

The impact of fungal infection on the growth of the *Rhizobium* strains (*Cicerarientinum* L)

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ABSTRACT

Four strains of *Rhizobium* were isolated from five different localities from Pravara area and labeled as R₁, R₂, R₃, and R₄. These isolates were tested for different fungicides with different concentrations. From the experimental finding it was interesting to note that the *Rhizobium* strains showed variation in growth to different concentration of fungicides.

Keywords –*Rhizobium*, *Strains*, *Fungicide*, *Concentrations*

INTRODUCTION

Legumes are ecologically unique, because of their ability to fix atmospheric nitrogen with the help of *Rhizobium* associated with the nodules on their roots and stems. The biological nitrogen fixation of legumes with the help of bacterial symbiotic is exploited in agriculture by using legume crops as green manure. Legume green manure comes from freshly collected green materials of selected herbs, shrubs and trees, which are rich in nitrogen and easily decomposable. Legume host plants roots that forming the root-nodules of host legumes or in close association with the plant roots. The symbiotic relationship results in huge quantities of N₂-fixation throughout the world and any adverse effect on Rhizobia results in reduced rates of biological N₂-fixation.

Cicerarientinum (L.) being rich in protein have pivotal place in the dietary of the people in semi arid tropics particularly in India. Chickpea belongs to family leguminosae and have a high efficiency of fixing atmospheric Nitrogen in the soil which in turn enriches the soil. Its root

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system is spreading type and is capable of penetrating deep, restricting the soil erosion for a considerable extent. The crop is desirable for crop rotations and fits well in various cropping systems. Many factors influence the growth of N₂-fixing rhizobia. The effects of temperature, light, moisture, soil pH, salinity, antibiotics and nutrition on rhizobia are well documented. In addition, fungicides may also influence the growth of rhizobia. Fungicide used as seed treatment to improve early plant emergence and to control the early attack by the pests. Some reports showed little damage, which may reflect the considerable variation within and in between various groups of *Rhizobium* in their sensitivity to fungicides. Sometimes these agrochemicals negatively affect the growth and multiplication of fungi and bacteria and consequently cause the disturbance of the natural soil microbial balance. Fungicides may harmfully affect the non-target soil microflora. In modern agriculture, fungicides applied as seed dressings protect germinating seeds and young seedlings against fungal pathogens. Fungicides used to increase crop yields by eliminating the harmful effects of non-target microbes. The interaction between these organisms and the fungicides can be considered as general and the often harmful interactions of *Rhizobium* and fungicides living in symbiosis with leguminous plants should be taken into consideration. The sensitivity of rhizobia to a number of fungicides is known. In contrast, some fungicides have less pronounced toxicity, and *rhizobia* are able to tolerate the effects of fungicides hence efforts were made to investigate the fungicide tolerance against different local strains of *Rhizobium*.

MATERIALS AND METHODS:

A) Isolation of *R. japonicum*

In laboratory root systems were separated and were washed with tap water to remove adhering soil particles. Two to four healthy pinkish root nodules were selected from each root system with the help of sterilized forceps. Selected nodules were surface sterilized with 0.1% mercuric chloride solution for 3 minutes then serially washed with sterilized distilled water two to three times to remove toxic residue of mercuric chloride. The sterilized root nodules were crushed in 5 ml. sterilized distilled water in sterile test tube in order to get "ozone" of *R. japonicum*. 1 ml. suspension was spread over Congo red Yeast Extract Mannitol Agar (CRYEMA) medium. (Tilak, 1993). Inoculated plates were incubated at 25 ± 2 °C for 3 to 5 days

whitishtranslucent growth was occurred on medium. After confirmation of theirbiochemicalcharactersand morphological characteristicsthesecultureswere transferred on pure yeast extract mannitol agars slants and were maintained by transfer and retransfer.

B) Fungicidal resistance:

The efficiency of isolates was studied against different fungicides like Bavistin, SAAF, Antracol, Mergeratat differentppm concentrations i.e.100ppm, 200ppm,300 ppm., 400 ppm, 500 ppm by food poisoning technique. Equalvolume of sterile nutrient medium and selected concentrations of fungicides were mixed in petridishes. After solidification ofplates a loopful isolate were inoculated on plates aseptically and incubatedatroom temperature.

RESULTS AND DISCUSSION:

Table No-1.Effect of Bavistin on different strains of *Rhizobium*

Strain	Bavistin - (Concentration in ppm)				
	100	200	300	400	500
R ₁	+	++	++	++	++
R ₂	+	+	++	++	+
R ₃	++	+++	+	+	++
R ₄	+	++	++	+++	+
Control	+	+	+	+	+

The result summarised in Table No.1 suggested that in BavistinLuxurient growth observed in the R₃ strain at 200 ppm and in R₄ strain at 400 ppm. Moderate growth observed in the R₁ strain at500 ppm & in R₂ strain300 & 400 ppm. & in R₃ strain at 100 & 500 ppm, In R₄ strain at 200 & 300 ppm. Poor growth observed in the R₁ strain at 100 ppm in R₂ strain at 100, 200& 500ppm, R₃ strain at 300, 400 ppm. In R₄ strain 100 & 500 ppm as compare to control.

TableNo : 2 – Effect of SAAF on different strains of *Rhizobium*

Strain	SAAF- (Concentration in ppm)				
	100	200	300	400	500
R ₁	+	++	++	+++	++

R ₂	++	+++	+	++	+
R ₃	+	++	+++	+	+
R ₄	++	+	+++	++	+
Control	+	+	+	+	+

The result summarised in Table No-2 suggested that in SAAF Luxurient growth observed in the R₁ strain at 400 ppm, in R₂ strain at 200 ppm In R₃ strain at 300 ppm, in R₄ strain at 300 ppm. Moderate growth observed in R₁ strain at 200, 300& 500 ppm, in R₃ strain at 200 ppm and 400 ppm. In R₃ strain at 200 ppm. In R₄ strain at 100&400ppm. Poor growth observed in R₁ strain at 100ppm, in R₂ strain300 & 500 ppm, in R₃ strainat 100, 200&500 ppm, in R₄ strain at 200 &500 ppmas compare to control.

TableNo : 3 -Effect of Merger on different strains of *Rhizobium*

Strain	Merger - (Concentration in ppm)				
	100	200	300	400	500
R ₁	++	+++	++	+	+
R ₂	++	+	+	++	+
R ₃	+	++	++	+++	+
R ₄	+	++	+++	+++	++
Control	+	+	+	+	+

The result summarized in Table No.4 suggested that in Merger Luxurient growth observed in R₁ strain at 200 ppm, in R₃ strain of 400ppm. In R₄ strain at 300&400 ppm. Moderate growth observed in R₁ strain of 100 &300 ppm, in R₂ strain 100 & 200 ppm, in R₃ strain 200, 300 ppm, in R₄ strain 200 & 500 ppm. And the poor growth in R₁ strain at 400 & 500 ppm, in R₂strain at 200,300 & 500 ppm, in R3 strain at 100 & 500 ppm in R₄ strain at 100 ppm as compare to control.

TableNo : 4 - Effect of Antracoln different strains of *Rhizobium*

Strain	Antracol - (Concentration in ppm)				
	100	200	300	400	500
R ₁	++	+	+++	+	++
R ₂	+	++	++	+++	+

R ₃	++	+	+	++	+
R ₄	+++	++	+	++	++
Control	+	+	+	+	+

The result summarized in Table No. 3 suggested that in AntracolLuxurient growth observed R₁ strain at 300ppm, in R₂ strain at 400ppm. Moderate growth observed in the R₄strain at 100& 500 ppm, in R₂ strain at 200 & 300 ppm in R₃ strain at 100 & 400 ppm, in R₄ strain at 200,400 & 500 ppm And the poor growth observed In the R₁ strain at 200 & 400ppm. In R₂ strain 100 & 500 ppm, in R₃ strain at 200, 300 &500 ppm and in R₄ strain at 300 ppm, as compare to control.

+ = Poor growth, ++ = Moderate growth, +++= Luxurient growth

CONCLUSION: The isolated local strains of rhizobium showed variation in growth as per the ppm. concentrations of fungicides.

REFERENCES:

- [1] Aamil M. Zaidi A and Khan M. S. 2004. Fungicidal impact on chickpea Mewrhizobiemsymbiosis. Journal of Enviroment Science Health #39(5-0):775-90
- [2] Jennifer fox 2007. Pesticides reduce symbiotic efficiency of nitrogen fixing Thizobiaandhostplants(ProceedingsofNationalAcademyofSciences,USA104/24) 10282-7.
- [3] Ghosh I K, Tyagi M K and Dushan J S 2003 Rhizobial compatibility againstlethal doses of pesticides in greengrais with different methods of inoculation Indian Journal of Agricultural Research.2003.(37):120 –123
- [4] CueneNFD.andDatAandGoeye M. 2003, Nodulation and nitrogen fixation of Fieldgrowncommonbean(*Phaseolusvulgaris*)asinfluencedbyfungicideseedtreatment.Afric anJournalofBiotechnology2(7) 198-201.
- [5] R K Hynes and Can J Microbiol. 2001 *Rhizobium* population dynamics in thepea rhizosphere of rhizobial inoculant strain applied in different formulations AgBiologicals, Saskatoon, SK, Canada. Russ47(7):595-600
- [6] Samrudhi R. Sharma¹, N. Kameswara Rao², Trupti S. Gokhale¹ and Shoaib Ismail²

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- 2012 Isolation and characterization of salt-tolerant rhizobia native to the desert soil of United Arab Emirates Emir. J. Food Agric. 2013. 25(2):102-108
- [7] Pooja Agrawal and Shruti Shukla, 2016 Diversity of root nodule bacteria from leguminous crops Department of Microbiology and Biotechnology, Sagar, India 2016 Volume 68(1):195-205.
- [8] Appunu, C and Dhar, B., 2006. Phage typing of indigenous Soybean-Rhizobia Relationship of a phage group of strains for their symbiotic and symbiotic nitrogen fixation. Ind. J. Exp. Biology. (12):1006-1144.
- [9] Agha, S.K., Oad, F.C. and Buriro, U.A., 2004. Yield and yield components of Inoculated and uninoculated soybean under varying nitrogen level. Asian J. PI. Sci., 3(3):370-371.
- [10] Anthraper A and Dubois JD (2003). The effect of NaCl on growth, N₂ fixation (acetylene reduction), and percentage of nitrogen in *Leucaena leucocephala* (Leguminosae) Var. K 8. Am J Bot. 90(5):683-Q92.
- [11] Kalpana Palghadmal, Fungicide tolerance against strains of *Bradyrhizobium japonicum* Flora and Fauna (Jhansi) 2016 Vol.22 No.2 pp.215-220 ref.11
- [12] Cenap C and Çiğdem K, Esat C. Fungicide, antibiotic, heavy metal resistance and Salt tolerance of root nodule isolates from *Vicia palaestina*. African J Biotechnology, 2011; 10(13): 2423-2429.
- [13] Philip EL. The Effect of herbicides and fungicides on legume *Rhizobium* symbiosis. Chapter 9. In: Pesticide Interactions in Crop Production: Beneficial and Deleterious Effects (Editor, Altman J.) 2018; 591.
- [14] J.R. Rathjen, M.H. Ryder, I.T. Riley, T.V. Lai, M.D. Denton, Impact of seed-applied pesticides on rhizobial survival and legume nodulation, Journal of Applied Microbiology, 10.1111/jam.14602, 129, 2, (389-399), (2020).
- [15] Bouchmouch, I. Souad-Moushine, B. Brahada, F. Aurag, J. 2005. Influence of Host cultivars And *Rhizobium* sp. on the growth and symbiotic performance of *Phaseolus vulgaris* under salt stress J. Plant Physiol. 162(10):1103-13.
- [16] Shahid M and Khan MS 2018 Fungicide tolerant *Bradyrhizobium japonicum* mitigate

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toxicity and enhance greengram production under hexaconazole stress Aligarh Muslim University, Aligarh 2002, Uttar Pradesh, India. 2019 Apr; 7: 8:92-108.

- [17] Chakraborty, M. Khan, H. and Ramkrishan, K. 2003. Symbiotic properties of Hypermotile mutants of *Bradyrhizobium sp. (Vigna acanitifolia)* Indian Journal of Microbiology. 43(2): 101-105.