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RESEARCH ARTICLE

Antagonistic effect of Rhizobium leguminosarum, Pseudomonas fluorescens and Bacillus subtilis in Cowpea [Vigna unguiculata (L.)Walp.]under in vitro condition

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Abstract

Brown blotch disease is caused by Colletotrichum truncatum Schw. in Cowpea plants.

The present investigation deals with the treatment of seeds with different Plant growth promoting rhizobacteria(PGPR) such as Rhizobium leguminosarum, Pseudomonas fluorescens and Bacillus subtilis as single and dual innoculants over a period of 20,40 and 60 days. The disease incidence and severity were reduced by the efficiency of the PGPR

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Introduction

Different bacterial genera are vital components of soils. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production. They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure (Ahemad. M. and M. Kibret, 2014) Indeed, the bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilising the nutrients compared to those from bulk soil.

Hence, diverse symbiotic (Rhizobium, Bradyrhizobium, Mesorhizobium) and non-symbiotic (Pseudomonas, Bacillus, Klebsiella, Azotobacter, Azospirillum, Azomonas), rhizobacteria are now being used worldwide as bio-inoculants to promote plant growth and development. Agrochemical treatment may result in environmental impact and pose a threat to humans and animals. As a result, there has been an increase in research on potential PGPR agents, aimed at finding a definitive solution or at least at reducing pesticide use in the treatment of phytopathogenic diseases.

PGPR, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals.

Cowpea, which is commercially grown throughout India for its long green pods as a vegetable, for its seeds as pulse and for its foliage as fodder, is an important component of the farming system of the arid and humid topics. As one of the food legumes, it is an important source of nutrients and provides high quality, inexpensive protein to diets based on cereal grains and starchy foods.

Colletotrichum truncatum Schw. causes brown blotch, a major disease of cowpea (Fig. 1). The fungus attacks all parts of the plant: seedlings, hypocotyl, stems, penduncles, flowers, leaves and pods. Yield loss of up to 75% due to brown blotch disease in cowpea has been reported (Bankole and Adebanjo, 1996).

In view of the problems and potential solution stated above to work out suitable PGPR control of diseases of cowpea and to evaluate the efficiency of three different microorganism in reducing disease incidence.

MATERIALS AND METHODS

Seed materials

The seeds of Cowpea [Vigna unguiculata(L.)Walp.]var. CO7 were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Bio-control agents

Different PGPR (R.leguminosarum., P. fluorescens and B. subtilis) were isolated the rhizosphere of healthy Cowpea and Soybean plants from Vadakkumangudi village, Cuddalore district, Tamil Nadu.

Location of experimental site

Field experiments were conducted in Agricultural Land, Vadakkumangudi, Cuddalore district located within 4 km from the Annamalai University, Tamil Nadu. The studies were conducted during January 2014 to April 2014. The experimental site was situated at 11°24′ N latitude and 79°41′ E longitude with an altitude of 5°79′ M above Mean Sea Level (MSL).

Mode of Action

A dual-culture test was conducted to examine, whether R. leguminosarum., P. fluorescens and B. subtilis could antagonize the growth of plant fungal pathogens. A mycelial disc, 0.5 cm in diameter of a pure culture of fungal pathogen was placed in the center of a PDA (Potato Dextrose Agar) plate and then R. leguminosarum., P. fluorescens and B. subtilis was inoculated in three symmetrical spots around the mycelial disc. The plates were incubated for 7-10 days at 28ºC. The plates were scanned once a day to monitor the formation of an inhibition zone and the growth of fungal pathogens. The width of the inhibition zones were measured and then average was found. A cell-free supernatant of R. leguminosarum., P. fluorescens and B. subtilis was prepared by inoculating in the improved SM medium (Sodium Succinate medium) (Sayyed and Chincholkar, 2006)for 48 h at 37ºC and then centrifuging at 12,000 rpm for 10 min. After mixing with PDA medium, the mixture was poured onto plates. After solidification, fungal mycelial disc of0.5 cm was placed in the center of each petridish. PDA medium containing 500µg ml^{-1} of 75% chlorothalonil wettable powder was used as a positive control (CK^{+}). The plates were incubated at 28ºC and observed once a day to monitor the mycelial growth of the fungal pathogens. The fungal colony diameters were measured and then compared to the diameters of the same pathogens grown on the control plates. The radial growth of the pathogen and percentage reduction over control was calculated by using the formula (Vincent, 1947) as follow.

$$\text{Percentage reduction over control} = \frac{C-T}{C} \times 100$$

Where, C = Mycelial growth of the pathogen in control (mm) and

T = Mycelial growth of the pathogen in dual plate (mm)

Suppression of Brown Blotch disease

To study the influence of PGPR agents, approximately after55 days, the percent of diseased plants per treatment was scored on the basis of Brown Blotch disease symptoms. The experiments were conducted at least three times for all PGPR treatments.

Disease index (Wheeler, 1969)

The Cowpea plants were analyzed for the incidence of disease during the 20, 40 and 60th day samples during the treatment. The percentage of the disease incidence was assessed by using the following formula.

$$\text{Percentage of disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{X } 100$$

RESULTS AND DISCUSSION

Disease incidence of Cowpea plants in field experiment(%)

In this study, higher disease incidence was recorded in control for Brown Blotch (62.67 %). The incidence of Brown Blotch diseases was less in dual inoculants than in single inoculant treated fields (Table 1, Fig. 2).

Disease severity of Cowpea plants in field experiment (%)

In Cowpea, higher disease severity was recorded in control (8.00, 41.45 and 68.33 %) at 20, 40 and 60th days. The incidence of disease severity was less in dual inoculations particularly T_4–R. leguminosarum+P. fluorescens (25.23 and 46.60 %) followed by R. leguminosarum +B. subtilis (28.01 and 51.61 %) and P. fluorescens+B. subtilis (29.57 and 53.54 %) when compared to single inoculations at 40 and 60th days (Table 2)

Table 1. Suppression of Brown Blotch diseases of Cowpea plants treated with different PGPR

Treatments	Brown Blotch disease	
	Disease incidence (%)	Reduction over control (%)
T_0 – Control	61.73±1.49	-
T_1 - Rhizobium leguminosarum	37.66±2.43	39.05
T_2 - Pseudomonas fluorescens	36.55±2.18	39.95
T_3 - Bacillus subtilis	38.30±0.66	39.48
T_4 – R. leguminosarum + P. fluorescens	28.21±1.84	52.94
T_5 – R. leguminosarum + B. subtilis	31.84±1.62	50.13
T_6 – P. fluorescens + B. subtilis	31.72±1.77	46.25

Table 2. Disease severity (%) of Cowpea plants treated with different PGPR

Treatments	Cowpea		
	20th day	40th day	60th day
T_0 – Control	8.00±0.398	41.45±0.98	68.33±1.639
T_1 - Rhizobium leguminosarum	-	33.13±5.588	53.07±1.055
T_2 - Pseudomonas fluorescens	-	35.34±1.368	52.22±2.106
T_3 - Bacillus subtilis	-	35.86±0.850	54.44±1.472
T_4 – R. leguminosarum+ P. fluorescens	-	25.23±0.163	46.60±1.727
T_5 – R. leguminosarum + B. subtilis	-	28.01±0.356	51.61±1.411
T_6 – P. fluorescens + B. subtilis	-	29.57±0.152	53.54±1.226

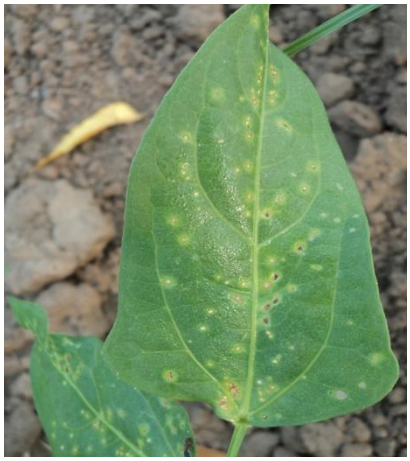

Fig. 1 Brown Blotchdisease **Fig. 2 Cowpea plants treated with different PGPRs**

CONCLUSION

Results of the present study have shown that the disease severity caused by Colletotrichum truncatumin cowpea is reduced when treated with different PGPR agents as single and dual inoculants.

REFERENCES

Ahemad.M. and M. Kibret, 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University Science, 26: 1–20.

Bankole, S.A. and A. Adebanjo, 1996. Bacterial isolates in controlling wet rot disease of cowpea caused by Pythiumaphanidermatum. J. Plant Protect. Tropics., 11: 37-43.

Sayyed, R.Z. and S.B. Chincholkar, 2006.Purification of siderophores of Alcaligenesfaecalis on amberlite XAD.Bioresour.Technol., 97: 1026- 1029.

Vincent, J.M., 1947. Distortion of fungal hyphae in the presence of certain inhibitors.Nature, 150: 850.

Wheeler, B.E.J., 1969. An Introduction to Plant Diseases.John Wiley and Sons Limited, London. p. 301.