Research Paper :

Identification of bacterial isolates for their antifungal activity against *Fusarium oxysporum* f. sp. *carthami*

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International Journal of Plant Protection, Vol. 4 No. 1 (April, 2011) : 158-160

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SUMMARY

Safflower suffers heavily from wilt incited by *Fusarium oxysporum* f. sp. *carthami* in Maharashtra State especially in Marathwada region. Present study was conducted to evaluate the local bacterial isolates, isolated from rhizosphere soil, for their antifungal activity against the pathogen in the laboratory by dual culture technique. In all, promising 12 bacterial isolates were evaluated for their antifungal activity. Results indicated that, all the bacterial isolates suppressed the pathogen. Maximum antifungal activity has been shown by bacterial isolate B1 followed by *Pseudomonas* sp. (B_6 and B_5).

Kurundkar, B.P. and Mahajan, S.B. (2011). Identification of bacterial isolates for their antifungal activity against *Fusarium oxysporum* f. sp. *carthami*. *Internat. J. Pl. Protec.*, **4**(1): 158-160.

Key words : Nutrient agar, Antifungal activity, Bacterial isolates, Wilt, inhibition, Over run

Received : ***December, 2010***
Accepted : ***February, 2011***

Safflower is one of the important oilseed crops of India. Country has highest acreage with largest production of safflower grains (Anonymous, 2001). Crop is mainly grown under rainfed conditions in various soils mainly of Andhra Pradesh, Karnataka and Maharashtra states. Crop suffers from various biotic and abiotic stresses resulting in substantially poor yields. Wilt caused by *Fusarium oxysporum* f. sp. *carthami* has recently became one of the serious problems of the crop and losses to the tune of 61 % have been reported (Anonymous, 2001). Since, the disease is seed and soil borne and the crop is low input crop, is difficult to manage through cultural and chemical means. Resistant varieties for commercial cultivation are not available. Therefore, cultivators are actually defenseless against the melody and in some hot spot areas of Marathwada region have opted for alternate crop. Considering all the aforesaid aspects, it was thought worth while to evaluate local bacterial isolates for their efficacy in reducing growth of *F. oxysporum* f. sp. *carthami* to be considered for biological management.

MATERIALS AND METHODS

Rhizosphere samples from different locations of Marathwada region were collected for isolation of antagonistic bacterial organisms. Isolation of bacterial organisms was carried out by dilution and pour plate method on Nutrient agar (NA), King's *et al*., B and Soil extract agar media under aseptic conditions. Well isolated colonies of bacterial organisms were picked by inoculating needle under aseptic condition and were transferred to plate containing sterilized NA medium under aseptic conditions. Purification of bacterial organisms was carried out by streaking. These were identified by studying colony characters, pigmentation on media, morphology of bacterial cells, gram reactions and growth on specific media. After expelling common contaminants, 10 bacterial organisms were selected to study their antagonistic effect against *Fusarium oxysporum* f. sp. *carthami* in dual culture on NA. For this, *F. oxysporum* f. sp. *carthami* and test organisms were separately inoculated to sterilized Petriplates (90 mm diameter) containing sterilized NA. A week old growth of these organisms was used for testing in dual culture. A 5 mm disc of the *F. oxysporum* f. sp. *carthami* was placed

at one end and the test organism was placed at opposite end of Petriplate (10 mm from periphery) by streaking and the plates were incubated in an incubator at 25 ± 1^0C. Control plates were inoculated with *F. oxysporum* f. sp. *carthami* only. There were three replications per treatment. Observations regarding radial growth of *F. oxysporum* f. sp. *carthami* and growth of test organisms were recorded at 7 and 9 days after incubation to study antagonistic effect.

RESULTS AND DISCUSSION

Growth of *F. oxysporum* f. sp. *carthami* as influenced by bacterial isolates is presented in Table 1. At 5 and 7 days after incubation, all the bacterial isolates except *Pseudomonas* sp. (B_9 and B_{10}), respectively have recorded significantly less growth of the fungus than as compared to control.

At 5 days after incubation, minimum growth of fungus was observed in bacterial isolates B_4 and *Pseudomonas* sp. (B_5) which were at par with fungus growth in bacterial isolates B_1 and *Pseudomonas* sp. (B_6) was and significantly less than fungus growth in all the other bacterial isolates. It was followed by fungus growth in B_1 and *Pseudomonas* sp. (B_6) which was at par with fungus growth in *Pseudomonas* sp. (B_2) and significantly less than fungus growth in remaining bacterial isolates. Growth of fungus in isolate B_7, *Pseudomonas* sp. (B_3 and B_8) was significantly less than fungus growth in *Pseudomonas* sp. (B_9 and B_{10}).

At 7 days after incubation, minimum growth of fungus was observed in bacterial isolate B_1, which was significantly less than all the other bacterial isolates. It was followed by growth of fungus in *Pseudomonas* sp. (B_6 and B_5) which was significantly less than other remaining isolates. Fungus growth in *Pseudomonas* sp. (B_8) was significantly less than fungus growth in *Pseudomonas* sp. (B_2, B_3 and B_9), B_7 and *Pseudomonas* sp. (B_{10}). Fungus growth in B_4 was significantly less than fungus growth in *Pseudomonas* sp. (B_9, B_3 and B_{10}). Also, fungus growth in *Pseudomonas* sp. (B_2) was significantly less than fungus growth in *Pseudomonas* sp. (B_9 and B_3) and was significantly less than fungus growth in *Pseudomonas* sp. (B_{10}).

At 3 days after incubation, fungus growth in bacterial isolate B_7 was slightly suppressed. However, at 5 and 7 days after incubation, growth of fungus was suppressed from 37.97 in *Pseudomonas* sp. (B_9) to 67.52 in B_4 and *Pseudomonas* sp. (B_5) and from 5.05 (*Pseudomonas* sp. (B_{10}) to 65.81 per cent (B_1), respectively.

Growth of bacterial isolates significantly differed at all the observation periods (Table 2). At 3 days after incubation, maximum growth of bacteria was observed in isolate 4 which was significantly superior to other isolates. It was followed by *Pseudomonas* sp. (B_5 and B_6), B_1, *Pseudomonas* sp. (B_8, B_3 and B_2), the preceding treatment being significantly superior to succeeding treatment. All these aforesaid treatments recorded significantly higher growth than isolates B_7 and *Pseudomonas* sp. (B_9 and B_{10}).

At 5 days after incubation, maximum growth of bacteria was observed in isolate B_1, which was

Table 1 : Growth of *Fusarium oxysporium* f. sp. *carthami* in bacterial isolates on nutrient agar medium

Sr. No.	Bacterial isolates	Growth of *Fusarium oxysporium* f. sp. *carthami* (mm)			Per cent increase (+) or decrease (-) over control		
		Days after incubation					
		3	5	7	3	5	7
1.	B_1	5.77	8.88	9.90	0.00	-66.27	-65.21
2.	*Pseudomonas* sp. (B_2)	6.44	10.00	18.66	11.61	-62.02	-29.13
3.	*Pseudomonas* sp. (B_3)	6.88	12.25	21.33	19.23	-53.47	-18.98
4.	B_4	5.88	8.55	17.73	1.90	-67.52	-32.66
5.	*Pseudomonas* sp. (B_5)	6.00	8.55	12.55	3.98	-67.52	-52.33
6.	*Pseudomonas* sp. (B_6)	7.21	8.88	11.77	24.95	-66.27	-55.29
7.	B_7	5.10	11.77	19.33	-11.61	-55.29	-29.58
8.	*Pseudomonas* sp. (B_8)	6.55	12.25	16.55	13.51	-53.47	-37.14
9.	*Pseudomonas* sp. (B_9)	6.55	16.33	20.00	13.51	-37.97	-24.04
10.	*Pseudomonas* sp. (B_{10})	5.88	15.66	25.00	1.90	-40.52	-5.05
	Control	5.77	17.66	26.33			
	S.E. ±	0.43	0.47	0.62			
	C.D. (P=0.05)	NS	1.40	1.81			

NS=Non-significant

Table 2 : Growth of bacterial isolates on nutrient agar medium

Sr. No.	Bacterial isolate	Growth of bacteria Days after incubation		
		3	5	7
1.	B_1	30.55	71.88	77.77
2.	*Pseudomonas* sp. (B_2)	3.00	8.32	8.99
3.	*Pseudomonas* sp. (B_3)	17.88	24.10	30.77
4.	B_4	51.77	61.44	77.28
5.	*Pseudomonas* sp. (B_5)	45.10	65.88	77.44
6.	*Pseudomonas* sp. (B_6)	35.21	66.88	77.77
7.	B_7	1.44	2.77	4.77
8.	*Pseudomonas* sp. (B_8)	27.10	58.44	72.55
9.	*Pseudomonas* sp. (B_9)	1.00	2.20	5.88
10.	*Pseudomonas* sp. (B_{10})	1.00	2.22	3.00
	S. E. $\pm$	0.43	0.70	0.45
	C.D. (P=0.05)	1.26	2.07	1.33

significantly superior to other isolates. It was followed by *Pseudomonas* sp. (B_6 and B_5), which were significantly superior to rest of the isolates. In order of merit, growth of bacteria was in B4, *Pseudomonas* sp. (B_8 and B_3) and *Pseudomonas* sp. (B_2), the preceding treatment being significantly superior to succeeding treatment. All the aforesaid treatments had significantly higher bacterial growth than isolate B7 and *Pseudomonas* sp. (B_9 and B_{10}).

At 7 days after incubation, growth of bacteria in isolates B_1, *Pseudomonas* sp. (B_6 and B_5) and B_4 was significantly more than rest of the isolates. These were followed by *Pseudomonas* sp. (B_8, B_3 and B_2) in order of merit, the preceding treatment being significantly superior to succeeding treatment. Bacterial growth in isolate B_7 and *Pseudomonas* sp. (B_9) was significantly superior than the bacterial growth in *Pseudomonas* sp. (B_{10}). Various workers in past reported usefulness of various bacterial isolates against *Fusarium* species. These include *Pseudomonas fluorescens* (Sakthivel and Gnanamanickam, 1986; Rajendran and Ranganathan, 1996 and Ventura *et al*., 1988) and *Bacillus subtilis* (Hervas *et al*., 1998). Observations of present study are on parallel line with the same. The various reasons assigned for the suppression of fungal growth include production of secondary metabolites, antibiotics and siderophores (Lim *et al.,* 1999) which seems to be applicable to the present study also.

Besides some of the bacterial isolates of present study grew fast on Nutrient agar medium (B_1, B_6, B_5 and B_4)and thus put forth competition to *Fusarium oxysporum* f. sp. *carthami*. The bacterial organisms *viz.* B_1, B_6, B_5 have emerged out as having fairly good antagonism against *F. oxysporum* f. sp. *carthami* and need to be evaluated in detail in future study.

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