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Suppression of *Rhizoctonia solani* Root Rot Disease of Clusterbean (*Cyamopsis tetragonoloba*) and Plant Growth Promotion by Rhizosphere Bacteria

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ABSTRACT
Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub) is a kharif legume crop grown under arid zone. Root rot is the major disease in clusterbean caused by *Rhizoctonia solani* during rainy season and may result upto 21-60% losses at pre-and post-emergence stages. Rhizobacterial isolates were tested in this study for use as biological control agent for suppression of the root rot disease and to minimize the use of fungicides for disease control. Fifty five rhizobacterial isolates obtained from clusterbean rhizosphere soil were screened for antagonistic interactions against *Rhizoctonia solani* on PDA medium plates. Rhizobacterial isolates HCS2, HCS4, HCS30, HCS36, HCS43 and HFS12 showed significant antagonistic activity and inhibited the growth of fungi on PDA medium plates. Forty percent of these rhizobacterial isolates utilized ACC on minimal medium plates and five rhizobacterial isolates HCS16, HCS35, HCS42, HCS43 and HFS5 showed significant growth on ACC supplemented plates. Inoculation of *Bradyrhizobium* isolate GSA11 with *Pseudomonas* isolate HCS36/*Bacillus* isolate HCS43 and *R. solani* formed maximum 47 nodules plant^{-1} and increased shoot dry weight by 306.3 and 281.5%, respectively as compared to uninoculated control at 30 days of plant growth under pot house conditions. At 60 days of plant growth, coinoculation of *Rhizobium* isolate GSA110 with *Bacillus* isolate HCS43 and *R. solani* formed 50 nodules plant^{-1} and caused 108.9% increase in shoot dry weight in comparison to uninoculated control. Maximum 140.26% increase in shoot dry weight, nodule number (54 nodules plant^{-1}) and nodule weight (346.6 mg plant^{-1}) was observed by coinoculation of *Pseudomonas* isolate HCS36 with *Rhizobium* strain GSA110 and fungus *R. solani*. Coinoculation of *Bacillus* isolate HCS43 and *Pseudomonas* isolate HCS36 with *Bradyrhizobium/Rhizobium* isolates also showed 66.7 and 83.7% disease control. Thus, *Bacillus* isolate HCS43 and *Pseudomonas* isolate HCS36 could be further tested for disease control and plant growth stimulation under field conditions.

Key words: Clusterbean, *Pseudomonas*, *Bacillus*, *Rhizobium*, *Rhizoctonia solani* ACC utilization, disease suppression, nodulation, plant growth

INTRODUCTION

Pathogenic microorganisms cause various plant diseases that usually weaken or destroy plant tissues and reduce crop yields varying from 25-100% (Frisvad and Samson, 1991). Root diseases are estimated to cause 10-15% yield losses annually in the world (Bajoria *et al*., 2008). *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank). Donk is an ecologically diverse soilborne fungus that causes root rot disease on clusterbean plants. Fungicide bavistin sprays are usually done for controlling the disease. But indiscriminate use of agrochemicals for disease and pest

control has resulted into considerable pollution of soil, water and air. Moreover, wide spread use of agrochemicals also have undesirable effects on non-target organisms and possible carcinogenicity effects. Thus, their extensive use is environmentally unsafe and also uneconomical. Therefore, it is imperative to develop some alternate strategies for controlling plant diseases. Biological control using antagonistic microorganisms offers a low cost ecofriendly technology that reduces the number and activity of plant pathogens (Glick *et al*., 1999; Sindhu *et al*., 2009; Yang *et al*., 2014).

Rhizosphere bacteria (rhizobacteria) suppress/control the plant diseases by various mechanisms viz., production of antibiotics (Keel *et al*., 1992; Saraf *et al*., 2014), production of hydrolytic enzymes (Sindhu and Dadarwal, 2001), hydrocyanic acid (Sarhan and Shehata, 2014), stimulation of phytoalexins or flavonoid-like compounds in roots (Goel *et al*., 2001) or by production of siderophores, which chelate metal cations rendering them unavailable for pathogenic forms (Raaijmakers *et al*., 1995; Sahu and Sindhu, 2011). Certain microbial strains protect the plants against pathogens through induced systemic resistance (Kaiser and Hannan, 1989). Some rhizosphere bacteria possess the enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase that reduces the level of stress hormone ethylene production. Enzyme ACC deaminase has been reported in many soil microorganisms (Khandelwal and Sindhu, 2012; Glick, 2014). These ACC deaminase-containing bacterial strains were also found more effective biocontrol strains (Glick, 2004; Wang *et al*., 2000). Thus, use of biocontrol agents isolated from plants and soils holds a great promise to establish them in the rhizosphere to control various plant diseases without disrupting the ecological balance (Weller, 2007).

A major group of rhizobacteria with biological control potential are the *Pseudomonas* and *Bacillus* strains (Sundaramoorthy and Balabaskar, 2013; Sarhan and Shehata, 2014). These bacteria are ubiquitous in agricultural soils and possess many traits that make them well suited as biocontrol and growth-promoting agents (Sindhu *et al*., 2014). Saikia *et al*. (2004) screened 54 fluorescent *Pseudomonas* isolates obtained from broad bean rhizosphere for antagonism against *Macrophomina phaseolina* and *R. solani* and reported that *Pseudomonas aeruginosa* strain RsB29 caused suppression of *Fusarium* wilt and charcoal rot of chickpea, and promoted the plant growth of broad bean. Ramette *et al*. (2006) found that *Pseudomonas* populations growing in the rhizosphere soil of tobacco produced the biocontrol compounds viz., 2,4-diacetylphloroglucinol and hydrogen cyanide which were suppressive to root rot disease. *Pseudomonas fluorescens* strain CHA0 has been found to produce several secondary metabolites, notably HCN, 2,4-diacetylphloroglucinol, pyoluteorin and indole acetic acid (Keel *et al*., 1992). The combined application of *P. fluorescens* and *B. subtilis* exhibited highest reduction of tomato wilt disease and increased the dry weight of tomato plants up to 27% in comparison to the non-bacterized control (Sundaramoorthy and Balabaskar, 2013).

Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub) is a kharif legume crop grown under arid zone in India (Singh *et al*., 2001). It is primarily grown for seed, animal feed, fodder, vegetable and green manuring purposes. Clusterbean is a rich source of high quality galatomannan gum and protein rich (40-50%) guar meal as animal feed. Seed gum is used in various industries such as textiles, paper, cosmetics, explosives and food processing. Besides the gum preparation, clusterbean is emerging as a potential source of vegetable protein for human beings. However, the root rot disease of clusterbean during rainy season may result up to 21-60% plant loss at pre-and post-emergence stages (Bajoria *et al*., 2008). Moreover, the information available on the antagonistic effect of rhizobacteria against *R. solani* is very scanty. Therefore, attempts were made in this study to develop biological control agents against root rot causing fungi *R. solani.*

MATERIALS AND METHODS

Isolation of bacteria from rhizosphere soil: Rhizosphere soil samples were collected from different fields of clusterbean grown in CCS Haryana Agricultural University, Hisar farm at 45 and 60 days of plant growth. The serial dilutions of the composite rhizosphere soil samples (up to 10^{-4}) were plated on King's B agar medium. *Pseudomonas* and *Bacillus* colonies were selected based on morphological and pigment production characteristics after 3 days of incubation at 28±2°C. Purified colonies of bacteria were transferred on Luria Bertani (LB) agar medium slopes. *Rhizobium/Bradyrhizobium* strains were obtained from Department of Microbiology, CCS H.A.U., Hisar. Phytopathogenic fungus *R. solani* was obtained from the Department of Plant Pathology, CCS H.A.U., Hisar. Liquid cultures of all the isolates were preserved in 50% glycerol at -20°C.

Screening of rhizobacterial isolates for utilization of ACC: The medium plates were prepared with minimal medium (Dworkin and Foster, 1958) supplemented with 3 mM ACC (Penrose and Glick, 2003) or ammonium sulphate (2 g L^{-1}). A loopful of 48 h old growth of *Pseudomonas* or *Bacillus* culture was spotted on the medium plates and incubated at 28±2°C for 2-5 days. The growth of different bacterial isolates on ACC supplemented medium plates was recorded (Khandelwal and Sindhu, 2012). The cultures showing good growth on ACC supplemented medium plates and capable of utilizing ACC as nitrogen source, were scored as ACC^{+}.

Growth inhibition of *Rhizoctonia solani* by rhizobacterial isolates: The antagonistic interactions of rhizobacterial isolates with phytopathogenic fungus *R. solani* were studied by the spot test method on Potato Dextrose Agar (PDA) medium plates (Sindhu *et al*., 1999). *Rhizoctonia solani* was grown on PDA slants for 4 days and spore suspension was harvested in 3 mL sterilized water. Fungal spore suspension (3.0 mL) was added into sterilized PDA medium, mixed uniformly and plated. Growth suspension (5 µL) of 48 h old rhizobacterial cultures was spotted on spore suspension-containing plates.

The inhibition of growth of *R. solani* by the spotted rhizobacterial isolates was recorded after 4 days of incubation at 28±2°C.

Coinoculation studies of selected rhizobacterial isolates with *Bradyrhizobium/Rhizobium* for nodulation and plant growth: Three rhizobacterial isolates i.e., HCS5 (control), HCS36 and HCS43 (with antagonistic activity) were used for coinoculation with symbiotically effective *Bradyrhizobium/Rhizobium* strains in clusterbean variety HG563 (susceptible to root rot disease). The earthern pots of 10 kg capacity were filled with sandy loam soil and river sand mixed in 70:30 ratio. *Pseudomonas* and *Bacillus* strains were grown on LB medium for 2 days and *Bradyrhizobium/Rhizobium* strains were grown on YEMA medium slopes for 7 days. The growth suspension of each *Pseudomonas/Bacillus* and *Bradyrhizobium/Rhizobium* cultures was made in 5 mL of sterilized water.

Seeds of clusterbean were inoculated either alone (with 10 mL of growth suspension) or as biomix (obtained from mixing of 5 mL growth of each bacteria in 1:1 ratio, v/v). The viable count in the broth was kept 10^8-10^9 cells mL^{-1} and 10 g seeds were inoculated with 10 mL of bacterial growth suspension and having 10^7-10^8 cells mL^{-1} of growth suspension. There were 20 treatments in this experiment and each treatment had three replications. Growth of 4 days-old *R. solani* was harvested from PDA plates and fungal growth suspension was prepared in sterilized saline water. Fungal growth suspension (100 mL) was mixed in the 10 kg soil: sand mixture in earthern pots with treatments T_7, T_{10}, T_{11}, T_{14}, T_{15}, T_{18}, T_{19} and T_{20}. The growth suspension of fungus was inoculated on the roots of clusterbean plants in the *R. solani* treatments only. Uninoculated seeds were sown as control. The plants were grown in the pot house under day light conditions during the month of May-June 2013. Sloger's nutrient solution was added in the pots as and when required (Sloger, 1969). The plants were uprooted at 30 and 60 days of plant growth and observations were taken for nodule number, nodule fresh weight, plant dry weight and disease index. After washing with tap water, nodules were detached from the roots and dried in the folds of filter paper. The nodules were counted and weighed. Shoot portions of the plants were dried in oven at 90°C for 24 h and weighed.

Disease index and reduction in disease: On the basis of symptoms observed percent disease index, percent final stand and percent disease control were calculated by following equation:

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

$$\text{Disease control (\%)} = \frac{\text{100 (–) DI in treatment}}{\text{Disease incidence in control}} \times 100$$

Disease control and disease incidence were recorded after 30 and 60 days of sowing. It was calculated on the average of six plants grown per pot.

Statistical analysis: Completely Randomized Design (CRD) was used for experimental data analysis. All determinations were carried out in triplicate and data represented are average values of three replications. Standard Error of Means± (SEM) values were calculated to determine the significant differences between treatment means. The C.D. and C.V. values represent coefficient of deviation and coefficient of variation, respectively.

RESULTS

Fifty five bacterial isolates representing *Pseudomonas* and *Bacillus* (based on morphological and pigment production characteristics) were selected from rhizosphere soil at 45 and 60 days of culsterbean growth. Bacterial counts in the rhizosphere soil ranged from $1.7\times18.4\times10^5$ colony forming units (CFU) g^{-1} soil at 45 days of plant growth. At 60 days of plant growth, bacterial counts increased and ranged from 19.2-76.8×10^5 CFU g^{-1} soil. Similarly, Baig *et al*. (2002) isolated 105 bacteria from rhizosphere and rhizoplane of groundnut. Out of these, 67% isolates were from the rhizosphere and 33% were from the rhizoplane. *Pseudomonas* was found as the most predominant (42%) followed by *Bacillus* (28%) and *Enterobacter* (21%). Results of oxidase test, catalase test, Gram and spore staining showed that 24 rhizobacterial isolates belonged to *Pseudomonas* sp. and 31 isolates were found *Bacillus* (data not shown).

Screening of bacterial isolates for ACC utilization: All the 55 rhizobacterial isolates belonging to *Pseudomonas* and *Bacillus* were screened for utilization of ACC on the minimal medium (Dworkin and Foster, 1958) plates supplemented with ammonium sulphate or 3 mM ACC. Twenty two isolates (40.0%) showed growth on ACC supplemented plates (Table 1, Fig. 1). Five rhizobacterial isolates HCS16, HCS35, HCS42, HCS43 and HFS5 showed significant growth on ACC supplemented plates. Three isolates HCS26, HCS33 and HCS34 showed good growth on ammonium sulphate plate and no growth on ACC supplemented plate. Isolates HCS6, HCS13, HCS20, HCS41, HCS44, HFS2, HFS3 and HFS6 showed more growth on ammonium sulphate plate than to ACC supplemented plate. Two isolates HCS36 and HCS40 showed significant growth on ammonium sulphate plate and good growth on ACC supplemented plate whereas, another isolate HCS16 showed significant growth on both ammonium sulphate and ACC supplemented plates. Ten cultures i.e., HCS1, HCS9, HCS11, HCS18, HCS19, HCS28, HCS29, HCS32, HCS37 and HFS8 did not grow on both type of plates and they may require some amino acid for growth.

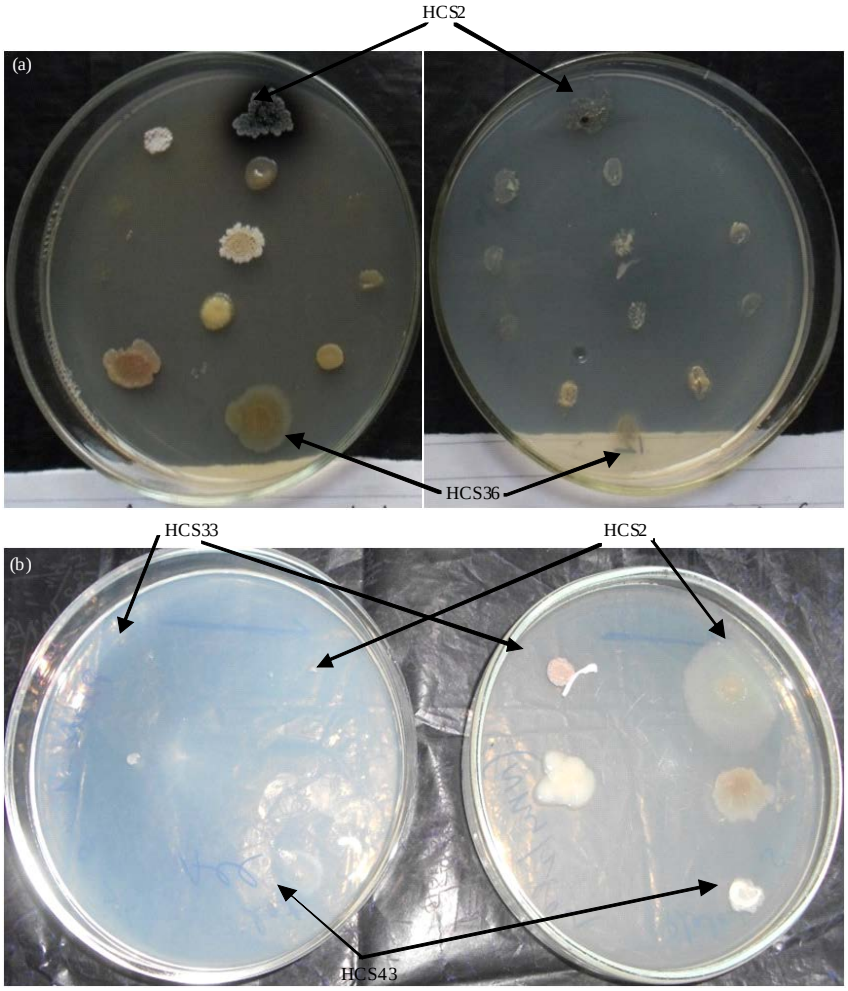

Fig. 1(a-b): Growth of rhizobacteria isolates on Dworkin and Foster minimal medium containing either ACC or ammonium sulphate, (a) Medium+Ammonium sulphate plate; Medium+ACC plate and (b) Medium+ACC plate; Medium+Ammonium sulphate plate

Table 1: ACC utilization by rhizobacterial isolates on minimal medium supplemented with ACC or ammonium sulphate

Rhizobacterial isolate No.	ACC utilization pattern
Growth of rhizobacterial isolates on ACC supplemented plates	
HCS16, HCS35, HCS42, HCS43, HFS5	+++
HCS3, HCS6, HCS7, HCS13, HCS20, HCS24, HCS25, HCS36,	++
HCS40, HCS41, HCS44, HFS2, HFS3, HFS4, HFS6, HFS9, HFS12	+
HCS1, HCS2, HCS4, HCS5, HCS9, HCS10, HCS11, HCS12, HCS14, HCS15, HCS17, HCS18, HCS19, HCS21, HCS22, HCS23, HCS26, HCS27, HCS28, HCS29, HCS30, HCS31, HCS32, HCS33, HCS34, HCS37, HCS39, HFS1, HFS7, HFS8, HFS10, HFS11, HFS14	-
Rhizobacterial isolate no.	**Ammonium sulphate utilization pattern**
Growth of rhizobacterial isolates on ammonium sulphate supplemented plates	
HCS17, HFS7	+
HCS31, HFS4, HFS9, HFS12	++
HCS4, HCS6, HCS12, HCS13, HCS15, HCS20, HCS26, HCS27, HCS33, HCS34, HCS39, HCS41, HCS41, HCS43, HCS44, HFS1, HFS2, HFS3, HFS5, HFS6	+++
HCS3, HCS5, HCS7, HCS10, HCS21, HCS22, HCS24, HCS25, HCS30, HCS42, HFS10, HFS14	++++
HCS2, HCS14, HCS16, HCS23, HCS35, HCS36, HCS40, HFS5	+++++
HCS1, HCS9, HCS11, HCS18, HCS19, HCS28, HCS29, HCS32, HCS37, HFS8	-

Growth of bacterial isolates was tested on minimal medium (Dworkin and Foster, 1958) supplemented with ammonium sulphate (2 g L^{-1}) or 3 mM ACC. On the basis of colony size after 2-5 days of incubation at 28±2°C, the growth of the isolates was scored as: -: No growth, +: Little growth, ++: Moderate growth, +++: More growth, ++++: Significant growth, +++++: Maximum growth

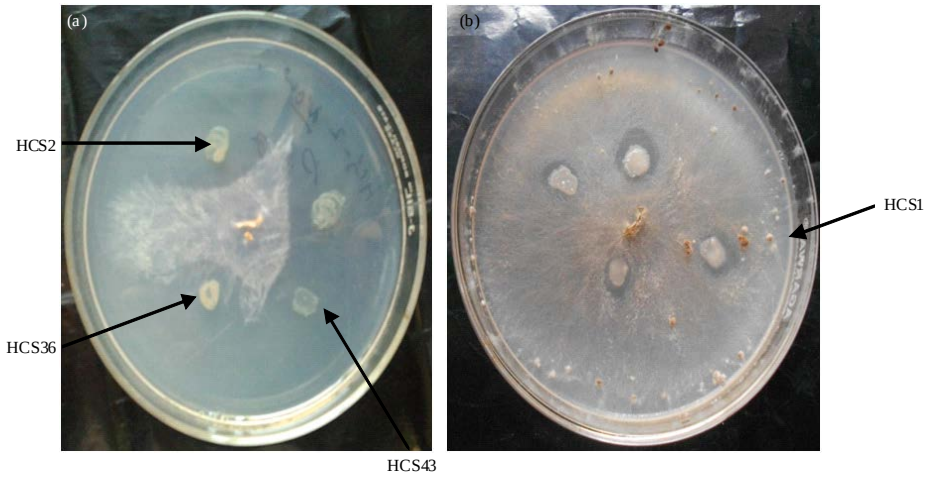

Fig. 2(a-b): Rhizobacterial isolates showing antifungal activity against *Rhizoctonia solani*

Table 2: Antifungal activity of *Pseudomonas* and *Bacillus* isolates against *Rhizoctonia solani*

Rhizobacterial isolates	Zone of inhibition (mm)
HCS12, HCS13, HCS39, HFS8, HFS10	3.0
HCS2, HCS4, HCS30, HCS36, HCS43, HFS12	5.0
HCS1, HCS3, HCS5, HCS6, HCS7, HCS9, HCS10, HCS11, HCS14, HCS15, HCS16, HCS17, HCS18. HCS19, HCS20, HCS21, HCS22, HCS23, HCS24, HCS25, HCS26, HCS27, HCS28, HCS29, HCS31, HCS32, HCS33, HCS34, HCS35, HCS37, HCS40, HCS41, HCS42, HCS44, HFS1, HFS2, HFS3, HFS4, HFS5, HFS6, HFS7, HFS9, HFS11, HFS14	-

Antagonistic activity of rhizobacterial isolates was tested on the basis of growth inhibition of fungal pathogens on PDA medium plates by spot test method (Sindhu *et al*., 1999)

Screening of rhizobacterial isolates for antagonistic activity against fungal pathogen under *in vitro* conditions: Antagonistic activity of all the fifty five rhizobacterial isolates was studied by observing the zone of inhibition of fungal growth on PDA plates. The zone of fungal growth inhibition varied with different isolates tested. *Pseudomonas/Bacillus* isolates HCS2, HCS4, HCS30, HCS36, HCS43 and HFS12 showed large inhibition zone (5.0 mm) of fungal growth. (Table 2, Fig. 2). Five isolates HCS12, HCS13, HCS39, HFS8 and HFS10 showed 3.0 mm inhibition zone. Isolate HCS2 showed largest inhibition zone. Remaining forty four isolates did not inhibit the growth of fungus *R. solani* on PDA medium plates. Ten isolates i.e., HCS2, HCS4, HCS12, HCS13, HCS30, HCS36, HCS39, HCS43, HFS10 and HFS12 showed ACC utilization as well as antifungal activity. Cultures HCS4, HCS36 and HCS43 showed more ACC utilization as well as antifungal activity than other isolates.

Coinoculation studies of *Rhizobium/Bradyrhizobium* and rhizobacterial isolates for disease control and plant growth of clusterbean: Selected rhizobacterial isolates were tested for disease control using wilt susceptible clusterbean variety HG563 under pot house conditions. Clusterbean seeds inoculated with rhizobacterial isolates were grown in pots (with three replications of each treatment) and fungal growth suspension was also inoculated in some treatments. Inoculation of *Bradyrhizobium* strain GSA11 and *Rhizobium* isolate GSA110 increased shoot dry weight by 186.33% and 169.63%, respectively as compared to uninoculated control at 30 days of plant growth (Table 3). Coinoculation of *Rhizobium* strain GSA110 and *Bacillus* HCS43 formed 42 nodules plant^{-1} with 209.6 mg nodule weight and shoot dry weight was increased by 207.7% as compared to uninoculated plants. *Bradyrhizobium* strain GSA11 and *Pseudomonas* isolate HCS36 along with fungus caused 306.3% increase in shoot dry weight in comparison to control uninoculated plants. Similarly, coinoculation of *Bradyrhizobium* strain GSA11 and *Bacillus* isolate HCS43 along with *R. solani* showed maximum stimulatory effect on nodule formation (47 nodules plant^{-1}) and nodule weight (225.4 mg plant^{-1}) and resulted in 281.5% gains in shoot dry weight. In fungus-inoculated treatments, disease caused by *R. solani* was effectively controlled by coinoculation of *Rhizobium/Bradyrhizobium* with rhizobacterial isolates HCS36 and HCS43.

At 60 days of plant growth, nodulation performance of *Bradyrhizobium* strain GSA11 and *Rhizobium* strain GSA110 significantly increased and their inoculation resulted in 112.3 and 103.3% gains in shoot dry weight, respectively (Table 4). Coinoculation of *Pseudomonas* isolate HCS36 and *Rhizobium* strain GSA110 formed 48 nodules plant^{-1} and 317.2 mg plant^{-1} nodule weight was observed. Coinoculation of *Rhizobium* strain GSA110 with *Bacillus* isolate HCS43 along with *R. solani* formed 50 nodules plant^{-1} and caused 108.9% increase in shoot dry weight in comparison to uninoculated control. Maximum 140.26% increase in shoot dry weight, nodule number (54 nodules plant^{-1}) and nodule weight (346.6 mg plant^{-1}) was observed by coinoculation of *Pseudomonas* isolate HCS36 with *Rhizobium* strain GSA110 and fungus *R. solani* (Fig. 3). Coinoculation of *Bacillus* isolate HCS43 along with *Rhizobium* strain GSA110 also reduced the

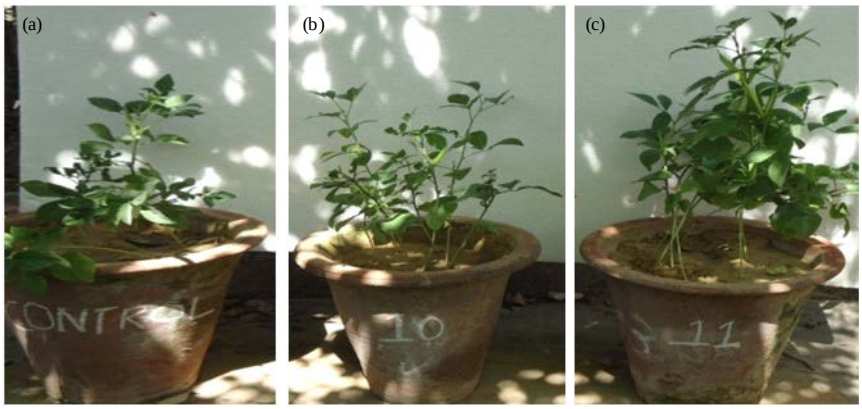

Fig. 3(a-c): Effect of inoculation of *Bradyrhizobium* GSA11 with rhizobacterial isolate HCS36 for plant growth enhancement under pot house conditions at 60 days of plant growth. (a) Control (uninoculated), (b): *Rhizoctonia solani* and (c) HCS43+GSA11+*R. solani*

Table 3: Symbiotic effectiveness and disease control of bacterial isolates on coinoculation of clusterbean at 30 days of plant growth

Treatments	No. of nodules plant^{-1}	Nodule weight (mg plant^{-1})	Shoot dry weight (mg plant^{-1})	Disease incidence (%)	Disease control (%)
T_1: Control (uninoculated)	2±0.57	5.6±0.64	105.4±4.01	8.6	-
T_2: HCS36 (*Pseudomonas*)	4±1.15	15.3±0.41	149.6±3.69	-	-
T_3: HCS5 (*Bacillus*)	3±1.15	14.1±0.30	197.2±4.04	-	-
T_4: HCS43 (*Bacillus*)	2±0.57	8.2±0.90	185.1±2.92	-	-
T_5: GSA11 (*Bradyrhizobium*)	29±1.52	157.2±4.80	301.8±4.89	-	-
T_6: GSA110 (*Rhizobium*)	31±2.08	168.6±6.82	284.2±4.12	-	-
T_7: *R. solani*	5±1.15	13.4±2.65	172.6±3.80	83.7	-
T_8: HCS36+GSA11	39±2.64	192.6±3.52	379.2±7.76	-	-
T_9: HCS36+GSA110	35±1.15	186.2±4.60	400.6±5.34	-	-
T_{10}: HCS36+GSA11+*R. solani*	47±2.08	217.8±5.71	428.2±6.46	8.3	92.7
T_{11}: HCS36+GSA110+*R. solani*	38±1.15	193.4±9.44	358.6±4.17	16.3	83.7
T_{12}: HCS5+GSA11	28±1.15	154.6±5.81	290.4±3.27	-	-
T_{13}: HCS5+GSA110	36±2.40	184.2±2.65	352.4±6.76	-	-
T_{14}: HCS5+GSA11+*R. solani*	26±1.15	147.2±6.58	246.8±2.28	92.7	8.3
T_{15}: HCS5+GSA110+*R. solani*	31±2.64	162.6±2.12	258.2±4.95	92.7	8.3
T_{16}: HCS43+GSA11	35±1.52	194.4±4.25	286.4±5.27	-	-
T_{17}: HCS43+GSA110	42±2.64	209.6±9.97	397.2±3.41	-	-
T_{18}: HCS43+GSA11+*R. solani*	47±1.52	225.4±4.01	412.6±4.83	16.3	83.7
T_{19}: HCS43+GSA110+*R. solani*	41±2.08	198.6±6.80	394.2±2.43	16.3	83.7
T_{20}: *R. solani*+Bavistin	3±0.57	10.4±2.44	226.8±2.76	-	100.0
C.D.	4.84	14.46	13.16		
C.V.	11.16	6.56	2.72		

Values given are average value of three plants. Disease incidence is the % of plants infected and disease control is the % reduction of diseased plants after inoculation with bacteria. The values of nodule fresh and shoot dry weight are calculated as per plant basis. C.D. and C.V. values represent coefficient of deviation and coefficient of variation, respectively. SEM (Standard Error of Means) values are represented as (±)

browning of collar root and caused 83.7% disease control (Table 4, Fig. 4) and 108.91% increase in shoot dry weight was observed. Treatment with bavistin in fungus-inoculated soils caused 92.7% reduction in disease appearance and resulted in 46.7% increase in shoot dry weight as compared to uninoculated control.

DISCUSSION

Previous studies have established that some rhizobacterial strains could serve as a useful biofertilizer and biocontrol agents for various crops (Sindhu *et al*., 2010). Antagonistic bacteria have also been found to promote the growth of different crops and termed as plant growth-promoting rhizobacteria. Inoculation with rhizobacteria having ACC deaminase activity is an efficient strategy used for lowering the level of stress hormone ethylene to minimize its adverse effect on plant growth. Several bacterial strains that can utilize ACC as a sole source of nitrogen have been isolated from rhizosphere soil samples and subsequently used for inoculation (Glick, 2004). In this study, 40.0% rhizobacterial isolates showed growth on ACC supplemented plates (Table 1, Fig. 1), indicating that these bacteria possess ACC deaminase activity. Five rhizobacterial isolates HCS16, HCS35, HCS42, HCS43

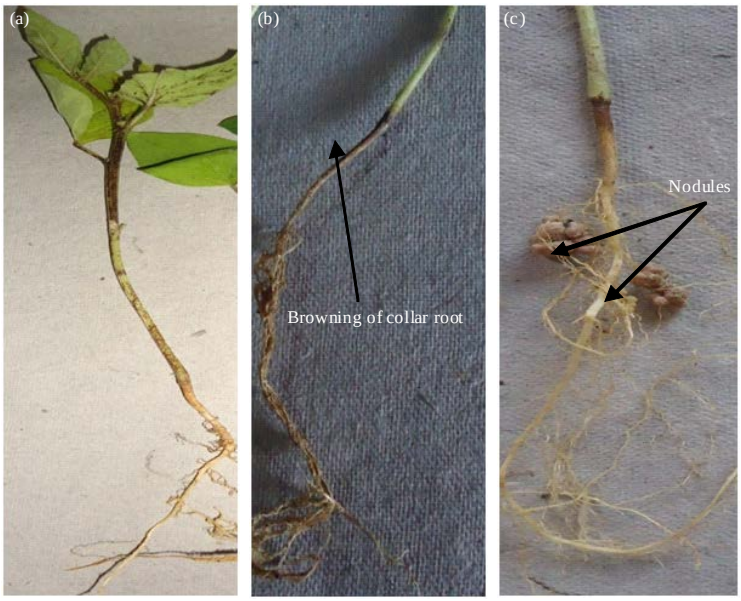

Fig. 4(a-c): Effect of inoculation of *Bradyrhizobium* and rhizobacterial isolates for disease control under pot house conditions at 60 days of plant growth

Table 4: Symbiotic effectiveness and disease control of bacterial isolates on coinoculation of clusterbean at 60 days of plant growth under pot house conditions

Treatments	No. of nodules plant^{-1}	Nodule weight (mg plant^{-1})	Shoot dry weight (mg plant^{-1})	Disease incidence (%)	Disease control (%)
T_1: Control (uninoculated)	9±1.00	38.2±4.14	242.4±4.46	16.3	-
T_2: HCS36 (*Pseudomonas*)	8±0.57	34.7±5.25	386.2±4.54	-	-
T_3: HCS5 (*Bacillus*)	10±1.52	45.6±5.93	364.2±5.00	-	-
T_4: HCS43 (*Bacillus*)	8±1.00	29.6±2.12	391.4±5.74	-	-
T_5: GSA11 (*Bradyrhizobium*)	36±3.21	315.6±4.13	514.6±3.41	-	-
T_6: GSA110 (*Rhizobium*)	45±2.08	329.4±3.61	492.8±3.77	-	-
T_7: *R. solani*	11±1.52	52.6±2.29	266.4±6.27	100.0	-
T_8: HCS36+GSA11	43±3.05	296.4±4.15	576.2±5.61	-	-
T_9: HCS36+GSA110	48±3.05	317.2±5.41	532.6±2.61	-	-
T_{10}: HCS36+GSA11+*R. solani*	42±2.08	302.4±3.01	558.2±4.96	16.3	83.7
T_{11}: HCS36+GSA110+*R. solani*	54±2.08	346.6±7.15	582.4±5.25	16.3	83.7
T_{12}: HCS5+GSA11	32±3.05	272.4±3.86	425.6±3.44	-	-
T_{13}: HCS5+GSA110	41±2.64	288.2±9.02	412.4±4.42	-	-
T_{14}: HCS5+GSA11+*R. solani*	34±2.64	296.8±6.97	365.2±4.11	83.7	16.3
T_{15}: HCS5+GSA110+*R. solani*	34±3.60	302.4±5.57	378.2±5.95	83.7	16.3
T_{16}: HCS43+GSA11	42±1.52	278.6±3.41	388.4±3.08	-	-
T_{17}: HCS43+GSA110	41±3.05	266.4±5.68	456.2±5.21	-	-
T_{18}: HCS43+GSA11+*R. solani*	46±3.21	302.6±4.87	492.6±4.61	33.3	66.7
T_{19}: HCS43+GSA110+*R. solani*	50±2.64	315.2±6.59	506.4±5.41	33.3	66.7
T_{20}: *R. solani*+Bavistin	12±2.08	56.8±4.78	355.6±4.86	8.3	92.7
C.D.	6.98	14.86	13.59		
C.V.		13.06	4.00	1.89	

Values given are average value of three plants. Disease incidence is the % of plants infected and disease control is the % reduction of diseased plants after inoculation with bacteria. The values of nodule fresh and shoot dry weight are calculated as per plant basis. C.D. and C.V. values represent coefficient of deviation and coefficient of variation, respectively. SEM (Standard Error of Means) values are represented as (±)

and HFS5 showed significant growth on ACC supplemented plates. Thanananta *et al*. (1997) reported that 6 *Pseudomonas* strains, out of total 55 bacterial isolates, were capable of growing on Dworkin and Foster minimal medium and showed the highest efficiency of ACC utilization. Similarly, the screening of 563 bacteria isolated from the roots of pea, lentil and chickpea showed that only 5% isolates showed ACC deaminase activity and 7% isolates were capable of indole acetic acid production (Hynes *et al*., 2008). Husen *et al*. (2009) observed that 11 out of total 13 *Pseudomonas* isolates possessed ACC deaminase activity and increased root development of soybean. Khandelwal and Sindhu (2012) found that 38.9% *Pseudomonas* isolates obtained from clusterbean rhizosphere showed good growth on ACC supplemented plates. Chaudhary and Sindhu (2015) observed that 44% *Mesorhizobium* strains possess the ACC utilization ability. Penrose and Glick (2003) suggested that rhizobial or *Pseudomonas* strains that are intended for use as inoculants of

host legumes should first be selected/ tested for the presence of a functional ACC deaminase to get better crop productivity and disease control.

Screening of rhizobacterial isolates for fungal growth inhibition showed that 20% cultures possess the ability to inhibit pathogenic fungi *R. solani* under cultural conditions (Table 2). Rhizobacterial isolates HCS2, HCS4, HCS30, HCS36, HCS43 and HFS12 showed 5.0 mm fungal growth inhibition zone (Fig. 2). Five isolates HCS12, HCS13, HCS39, HFS8 and HFS10 showed 3.0 mm inhibition zone. Similar growth inhibition of *R. solani* has been reported by *P. fluorescens* (PS-4) strain and the antagonistic potential was attributed to the production of antibiotics and siderophores or due to induced systemic resistance (Kaiser and Hannan, 1989). Siddiqui *et al*. (2001) showed that *Pseudomonas aeruginosa* and *Bacillus subtilis* strains produced growth inhibition zones by inhibiting the radial growth of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Rhizoctonia solani*. Coombs *et al*. (2004) evaluated 38 stains belonging to *Streptomyces* and *Microspora* for their ability to produce antifungal compounds *in vitro* against *G. graminis* var. *tritici*, *R. solani* and *Pythium* sp. They observed that 64% cultures exhibited antifungal activity under cultural conditions. Similarly, *Pseudomonas aeruginosa* strain isolated from Botanical Garden at Udaipur, Rajasthan was used as biocontrol agent against three phytopathogenic fungi *Fusarium moniliformae*, *Alternaria solani* and *Helminthosporium halodes* (Sharma *et al*., 2007). Karuppiah and Rajaram (2011) reported that eight *Bacillus* sp., out of 63 different *Bacillus* isolates, exhibited plant growth promoting activities and six of these *Bacillus* isolates also inhibited the growth of *Penicillium* sp., *Cercospora* sp. and *Fusarium oxysporum*. Dua and Sindhu (2012) isolated antagonistic bacteria from the rhizosphere soil of wheat and sixteen bacterial isolates were found to inhibit the growth of *R. solani*. Growth inhibition zone varied from 6-15 mm by different rhizobacterial isolates. Growth inhibition of the pathogenic fungi was also observed by using culture filterates of antagonistic rhizobacterial isolates.

Inoculation of *Bradyrhizobium* isolate GSA11 and *Rhizobium* isolate GSA110 on clusterbean seeds increased shoot dry weight by 186.33 and 169.63%, respectively as compared to uninoculated control at 30 days of plant growth (Table 3). Coinoculation of *Bradyrhizobium* isolate GSA11 with *Pseudomonas* isolate HCS36/*Bacillus* isolate HCS43 and *R. solani* formed maximum 47 nodules plant^{-1} and increased shoot dry weight by 306.3 and 281.5%, respectively as compared to uninoculated control at 30 days of plant growth under pot house conditions. Disease caused by *R. solani* was effectively controlled on coinoculation of *Rhizobium/Bradyrhizobium* with rhizobacterial isolates HCS36 and HCS43 in fungus inoculated soil. At 60 days of plant growth, coinoculation of *Pseudomonas* isolate HCS36 and *Rhizobium* strain GSA110 formed 48 nodules per plant and 317.2 mg plant^{-1} nodule weight was observed (Table 4, Fig. 3). Coinoculation of *Bacillus* isolate HCS43 along with *Rhizobium* strain GSA110 reduced the browning of collar root and caused 83.7% disease control (Fig. 4).

In earlier studies, a significant correlation was found between *in vitro* ACC deaminase activity of the rhizobacteria and growth promoting activity of these bacteria on maize under axenic conditions and on nodulation of mung bean (*Vigna radiata* L.) under natural pot and field trials (Shaharoona *et al*., 2006). Ma *et al*. (2003) found that ACC deaminase lacking mutant of *Rhizobium leguminosarum* bv. *viciae* strain 128C53K formed approximately 25% fewer nodules than the wild-type strain on *Pisum sativum* L. cv. Sparkle and also produced approximately 30% less plant biomass. Afsharmanesh *et al*. (2010) evaluated the antagonistic potential of *Pseudomonas fluorescens* strain UTPF5 against *R. solani* AG-4 in bean and showed that strain UTPF5 could inhibit the growth of *R. solani* both *in vitro* and *in vivo* and suppressed the disease by 33.34 and 14.29% in soil drenching and seed treatment, respectively. Susilowati *et al.* (2011) reported that *Pseudomonas* sp. CRB inhibited the growth of the pathogenic fungi i.e., *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani*, approximately 11.1-60.0% *in vitro*. Seed coating with the *Pseudomonas* sp. CRB accomplished disease suppression in plant about 14.3-100% in sterile soil condition and 5.2-52.6% in non sterile soil condition.

Abeysinghe (2009) reported that a combination of two compatible biological control agents, *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 showed significant plant protection of *Solanum melongena* and *Capsicum annuum* for control of damping-off disease caused by *Rhizoctonia solani*. Dua and Sindhu (2012) showed that inoculation of *R. solani* in wheat caused 85-90% root rot disease at 60-90 days of plant growth. Whereas, coinoculation of antagonistic *Pseudomonas* isolates WPS3 or WPS90 in *R. solani*-inoculated plants caused 88.9 and 66.7% disease control, respectively at 90 days of plant growth in the pots. Coinoculation of *Pseudomonas* isolate WPS3 or WPS90 with *R. solani* enhanced 115 and 98% plant dry weight in comparison to uninoculated plants at 90 days of plant growth. Similar, synergistic effects have been observed on nodulation and plant growth of other legumes by dual inoculation of *B. japonicum* and *P. fluorescens* in soybean (Li and Alexander, 1988), *R. leguminosarum* with an antibiotic-producing *P. fluorescens* strain F113 in pea (Andrade *et al*., 1998) and *Bradyrhizobium/Mesorhizobium* strains with *Pseudomonas* sp. in green gram and chickpea (Goel *et al*., 2000; Sindhu *et al*., 2002). Combined application of *Pseudomonas* and *Bacillus* strains has also been found to control effectively the tomato wilt and damping-off disease in alfalfa (Sundaramoorthy and Balabaskar, 2013; Sarhan and Shehata, 2014). Thus, it appears that use of mixtures or combinations of biocontrol agents may be more effective method to improve biological control (Spadaro and Gullino, 2005) leading to enhanced crop productivity. For realistic inoculation effects of this study, *Bacillus* isolate HCS43 and *Pseudomonas* isolate HCS36 needs to be evaluated under the field conditions for disease control and plant growth stimulation of clusterbean.

CONCLUSION

Pseudomonas and *Bacillus* isolates obtained from the rhizosphere soil of clusterbean were found to inhibit the growth of root rot-causing fungi *Rhizoctonia solani* on PDA medium plates. Seed inoculation of antagonistic *Bacillus* isolate HCS43 and *Pseudomonas* isolate HCS36 caused 66.7-83.7% reduction of root rot disease in clusterbean under pot house conditions. Coinoculation of *Pseudomonas/Bacillus* isolates with *Bradyrhizobium/Rhizobium* strain GSA11 or GSA110 in clusterbean enhanced the nodule number, nodule fresh weight and plant dry weight as compared to *Bradyrhizobium/Rhizobium*-inoculated or uninoculated control plants and also suppressed the root rot disease under pot house conditions. These results suggested that rhizosphere bacteria from the soil could minimize/replace the use of fungicides and nitrogenous fertilizers to reduce the pollution of soil and environment.

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