagonistic *Bacillus* isolates HCA76 and SYB101 was studied with *Mesorhizobium* strains MBD26 and KR48 on chickpea growth. At 90 days after showing (DAS), coinoculation of MBD26+RCA3 and KR48+RCA7 showed significant increase in root and shoot dry weight of chickpea under chillum jar conditions. *Bacillus* isolates i.e. HCA61, RCA3 and RCA7 coinoculated with *Mesorhizobium* strains showed 100% disease control of *F. oxysporum* at different stages of chickpea growth under pot house conditions. At 60 DAS, coinoculation of *F. oxysporum*+KR48+SYB101 showed significant increase (155.4%) in SDW followed by coinoculation of *F. oxysporum*+KR48+RCA7 (124%) and *F. oxysporum*+MBD26+RCA7 (121.1%), whereas at 75 DAS, 173.3% increase in SDW followed by 130.9 and 128.3% increase was observed in these treatments under pot house conditions. At 90 DAS, coinoculation of *F. oxysporum*+KR48+SYB101 caused 149 and 111% increase in SDW and RDW of chickpea followed by *F. oxysporum*+KR48+RCA7 (129.5, 96.9%) and *F. oxysporum*+KR48+RCA3 (107.7, 77.2%), respectively. *Bacillus* isolates RCA3 and RCA7 could further be assessed for their plant growth promoting and disease control potential under field conditions for their subsequent application as biofertilizer and biocontrol agents.

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11. Participated in Workshop on Capacity Building on IPR instruments on May 6, 2017 organized by Directorate of Research, CCS Haryana Agricultural University, Hisar and Department of Science and Technology, Panchkula.

(k) Papers/book chapters published

1. **Sehrawat, A**., Suneja, S., Yadav, A. and Anand, R.C. (2015) Influence of different additives on shelf life of rhizobial inoculants for mungbean (*Vigna radiata* L.). International Journal of Recent Scientific Research. 6(5): 4338-4342.
2. **Anju Sehrawat** and S.S Sindhu (2015) Characterization of *Bacillus* isolates from chickpea rhizosphere antagonistic to *Fusarium oxysporum* and their effect on seed germination of chickpea **In:** 56rd Annual Conference of AMI and International symposium on Emerging discoveries in Microbiology and its interdisciplinary relevance, held at School of Life Sciences, Jawaharlal Nehru University, New Delhi. (Abstract book) AMP55.
3. Satyavir S. Sindhu, Manisha Phour and **Anju Sehrawat** (2016) Streptokinase production by microorganisms. e-Pathshala pp. 1-11.
4. Satyavir S. Sindhu, **Anju Sehrawat** and Manisha Phour (2016) Tyrosinase production by microbes and its utility. e-Pathshala pp. 1-13.
5. Sindhu, S.S., Parmar, P., **Phour, M.** and Sehrawat, A. (2016) Potassium solubilizing microorganism (KSMs). Meena, V.S., Verma, J.P., Maurya, B.R. & Meena, R.S. (eds.). Springer-Verlag, Berlin, Heidelberg.
6. **Anju Sehrawat** (2016-17) Biological control of plant pathogens. College Magazine, COBS&H, CCS Haryana Agricultural University, Hisar.
7. **Sehrawat, A.**, Yadav, A., Anand, R.C., Kukreja, K. and Suneja, S. (2017) Enhancement of shelf life of liquid biofertilizer containing *Rhizobium* sp. infecting mungbean (*Vigna radiata* L.). Legume Research ISSN: 0250-5371.
8. Sindhu, S. S., **Sehrawat**, **A**., Sharma, R. and Dahiya, A. (2017) Biopesticides: use of rhizosphere bacteria for biological control of plant pathogens. Defence Life Science Journal 1: 135-148.
9. Satyavir S. Sindhu, **Anju Sehrawat**, Ruchi Sharma and Aakanksha Khandelwal (2017) Biological control of plant pathogens and insect pests for sustainable agriculture. In: Advances in Microbiology (Adhya, T.K. eds). Springer.
10. S.S. Sindhu, Aakanksha Khandelwal, Manisha Phour and **Anju Sehrawat** (2017) Bioherbicidal potential of rhizosphere microorganisms for ecofriendly weeds management. In: Agriculturally important microbes for sustainable agriculture. Vol. 2; Applications in crop production and protection (Meena, VS, Mishra, PK, Bisht, JK and Pattanayak, A, eds.). Springer.
11. **Anju Sehrawat**, Ruchi Sharma and Satyavir S. Sindhu (2017) Application of biofertilizers and biopesticides in crops to minimize the use of agrochemicals. In: Lab to Land: Innovations in Science. Proceedings of the seminar held at RKSD College, Kaithal on 27.01.2017.
12. Satyavir S. Sindhu and **Anju Sehrawat** (2017) Rhizosphere microorganisms: application of plant beneficial microbes in biological control of weeds. In: Agriculturally beneficial microorganisms for sustainable agriculture (Panpatte, DG, Jhala, VK, Vyas, RV and Shelat, HN eds.). Springer.

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UNDERTAKING OF COPYRIGHT

I, **Anju Sehrawat**, Admission No. 2013BS14D, undertake that I give copyright to the CCS HAU, Hisar of my thesis entitled **"Fusarium wilt management and growth stimulation of chickpea (*Cicer arietinum* L.) by inoculation with antagonistic *Bacillus* species"**. I also undertake that patent, if any, arising out of the research work conducted during the program shall be filled by me only with due permission of the competent authority of CCS HAU, Hisar.

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