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Biocontrol Potential of Non-pathogenic *Fusarium* Isolates against the *Fusarium* Wilt of *Chrysanthemum*

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A B S T R A C T

Non-pathogenic *Fusarium* isolates were isolated and five isolates were selected for their biocontrol activity against *Fusarium* wilt of chrysanthemum. Different methods of in vitro tests were conducted, under the dual plate method the isolate UASB NPF-III and UASB NPF-I inhibited the pathogen by 64.17 % and 60.44 % respectively at seventh day of incubation. The cell free culture filtrates of non pathogenic *Fusarium* isolates UASB NPF-III and UASB NPF-I were found effective in controlling the pathogen by 26.36 % and 24.34 % at seventh day of inoculation. Similarly, the methanol and ethyl extract of culture filtrate of UASB NPF-III also have some effects on the pathogen and the inhibition per cent was about 41.81 % and 39.99 % respectively.

Introduction

Chrysanthemum is one of the three best merchandisable floriculture crops, globally cultivated for cut as well as loose flowers and also as pot plants. In the global market, The Netherlands stands first in *Chrysanthemum* production followed by Germany and the UK. Chrysanthemum (family Compositae) includes about 200 species producing flowers of different types and about 20,000 diverse varieties of *Chrysanthemum* are grown worldwide, out of which nearly 1000 varieties are cultivated in India. It is commercially cultivated in Maharashtra (Pune, Nasik and Ahmednagar); Karnataka (Bengaluru, Kolar, Dharwad, Belgaum and Tumkur); Rajasthan (Udaipur, Jaipur Ajmer, Jaipur and Kota); Gujarat (Anand, Vadodara, Surat, Navsari and Valsad); Haryana (Ambala, Gurgaon and Faridabad); West Bengal (Calcutta and adjoining areas); Delhi; Uttar Pradesh and Tamil Nadu. Chrysanthemum flowers have immense demand both in national and in global markets which has consequently resulted in the increase in the area of cultivation. Successful cultivation of Chrysanthemum plant is affected by numerous bacterial, fungal and viral diseases (Bhattacharjee and De, 2003).

The microorganisms in the rhizosphere were

ideal for use as biocontrol agents, since the rhizosphere provides the front line defense for roots against attack by pathogens. The biological control agent may operate primarily in the host tissue, thereby indicating a resistance response of the host, transmitting factors rendering the pathogens avirulent. These interactions mediated by environment and have an overriding impact on determining whether biocontrol operates in a system (Papavizas and Lumsden, 1980). *Fusarium* wilt of Chrysanthemum caused by *Fusarium oxysporium* f. sp. *chrysanthemi* is considered as one of the most wide spread and destructive disease, causing infection and loss from nursery to flowering stage. The disease was most severe in the warm climates (Locke *et al.*, 1985). Chrysanthemum wilt caused by *Fusarium* is difficult to control because, the pathogen persistency in the soil and low availability of resistant varieties for the cultivation (Garibaldi *et al.*, 2009). The aspects of epidemiology, pathogenesis and biological control measures of *Fusarium oxysporum* causing wilt disease of Chrysanthemum are studied. The present study was done on the biocontrol of *Fusarium* wilt of Chrysanthemum growing regions of southern districts of Karnataka.

Materials and Methods

Collection of soil samples and disease samples

The soil samples were collected from chrysanthemum growing regions in the southern districts of Karnataka *viz.*, Bengaluru, Chamarajanagar, Chikkaballapur, Chikkaballapur, Kolar and Mysore. Rhizosphere soils of the chrysanthemum plants were collected in polythene bags and brought to the laboratory for the isolation of non-pathogenic *Fusarium*. During the collection of soil samples from chrysanthemum fields, diseased samples of chrysanthemum plants infected with *Fusarium* wilt was collected for isolation of pathogen.

Totally 67 isolates was isolated and screened. Among those isolates, five *Fusarium* isolates BAF6, BNF10, CKF4, CGF1 and CCF3 was selected for further studies. The above 5 *Fusarium* isolates was named as BAF6 – UASB NPF-I; BNF10- UASB NPF-II; CKF4- UASB NPF-III; CGF1- UASB NPF-IV and CCF3- UASB NPF-V.

In vitro evaluation of non-pathogenic *Fusarium* isolates against wilt pathogen of chrysanthemum

Dual plate technique

The antagonistic potential of the non-pathogenic *Fusarium* isolates against wilt pathogen *Fusarium oxysporum* was conducted by dual culture method (Dennis and Webster, 1971a) on PDA medium. Fifteen ml of PDA medium was poured into sterile Petri plate and allowed for solidification. Five mm agar disc of fungal pathogen was cut with a sterile cork borer and placed on PDA plate one cm away from the edge. Similarly, non-pathogenic *Fusarium* isolate was placed on the other side, *i.e.*, at an angle of 180^0. Plates without non-pathogenic *Fusarium* isolate were served as the pathogen control. The plates were incubated at 28 ± 1^0C for seven days. Each treatment was replicated thrice. The extent of antagonistic activity by non-pathogenic *Fusarium* isolates was recorded on fourth and seventh day after incubation by measuring growth of pathogen in dual culture plate and control plate.

The per cent inhibition of pathogen was calculated as suggested by Vincent (1927).

W] $$I = \frac{(C - T)}{C} \times 100$$

I=Per cent inhibition
C=Growth of fungal plant pathogens in control (cm)
T=Growth of fungal plant pathogens in dual culture plate (cm)

Effect of cell free culture filtrates of non-pathogenic *Fusarium* isolates against the *Fusarium* wilt of chrysanthemum

The effect of cell free culture filtrates of non-pathogenic *Fusarium* isolates was determined by following the methods of Dennis and Webster (1971c). The isolates was inoculated in 100 ml potato dextrose broth and incubated at 28±1 °C for 10 days. The cultures were then filtered through Whatman No. 1 filter paper. The culture filtrate was added at the concentration of 10% to the molten PDA medium while pouring to the Petri plates and allowed for solidification. Then the plates were inoculated with 5mm mycelial disc of pathogen *Fusarium* spp. and incubated at 28±1°C for seven days. Control plates were maintained without adding the culture filtrate. Colony diameter of the pathogen and inhibition of the mycelial growth was observed.

Similarly, culture broth of the isolates was extracted with organic solvents such as methanol and ethyl acetate. Organic solvents were added to whole culture broth in 1:1 proportion and incubated at 100 rpm for 30 minutes. Then, the crude culture filtrate was filtered and concentrated (Sowparthani and Kathiravan, 2011). These crude extracts were tested against the pathogen by following the procedure as mentioned above.

Results and Discussion

Potential of biocontrol of non pathogenic *Fusarium* isolates against the chrysanthemum *Fusarium* wilt pathogen was conducted under *in vitro* condition by dual culture method. The results are presented in Table 1. On fourth day after inoculation, colony diameter of the pathogen was found lesser in the isolate UASB NPF-III (1.23 cm) which was followed by the isolate UASB NPF-I (1.9 cm), whereas in the pathogen control plate, the diameter of the colony of 4.4 cm was recorded. The higher inhibition of the pathogen was observed in the isolate UASB NPF-III (71.98 %) followed by the isolate UASB NPF-I (56.79 %) which were statistically significant with each other. The lowest inhibition (45.40 %) was shown by the isolate UASB NPF-IV.

On the seventh day after inoculation, the lower colony diameter of pathogen was recorded in the isolate UASB NPF-III (2.23 cm) which was followed by the isolate UASB NPF-I (2.46 cm).in the pathogen control plate the diameter of the colony was 6.23 cm. The highest inhibition of 64.17 % was showed by the isolate UASB NPF-III followed by the isolate UASB NPF-I (60.44 %). Significant differences were observed among the non-pathogenic *Fusarium* isolates in the antagonistic potential (Figure 1).

From the above *in vitro* studies it was revealed that the isolates UASB NPF-III and UASB NPF-I are fast growing and effective compared to pathogen culture and are capable in competing with the pathogen for nutrients and space and thereby suppressing its growth. Similar studies of antagonistic activity of non-pathogenic *Fusarium* cultures against the *Fusarium* wilt of tomato was done by Patil *et al.*, (2011). The inhibition of pathogen was found to the extent of 32-40 % on testing with the non-pathogenic *Fusarium* isolates Fu3, Fu4, Fu24 and Fu25.

The cell free culture filtrate of non-pathogenic *Fusarium* isolates was tested against the *Fusarium* wilt pathogen of chrysanthemum under *in vitro* conditions. Significant

differences were found when the cell free culture filtrate was used. At fourth day of inoculation, the lower colony diameter of the pathogen was observed to be 2.93 cm in the isolate UASB NPF-III followed by the isolate UASB NPF-I (2.96 cm). The control plate was observed to have the colony diameter of 4.46 cm. The higher 34.28 % inhibition of pathogen was recorded in the isolate UASB NPF-III followed by the isolate UASB NPF-I (24.34 %).

At seventh day after inoculation, the less colony diameter of pathogen was recorded in the isolate UASB NPF-III (4.83 cm) which was followed by the isolate UASB NPF-I (4.97 cm) in the pathogen control plate the diameter of the colony was 6.57 cm. The highest inhibition of 26.36 % was showed by the isolate UASB NPF-III followed by the isolate UASB NPF-I (24.24 %). The results are represented in the Table 2.

Even less inhibition of pathogen was recorded in the above studies when compared to dual culture technique, this test also showed that the non-pathogenic *Fusarium* isolates may produce bioactive compounds and is responsible for the inhibition of pathogen. Similar studies was done by Thongkamngam and Jaenaksorn (2016) tested the potential of culture filtrate (CF) of non-pathogenic *Fusarium oxysporum* (F221-B) against plant pathogenic fungi namely, *Curvularia* sp. *F. semitectum*, *F. oxysporum* f. sp. *lactucae*, *Rhizoctonia* spp. and *R. solani in-vitro* and *Fusarium* root rot disease in hydroponics. The cell free culture filtrate at all test concentrations revealed the greatest spore germination inhibition 100% over control against the fungal pathogens tested.

Table.1 Effect of non-pathogenic *Fusarium* isolates on the growth of pathogenic *Fusarium* spp. under *in vitro* condition using dual plate technique

Isolates	Mean colony diameter on 4th day (cm)		% Inhibition	Mean colony diameter on 7th day (cm)		% Inhibition
	Pathogen	NPF		Pathogen	NPF	
UASB NPF-I	1.90	4.17	56.79^{b}	2.46	5.20	60.44^{b}
UASB NPF-II	2.06	4.10	53.00^{bc}	2.63	4.70	57.76^{bc}
UASB NPF-III	1.23	3.77	71.98^{a}	2.23	5.73	64.17^{a}
UASB NPF-IV	2.4	4.33	45.40^{d}	2.76	5.30	55.62^{c}
UASB NPF-V	2.23	4.06	49.21^{cd}	2.73	5.10	56.15^{c}
Pathogen	4.4	---	---	6.23	---	---
S.Em	---	---	1.878	---	---	0.867
CD@ 5%	---	---	5.916	---	---	2.731

Table.2 Effect of cell free culture filtrate of non-pathogenic *Fusarium* isolates on the growth of pathogenic *Fusarium* spp. under *in vitro* condition

Isolates	Mean colony diameter on 4th day (cm)	% Inhibition	Mean colony diameter on 7th day (cm)	% Inhibition
UASB NPF-I	2.96	33.56[a]	4.97	24.34[ab]
UASB NPF-II	3.06	31.32[ab]	5.10	22.29[abc]
UASB NPF-III	2.93	34.28[a]	4.83	26.36[a]
UASB NPF-IV	3.23	27.58[c]	5.36	18.23[c]
UASB NPF-V	3.17	29.04[bc]	5.23	20.26[bc]
Pathogen	4.46	---	6.57	---
S.Em	--	1.167	--	1.529
CD@ 5%	--	3.676	--	4.819

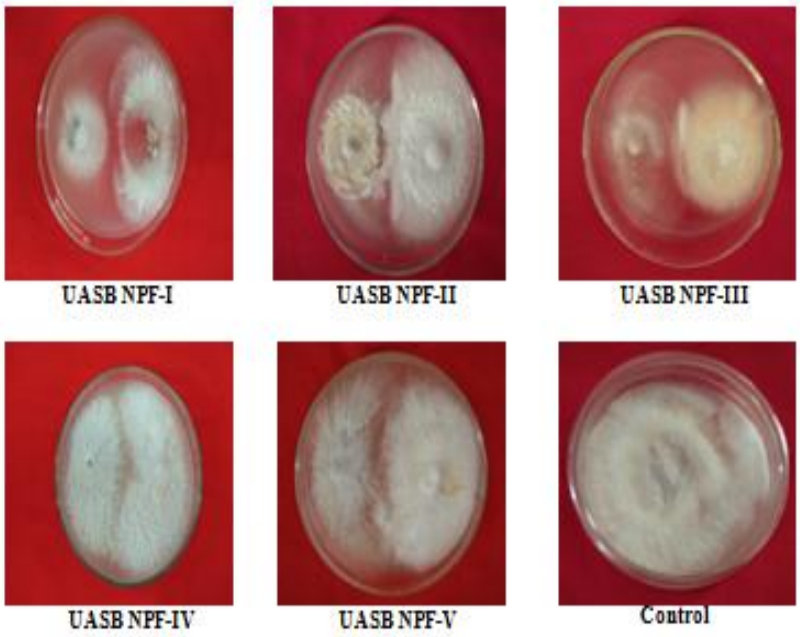
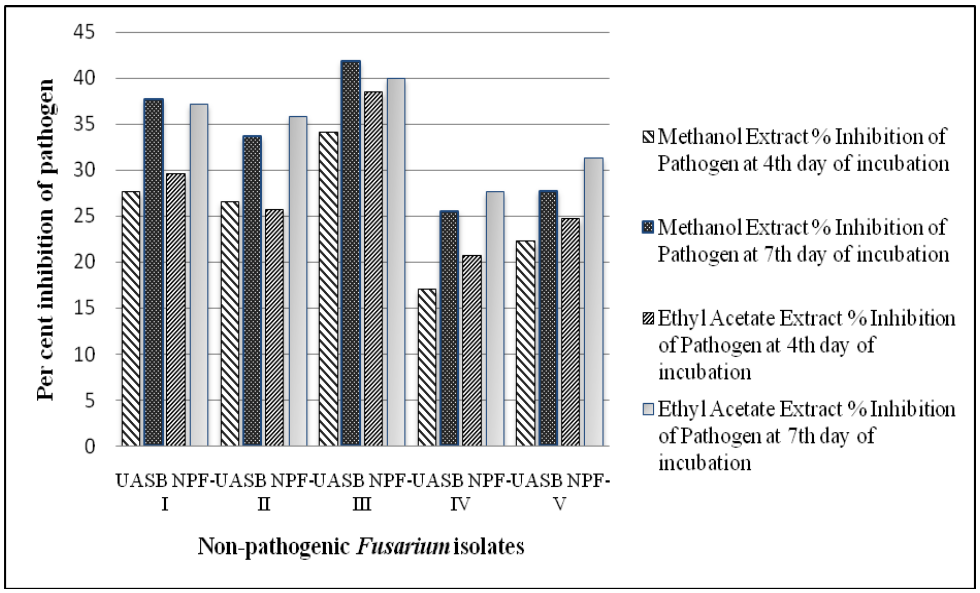


Figure 1: Non-pathogenic *Fusarium* isolates effective against chrysanthemum wilt pathogen by dual plate method

Fig.2 Effect of methanol and ethyl extracts of the culture filtrates of non-pathogenic *Fusarium* isolates on the growth of pathogenic *Fusarium* spp. under *in vitro* condition



The methanol and ethyl acetate extracts of the culture filtrates of non-pathogenic *Fusarium* isolates was tested against the pathogen (Fig 2). The inhibition of pathogen was more (34.04 % and 41.81 %) in the methanol extract of the isolate UASB NPF-III during fourth and seventh day of inoculation respectively and comparatively lesser inhibition was recorded (17.04 % and 25.47 %) in the isolate UASB NPF-IV during fourth and seventh day of inoculation respectively. Similarly, the inhibition of pathogen was more (38.52 % and 39.99 %) in the ethyl acetate extract of the isolate UASB NPF-III during fourth and seventh day of inoculation respectively and comparatively lesser inhibition was recorded (20.73 % and 27.72 %) in the isolate UASB NPF-IV during fourth and seventh day of inoculation respectively. This is in evidence with the findings of Islam *et al.*, (2018) conducted *in vitro* study of biocontrol potential of culture filtrate and ethyl acetate crude extract of rhizospheric *Pseudomonas aeruginosa* inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* by 56.66 and 25.0%, respectively.

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