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Evaluation of Antagonistic Biocontrol Agents Isolated from Maize Phyllosphere against *Exserohilum turcicum*

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ABSTRACT

Maize is the third most important cereal crop in India after rice and wheat. Diseases play an important role in the yield losses in maize. Among the diseases, the Turcicum leaf blight, caused by *Exserohilum turcicum* is the most destructive and prevalent disease affecting the yield by the reducing photosynthetic ability. As the use of fungicides may harm the environment, the present study was carried out focusing on the biological control of the disease. Pathogen may be controlled by different microbes that exist along with it. The term epiphyte is used to denote the phyllospheric organisms which are isolated from the maize using selective media. Ten isolates of *Bacillus*, *Pseudomonas*, Actinomycetes and Methylotrophic bacteria are obtained from two different loca tions. The antagonistic potential of these bacteria is evaluated by dual culture technique *in vitro*. Among various isolates of bacteria, *Bacillus* (B 19003), *Pseudomonas* (P 19001), Actinomycetes (A 19002) have shown a good antagonistic potential. Different microscopic observations like formation of chlamydospores, granulation of hyphae were recorded.

Keywords: *Biocontrol,Exserohilum, Isolation, Maize, Phyllosphere.*

Maize (*Zea mays* L.), a C4 grass belonging to the family Poaceae, is considered as the queen of cereals because of its highest genetic potential among the cereals. It is grown throughout the year and predominantly as *kharif* crop with 85 percent of the area under cultivation in the season. It accounts for 9 percent of total food grain production in India. In recent times, maize is slowly occupying the area under rice fallow pulse resulting in increased area under maize cultivation. However, there are some production constraints that result in the decreased yield. Considering its importance, the losses in the yield of maize is a matter of concern where the diseases constitute the major constraint limiting the production. The major diseases of maize are four foliar diseases, two pre-flowering and three post-flowering stalk rots, four downy mildews and two sheath diseases (Payak and Sharma, 1982).

Among the foliar diseases, the turcicum leaf blight which is also called as Northern leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Helminthosporium turcicum* Pass.) is the most destructive and prevalent disease affecting the yield by the reduced photosynthetic ability (Pant *et al*., 2001).

The disease is prevalent in almost all the maize growing areas of India. Epidemics of Turcicum leaf blight cause severe losses in the grain yield and these losses may vary from 25 to 90 percent depending on the severity of the disease (Chenulu and Hora, 1962). It causes a serious problem in the states of Karnataka, Himachal Pradesh, Uttar Pradesh, Uttarakhand, Orissa, Andhra Pradesh and North Eastern Hill states.

The disease can be controlled by use of fungicides, botanicals and biological control methods. The use of native biocontrol agents that coexist with the pathogen in disease management is considered as the eco-friendly and sustainable approach. As the use of fungicides may harm the environment, the present study is taken up focusing on the biological control of the disease.

MATERIAL AND METHODS

The present study was carried out at the Department of Plant Pathology, Agricultural College, Bapatla.

Sample collection

The samples were collected from two locations, *viz*. from college and college farm. The samples for isolating phyllospheric organisms were characterized by the deep green and disease free leaves from upper part of maize plants. Rhizospheric soil sample was collected from the rhizosphere of healthy maize plant about 5-6 cm deep using a soil auger.

Isolation of phyllospheric biocontrol agents

For isolation of *Pseudomonas*, *Bacillus* and Actinomycetes, Pseudomonas Agar, Bacillus Differentiation Agar and Actinomycetes Isolation Agar were used respectively. For isolation of methylotrophic bacteria, Ammonium Salt medium was prepared and used. In order to isolate biocontrol agents, the samples that were collected from the two locations were imprinted by cutting the leaves to the shape of Petri plate onto their respective selective media. Then inoculated Petri plates were incubated at 25^0C for three days.

Isolation of native biocontrol agents from soil

The rhizospheric soil sample that was collected from the rhizosphere of the healthy maize plant was weighed a gram and added to a 10 ml sterilized deionised water to make a soil solution. The solution was diluted to 10^{-6}. The solution samples were mixed properly by shaking for 5 minutes and one ml of aliquot was taken and transferred to 9 ml water blank containing sterile deionised water. The suspension was stirred for one minute, before it was further diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} for isolation of Actinomycetes and *Bacillus subtilis*. 20 ml of molten Actinomycetes Selective medium and Bacillus Differentiation Agar were poured into Petri plates and 0.1 ml of dilutions from 10^{-4} to 10^{-6} were transferred onto the solidified Agar plates aseptically.

Identification of the biocontrol agents

After the incubation period, *Pseudomonas* was identified by the presence of fluorescence when viewed under UV lamp. *Bacillus subtilis* was differentiated from *Bacillus cereus* by the characteristic bright yellow coloured colony. Actinomycetes, Methylotrophic bacteria were identified by the characteristic growth habit *i.e* filamentous growth and by the presence of pink coloured colonies respectively.

Maintenance of cultures

First identified bacterial cultures were sub-cultured onto their respective selective media and then on Nutrient Agar. Then loopful of each isolate was transferred on to NA slants aseptically and incubated for two days at 26^0C. The cultures thus obtained were stored in refrigerator at 4^0C and revived monthly for further studies.

***In vitro* efficacy of biocontrol agents against *E. turcicum*:** Ten isolates of bacteria that were obtained earlier were evaluated for the presence of antagonistic potential by dual culture technique (Dhingra and Sinclair, 1985). One day old bacterial cultures and five days old *E. turcicum* were used for the dual culture. A loopful of each bacterial isolate was streaked on two ends of the Petri plate leaving one cm from the periphery and a mycelia disc of five mm diameter were inoculated on to PDA plate. The plates were incubated at 25^0C. the experiment was laid in three replications and 11 treatments including pathogen control. Observations on radial growth were recorded every 24 h until zone of inhibition was clearly seen. The per cent growth inhibition of the test pathogen over control was calculated according to the given formula

$$\text{Per cent growth inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} X100$$

RESULTS AND DISCUSSION

Three isolates *viz*. A 19002, B 19003 and P 19001 were found equally effective against *E. turcicum* showing the maximum percent inhibition 43.09, 39.78, 42.54 respectively. Similar findings have been reported by Rai and Rajesh Singh (2013). These isolates have shown a clear zone of inhibition, demarcating the pathogen culture. The inhibition zone observed in case of these potential isolates ranged from 0.2 to 0.4 cm. The next best potential isolates found were P 19003 and B 19002 with per cent inhibition of 28.18 and 19.89 respectively. But no zone of inhibition was observed in these isolates instead the pathogen has overgrown the bacterial culture. The least per cent inhibition was observed in case of B 19001 and A 19001 isolates with 2.76 per cent (Table.1).

Microscopic observations

Different microscopic observations were recorded for the potential isolates. P 19001 isolate induced the process of hyphal anastomosis. In B 19003 isolate bacterial cells colonized the hyphae of *E. turcicum*. A 19002 isolate restricted the growth of pathogen to a great extent such that it induced the formation of chlamydospores.

Table 1: Efficacy of maize biocontrol agents against Exserohilum fussicum

T.No.	Treatments	Radial Growth (in cm)	% Inhibition	Zone of Inhibition (in cm)	Over groth of the Pathogen
T_1	B 19001	5.87(2.42)*[d]	2.76	-	+
T_2	B 19002	4.83(2.20)[bc]	19.89	-	+
T_3	B 19003	3.63(1.90)[a]	39.78	0.2	-
T_4	P 19001	3.47(1.86)[a]	42.54	0.4	-
T_5	P 19002	5.03(2.24)[c]	16.57	-	+
T_6	P 19003	4.33(2.08)[b]	28.18	-	+
T_7	A 19001	5.87(2.42)[d]	2.76	-	+
T_8	A 19002	3.43(1.85)[a]	43.09	0.5	-
T_9	PPMB 19001	5.43(2.33)[cd]	9.94	-	+
T_{10}	PPMB 19002	5.07(2.25)[c]	16.02	-	+
T_{11}	Control	6.03(2.46)[d]	0.00	NA	NA
	SEm ±	0.048			
	CD ($P \leq 0.05$)	0.140			
	CV (%)	3.798			

*figures in parenthesis are square root transformed values + indicate overgrowth by the pathogen, - indicate no overgrowth of the pathogen

CONCLUSION

The present study suggested that the use of native phyllospheric bio-control agents could effectively control the disease. Maize Turcicum leaf blight can be controlled by using biocontrol agents in addition to the regular use of fungicides. Hence, the disease can be managed integratedly leading to sustainability in agriculture.

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