Chickpea Endophytic Bacteria Inhibiting Dry Root Rot Fungus *Rhizoctonia bataticola*

D.Ganga Bhavani, A. Muni Kumari* and A.Kiran Kumar

Abstract— Chickpea *(Cicer arietinium)* is the world's most important pulse crop with a production of about 9.2 million tons worldwide. India is the largest producer of Chickpea with 75% of world's production and an average of about 15-20 quintals per hectare. Despite of high production, yields of Chickpea were low due to many biotic and abiotic constraints. More than 50 diseases have so far been reported on Chickpea. Dry root rot caused by the fungus *Rhizoctonia bataticola* is causing severe damage in many chickpea growing regions. This fungus is polyphagous, seed and soil borne. Chemical control is not an effective practice and also environmentally hazardous. In the present scenario biological control of dry root rot of chickpea offers a great promise. In last decades endophytic bacteria attracted more and it is a novel resource in biological control of plant diseases. Infected plants of JG 62 were collected from experimental plot of Regional Agricultural Research Station Nandyal and fungi was isolated on PDA and grown at 30^0C. The fungi appear greyish to black. Commonly growing variety JG11 chickpea Plants were collected from the fields of Kadapa and Kurnool. Endophytic bacteria were isolated from the roots of these plants using phosphate buffer. Individual colonies were streaked on plates of Nutrient Agar media and Tryptic Soy Agar media. Anti microbial assay was performed by dual culture method. Twenty three isolates were tested against *Rhizoctonia bataticola* among them five isolates were showing good inhibition. Maximum (81 %) inhibition was recorded by the isolate B5.

Index Terms— chickpea, important pulse, dry root rot, *Rhizoctonia bataticola*, polyphagous, biological control, endophytic bacteria.

1 INTRODUCTION

Chickpea (*Cicer arietinum L)* is an important crop among pulses. India is the largest chickpea producing country accounting for about 75% of global chickpea production and it is cultivated in an area of about 8.56 million hectors with a production of about 7.35 million tons with productivity of about 858 kg per hectare and average of 15-20 quintal per hectare [1] In Andhra Pradesh it is grown in an area of 0.58 million hactares with a productivity of 1141 kg per hectare despite of high production rate yields of chickpea are getting low due to many biotic and abiotic constraints. Morethan 50 diseases have so far been reported on chickpea [2]. Chickpea disease may cause yield losses of upto 100% depending on time of infection [3]. Among the several diseases 10-35% of the yield loss is due to wilt and dry root rot diseases [4]. Among them dry root rot caused by *Rizoctonia Balaticola* is becoming most severe in chickpea growings regions of India [5]. Dry root rot is caused by a necrotic fungus *Rhizoctonia bataticola* (Taub.) Butler (Pycnidal stage; *Macrophomia phaseolina* (Tassi) Goid) and is an important component of the disease complex that causing root rots and seedling blights in many grain legumes [6]. As *Rizoctonia Batalicola* is a soil born and has a wide host range and survive in soil a longer periods through Sclerotia chemical control is not so promising [7]. Repeating use of chemicals causes the development of Pathogen resistance and environmentally hazardous, all these lead towards biocontrol approach.

In the last decades, endophytic bacteria have attracted more and more concerns as novel resource in biological control of plant diseases and in promotion of plant growth [8]. Endophytic bacteria are those which colonize the internal tissue of the plant without causing any infection or negative effect on plant [9]. Many reports have also shown that endophytic microorganism have the capacity to inhibit plant pathogens [10].

2 MATERIALS AND METHODS

2.1 Collection of plant samples:

Diseased plants of variety JG 62 were collected from RARS Nandyal for the isolation of Dry Root Rot causing fungi *Rhizoctonia bataticola.* For isolation of endophytic bacteria healthy plants of commonly growing variety JG 11 were selected from fields of Balapanur in Kurnool District and shivaji Nagar in Kadapa Districts.

2.2 Isolation of pathogen:

Roots of diseased plants were taken and cut into 1cm long. Tissue was surface sterilized for 2times with 1% sodium hypochlorite for 30sec and then washed with sterile distilled water for 2 times then aseptically plated on Potato Dextrose Agar medium (PDA).

2.3 Isolation of Endophytic bacteria:

Endophytic bacteria were isolated from healthy roots of chickpea. Rots were thoroughly washed under running tap water to remove the traces of soil. Roots were seperated from plant and cut into 2-3cm long. Then surface sterilized for 3-5 times with 2% sodium hypochlorite for 2 times with 70% ethanol and 4 times in sterile distilled water and 1ml of last wash was plated on Tryptic Soya Agar medium(TSA) for sterility check. The tissue is macerated in 9ml of phosphate buffer and 1 ml of macerate is serially diluted upto 10^{-6} dilutions and 1ml of each dilution is pour plated on TSA, Nutrient Agar Media (NAM), Luria Ber-

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tani Agar (LB) plates. Single colonies were isolated and streaked on TSA plate and glycerol stocks were prepared and stored at -20° C for further use.

2.4 Antagonism of Endophytic bacteria against Dry Root Rot pathogen (*Rhizoctonia bataticola):*

Isolated endophytic bacteria were tested for their anti-fungal activities against R*hizoctonia bataticola* by dual culture method [11]. Fungi were inoculated at one end of the PDA plate and at the other end endophytic bacteria were streaked. The plate with only fungi inoculated on one end without bacteria is kept as control. These plates were incubated at 30^{0}C for 7days and radial growth is recorded. Percentage of inhibition is calculated by using the formula

$$\text{Inhibition \%} = \frac{(C) - (T)}{(C)} \times 100$$

(C) = Radial growth in control
(T) = Radial growth in dual culture

2.5 Characterization of bacterial isolates:

Morphological and physico-chemical characteristics of Antagonistic bacteria were done such as Grams staining [12], Motility [12], Indole test, Methyl Red Test, Voges-Proskauer Test [13].

3 Results

Isolation of pathogen:

The isolated fungal colonies were greyish to black in colour producing cottony mycelia and numerous jet black microsclerotia.

Isolation of bacteria:

Total of 63 isolates were obtained. Most of them are creamy white, translucent, round, highly gummy and forming convex colonies.

Antagonism of Endophytic bacteria against Dry Root Rot pathogen (*Rhizoctonia bataticola):*

All the 63 isolates were tested for their antagonistic properties. Out of them five isolates were showing significant inhibition. The isolates B5 is showing the most significant inhibition with 81%, K1 with 77%, K2 with 75%, C2 and A8 with 74%.

Characterization of bacterial isolates:

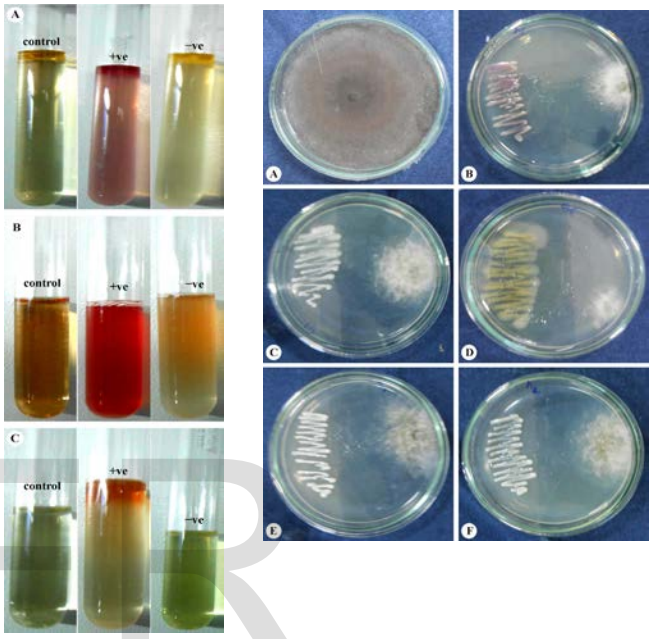
The isolates B5, K1, K2, C2, A8 were showing different results for biochemical tests. (Table 1)

Table 1 Biochemical chacteristics

Isolates	1	2	3	4	5	6	7
B5	+	bacilli	+	+	-	-	-
K1	-	cocci	+	-	+	-	-
K2	-	cocci	-	-	+	+	-
C2	-	bacilli	+	+	+	+	+
A8	-	cocci	+	+	+	-	+

1. Grams test 2. Shape 3. Motility 4. Catalase 5. Indole test 6. Methyl Red test 7. Voges Prosekeur test.

Invitro antagonism against pathogen *Rhizoctonia bataticola.* (A) *Rhizoctonia bataticola* alone on PDA. (B) in the presence of endophytic bacterial isolate A8 (c) inhibited by C2 (D) B5 (E) K1 (F) K2



Biochemical tests:

a. Indole Test
b. Methyl Red Test
c. Voges prosekur Test

Phylogenetic analysis:

Phylogenetic analysis was carried out for the most significant antagonistic strain B5. The isolate B5 is showing 99% similarity with the *Pseudomonas aeruginosa* PAO1(FIG 1).

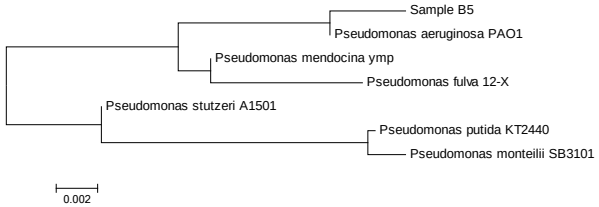
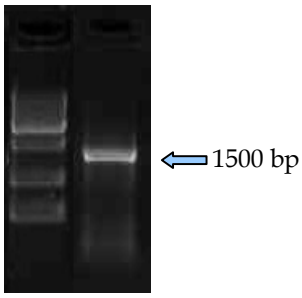


Fig. 1. Phylogenetic tree for the isolate B5



1.2% Agarose gel showing single 1.5kb of 16S rDNA amplicon

4 Conclusion

The present experiment showed that chickpea plants were found to have most diverse spectrum of endophytic bacteria. Fungal colonies were greyish to black in colour producing numerous jet black microsclerotia. Presence of these features confirms the characteristics of *Rhizoctonia bataticola* as have been decribed by other workers [15]. Out of 63 isolates obtained maximum numbers of isolates were showing antagonistic effects on the fungi. The five isolates, B5 with 81%, K1 with 77%, K2 with 75%, C1 and A5 with 74% were most promising results and this shows that these bacteria may involve in controlling the pathogen. They can be used as biocontrol agents. Environmental *P. aeruginosa* isolates have been considered as potential biological control agents or inducers of systemic acquired resistance and also somestrains have been reported as plant-growth promoting rhizobacteria [16]. Some reports also say that pseudomonas aeruginosa effectively inhibited the growth of some plant pathogens like S*clerotina sclerotium [17]*

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