

Effect of Bacterial Isolates Against *Fusarium* Wilt in Chick Pea

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Abstract A study was taken up to know the performance of native bacterial isolates for the control of wilt disease in chick pea. Nine bacterial isolates were selected to see the performance of its antagonistic activity against wilt disease in chick pea. Highest germination percent was observed in the treatment BCOH 3 inoculated plants, treatment T₆-BCOH 6 was par with others. The yield parameters like number of pods per plant was maximum in T₆ BCOH 6, it was significantly superior and the yield per ha was also maximum in BCOH 6. The application of microbial in-

oculants promote plant growth and in turn increases the yield of the crop. Disease incidence at 30 DAS was highest in control. At 65 DAS, the disease incidence was almost stalled in T₆ BCOH 6. In the present study BCOH 6 has performed well and hence this can be mass multiplied and can be used as a Microbial inoculants for production of chick pea in Hassan district.

Keywords Biocontrol, Bacterial isolates, Chick pea, Disease incidence.

Introduction

Plant pests (harmful insects, parasitic weeds and plant pathogens) are among the most important biotic agents causing serious losses and damages to agricultural crops. Plant pests need to be controlled to ensure food, feed and fiber production quantitatively and qualitatively. A number of different strategies are currently being employed to manage and control plant pests [1, 2]. Beyond good agronomic and cultural practices, growers often rely heavily on chemical pesticide application. However, the environmental pollution caused by excessive use of agrochemicals has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Today, there is political pressure to remove the most hazardous chemicals from the market. In this regard there is a need for the development of non-chemical alternative methods to manage plant pests. Biological con-

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Table 1. Effect of bacterial inoculants on the germination percent of chick pea. DAS : Days after sowing.

Treatments	Germination Percent (%)		
	7 DAS	14 DAS	21 DAS
T ₁ -BCOH-1	88.20	90.30	90.00
T ₂ -BCOH-2	88.50	93.50	92.33
T ₃ -BCOH-3	89.50	96.50	93.33
T ₄ -BCOH-4	85.00	92.00	88.00
T ₅ -BCOH-5	85.50	91.10	87.33
T ₆ -BCOH-6	84.30	94.20	90.00
T ₇ -BCOH-7	85.60	92.60	91.67
T ₈ -BCOH-8	84.00	93.50	88.67
T ₉ -BCOH-9	85.40	91.40	87.67
T ₁₀ -Neem Cake	84.50	93.50	88.33
T ₁₁ - <i>Trichoderma</i> sp.	85.13	89.10	88.67
T ₁₂ - <i>Pseudomonas</i> sp.	83.00	91.37	88.00
T ₁₃ -Carbendazim	85.76	89.70	88.00
T ₁₄ -Control	81.83	90.30	84.00
SEM ±	1.55	1.55	1.79
CD at 5%	4.51	4.51	5.19

trol of plant diseases has been considered a viable alternative to manage plant diseases. Biocontrol is the inhibition of growth, infection or reproduction of one organism using another organism.

Chickpea is a cool season legume crop and is grown in several countries worldwide as a food source. Seed is the main edible part of the plant and is a rich source of protein, carbohydrates and minerals especially for the vegetarian population. Chickpea is the third most important food legume crop and India is the largest producer contributing to 65% of world's chickpea production. The Desi type chickpea contributes to around 80% and the Kabuli type around 20% of the total production.

Fusarium wilt is the most important disease of chickpea and is wide-spread in chickpea growing areas of Asia, Africa, Southern Europe and the America. Annual yield losses due to wilt have been estimated at 10-90%. The causative agent of this disease has been classified as *Fusarium oxysporum* f. sp. ciceris, is internally seed borne and is found as chlamydospore-like structures in the hilum region of the seed. Infected seed plays an important role in long distance dispersal and in transmitting the disease to new areas. Once the inoculum is established in soil, it is difficult to eradicate as the chlamydospores survive

in the soil for at least 6 years, and under favorable conditions germinate and infect the seedlings through tender roots. The pathogen can also survive in infected crop residues buried in the soil and other species can also be affected under artificial inoculation conditions.

Chick Pea is also known as Bengal gram, is one of the major pulse crop which is extensively grown in Indian conditions and nutritionally rich in protein. Since Bengal gram is a *rabi* crop, easy to cultivate, requires less cost of cultivation. It is highly susceptible to many of the soil borne diseases especially wilt which is our major interested disease of research to know which treatment will better overcome the disease *Fusarium* wilt caused by *Fusarium* sp. It causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop. Hence experiment was conducted, to know the efficacy of different bacterial isolates for integrated disease management practices in the management of chickpea wilt.

Bacteria like *Bacillus* and *Pseudomonas* are of increasing interest as it is antagonistic soil microorganisms and attack a wide range of plant pathogens [3]. Some of the important pathogens are controlled by *Bacillus* and *Pseudomonas* are *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* spp., *Pythium* spp. and *Paytophthora* spp. [4]. One of the main reason for the failure of biocontrol is the selection of biocontrol agents, in this regard there are various reports which indicated the native biocontrol agents showed better performance than the introduced one. In this regard this study was taken up to know the performance of native bacterial isolates for the control of wilt disease in chick pea.

Materials and Methods

This study was taken up to screen the native Bacterial isolates against *Fusarium* pathogen of chick Pea in the filed condition at College of Agriculture, Hassan, Karnataka state, India.

Soil samples were collected from rhizosphere soils of different locations in Hassan district. The Bacterial

Table 2. Effect of bacterial inoculants on growth parameters of chick pea. DAS : Days after sowing.

Treatments	Number of Branches			Plant Height (cm)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁ -BCOH-1	2.67	3.33	3.33	15.33	26.33	36.00
T ₂ -BCOH-2	3.33	3.67	3.67	16.00	28.00	31.00
T ₃ -BCOH-3	2.00	4.00	3.33	14.00	22.33	32.33
T ₄ -BCOH-4	3.00	3.00	3.00	13.67	20.67	36.00
T ₅ -BCOH-5	3.33	4.00	4.00	15.00	24.00	33.00
T ₆ -BCOH-6	2.00	4.33	5.00	16.00	30.67	40.00
T ₇ -BCOH-7	2.67	3.00	3.00	14.00	24.33	35.00
T ₈ -BCOH-8	2.33	3.33	4.33	14.00	25.33	33.33
T ₉ -BCOH-9	3.00	3.00	3.00	14.67	26.00	34.67
T ₁₀ -Neem Cake	1.33	3.33	3.33	13.00	27.33	31.33
T ₁₁ - <i>Trichoderma</i> sp.	3.00	4.00	4.33	15.33	23.33	32.67
T ₁₂ - <i>Pseudomonas</i> sp.	2.00	3.00	3.00	14.00	24.67	31.67
T ₁₃ -Carbendazim	1.70	3.00	2.70	12.70	25.00	31.70
T ₁₄ -Control	2.00	3.00	3.00	14.30	25.30	32.70
SEm ±	0.51	0.45	0.54	2.12	1.81	1.81
CD at 5%	1.47	1.32	1.57	2.36	5.25	5.26

antagonists were isolated from rhizosphere soils of apparently healthy plants by dilution plate technique on Nutrient Agar medium. Isolates were maintained in nutrient agar and nutrient broth by periodically sub culturing. The bacterial isolates were characterized morphologically by examining the colony morphology, cell shape and Gram's reaction [5].

A field experiment was conducted to test the efficacy of bacterial isolates for controlling *Fusarium* wilt of chickpea. The Variety used is JG-11 (Jawahar gram), totally 14 treatments with 3 replication was used and the design was RCBD, the size of the plot was 4 m² with 12 × 4 cm spacing. The treatment was imposed fifteen days after germination. The bacterial inoculants were mass multiplied by using Potato dextrose agar medium, 100 ml of the different bacterial broth culture was drenched to each plot. One kilogram of neem cake was applied to each plot for treatment No. 10. The standard biocontrol agents viz., *Trichoderma* and *Pseudomonas* were procured from Department of Plant Pathology, UAS, GKVK, Benagaluru, here 10 gram of the biocontrol agents was mixed with 100 ml of water and it was applied to the each plot for treatments no 11 and 12 respectively. Hundred ml of Carbendazim was drenched to the plants 15 days after germination at the rate of one gram/l of, for three times at an interval of 15 days. One plot was maintained as

a control with out giving any treatments. All the agronomical practices were done as per the procedure.

The germination percent, morphological parameters, yield parameters and the disease incidence of the experiment was recorded with different intervals by using standard methodology.

Results and Discussion

Experiment was conducted, to know the efficacy of different bacterial isolates for integrated disease management practices in the management of chickpea wilt and the result is as followed. Highest germination percent at seven day after sowing (DAS) was observed in the treatment T₃ where BCOH-3 Microbial inoculants was inoculated (89.5%) and it was on par with T₁ BCOH-1 (88.20%) and T₂ BCOH-2 (88.50%) (Table 1). There was significant difference between the above treatments compared to the control (81.83%) and treatment T₁₃ where carbendazim was drenched (85.76%). The same trend was observed at 14 DAS and treatment T₆-BCOH 6 was no par with T₁, T₂ and T₃. At 21 DAS the germination percent was decreased in all the treatments. There was significant difference between T₁ (90.00%), T₂ (92.33%) and T₃ (93.33%) compared to the control (84.00). The reason

Table 3. Effect of bacterial inoculants on yield parameters of chick pea. DAS : Days after sowing.

Treatments	Number of pods per plant		Weight of 100 seeds (g)	Yield per hectare (kg)
	50 DAS	60 DAS		
T ₁ -BCOH-1	37.00	49.33	23.37	405.00
T ₂ -BCOH-2	41.00	53.00	23.53	378.17
T ₃ -BCOH-3	38.67	54.00	23.67	407.67
T ₄ -BCOH-4	35.00	49.33	23.67	408.83
T ₅ -BCOH-5	39.33	53.00	23.63	362.50
T ₆ -BCOH-6	53.33	65.00	23.47	411.10
T ₇ -BCOH-7	42.67	54.67	23.23	387.50
T ₈ -BCOH-8	45.00	56.00	23.53	348.83
T ₉ -BCOH-9	38.33	57.00	23.53	401.33
T ₁₀ -Neem Cake	46.67	55.67	23.87	403.97
T ₁₁ - <i>Trichoderma</i> sp.	41.00	52.00	23.40	388.60
T ₁₂ - <i>Pseudomonas</i> sp.	35.00	48.00	23.50	355.63
T ₁₃ -Carbendazim	36.30	50.30	23.40	405.40
T ₁₄ -Control	30.70	47.30	23.50	380.60
SEm ±	4.07	3.30	0.19	15.87
CD at 5%	11.84	9.59	0.54	46.12

for more germination could be production of growth regulators by microbial inoculants. There are some reports which states that the Bacterial inoculants helps in increasing the germination. Similar results was also observed [6, 7].

At 30 DAS the number of branches was highest in T₂-BCOH-2 (3.33) and T₅-(BCOH-5) and the lowest was observed in T₁₀ (1.33) where neem cake was treated (Table 2). At 45 DAS highest branches was observed in T₆ BCOH-6 (4.33) and it was on par with T₃ BCOH-3 (4.00). The lowest was observed in control plots and the carbendazim treated plots (3.00). The same trend was observed at 60 DAS. The plant height at 30 DAS was highest in T₆ BCOH-6 (16.00 cm) and T₂ BCOH-2 (16.00 cm), here significant difference was observed when compared to plants treated with carbendazim (12.70 cm), the control plant showed 14.30 cm height. At 45 DAS, T₆ BCOH-6 showed maximum height (30.67 cm), the carbendazim treated plants showed 25.00 cm followed by control plots (25.30 cm), which was significantly differed from treatment T₆. The same trend was observed at 60 DAS, T₆ BCOH-6 (40.00 cm), T₁₃ carbendazim treated showed 31.70 cm and the control plants showed 32.70 cm height. The microbial inoculants acts as plant growth promoter, which produces the plant growth hormone viz., Auxin,

Table 4. Effect of bacterial inoculants on the disease incidence of chick pea. DAS : Days after sowing.

Treatments	Percent Disease Incidence (%)			
	30 DAS	45 DAS	60 DAS	75 DAS
T ₁ -BCOH-1	2.81	5.80	6.70	7.70
T ₂ -BCOH-2	3.11	3.10	5.50	7.00
T ₃ -BCOH-3	2.51	3.70	5.00	5.60
T ₄ -BCOH-4	4.93	5.17	5.83	7.70
T ₅ -BCOH-5	1.32	1.78	3.36	3.40
T ₆ -BCOH-6	3.55	4.51	5.40	5.68
T ₇ -BCOH-7	6.72	7.12	8.36	8.76
T ₈ -BCOH-8	2.04	7.03	10.20	12.30
T ₉ -BCOH-9	1.40	3.71	5.60	8.62
T ₁₀ -Neem Cake	2.50	4.20	5.80	7.90
T ₁₁ - <i>Trichoderma</i> sp.	1.40	3.10	6.90	7.60
T ₁₂ - <i>Pseudomonas</i> sp.	2.03	3.82	6.91	7.80
T ₁₃ -Carbendazim	4.20	6.40	8.40	9.10
T ₁₄ -Control	6.80	11.70	18.10	19.20
SEm ±	1.29	1.75	2.51	2.61
CD at 5%	3.75	5.09	7.29	7.60

Gibberlin by this the growth of the plant will increase [8—11].

The yield parameters like number of pods per plant at 50 DAS was maximum in T₆ BCOH-6 compared to all other treatments and it was significantly superior, the control plants showed the lowest 30.70 pods/plant (Table 3). At 60 DAS the same trend was observed, T₆ BCOH-6 was maximum 65.00 and it was on par with T₉ BCOH-9 inoculant (57.00). The lowest was observed in control plants (47.30), the carbendazim treated plants showed 50.30 pods/plant which was very much lower than T₆ BCOH-6. The weight of 100 seeds was almost similar in all the treatments. The highest was observed in treatment T₁₀ neem cake treated plants (23.87 g) and the lowest was observed in treatment T₇ BCOH-7 (23.23 g). Finally these treatment were compared based on the yield per hectare. The results showed significantly difference between some of the microbial inoculated plants compared to control (380.60 kg/ha) and carbendazim treated plants (405.40 kg/ha). The maximum yield was observed in T₆ BCOH-6 (411.10 kg/ha). The application of Microbial inoculants promote plant growth and in turn increases the yield of the crop [8, 9].

Disease incidence at 30 DAS was highest in control (6.80%) and the lowest was observed in T₅ BCOH-

5 (1.32%), it was on par with T₉ BCOH-9 (1.40%) and T₁₁ *Trichoderma* treated plants (1.40%) (Table 4). Treatment T₆ BCOH-6 also showed minimum disease incidence (3.55%). The same trend was observed at 45 DAS. At 66 DAS, the disease incidence was almost stalled in T₆ BCOH-6 when compared to other treatment, highest disease incidence was observed in control plants (18.10%). The same trend was also observed at 75 DAS. Application of Microbial inoculants viz., *Bacillus*, *Pseudomonas* and *Trichoderma* either singly or in combination control with and rot diseases in various crops [5, 6] which can thrive well in the existing environment for the control of the pathogen by adopting various biocontrol mechanisms. These Microbial inoculants not only control the diseases efficiently but also promote plant growth [8, 9, 12, 13].

Plant pests are among the most important biotic agents causing serious losses and damages to agricultural crops. However, the environmental pollution caused by excessive use of agrochemicals has led to considerable changes in soil leading to environmental degradation. Hence we have to look into the organic farming, particularly using native microbes as an biocontrol agent and biofertilizers. In the present study BCOH-6 has performed well and hence this can be mass multiplied and can be used as a Microbial inoculants for chick pea in Hassan district.

References

- Heydari A (2007) Biological control of Turfgrass Fungal diseases. In: Turfgrass management and physiology. Pessarathi M (ed.). CRC Press, Florida, USA.
- Heydari A, Maisaghi IJ, Balestra GM (2007) Pre-emergence herbicides influence the efficacy of fungicides in controlling cotton seedling damping off in the field. Int J Agric Res 2 : 1049—1053.
- Umashankar N, Devakumar AS, Reveendra HR, Krishnamurthy R (2010) Biological control of *Fusarium* Wilt in tomato. Environ Ecol 28 : 1111—1115.
- Muthuraju R, Umashankar N, Nagaraju K (2006) Biological control of Rot (*Phytophthora infestans*) on tomato by selective antagonists. J Soil Biol and Ecol 26 : 85—93.
- Umashankar N, Umashankar Kumar N (2015) Isolation and Screening of Bacterial Isolates against *Fusarium* sp. Int J Retailing and Rural Business Perspective. Pezzottaite J 4 : 1432—1436.
- Das AC, Kole SC (2006) Effect of some root associative bacteria on germination of seeds, nitrogenase activity and dry matter production by rice plants. J Crop and Weed 2 : 47—51.
- Singh SK, Pancholy A, Jindal SK, Pathak R (2011) Effect of plant growth promoting rhizobia on seed germination and seedling traits in *Acacia* Senegal. Ann For Res 54 : 161—169.
- Umashankar N, Venkateshamurthy P, Devakumar AS, Devagiri GM, Sathish KM (2011) Studies of different microbial inoculants on the growth of cardamom in nursery conditions. Environ Ecol 29 : 1476—1472.
- Umashankar N, Venkateshamurthy P, Devagiri GM, Devakumar AS, Sathish KM (2011) Effect of microbial inoculants on the growth of coffee in nursery condition. Environ Ecol 29 : 1419—1422.
- Ghilavizadeh A, Darzi MT, Hadi MHS, Rejali F (2012) Influence of plant growth promoter bacteria and plant density on yield components and seed yield of ajowan (*carum copticum*). Int J Agri Crop Sci 4 : 1255—1260.
- Alemu F, Alemu T (2015) *Pseudomonas fluorescens* isolates used as a plant growth promoter of Faba bean (*Vicia faba*) *in vitro* as well as *in vivo* study in Ethiopia. Am J Life Sci 3 : 100—108.
- Nguyen MT, Ranamukhaarachchi SL (2010) Soil-borne antagonists for biological control of bacterial wilt disease caused by *Ralstonia solanacearum* in tomato and pepper. J Pl Pathol 92 : 395—406.
- Purnawatil A, Rochdjatun I, Sastrahidayat, Abadi AL, Hadiastono T (2014) Endophytic bacteria as biocontrol agents of tomato bacterial wilt disease. J Trop Life Sci 4 : 33—36.