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Research Article

INFLUENCE OF MICROBIAL CONSORTIA ON WILT SUPPRESSION AND YIELD PARAMETERS OF REDGRAM (*Cajanus cajan* L.)

AVINASH M.*, NAGARAJU K. AND LOHITH N.
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru, 560065, Karnataka, India
*Corresponding Author: Email - avi.mukhtedar@gmail.com

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Abstract- A field experiment was conducted to evaluate the efficiency of rhizosphere isolates as consortia to control wilt disease. Three rhizosphere isolates (RGB8, RGP7 and RGT4) were selected as efficient biocontrol agents based on their antagonistic activity against the pathogen and used for the development of microbial consortia. The results revealed that treatments inoculated with all the three isolates along with recommended dose of fertilizers (T8: RGB8+ RGP7+ RGT4+ RDF), showed significantly higher disease suppression and higher seed yield compared to all other treatments.

Keywords- *Biocontrol agents, Fusarium udum, Wilt, Antagonistic activity, Seed yield*

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Introduction

Pigeonpea (*Cajanus cajan* L.) is widely grown by small farmers in the semi-arid tropics as a backyard subsistence crop. It is also called as Redgram, Arhar and Tur. It is mainly cultivated and consumed in developing countries of the world and produced commercially in India, Myanmar, Kenya, Malawi, Uganda and few countries of Central America. It is the second important pulse crop after chickpea in India. It is grown in a wide range of agro-ecological conditions, its deep rooting and drought tolerant characters make it especially useful crop in the area of less and uncertain rainfall and on the lighter soils. It is widely grown in India and India is the largest producer and consumer of Redgram in the world. Redgram is a protein rich staple food and grown mainly as pure *kharif* monocrop or intercropped with Greengram, Blackgram.

Fusarium wilt is the economically devastating fungal disease of Redgram and is common throughout India. It is prevalent in Andhra Pradesh, Maharashtra, Madhya Pradesh, Uttar Pradesh and Bihar. It is susceptible to wilt pathogen throughout its development stages. However, the symptoms are more pronounced and the damage is greater at flowering and pod formation stage. Wilting of seedlings and grown up plants as if they have suffered from water shortage although there is plenty of moisture in the field, is the main symptom. A voluminous work has been done on *F. udum* Butler in India and abroad. The pathogen is a soil and seed borne. The genus Fusarium has wide host range and survives for long time in field in the absence of host plant. Therefore, chemical control is not satisfactory, adequate and non-economical as a long-term solution. Considering, the crop health and economic losses, the alternative to this is to explore the possibility of improving genetical disease resistance and integration of chemical and biological control, which can be successfully adopted in modern agriculture.

Material and methods

Isolation of bacteria and fungi from healthy redgram rhizosphere

Serial dilution technique was followed to isolate antagonistic bacteria and fungi in this experiment [1]. 'Soil sample were collected from' rhizosphere of healthy redgram plants. The soil was dried under shade and then used for serial dilution. Diluted suspension of 10-6 for bacteria and 10-4 dilution for fungi was inoculated by pour plate method. Petri plates were incubated at 27°C for 24h. After incubation period the plates were observed for growth of colonies. Isolated and well developed colonies were selected and used for purification of bacterial and fungal isolates. Nutrient agar medium was used for isolation of bacteria and potato dextrose agar was used for fungi. Rhizosphere isolates were subjected to screening against *Fusarium udum* isolated from wilt affected redgram plant under in vitro conditions by two methods such as dual plate assay and mycelial dry weight reduction assay.

Compatibility test of the selected biocontrol agents

Two bacterial (RGB8 and RGP7) and one fungal (RGT4) biocontrol agents were selected as best biocontrol agents amongst forty bacterial and ten fungal isolates respectively. These three isolates were checked for their compatibility by following the technique described by Fukui *et al*. (1994) [2]. Bacterial cultures were streaked on single plate in such a way that the streaks touches each other. For bacterial and fungal agents on one side of plate bacteria was streaked and on other side fungal disc was placed. The plates were incubated at 30°C for 48-72 h. The compatibility was tested by overgrowth or by inhibition of the test organisms and observations were recorded. The results found that isolates were compatible with each other.

Preparation of bio inoculants in small scale for field experiments

Nutrient broth for bacterial inoculants was prepared in conical flasks and the medium was sterilized at 121°C for 30 minutes. The conical flasks were inoculated with 1ml of the standard inoculum of selected bacterial isolates and for fungal isolate potato dextrose broth was prepared and fungal mycelial disc was inoculated. The conical flasks were incubated at 28°C (±2) on rotary shaker for 24h. Liquid inoculum was used for both field and greenhouse experiments.

Treatment details for field experiments:

Treatment	Particulars
T_1	RGB8 + RDF
T_2	RGP7 + RDF
T_3	RGT4 + RDF
T_4	RGB8 + RGP7 + RDF
T_5	RGB8 + RGT4 + RDF
T_6	RGP7 + RGT4 + RDF
T_7	RGB8 + RGP7 + RGT4
T_8	RGB8 + RGP7 + RGT4 + RDF
T_9	RDF only
T_{10}	Control

Note: RGB- Redgram Bacillus isolate, RGP- Redgram Pseudomonas isolate, RGT- Redgram Trichoderma isolate, RDF- Recommended dose of fertilizer.

Control of wilt by biocontrol agents: biological control efficacy

Biological control efficacy was calculated using the following formula given by Guo *et al*. (2004).

BCE = (DIPC-DIT/ DIC) x 100

Where,

DIPC-Disease incidence in control,

DIT- Disease incidence in treatment group

Seed yield: The air dried pods from net plot area were threshed, cleaned and the weight of the seeds was recorded. Based on the yield from net plot, the seed yield per hectare was calculated and expressed in kg ha-1.

Results and discussion

Biocontrol efficiency of rhizosphere isolates against wilt causing pathogen of redgram (*Cajanus cajan* L.) under field conditions

As the main objective of research the wilt disease control was carried out using different combinations of biocontrol agents in field and observations were made. The results obtained were presented in [Table-1].

Disease incidence was observed in all treatments at regular intervals of plant growth and it was found that there is no visual observations of wilt disease was observed at initial days of plant growth (30 DAS) and at the time of harvest the plants showed resistance to disease, hence there is no sign of disease in this stage. As typical wilt symptoms the disease appears during the flowering and grand growth stages of plant, the observations were recorded as number of wilt affected plants at 60 and 90 DAS, there after biocontrol efficiency was calculated. The results revealed that treatment T8 which was treated with consortia of all the three biocontrol agents (*Bacillus* sp., Pseudomonas sp. and *Trichoderma* sp.) along with recommended dose of fertilizers, showed significantly higher disease suppression (78.57% and 63.75% at 60 and 90 DAS) compared to all other treatments followed by T7 (69.05% and 57.36% at 60 and 90 DAS) which had consortia alone (no RDF). The control of wilt disease may be due to the combined effect of biocontrol agents by the production of antimicrobial compounds, inducing systemic resistance to the plants and competition with pathogen for nutrients and space in the rhizosphere which may reduce the virulence of pathogen there by increased disease control was observed.

Table-1 *Biocontrol efficiency of rhizosphere isolates against wilt causing pathogen of redgram (Cajanus cajan L.) under field conditions*

Treatments	Biocontrol efficiency (%)	
	60 DAS	90 DAS
T_1: RGB8 + RDF	47.62^{cd}	27.64^{f}
T_2: RGP7 + RDF	36.51^{de}	25.56^{f}
T_3: RGT4+ RDF	47.62^{cd}	34.03^{de}
T_4: RGB8+ RGP7 + RDF	57.94^{bc}	31.81^{ef}
T_5: RGB8+ RGT4+ RDF	58.73^{bc}	38.19^{cd}
T_6: RGP7+ RGT4+ RDF	52.38^{c}	40.42^{c}
T_7: RGB8+ RGP7+ RGT4	69.05^{ab}	57.36^{b}
T_8: RGB8+ RGP7+ RGT4+ RDF	78.57^{a}	63.75^{a}
T_9: RDF only	31.75^{e}	14.72^{g}
T_{10}: Control	--	--

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT).

Results were in accordance with the work done by Mishra *et al*. (2013) [3], where they evaluated individual and mixed formulations of fungal isolate PBAT43 (*T. harzianum*) and bacterial isolate PBAP27 (*P. fluorescens*) for their relative bio efficacy under glass house and field conditions. Mixed formulation exhibited increase in seed germination ranges from 25.5-72.11% and disease control 47.68-76.00% in different crops as compared to control. The widely recognized mechanisms of biocontrol mediated by PGPB are competition for an ecological niche or a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens [4] and/or abiotic stresses.

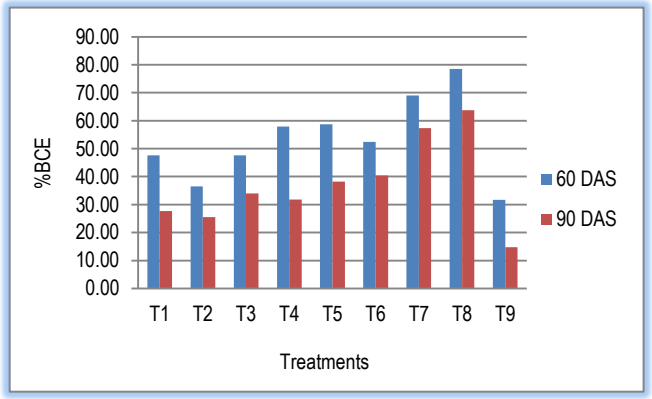

Fig-1 Effect of biocontrol agents on biocontrol efficiency against Fusarium wilt of redgram (*Cajanus cajan* L.) under field conditions.

Effect of biocontrol agents on yield parameters

Effect of applied biocontrol agents on yield parameters like number of pods per plant, pod weight per plant, seed weight per plant and seed yield was recorded among different treatments; the results obtained were presented in [Table-2].

Treatment which had recommended dose of fertilizer along with all the three microorganisms (T8) recorded significantly more number of pods per plant (115.33) which had more pod weight per plant (155.30g/plant). When seeds were separated from pods T8 recorded significantly more seed weight and yield per hectare (44.21g/plant and 1403.49kg/ha) than all other treatments. Control treatment recorded lowest seed yield (994.71kg/ha) compared to other treatments. Microorganism known to help in plant growth promotion and disease resistance, leading to increased yield of plants without affecting the soil health. Results were in agreement with Ade *et al*. (2018) [5] who reported that highest seed yield (kg ha-1) obtained due to dual seed inoculation of *Rhizobium* + PSB (2660 kg ha-1) followed by PSB (2279 kg ha-1) and *Rhizobium* (1921 kg ha-1). Combined effect *Rhizobium* + PSB improved N and P status of soil and ultimately increased N and P uptakes which enhanced yield attributes and yield of crop. Phosphorus improved in the rate of symbiotic N fixation and it turn, stimulates the growth and number of pods plant-1. Ade *et al*. (2018) [6] also reported that higher number of pods per plant were obtained due to dual seed inoculation of *Rhizobium* + PSB. It was found significantly superior over *Rhizobium* or PSB inoculation alone. Seed inoculation of PSB and *Rhizobium* was remaining at par with each other.

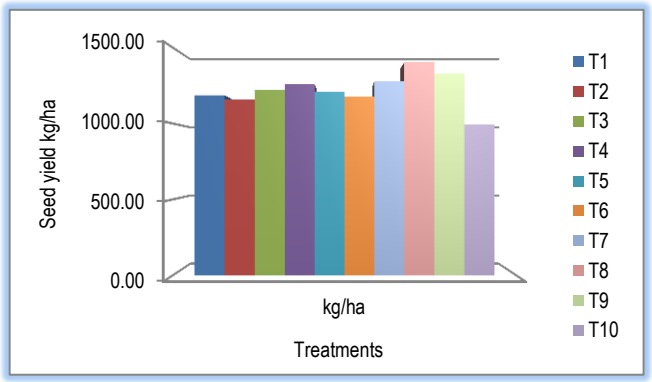

Fig-2 Effect of biocontrol agents on seed yield of redgram (Cajanus cajan L.) under field conditions.

Table-2 *Effect of biocontrol agents on yield parameters of redgram (Cajanus cajan L.) under field conditions*

Treatments	No. of pods/plant	Pod weight (g/plant)	Seed weight (g/plant)	Seed yield (kg/ha)
T_1: RGB8 + RDF	96.50^{cd}	139.50^{c}	37.33^{ef}	1185.08^{ef}
T_2: RGP7 + RDF	96.67^{cd}	150.60^{ab}	36.50^{f}	1158.73^{f}
T_3: RGT4+ RDF	97.33^{c}	148.50^{ab}	38.50^{cde}	1222.22^{cde}
T_4: RGB8+ RGP7 + RDF	97.00^{c}	145.30^{bc}	39.66^{cd}	1258.92^{cd}
T_5: RGB8+ RGT4+ RDF	98.17^{c}	139.30^{c}	38.05^{def}	1207.94^{def}
T_6: RGP7+ RGT4+ RDF	104.50^{b}	147.60^{b}	36.99^{ef}	1174.29^{ef}
T_7: RGB8+ RGP7+ RGT4	109.33^{b}	145.00^{bc}	40.27^{bc}	1278.28^{bc}
T_8: RGB8+ RGP7+ RGT4+ RDF	115.33^{a}	155.30^{a}	44.21^{a}	1403.49^{a}
T_9: RDF only	105.15^{b}	151.50^{ab}	41.83^{b}	1327.86^{b}
T_{10}: Control	91.67^{d}	126.00^{d}	31.33^{g}	994.71^{g}

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT).

Conclusion

The present study revealed that use of microbial consortia along with recommended dose of fertilizer, significantly reduced the disease incident and also improved plant yield parameters. As wilt pathogen is soil borne chemical control of disease will reduce the soil health and inhibits native microflora. So use microbial consortia along with recommended dose of fertilizers can be recommended as ecofriendly measure of disease control and also used for increased yield.

Application of research: Microbial consortia along with recommended dose of fertilizers can be recommended as ecofriendly measure of disease control and also used for increased yield.

Research Category: Crop improvement

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Study area / Sample Collection: Department of Agricultural Microbiology, UAS, Bengaluru, 560065, Karnataka, India

Cultivar / Variety / Breed name: Pigeonpea (*Cajanus cajan* L.)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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