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Influence of antagonistic bacteria on growth of *Fusarium oxysporum f.sp. cumini* and *Alternaria brunsii in vitro*

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Abstract
The experiment was carried out during 2017-18 and 2018-19 at ICAR-NRCSS, Ajmer (Raj.). The maximum growth inhibition of *Fusarium oxysporium f. sp. cumini* exhibited by bacterial isolate DCU-451 (*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168) in both qualitative (61.2%) and quantitative assay (79.9%) while the maximum growth inhibition of *Alternaria brunsii* exhibited by bacterial isolate DCU-251 (*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165) in both qualitative (54.2%) and quantitative assay (81.8%).

Keywords: Antagonistic bacteria, *Fusarium oxysporium f. sp. cumini* and *Alternaria brunsii*

Introduction
Cumin (Cuminum cyminum L.) belongs to the family Umbellifereae and is believed to be a native of the Mediterranean and Near Eastern regions. It is mainly cultivated in India, Egypt, Libya, Iran, Pakistan and Mexico. Cumin is cultivated on 264 thousand hectares with a production of 108.7 thousand tones in the country [1]. In India, cumin is mainly cultivated in the states of Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh and Bihar. Among these Rajasthan contribute maximum area as well as production. Major cumin cultivation areas in the state of Rajasthan are Jalore, Barmer, Nagaur, Jodhopur, Pali, Ajmer and Tonk districts [2].

The seeds are used as a condiment or spice in curries, pickles and in cooking. In view of the great economic importance of cumin, it is imperative that a coherent and comprehensive programmer be meticulously undertaken on the study of wilt and blight diseases of cumin with a view to control these diseases. This can be achieved only through a pave way for devising management strategies through bio-control. The main objective of the present study was disease control with eco-friendly environment by investigating host-pathogen interaction under *in vitro* conditions. Seed spices are often treated with insecticides and/or fungicides to protect the seeds and seedlings from harmful insects and soil microorganisms during germination and early seedling development. Chemically treated seeds are inoculated with rhizobia which may be adversely affected by these chemicals [3]. Since large numbers of viable rhizobia are required to bring about effective nodulation, compatibility or survival of rhizobia on treated seeds is a major of concern [4]. Fungicides markedly affect the saprophytic soil microbial population as equally as the pathogens they are designed to control. The degree of inhibition and the duration vary with the chemical, the soil and the environmental conditions [5]. Fungicides differ in their effects on the growth and survival of *Rhizobium* and *Bradyrhizobium* strains depending on the strain and the concentration of the fungicide [6].

Materials and Methods
Screening of antagonistic rhizobacteria
Antagonistic activity of the bacterial isolates against *Fusarium oxysporum sp. cumini* and *Alternaria burnsii* was evaluated based on dual plate technique. 15 ml of potato dextrose agar was poured into sterile petri dish. 5 mm mycelia plug of the test fungus was inoculated at the centre of plate. Antagonistic rhizobacterial isolates were streaked 3 cm apart from the fungal inoculum and plates were incubated at 28 ºC for 15 days and in this interval radial growth of the test fungus was measured and % growth inhibition was calculated using the formula:

% Inhibition= (R – r)/R×100

Where, r is the radius of the fungal colony opposite the bacterial colony and R, is the maximum radius of the fungal colony in absence of the bacterial colony.

Fungal biomass inhibition in liquid medium

Quantitative evaluation of antagonistic potential of isolates against *Fusarium oxysporum* and *Alternaria burnsii* were carried out in broth medium. A volume of 1 ml of overnight bacterial culture and a disc of test fungus (5 mm) from a well grown fungal colony on PDA plates were inoculated in 50 ml broth of potato dextrose media in 250 ml conical flasks at 25 ºC. Broth medium inoculated only with fungus served as control. The difference in dry weight between treatment (pathogen+antagonistic bacteria) and control (pathogen only) cultures were recorded by passing 5 days grown dual cultures through pre weighed filter paper (Whatmann No.1). The filter papers were dried for 24 hrs at 70ºC and weighed. The% reduction in weight of the test fungus was calculated using formula:

% reduction in weight = (w1-w2)/ w1×100

Where, w1 represents the weight of the test fungus in control flask and w2 with the bacterial antagonists.

Results and Discussion

***In vitro* screening for antagonistic rhizobacteria**

Twenty isolates were found antagonistic to *Fusarium oxysporum*, 14 to *Alternaria brunsii* and 5 to both *Fusarium oxysporum* and *Alternaria brunsii*. However, 29 isolates were screened for antagonists against *Fusarium oxysporum sp. cumini* and *Alternaria brunsii* listed in Table-1.

Table 1: Screening on the basis of antagonistic activity against cumin fusarium and Alternaria

S.N.	Antagonistic isolates	Source	*Fusarium oxysporum f. sp. cumini*	*Alternaria brunsii*
1.	DCU-22	Cumin Rhizospheric soil	+	-
2.	DCU-159	Cumin Root	+	+
3.	DCU-181	Cumin Root	-	+
4.	DCU-184	Cumin Root	+	-
5.	DCU-188	Cumin Rhizospheric soil	+	+
6.	DCU-251	Cumin Rhizospheric soil	+	+
7.	DCU-252	Cumin Root	+	-
8.	DCU-253	Cumin Rhizospheric soil	-	+
9.	DCU-254	Cumin Rhizospheric soil	+	-
10.	DCU-258	Cumin Root	+	+
11.	DCU-259	Cumin Rhizospheric soil	+	-
12.	DCU-260	Cumin Root	+	+
13.	DCU-261	Cumin Rhizospheric soil	-	+
14.	DCU-262	Cumin Root	+	-
15.	DCU-351	Cumin Rhizospheric soil	-	+
16.	DCU-353	Cumin Rhizospheric soil	+	-
17.	DCU-354	Cumin Root	+	-
18.	DCU-360	Cumin Rhizospheric soil	-	+
19.	DCU-364	Cumin Rhizospheric soil	+	-
20.	DCU-366	Cumin Root	-	+
21.	DCU-371	Cumin Rhizospheric soil	+	-
22.	DCU-372	Cumin Root	+	-
23.	DCU-451	Cumin Rhizospheric soil	+	-
24.	DCU-453	Cumin Rhizospheric soil	-	+
25.	DCU-563	Cumin Rhizospheric soil	-	+
26.	DCU-567	Cumin Rhizospheric soil	+	-
27.	DCU-568	Cumin Rhizospheric soil	+	-
28.	DCU-570	Cumin Root	+	-
29.	DCU-651	Cumin Root	-	+

Qualitative assay for antagonists against *Fusarium oxysporum sp. cumini*

Out of 29 antagonists, 20 were inhibiting the growth of *Fusarium oxysporum sp. cumini*. The growth inhibition of *Fusarium oxysporum sp. cumini* in plate was found in the range of 26.2- 61.2% while that of in broth medium, ranged from 3.3-79.9%. DCU-451 (*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168) exhibited maximum growth inhibition in both qualitative and quantitative assay. Data of growth inhibition in plate assay were depicted in Table 2 (Plate. 1). Isolate DCU-451 showed maximum growth inhibition of *Fusarium oxysporum* (61.2%) followed DCU-188 *Pseudomonas aeruginosa* NRCSSDCU188 Accession no. MN192164 (57.5%), DCU-22 *Bacillus paramycoides* NRCSSDCU22 Accession no. MN192162 (51.2%), DCU-184 *Pseudomonas aeruginosa* NRCSSDCU184 Accession no. MN192163 (48.7%) and DCU-364 (47.5%).

Least growth inhibition was 26.2% due to antagonistic isolates DCU-254. Similarly [7] reported antagonistic effect of *Pseudomonas* isolates from chickpea rhizosphere against *Fusarium oxysporum* sp. *ciceris*, they observed that 14 out of 96 isolates were highly antagonistic to the phytopathogen and showed inhibition zone ranging from 5-7 mm under *in vitro* growth conditions. Growth inhibition of *Aspergillus niger* (66.5%) and *Fusarium oxysporum* (64.4%) has also been reported by [8] the present finding, [9] reported that rhizobacterial isolates Ps-17, Ps-14 (belonging to genera

Pseudomonas) and S-10 (producing characteristic pink pigmentation of *Serratia*) isolated from chickpea rhizosphere inhibited growth of *Fusarium oxysporum* sp. *ciceris* under *in vitro* dual plate assay by 42.8, 31.4 and 28.6% respectively.

Table 2: Qualitatively growth inhibition of *Fusarium oxysporum f.sp. cumini* by antagonistic bacteria

S.N.	Antagonistic isolates	*Fusarium oxysporum f.sp. cumini*		
		Radial growth of pathogen (mm)	Inhibit radial growth of pathogen (mm)	Growth inhibition%
1.	Control	8.0	-	-
2.	DCU-22	3.9 ± 0.14	4.1	51.2
3.	DCU-364	4.2 ± 0.17	3.8	47.5
4.	DCU-251	4.4 ± 0.19	3.6	45.0
5.	DCU-159	4.9 ± 0.08	3.1	38.7
6.	DCU-567	4.9 ± 0.17	3.1	38.75
7.	DCU-451	3.1 ± 0.14	4.9	61.2
8.	DCU-252	5.5 ± 0.11	2.5	31.2
9.	DCU-254	5.9 ± 0.08	2.1	26.2
10.	DCU-258	5.4 ± 0.15	2.6	32.5
11.	DCU-259	5.3 ± 0.12	2.7	33.7
12.	DCU-260	5.8 ± 0.09	2.2	27.5
13.	DCU-568	4.8 ± 0.18	3.2	40.0
14.	DCU-184	4.1 ± 0.16	3.9	48.7
15.	DCU-353	5.6 ± 0.12	2.4	30.0
16.	DCU-354	5.7 ± 0. 14	2.3	28.7
17.	DCU-570	5.4 ± 0.07	2.6	32.5
18.	DCU-262	4.4 ± 0.17	3.6	45.0
19.	DCU-371	4.7 ± 0.12	3.3	41.2
20.	DCU-372	5.3 ± 0.20	2.7	33.7
21.	DCU-188	3.4 ± 0.16	4.6	57.5

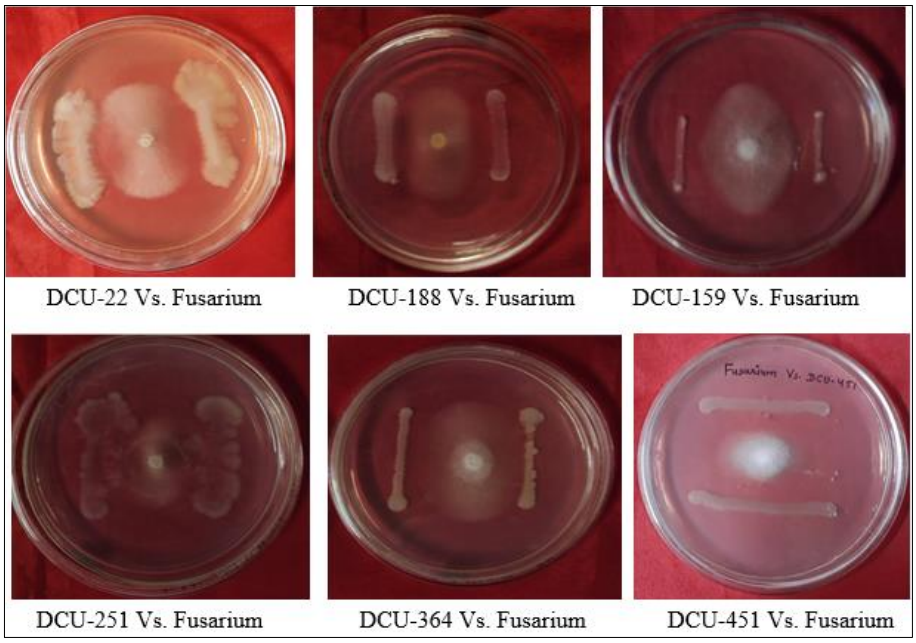

Plate 1: Growth inhibition of *Fusarium oxysporum f.sp. cumini* by antagonistic bacterial isolates

Biomass inhibition of *Fusarium oxysporum sp. cumini* in liquid medium

Selected 20 bacterial antagonists were evaluated for their ability to inhibit the mycelial proliferation of *Fusarium oxysporum* sp. *cumini* in liquid medium as this is a better method for evaluation of antagonistic efficiency since the liquid medium provides a better environment for interaction of antagonistic activities from all possible interacting sites. All the isolates showed varied ability to inhibit mycelial growth of the fungus and a notable reduction in mycelial biomass was observed as compared to the control. In confrontation assays in liquid media, the fungal proliferation was inhibited by DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168) isolates as indicated by decrease in dry weight of the fungal cultures grown with antagonists as compared to control. Data of fungal biomass inhibition were depicted in Table 3 (Plate 2). Reduction of fungal dry weight ranged from 30.3-79.9 percent. Maximum being observed with isolate DCU-451*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168 (79.9%) followed by DCU-188 (71.9%), DCU-251*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165 (70.0%), DCU-262 *Kosakonia oryzendophytica* strain NRCSSDCU262 Accession no. MN192166 (68.5%), DCU-364 (64.3%) and DCU-101 (61.7%). Least growth inhibition was 30.3% due to

antagonistic isolates DCU-254. Rhizo bacterial isolates have been reported for their inhibitory activity against plant pathogens and antibiosis is probably the best-known and the most important mechanism used to limit pathogen invasion in host plant tissues. Higher fungal biomass inhibition (79.9%) by isolate DCU-451 in liquid medium suggests the release of antibiotic compounds in close proximity of the pathogen thus confirming the antagonistic potential of this bacterium. Determination of *in vitro* antagonistic activities by estimating the reduction in fungal biomass has been used by several workers [10-11]. Variable responses in reduction of fungal biomass in broth culture as compared to dual plate assay has been reported as certain antifungal metabolites are induced or repressed by the absence or presence of pathogen [12-13] reported 75% reduction in dry weight of *Fusarium oxysporum* by *Pseudomonas aeruginosa* while in a similar study [11] recorded 41% reduction in biomass of *Fusarium oxysporum* by *Pseudomonas corrugata* in liquid media.

Table 3: Biomass reduction of *Fusarium oxysporum f. sp. cumini* by antagonistic bacteria

S.N.	Antagonistic isolates	*Fusarium oxysporum f. sp. cumini*		
		Dry biomass of pathogen (mg)	Inhibit dry biomass of pathogen (mg)	Growth inhibition%
1.	Control	264	-	-
2.	DCU-22	101	163	61.7
3.	DCU-364	94	170	64.3
4.	DCU-251	79	185	70.0
5.	DCU-159	177	87	32.9
6.	DCU-567	144	120	45.4
7.	DCU-451	53	211	79.9
8.	DCU-252	138	126	47.7
9.	DCU-254	184	80	30.3
10.	DCU-258	155	109	41.2
11.	DCU-259	176	88	33.3
12.	DCU-260	143	121	45.8
13.	DCU-568	179	85	32.1
14.	DCU-184	139	125	47.3
15.	DCU-353	166	98	37.1
16.	DCU-354	128	136	51.5
17.	DCU-570	162	102	38.6
18.	DCU-262	83	181	68.5
19.	DCU-371	163	101	38.2
20.	DCU-372	172	92	34.8
21.	DCU-188	74	190	71.9

Qualitative assay for antagonists against *Alternaria brunsii*

Out of 29 antagonists, 14 were selected to inhibit *Alternaria brunsii*. The growth inhibition of *Alternaria brunsii* in plate was found in the range of 24.2-54.2% while that of in broth medium, ranged from 24.1-81.8%. DCU-251 exhibited maximum growth inhibition in both qualitative and quantitative assay. Data of growth inhibition in plate assay were depicted in Table 4. DCU-251 (*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165) showed maximum growth inhibition of *Alternaria brunsii* (54.2%) followed DCU-181 *Pseudomonas argentinensis* NRCSSDCU181 Accession no. MN337284 (51.4%), DCU-453 *Pseudomonas aeruginosa* NRCSSDCU-453 Accession no. MN192169 (48.5%), DCU-651 *Bacillus pacificus strain* NRCSSDCU651 Accession no. MN192170 (47.1%) and DCU-351 (41.4%). Least growth inhibition was 24.2% due to antagonistic isolates DCU-366. [14] reported almost 100% inhibition of pathogen proliferation under dual culture technique when co-inoculated with *Bacillus* isolate (BC2) as compared to the test fungus alone suggesting the production of metabolites by bacteria which have inhibitory effect on pathogens.

Table 4: Qualitatively growth inhibition of *Alternaria brunsii* by antagonistic bacteria

S.N.	Antagonistic isolates	*Alternaria brunsii*		
		Radial growth of pathogen (mm)	Inhibit radial growth of pathogen (mm)	Growth inhibition%
1.	Control	7.0	-	-
2.	DCU-159	4.3 ± 0.14	2.7	38.5
3.	DCU-181	3.4 ± 0.18	3.6	51.4
4.	DCU-188	4.4 ± 0.09	2.6	37.1
5.	DCU-251	3.2 ± 0.12	3.8	54.2
6.	DCU-253	5.1 ± 0.20	1.9	27.1
7.	DCU-258	4.6 ± 0.17	2.4	34.2
8.	DCU-260	5.0 ± 0.08	2.0	28.5
9.	DCU-261	5.2 ± 0.15	1.8	25.7
10.	DCU-351	4.1 ± 0.19	2.9	41.4
11.	DCU-360	4.9 ± 0.11	2.1	30.0
12.	DCU-366	5.3 ± 0.21	1.7	24.2
13.	DCU-453	3.6 ± 0.16	3.4	48.5
14.	DCU-563	5.2 ± 0.18	1.8	25.7
15.	DCU-651	3.7 ± 0.12	3.3	47.1

Biomass inhibition of *Alternaria brunsii* in liquid medium

Selected 14 bacterial antagonists were evaluated for their ability to inhibit the mycelial proliferation of *Alternaria brunsii* in liquid medium as this is a better method for evaluation of antagonistic efficiency since the liquid medium provides a better environment for interaction of antagonistic activities from all possible interacting sites. All the isolates showed varied ability to inhibit mycelial growth of the fungus and a notable reduction in mycelial biomass was observed as compared to the control. Data of fungal biomass inhibition were depicted in Table 5 (Plate. 3). Reduction of fungal dry weight ranged from 24.1-81.8 percent. Maximum being observed with isolate DCU-251*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165 (81.8%) followed by DCU-181 *Pseudomonas argentinensis* NRCSSDCU181 Accession no. MN337284 (58.3%), DCU-351 *Pseudomonas aeruginosa* strain NRCSSDCU351 Accession no. MN192167 (49.7%), DCU-453 *Pseudomonas aeruginosa* NRCSSDCU453 Accession no. MN192169 (44.2%) and DCU-360 (42.8%). Least growth inhibition was 24.1% due to antagonistic isolates DCU-563. Similarly, [15] isolated eighteen bacteria of fluorescent pseudomonads and one of *Bacillus* from *Alternaria triticina* suppressive soils.

Table 5: Biomass reduction of Alternaria *brunsii* by antagonistic bacteria

S.N.	Antagonistic isolates	*Alternaria brunsii*		
		Dry biomass of pathogen (mg)	Inhibit dry biomass of pathogen (mg)	Growth Inhibition%
1.	Control	348	-	-
2.	DCU-159	211	137	39.3
3.	DCU-181	145	203	58.3
4.	DCU-188	219	129	37.0
5.	DCU-251	63	285	81.8
6.	DCU-253	241	107	30.7
7.	DCU-258	227	121	34.7
8.	DCU-260	256	92	26.4
9.	DCU-261	238	110	31.6
10.	DCU-351	175	173	49.7
11.	DCU-360	199	149	42.8
12.	DCU-366	215	133	38.2
13.	DCU-453	194	154	44.2
14.	DCU-563	264	84	24.1
15.	DCU-651	213	135	38.7

Plate 3: Reduction in biomass of *Alternaria brunsii* in Broth medium by bacterial isolates

Conclusion

The growth inhibition of *Fusarium oxysporum sp. cumini* in plate was found in the range of 26.2- 61.2% while that of in broth medium, ranged from 3.3-79.9%. DCU-451(*Pseudomonas aeruginosa* strain NRCSSDCU451 Accession no. MN192168) exhibited maximum growth inhibition in both qualitative and quantitative assay. The growth inhibition of *Alternaria brunsii* in plate was found in the range of 24.2-54.2% while that of in broth medium, ranged from 24.1-81.8%. DCU-251(*Pseudomonas aeruginosa* strain NRCSSDCU251 Accession no. MN192165) exhibited maximum growth inhibition in both qualitative and quantitative assay.

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