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EXPLOITING *RHIZOBIUM, AZOTOBACTER, AZOSPIRILLUM* AND *ACETOBACTER* BACTERIA AS BIO-FUNGICIDE AGAINST WILT CAUSING FUNGI

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Key word: *Antagonistic Rhizobium, Azotobacter, Azospirillum, Acetobacter, Fusarium oxysporium and Sclerotium rolifsii.*

Abstract–The current study was carried out at the Department of Agricultural Microbiology, College of Agriculture, Raipur, C.G., during the years 2021–2022. This study was set up with the general objective of investigating the interaction between *Fusarium* and plant nutrient mobilizing bacterial isolates namely Rhizo-L3, Rhizo-L4, Azoto-MW15, Azoto-Palak16, Azos-Palak13, Azos-Palak17, Aceto-Root18 and Aceto-RD15. These isolates were selected and tested to control wilt disease causing fungi. In this connection, these isolates were tested for different biochemical parameters viz.starch hydrolysis, catalase, urease, oxidase test etc. Further these isolates were evaluated for their ability to inhibit the mycelial biomass of wilt causing fungi *Fusarium oxysporium* and *Sclerotium rolfsii* in modified Martin broth and agar media. Results show that the maximum growth inhibition of *Fusarium oxysporium* (83.62%) Azoto-MW15 + Azoto-Palak16 exhibited followed by mixed cultures of Rhizo-L3 and Rhizo- L4 (79.40%), Aceto-RD15 + Aceto-Root18 (74.39 %) and Azos-Palak13 + Azos-Palak17 (72.99 %), while, in plate assay with *Sclerotium rolfsii* also showed inhibition of 56.44%, 53.19% and 52.21 % respectively.

INTRODUCTION

Wilt caused by *Fusarium oxysporium* is one of the most important diseases of pulse and oils seed crops grown in Chhattisgarh state which account towards almost total failure of some of the legume crops in the wilt affected areas. *Fusarium* wilt is caused by soil-borne fungus-*Fusarium oxysporum*, present in most of the major pulse crops growing areas and causing yield losses reaching sometimes as high as up to 90% (Jendoubi *et al.,* 2017; Sonatakke *et al*. 2020). *Fusarium* wilt infection was found to be a major chickpea and lentil illness in central and southern India (Ghosh *et al.,* 2013). Collar rot and root rot are the other associated disease occurring from pre emergence to the maturity of the crop and are commonly designated as chickpea or lentil wilt complex. *Sclerotium rolfsii* is also known to have a very wide host range. It is one of the most important causal organisms responsible for rotting in legumes. *Sclerotium rolfsii* is a non-specialized soil borne fungal pathogen of worldwide importance and has a host range of over 500 species (Punja, 1988). The fungi can attack the crop during any time from seedling to flowering stage and are comparatively more destructive at the seedling stage and also gradually turn the crop pale, droop and dry (Njambere and Chen, 2011). The disease causes appreciable loss in yield it is necessary to reduce the loss caused by this disease.

Huge quantity of fungicides is being used to reduce intensity of this disease. It often leads to atmospheric pollution and development of fungicidal resistant strains of the pathogen and the upset of biological equilibrium in soil. Now a day, as a component of integrated pest management (IPM), researchers have initiated to include bio-control of phyto-pathogens in the crop protection (Mukhopadhyay, 1987). Despite the proven applicability of rhizobia in combination with other beneficial bacteria to control diseases and improve productivity in legumes, research data are

insufficient regarding the Bacteria-*Fusarium*-Legume interaction. Therefore, there is a need to explore different rhizobacteria mixtures, which interact synergistically to improve sustainable legume production (Naseri, 2014, Naseri and Tabande, 2017). Keeping in view, the problem of the environmental pollution by use of chemicals in intensive agriculture, it is certainly useful to develop eco-friendly, effective and low cost biological agents to control wilt causing fungi. Therefore, this study is planned in order to exploit non-hazardous microbes like *Rhizobium, Azotobacter, Azospirillum* etc for their multi-dimensional use apart from their known activity of atmospheric nitrogen fixation.

MATERIALS AND METHODS

Morphological characterization of bacterial isolates

The pure culture of these isolates were tested for the following morphological properties in which different colour, forms, margin, elevation and gram's reaction were examined.

Biochemical characterization of bacterial isolates

The isolates were characterized using standard biochemical method as given in Bergey's Manual of Systematic Bacteriology (Peter, 2001) the urease test, catalase test, starch hydrolysis test and oxidase test.

Fungal biomass inhibition in broth media

In this study, the antagonistic potential of bacterial isolates against *Fusarium oxysporum* was quantitatively evaluated in the broth medium. Fresh bacterial and fungus cultures were inoculated in 250 ml conical flasks containing Martin modified media at 28 °C. As a control, a broth medium was inoculated only with the *Fusarium*. By passing 5 day-old dual cultures through pre-weighed filter paper (Whatmann No.1), the difference in fresh and dry weight of the fungal biomass among treatments (wilt causing the fungal pathogen+antagonistic bacteria) and control (the pathogen only) were recorded. The filter papers were weighed after drying for 24 hours at 60 °C. The fresh and dry weight of the test fungus was estimated.

Antagonistic effect of bacterial isolates

The antagonistic study was also carried out between isolates of *Rhizobium, Azotobacter, Azospirillium* and *Acetobacter* and the fungal culture of *Sclerotium rolfsii* on the agar plates. The plates were then incubated for 3-6 days and the antagonistic effect was studied by measuring the diameter (in mm) of the fungal growth in the plate.

Determination of % inhibition of fungal growth

$$\% \text{ fungal growth area inhibition} = \frac{\text{Total fungal growth area with Antagonistic bacteria}}{\text{Total fungal growth area}} \times 100$$

RESULTS AND DISCUSSION

Morphological characterization

All the 8 isolates were selected for morphological studied and were confirmed as *Rhizobium, Azotobacter, Azospirillum* and *Acetobacter* morphological characteristics and their gram's reaction (Table 1)

Biochemical characterization of bacterial isolates

A series of biochemical test were carried out for a better understanding of the physiochemical functions going on within the cell. The isolates *Rhizobium*, *Azotobacter*, *Azospirillum* and *Acetobacter* were tested for different biochemical properties. The isolates Rhizo-L 3, Rhizo-L 4, Aceto-RD 15 and Aceto-Root 18 found positive for urease test. In case

Table 1. Morphological characteristics of bacterial isolates

Bacterial isolates	Gram's reaction	Morphology character			
		Colour	Forms	Margin	Elevation
Rhizo-L3	-ve	White translucent	Circular	Entire, smooth	Convex
Rhizo-L4	-ve	White translucent	Circular	Entire, smooth	Convex
Azoto-MW15	-ve	White	Circular	Entire	Convex
Azoto-Palak16	-ve	White	Circular	Entire	Convex
Azos-Palak13	-ve	Pale green	Circular	Entire	Raised
Azos-Palak17	-ve	Pale green	Circular	Entire	Raised
Aceto-RD15	-ve	Yellowish	Circular	Smooth	Convex
Aceto-Root18	-ve	Yellowish	Circular	Smooth	Convex

➢ (+) = Positive, (-) = Negative

of catalase test the isolates Rhizo-L 3, Rhizo-L 4, Azos-Palak 13, Azos-Palak 17, Aceto-RD 15 and Aceto-Root 18 shows positive while isolates Rhizo-L3, Rhizo-L 4, Azoto-MW 15, Azoto-Palak 16, Azos-Palak 13, Azos-Palak 17, Aceto-RD 15 and Aceto-RD 15 found positive for starch hydrolysis test. Isolates Rhizo-L 3, Rhizo-L 4, Azoto-MW 15, Azoto-Palak 16 Aceto-RD1 5 and Aceto-RD1 5 were found positive for oxidase test. The results are close to findings of Soundarya Shree *et al.* (2022); Jain *et al.* (2021); Natarajan (2022); Kowser and Uddin (2015); Painkra *et al.* (2019).

Antagonistic relationship of *Rhizobium* isolates against *Fusarium oxysporium* and *Sclerotium rolfsii*

The data presented in Table 3 showed that on broth medium, maximum growth inhibition of *F. oxysporium* (79.40 %)by dual inoculation of *Rhizobium* isolates Rhizo-L3 + Rhizo-L4 (T_8), while, minimum inhibition in the fungal growth (59.87 %) was associated with Treatment T_6 (Rhizo-L3). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*F. oxysporium* alone), Similarly the data presented in Table 3 on agar plate, maximum growth inhibition of *S. rolfsii* (53.19 %) by dual inoculation of *Rhizobium* isolates Rhizo-L3 + Rhizo-L4 (T_8), while, minimum inhibition (41.22 %) in the fungal growth was associated with Treatment T_6 (Rhizo-L3). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*S. rolfsii* alone). Muthukumar and Suthinraj (2019) also reported that *Pseudomonas sp*. gave antagonistic test to mycelial growth of *Sclerotium rolfsii* accounting for 74.25% reduction in the mycelial growth over control. Similarly Saini *et al.* (2019) did evaluation of bacteria isolated from wheat rhizosphere for plant growth promoting attributes and antagonistic activity against *Sclerotium rolfssi* and found 60 percent inhibition in the fungal growth.

Antagonistic relationship of *Azotobacter* isolates against *Fusarium oxysporium* and *Sclerotium rolfsii*

The data presented in Table 4 showed that on broth

Table 2. Biochemical characterization of bacterial isolates

Bacterial isolates	Urease test	Catalase test	Starch hydrolysis test	Oxidase test
Rhizo-L3	+	+	+	+
Rhizo-L4	+	+	+	+
Azoto-MW15	-	-	+	+
Azoto-Palak16	-	-	+	+
Azos-Palak13	-	+	+	-
Azos-Palak17	-	+	+	-
Aceto-RD15	+	+	+	+
Aceto-Root18	+	+	+	+

➢ (+) = Positive, (-) = Negative

Table 3. Efficacy of different isolates of *Rhizobium* against the growth of *Fusarium oxysporium* and *Sclerotium rolfsii*

Treatmentdetail	Mycelial biomass of *F. oxysporium*		% Inhibition of *F. oxysporium* (Surface area)	Treatment detail %	Inhibition of *S. rolfsii* (Surface area)
	Wetwt.(g)	Drywt.(g)			
T_1 Control	0.000*	0.000*	0.000*	Control	0.000*
T_2*Fusarium*	1.803	0.018	0.000**	*Sclerotium*	0.000**
T_3 Rhizo-L3	0.000*	0.000*	0.000*	Rhizo-L3	0.000*
T_4 Rhizo-L4	0.000*	0.000*	0.000*	Rhizo-L4	0.000*
T_5 Rhizo-L3 + Rhizo-L4	0.000*	0.000*	0.000*	Rhizo-L3+Rhizo-L4	0.000*
T_6 *Fusarium* + Rhizo-L3	1.500	0.015	59.87	*Sclerotium*+Rhizo-L3	41.22
T_7 *Fusarium* + Rhizo-L4	1.370	0.014	70.21	*Sclerotium*+Rhizo-L4	46.60
T_8 *Fusarium* + Rhizo-L3 + Rhizo-L4	1.223	0.013	79.40	*Sclerotium*+Rhizo-L3 + Rhizo-L4	53.19
CD (0.5%)	0.035	0.001	1.84		1.23

➢ * Zero fungal growth due to No Fungal Inoculation

➢** Zero fungal inhibition because of No Antagonistic Bacterial

medium, maximum growth inhibition of *F. oxysporium* (83.62 %)by dual inoculation of *Azotobacter* isolates Azoto-MW15 + Azoto-Palak16 (T_8), while, minimum inhibition in the fungal growth (62.49 %) was associated with Treatment T_6 (Azoto-Palak16). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*F. oxysporium* alone), Similarly the data presented in Table 4 on agar plate, maximum growth inhibition of *S. rolfsii* (56.44 %) by dual inoculation of *Azotobacter* isolates Azoto-MW15 + Azoto-Palak16 (T_8), while, minimum inhibition (40.97 %) in the fungal growth was associated with Treatment T_6(Azoto-Palak16). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*S. rolfsii* alone) Muthukumar and Suthinraj (2019) also reported that *Pseudomonas sp*. showed antagonistic affect to mycelial growth of *Sclerotium rolfsii* accounting for 74.25% reduction over control. Similarly Saini *et al.* (2019) did evaluation of bacteria isolated from wheat rhizosphere for plant growth promoting attributes and antagonistic activity against *Sclerotium rolfssi* and found 60 per cent inhibition in the fungal growth.

Antagonistic relationship of *Azospirillum* isolates against *Fusarium oxysporium* and *Sclerotium rolfsii*

The data presented in Table 5 showed that on broth medium, maximum growth inhibition of *F. oxysporium* (72.99 %) by dual inoculation of *Azospirillum* isolates Azos-Palak13 + Azos-Palak17 (T_8), while, minimum inhibition in the fungal growth (52.29 %) was associated with Treatment T_7 (Azos-Palak17). It was clearly observed that T_8 gave

Table 4. Efficacy of different isolates of *Azotobacter* against the growth of *Fusarium oxysporium* and *Sclerotium rolfsii*

Treatment detail	Mycelial biomass of *F. oxysporium*		% Inhibition of *F. oxysporim*	Treatment detail	% Inhibition of *S. rolfsii*
	Wet wt.(g)	Dry wt.(g)	(Surface area)		(Surface area)
T_1 Control	0.000*	0.000*	0.000*	Control	0.000*
T_2 *Fusarium*	1.803	0.019	0.000**	*Sclerotium*	0.000**
T_3 Azoto-Palak16	0.000*	0.000*	0.000*	Azoto-Palak16	0.000*
T_4 Azoto-MW15	0.000*	0.000*	0.000*	Azoto-MW15	0.000*
T_5 Azot-Palak16 + Azoto-MW15	0.000*	0.000*	0.000*	Azot-Palak16 + Azoto-MW15	0.000*
T_6 *Fusarium*+ Azoto-Palak16	1.460	0.015	62.49	*Sclerotium* + Azoto-Palak16	40.97
T_7 *Fusarium* + Azoto-MW15	1.197	0.012	78.34	*Sclerotium* + Azoto-MW15	54.23
T_8 *Fusarium* + Azoto-Palak16 + Azoto-MW15	1.143	0.011	83.62	*Sclerotium* + Azoto-MW15+ Azoto-Palak16	56.44
CD (0.5%)	0.025	0.001	1.00		2.67

- * Zero fungal growth due to No Fungal Inoculation
- ** Zero fungal inhibition because of No Antagonistic Bacterial Inoculation

Table 5. Efficacy of different isolates of *Azospirillum* against the growth of *Fusarium oxysporium* and *Sclerotium rolfsii*

Treatment detail	Mycelial biomass of *F. oxysporium*		% Inhibition of *F. oxysporium*	Treatment detail	Inhibition of % *S. rolfsii*
	Wet wt.(g)	Dry wt.(g)	(Surface area)		(Surface araea)
T_1 Control	0.000*	0.000*	0.000*	Control	0.000*
T_2 *Fusarium*	1.803	0.018	0.000**	*Sclerotium*	0.000**
T_3 Azos-Palak13	0.000*	0.000*	0.000*	Azos-Palak13	0.000*
T_4 Azos-Palak17	0.000*	0.000*	0.000*	Azos-Palak17	0.000*
T_5 Azos-Palak13 + Azos-Palak17	0.000*	0.000*	0.000*	Azos-Palak13 +Azos-Palak17	0.000*
T_6 *Fusarium* + Azos-Palak13	1.547	0.015	64.28	*Sclerotium* +Azos-Palak13	44.80
T_7 *Fusarium* + Azos-Palak17	1.450	0.017	52.29	*Sclerotium* +Azos-Palak17	31.06
T_8 *Fusarium* + Azos-Palak13 + Azos-Palak17	1.373	0.014	72.99	*Sclerotium* +Azos-Palak13 + Azos-Palak17	45.71
CD (0.5%)	0.030	0.001	1.69		1.47

- * Zero fungal growth due to No Fungal Inoculation
- ** Zero fungal inhibition because of No Antagonistic Bacterial

significantly higher inhibition in the fungal growth over T_2 (*F. oxysporium* alone), Similarly the data presented in Table 5 on agar plate, maximum growth inhibition of *S. rolfsii* (45.71 %) by dual inoculation of *Azospirillum* isolates Azos-Palak13 + Azos-Palak17 (T_8), while, minimum inhibition (31.06 %) in the fungal growth was associated with Treatment T_7 (Azos-Palak 17). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*S. rolfsii* alone). Muthukumar and Suthinraj (2019) also reported that *Pseudomonas sp*. gave antagonistic test to mycelial growth of *Sclerotium rolfsii* accounting for 74.25% reduction in the mycelial growth over control. Similarly Saini *et al.* (2019) did evaluation of bacteria isolated from wheat rhizosphere for plant growth promoting attributes and antagonistic activity against *Sclerotium rolfssi* and found 60 per cent inhibition in the fungal growth.

Antagonistic relationship of *Acetobacter* isolates against *Fusarium oxysporium* and *Sclerotium rolfsii*

The data presented in Table 6 showed that on broth medium, maximum growth inhibition of *F. oxysporium* (74.39 %)by dual inoculation of *Acetobacter* isolates Aceto-RD15 + Aceto-Root18 (T_8), while, minimum inhibition in the fungal growth (57.32 %) was associated with Treatment T_6 (Aceto-RD15). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*F. oxysporium* alone), Similarly the data presented in Table 6 on agar plate, maximum growth inhibition of *S. rolfsii* (47.32 %) by dual inoculation of *Acetobacter* isolates Aceto-RD15 + Aceto-Root18 (T_8), while, minimum inhibition (38.24 %) in the fungal growth was associated with Treatment T_6 (Aceto-RD15). It was clearly observed that T_8 gave significantly higher inhibition in the fungal growth over T_2 (*S. rolfsii* alone). Muthukumar and Suthinraj (2019) also reported that *Pseudomonas sp*. gave antagonistic test to mycelial growth of *Sclerotium rolfsii* accounting for 74.25% reduction in the mycelial growth over control. Similarly Saini *et al.* (2019) did evaluation of bacteria isolated from wheat rhizosphere for plant growth promoting attributes and antagonistic activity against *Sclerotium rolfssi* and found 60 per cent inhibition in the fungal growth.

CONCLUSION

Results show that the maximum growth inhibition of *Fusarium oxysporium* (83.62%) exhibited in treatment T_8 by mixed culture of bacteria isolates i.e. Azoto-MW15 + Azoto-Palak16 followed mixed cultures of Rhizo-L3 and Rhizo- L4 (79.40%) similarly by dual culture of Aceto-RD15 + Aceto-Root18 (74.39%) in broth medium while, in plate assay with *Sclerotium rolfsii* also showed inhibition 56.44%, 53.19% and 47.32 % respectively.

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Compliance with ethical standards

Conflict of interest Author (s) declares that they do not have any conflict of interest.

Table 6. Efficacy of different isolates of *Acetobacter* against the growth of *Fusarium oxysporium* and *Sclerotium rolfsii*

Treatment detail	Mycelial biomass of *F. oxysporium*		% Inhibition of *F.oxysporium* (Surface area)	Treatment detail (Surface area)	% Inhibition of *S. rolfsii*
	Wet wt.(g)	Dry wt.(g)			
T_1 Control	0.000*	0.000*	0.000*	Control	0.000*
T_2 *Fusarium*	1.803	0.018	0.000**	*Sclerotium*	0.000**
T_3 Aceto-RD15	0.000*	0.000*	0.000*	Aceto-RD15	0.000*
T_4 Aceto-Root18	0.000*	0.000*	0.000*	Aceto-Root18	0.000*
T_5 Aceto-RD15 + AcetoRoot-18	0.000*	0.000*	0.000*	Aceto-RD15 + AcetoRoot-18	0.000*
T_6 *Fusarium* + Aceto-RD15	1.493	0.016	57.32	*Sclerotium* + Aceto-RD15	38.24
T_7 *Fusarium* + Aceto-Root18	1.427	0.014	64.86	*Sclerotium* + Aceto-Root18	42.39
T_8 F*usarium* + Aceto-RD15 + Aceto-Root 18	1.337	0.012	74.39	*Sclerotium* + Aceto-RD15 + Aceto-Root18	47.32
CD (0.5%)	0.037	0.001	1.57		1.81

- * Zero fungal growth due to No Fungal Inoculation
- ** Zero fungal inhibition because of No Antagonistic Bacterial

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