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Assessing the Biocontrol Potentials of *Azotobacter* Isolates against *Fusarium* Wilt of Chilli (*Capsicum annum* L.) Under *In vivo* Condition

Swapna[1*], K. Tamil Vendan[1], Mahadevaswamy[1], D. S. Aswathanarayana[2] and R. C. Gundappagol[1]

[1]Department of Agricultural Microbiology, [2]Department of Plant Pathology College of Agriculture, University of Agricultural Sciences, Raichur- 584104

*Corresponding Author E-mail: swapnav1357@gmail.com

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ABSTRACT

In the present investigation, four efficient Azotobacter isolates viz., AZT-R_7, AZT-Y_2, AZT-J_1 and AZT-G_4 were studied for bio efficacy against F. solani using chilli as a test crop under greenhouse condition. In chilli pot culture experiment, these four isolates along with Pseudomonas fluorescens, Bacillus subtilis, Trichoderma and Carbendazim were further tested under greenhouse conditions to assess their antagonistic activity against F. solani. The isolate AZT-R_7 recorded significantly less disease incidence of 14.66 per cent followed by Trichoderma sp (22.44 %) and carbendazim (25.12 %) while the plants from control treated with Fusarium solani recorded a significant increase in the disease incidence (38.15 %). AZT-R_7 recorded significantly increase in the activity of antioxidants, soluble protein, free phenol contents, increase in the growth and yield parameters and lower total sugar content.

Key words: *Azotobacter, Chilli, F. solani, Pseudomonas fluorescens, Bacillus subtilis and Trichoderma sp.*

INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the most important commercial spice crops of India. Chilli is used for different purposes such as vegetable, spice, condiments, sauce and pickles. The major chilli growing countries are India, China, Korea, Nigeria, U.S.S.R. Mexico *etc*.[6]. The important chilli growing states in India are Andhra Pradesh, Orissa, Maharashtra, West Bengal, Karnataka, Rajasthan and Tamil Nadu. Andhra Pradesh alone commands 46 per cent of the chilli production in India. India is the largest producer and consumer of chilli, grown over an area of 0.79 million ha with an annual production of 0.13 million tons with the productivity of 1.5 t/ha[2]. India ranks second among world's chilli exporting countries and has showed a steady decline in chilli trade due to domestic consumption.

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In Karnataka, northern Karnataka is an important chilli growing area and it is highly concentrated in the districts like Dharwad, Haveri, Koppal, Bellary, Raichur, Kalaburagi and Belgaum. Karnataka ranks second in area with 100.73 ('000 ha) and production of 107.00 ('000 MT) dry chilli after Andhra Pradesh[2]. The chilli area, production and productivity are in decreasing trend even though it is a highly profitable commercial spice and vegetable crop. Many factors operate in the successful cultivation, production, marketing and exporting of the quality chilli, of which diseases play an important role. Wilt diseases are caused by major fungal pathogens like *Fusarium solani*, *Sclerotium rolfsii*, *Rhizoctonia solani* and bacterial pathogen *Ralstonia solanacearum*. Amongst the wilt diseases of chilli, *Fusarium* wilt has become a serious problem in recent years in almost all chilli growing tracts of India, especially in black cotton soils leading up to 20 per cent yield loss[4]. The occurrence of the disease was first reported from New Mexico as early as 1919 as a rapid wilt. The disease is typically a soil borne one and the pathogens perpetuates in soil for a longer period of time. The chemical control of the soil borne diseases are frequently ineffective because of the physical and chemical heterogeneity of the soil which may prevent the effective concentration of the chemical from reaching the pathogen and also it is un economical and hazardous to soil health. Hence, best alternative measure is to look for potential biocontrol agents which colonize the rhizosphere such as *Pseudomonas fluorescens, Bacillus sp*. *Azotobacter sp.* and *Trichoderma sp.*

Among the commercial biocontrol agents, complex cyst forming *Azotobacter* isolates with high rhizosphere competency will overcome the limitations in commercial production. The present study aims at the selection of an efficient *Azotobacter* isolate with multiple biocontrol traits and development of *Azotobacter* inoculants with commercialization potential, a major challenge hindering the bioinoculant production technology.

MATERIAL AND METHODS

Pot culture experiment

Azotobacter inoculants *viz*., AZT-R_7, AZT-Y_2, AZT-J_1 and AZT-G_4 were prepared as per the standard procedure. The root portions of the chilli seedlings were dipped in the slurry (10^{-9} *Azotobacter* cells ml^{-1}) and planted in the pots containing the pathogen (20 g kg^{-1} soil). The uninoculated pot was served as control.

Plant parameters recorded

The efficient *Azotobacter* selected from *in vitro* studies were treated with the chilli seedlings and transplanted in sick pots, observation on Per cent Disease Incidence (PDI) and growth and yield of chilli was recorded at periodical intervals. Total sugars were estimated by Nelson somogy's method, soluble protein by Lowry's method and free phenols in plant samples by Folin Ciocalteu's reagent (FCR).

Antioxidant properties of chilli

One gram of the leaf sample was homogenized with 1 ml of 0.1 M sodium phosphate buffer (pH 7.0), used for the estimation of catalase and peroxidase enzymes at 40 DAP.

Catalase activity

The reaction mixture was made by mixing of 3 ml phosphate buffer (0.1 M, pH 7.0), two ml of H_2O_2 (2.5 mM) and 1 ml enzyme extract and incubated for 1 min and the reaction was stopped by adding 10 ml 0.7 N sulphuric acid. Then, the reaction mixture was titrated against 0.01 N $KMnO_4$ until a faint purple color persists for at least 15 sec. The enzyme activity was expressed as min^{-1} mg $^{-1}$ of protein[3].

Peroxidase activity

Leaves were extracted in 0.1 M phosphate buffer (pH 6.0) with the rate of 0.1 g leaves per 1 ml buffer and then centrifuged at 10,000 rpm at 4 ^{0} C for 5 min. Formation of tetraguaiacol was performed in a 3 ml reaction mixture containing: 1 ml of 0.1 M phosphate buffer (pH 6), 1 ml of 15 mM 2-Methoxyphenol (Guaiacol), one ml of 3 mM H_2O_2 and 15 µl of enzyme extract. Peroxidase activity was determined at 470 nm with a spectrophotometer. One unit of peroxidase

represents the amount of enzyme catalyzing the oxidation of 1 mol of Guaiacol in 1 min[5].

RESULTS AND DISCUSSION

Growth and yield parameters of chilli under pot culture experiment

Growth parameters *viz*, plant height, number of branches per plant, shoot and root dry weight and total dry matter production were found significantly increased in the plants treated with *Azotobacter* isolate AZT-R_7 and AZT-Y_2 compared to all other inoculants at both 40 and 60 DAP (Table 2 and Plate 1). Selvarathi *et al.*[15] also mentioned that addition of 3 per cent *Azotobacter* in the substrate increased the shoot length of tomato plants by 77 per cent. Similar findings were reported by Raheem *et al.,*[12] and Kanchana *et al.,*[8], showed that the growth, yield and quality parameters of chilli increased significantly with the inoculation of *Azotobacter* isolates.

Significant increase in the number of fruits per chilli was observed in the plants inoculated with *Azotobacter* isolates AZT-R_7 (27) followed by AZT-Y_2 (26) compared to all other inoculants. Similar findings were reported by Sharma and Thakur[16] Kanchana *et al.*[8].

Per cent disease incidence

The infected plants exhibited foliar yellowing. The affected plants wilted and dried up, but didn't fall on the ground. The affected plants showed a brown discoloration of the vascular system and cortical rot[10].

There was a significant increase in disease incidence with the control plants (38.15 %) as compared to all other inoculants. The isolate AZT-R_7 showed significant least incidence of 14.66 per cent, which on par with the *P. fluorescens* over the control followed by *Trichoderma* and carbendazim (Table 1). Similar results were reported by Ahmad e*t al.*, 2008, antifungal activity exhibited by isolates of *Azotobacter* (16.22 %), *P. fluorescens* (11.11 %) and *Bacillus* (10 %). The symptoms produced by *Fusarium* were found to be in agreement with Wahdatullah[19] on tomato, Shyla[17], Sachidananda[14] and Ramaprasad[13] in coleus.

Estimation of total sugars, soluble protein and free phenols

In control plants total sugar content was significantly increased up to 25.96 mg g^{-1} compared to all other treatments (Table 3). The increase in sugars may be because they provide structural material for defense responses in plants and act as signal molecules regulating the plant immune system. Also, sugars enhance oxidative burst at early stages of infection, increasing lignifications of cell walls, stimulate synthesis of flavanoids and induce certain PR proteins[9]. Significantly increase in the protein content was observed with AZT-R_7 (25.87 mg g^{-1}) compared to all other inoculants followed by AZT-Y_2 (24.72 mg g^{-1}). Similar results were observed by Prakash *et al.*[11] in leaves and seeds of *Amaranthus* infected with wilt disease. There was significant increase in the free phenols content with AZT-R_7 (18.40 mg g^{-1}) followed by AZT-Y_2 (16.07 mg g^{-1}) compared to all other inoculants. The result of this study are in agreement with those of Singh *et al.*[18], who studied biochemical basis of resistance in chickpea (*Cicer arietinum* L.) against *Fusarium* wilt and reported higher increase in phenolics in resistant ones than in susceptible after treatment with cultural filtrate, indicating that phenols reach an inhibitory level to the fungus in the resistant calli.

Antioxidant properties of chilli – Catalase and Peroxidase activity

Significantly higher antioxidant properties *viz*, catalase and peroxidase were observed in the plants inoculated with *Azotobacter* isolate, AZT-R_7 compared to all other inoculants (Fig. 1). Catalase activity and peroxidase activities were ranged between 54.17 to 69.13 $min^{-1}g^{-1}$ and 9.09 to 12.65 $min^{-1}g^{-1}$, respectively. Similar results were reported by Morkunas[9] showed an increase in catalase activity in infected tissue during interactions between chickpea and *F. ciceris*. Hanifei *et al.*[7] also reported significant increase in catalase activity few days after inoculation with *Fusarium*. These results were in harmony with those found by El-Borollosy and Oraby[5].

Table 1: Bioefficacy of *Azotobacter* isolates against *Fusarium* wilt of chilli

Sl. No.	Treatment	Disease incidence (%)
1	Control	38.16[a]
2	AZT-R_7	14.66[d]
3	AZT-G_4	24.41[c]
4	AZT-J_1	27.16[b]
5	AZT-Y_2	17.47[cd]
6	Ref. *Azotobacter*	23.50[c]
7	*B. subtilis*	25.84[b]
8	*P. fluorescens*	15.47[d]
9	*Trichoderma*	22.44[c]
10	Carbendazim (0.2 %)	25.12[bc]
SEm ±		00.81
CD (1 %)		02.62

Table 2: Growth and yield parameters of chilli as influenced by the inoculation of efficient *Azotobacter* isolates

Sl. No.	Treatment	Plant height (cm)	No. of branches per plant	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Total dry matter production (g plant^{-1})	No. of fruits per plant
1	Control	20.83[d]	09.00[d]	0.41[f]	0.11[cd]	0.52[c]	21[e]
2	AZT-R_7	38.16[a]	14.00[a]	0.78[a]	0.16[a]	0.98[a]	27[a]
3	AZT-G_4	36.50[ab]	12.00[c]	0.65[c]	0.13[abc]	0.76[abc]	24[bcd]
4	AZT-J_1	36.16[a]	13.00[bc]	0.52[e]	0.11[cd]	0.64[abc]	23[d]
5	AZT-Y_2	32.50[c]	13.00[a]	0.72[b]	0.15[ab]	0.75[ab]	25[abc]
6	Ref. *Azotobacter*	32.33[c]	12.00[b]	0.60[d]	0.12[bcd]	0.52[bc]	23[d]
7	*B. subtilis*	31.16[d]	12.00[c]	0.70[b]	0.14[abc]	0.85[ab]	24[cd]
8	*P. fluorescens*	33.00[cd]	13.00[bc]	0.40[f]	0.09[d]	0.61[bc]	26[ab]
9	*Trichoderma*	34.16[bc]	13.00[bc]	0.38[f]	0.08[d]	0.52[abc]	24[bcd]
10	Carbendazim (0.2 %)	33.83[c]	12.00[bc]	0.39[f]	0.08[d]	0.50[bc]	23[d]

Table 3: Total sugars, soluble protein and free phenol contents of chilli as influenced by the inoculation of efficient *Azotobacter* isolates

Sl. No.	Treatment	Total sugars (mg g^{-1})	Soluble proteins (mg g^{-1})	Free phenols (mg g^{-1})
1	Control	25.96[e]	14.00[g]	10.86[d]
2	AZT-R_7	20.40[a]	25.87[a]	18.40[a]
3	AZT-G_4	21.03[c]	19.94[cd]	12.66[c]
4	AZT-J_1	24.15[d]	19.69[cde]	12.89[c]
5	AZT-Y_2	22.04[b]	24.72[ab]	16.07[b]
6	Ref. *Azotobacter*	23.50[d]	18.38[e]	12.45[c]
7	*B. subtilis*	24.65[d]	18.80[de]	13.04[bc]
8	*P. fluorescens*	21.84[c]	24.57[b]	15.71[b]
9	*Trichoderma*	20.02[d]	23.56[b]	14.97[bc]
10	Carbendazim (0.2 %)	23.87[d]	15.57[f]	11.60[cd]
SEm ±		0.31	0.23	0.32
CD (1 %)		1.25	0.95	1.31

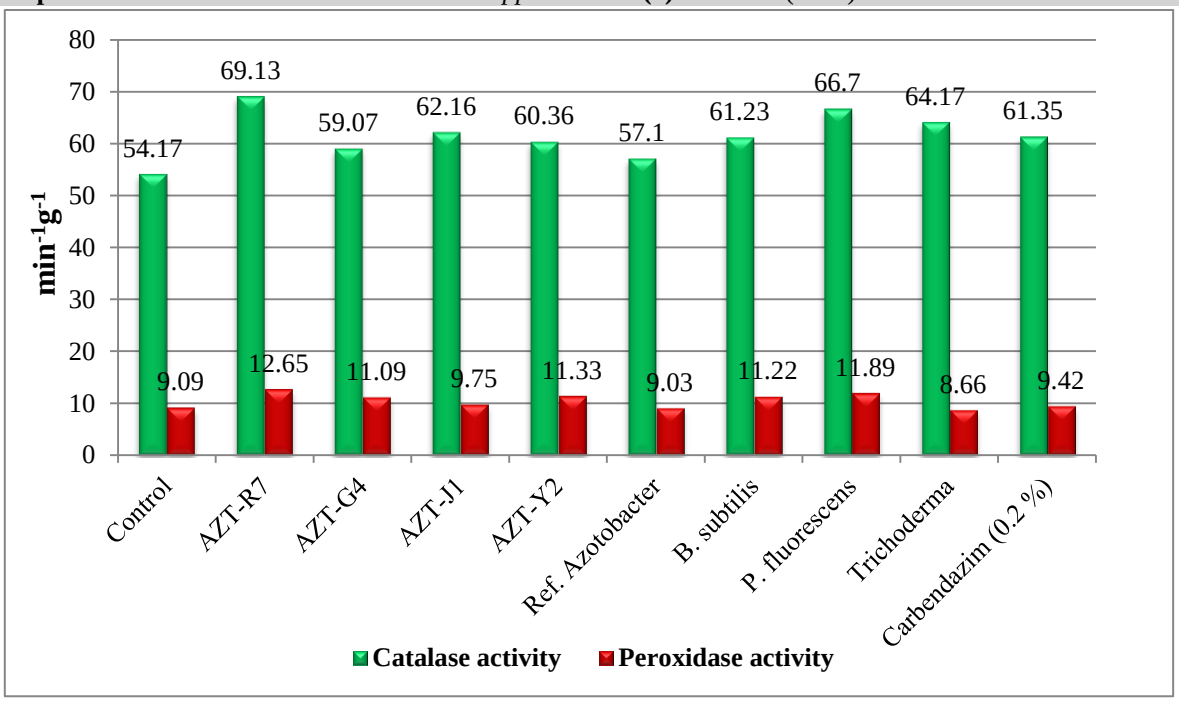

Fig. 1: Antioxidant properties of chilli as influenced by the inoculation of efficient *Azotobacter* isolates

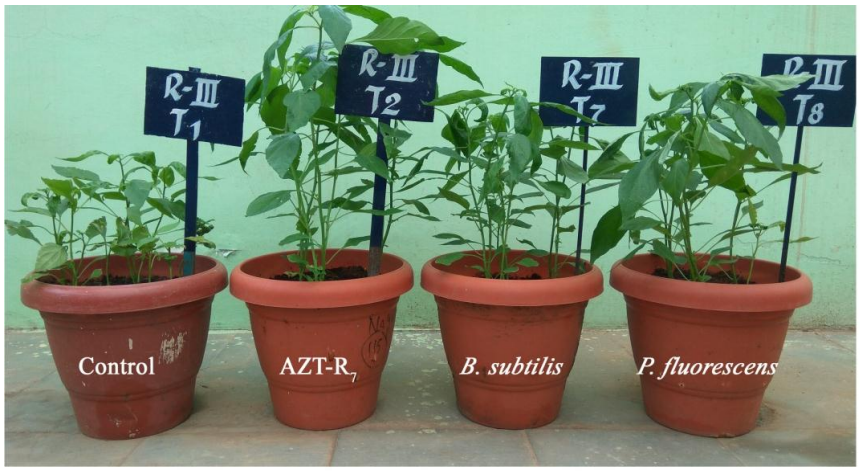

Plate 1: Influence of *Azotobacter* isolate AZT-R_7 on plant height of chilli

CONCLUSION

The role of AZT-R_7 in cultivation of chilli in sustainable agriculture is multifaceted and hence the use of this isolate would be a viable option to counteract *Fusarium solani* in cultivation of chilli as it has proved its biocontrol potential both under *in vitro* and *in vivo* in addition to fixing nitrogen and phytostimulation which would help save considerable amount of input cost in sustainable cultivation of chilli ultimately increasing the income of the farmers of Hyderabad Karnataka region.

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