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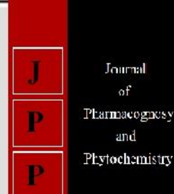

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Isolation and characterization of non-pathogenic strains of *Fusarium* spp. from the rhizosphere of chrysanthemum

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Abstract
The wilt of Chrysanthemum is caused by *Fusarium oxysporium* f. sp. *chrysanthemi.* As a biocontrol aspect the non-pathogenic *Fusarium* spp. were isolated and studied to test against the pathogenic *Fusarium* spp. Totally 67 isolates of *Fusarium* were isolated from the southern districts of Karnataka. Out of which, five isolates were found to be non-pathogenic *Fusarium* spp. by four different non-pathogenicity tests. The isolates are UASB NPF-I, UASB NPF-II, UASB NPF-III, UASB NPF-IV and UASB NPF-V. The isolates were observed for microconidia, macroconidia and chlamydospores. The microconidial size varied from 7×2.2 µm to 9.1×2.4 µm, macroconidial structure varied from 26×7.2 µm to 32×7.2 µm and the chlamydospores from 4.2 to 5.2 µm in diameter. Also, the *Fusarium* cultures were studied for their morphological and cultural characters on different culture media and they were recorded with different growth parameters like colony colour, colony diameter, pigmentation.

Keywords: Chrysanthemum, non-pathogenic, *Fusarium*, morphology

Introduction
Chrysanthemum (*Dendranthema grandiflora* Tzvelev.) is also known as 'Queen of East' or 'Autumn Queen' belongs to the Asteraceae family. The word Chrysanthemum is named by Carolus Linnaeus and derived from two Greek words, 'Chrys' meaning golden (the colour of the original flowers), and 'anthemon', meaning flower. There are about 160 species in the genus *Chrysanthemum*. It occupies a major position in ornamental horticulture and is one of the commercially exploited flower crops. In many countries, including India, United States and Japan it is considered as the number one crop. The genus, of which the present-day florist Chrysanthemum (*Chrysanthemum morifolium*) group belongs, has been renamed as 'Dendranthema' and the species as grandiflora (Anderson, 1987) [1].

Chrysanthemum is native to the northern hemisphere and is widely distributed in Europe and Asia. However, it is believed that, its origin is China (Carter, 1980) [3]. Japan, China, Holland, France, England, America and India are the major commercially producing countries. The Chrysanthemum has been recognized in India as one among the five important commercially potent flower crops by the All India Coordinated Floriculture Improvement Project (ICAR) and is most important flower grown on commercial scale with an area of 11050 hectare and production of 106760 million tons. In India, Karnataka is the most prominent chrysanthemum growing state mainly grown in Dharwad, Belgaum, Hassan, Bengaluru, Mysore and Malur with an area of 5100 ha and annual production of 61200 tons of flowers followed by the state's Tamil Nadu, Andhra Pradesh, Maharashtra, Rajasthan and Bihar.

Successful cultivation of Chrysanthemum plant is hindered by numerous bacterial, fungal and viral diseases (Bhattacharjee and De 2003) [2]. *Fusarium* wilt of Chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi* is one of the wide spread and destructive diseases, causing infection and loss from nursery to flowering stage. The disease is most severe in warm climates (Locke *et al.,* 1985) [9]. Plants infected with *Fusarium* wilt shows symptoms of drooping, yellowing and loss of turgidity of leaves; stunted growth and failure in production of normal buds and flowers. *Fusarium* wilt of Chrysanthemum is difficult to control because of the persistence of pathogen in the soil and low availability of resistant varieties for its cultivation (Garibaldi *et al.,* 2009) [6]. The present paper is intended to discuss the aspects of isolation and characterization of non-pathogenic *Fusarium* strains from the rhizosphere of Chrysanthemum growing regions of southern parts of Karnataka.

Material 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, 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.

Isolation of *Fusarium* wilt pathogen of Chrysanthemum

Fusarium wilt pathogen of chrysanthemum was isolated by tissue isolation method. Infected plant parts mainly collar region and roots of collected disease samples of the chrysanthemum plant were rinsed with running tap water and cut into small bits (2-3 mm). These root samples were surface sterilized with 3% sodium hypochlorite solution for two minutes, followed by washing with distilled water and finally surface sterilized with 70% alcohol for one minute. Surface sterilized root samples were finally rinsed with distilled water for three to four times. Surface sterilized root samples were inoculated on the solidified potato dextrose agar (PDA) plates and incubated at 28 °C for 2-3 days. Petri plates were observed for the fungal colonies growing on the infected root samples. Fungal colonies were sub cultured and microscopic observation of Macroconidia and Microconidia was done to confirm the *Fusarium* isolates. The confirmed *Fusarium* isolates were purified and further used for pathogenicity studies.

Evaluation of pathogenicity

The pathogenicity test was done to establish the ability of the isolates to produce typical symptoms of *Fusarium* wilt in chrysanthemum under the artificial inoculation condition. The test was carried out by using sterilized coco peat mixture in seedling trays. The commercial coco peat mixture was sterilized twice in a two days interval by autoclaving at 15 psi for 60 minutes. The sterilized coco peat was moistened to 60% with sterile water. Healthy chrysanthemum suckers were selected and their roots were treated with 1% sodium hypochlorite solution for 2 minutes, subsequently washed with sterile distilled water to remove the traces of sodium hypochlorite and were planted in the seedling trays. Twenty days old suckers were used to check the pathogenicity of the *Fusarium* isolates. One ml of the seven days old culture broth of the *Fusarium* isolates were drenched at the collar region of the suckers (Locke *et al.*, 1985) [9]. Suckers were observed for the development of the wilt symptoms. Based on the development of wilt symptoms, isolates were categorized as less virulent, moderate and highly virulent isolates. Highly virulent isolate was selected and used as pathogen control in further studies.

Isolation of non-pathogenic *Fusarium* spp.

Fusarium species were isolated by standard plate count technique using *Fusarium* selective media such as Selective *Fusarium* Agar (SFA) and Peptone PCNB Agar (PPA) (Nash and Snyder, 1962) [11]. The isolates were purified by establishing single spore culture by standard dilution plate method. Isolated *Fusarium* cultures were confirmed by observation of morphological characters like micro and macroconidia as per the descriptions of Leslie and Summerell (2006) [8] in *Fusarium* laboratory manual.

Screening of *Fusarium* cultures for non-pathogenicity

The isolates of *Fusarium* were screened for their non-pathogenicity by different methods (Patil *et al.,* 2011) [13].

A. Test tube assay

In this method 20 days old suckers of chrysanthemum grown on sterilized coco peat compost were uprooted and gently rinsed with running tap water followed by sterile water. Spore suspension of *Fusarium* was prepared by flooding water to seven days old cultures on PDA plates. To obtain 100 ml spore suspension, three Petri plate cultures were used and spore suspension was adjusted to 4-6 x 10^6 ml^{-1}. The resultant spore suspension was filtered through muslin cloth. Suckers were transferred into different test tubes containing the spore suspension of different isolates of *Fusarium* spp. so that roots were immersed in the suspension and cotton was placed around the plant at the mouth of the test tube. Ten suckers were treated for each isolate tested. The test tubes were incubated at ambient temperature. Based on the development of wilt symptoms isolates were categorized as non-pathogenic (where no wilt symptoms were observed up to 15 days of incubation) and least, moderate and highly pathogenic strains based on intensity of wilt symptoms appeared during incubation period.

B. Suckers dip in culture broth

The *Fusarium* isolates were cultured in potato dextrose broth for five days under shaking condition. The 20 days old suckers of chrysanthemum were dipped in the beaker containing *Fusarium* biomass for 5-6 hours followed by transplanting into plastic cups containing sterilized coco peat compost. Ten suckers were treated for each isolate tested. The observations on wilt symptoms were recorded till maximum of 50 per cent of suckers got wilted in 15 days after inoculation.

C. Drenching the suckers with culture broth

The *Fusarium* isolates under study were cultured in potato dextrose broth for five days under shaking condition. Twenty days old suckers of chrysanthemum raised in trays were drenched with one ml of fungal biomass broth at collar region of the suckers. Ten suckers were treated for each isolate tested.

D. Drenching the suckers with spore suspension

The spore suspension of the *Fusarium* isolates was prepared by using sterile water. The spore suspension at the concentration of 4-6 x 10^6 ml^{-1} was used to drench the collar region of the chrysanthemum suckers of 20 days old. Ten suckers were treated for each isolate tested. The observations on wilt symptoms were recorded till maximum of 50 per cent of suckers got wilted in 10 days after inoculation.

Host range studies of the selected *Fusarium* isolates

The selected non-pathogenic *Fusarium* isolates from the screening experiments were tested for host range studies *i.e.,* confirming their non-pathogenicity in other crop plants belonging to the Asteraceae family namely, China aster, Cosmos, Dahlia, Lettuce, Marigold, Sunflower and Zinnia. Seeds were treated with talc formulations of non-pathogenic *Fusarium* (2g/ 100 g of seeds). Seeds were sown in seedling trays and tested for wilt symptoms. Observations were recorded for wilting.

Microscopic observations of the *Fusarium* cultures

All the isolates of *Fusarium* spp. were studied for their spore behaviours like micro and macroconidial structures. Five mm mycelial disc from actively growing seven days old culture of *Fusarium* were inoculated on to the Pre solidified Petri plates of Potato dextrose Agar (PDA) and plates were incubated at 28 °C for ten days. Spores were taken on a clean glass slide and mixed thoroughly with lactophenol cotton blue along with a drop of sterile water so that, a uniform spread is obtained and then cover slips were placed on it. Spores were measured under high power objectives (40x) using ocular and stage micrometer. The size of the spores was determined and shape of the spores were noted. For micro and macroconidia, length and breadth of all the isolates. To observe the chlamydospores, 12 to 15 days old cultures were used.

Morphological and cultural characterization of isolated *Fusarium* spp.

All the isolates of *Fusarium* spp. were studied for differences in morphological and cultural characteristics. Different culture media such as Czapek's Agar (CDA), Malt Extract Agar (MEA), Oat Meal Agar (OMA), Potato Carrot Agar (PCA), Richard's Agar (RCA), Sabouraud's Dextorse Agar (SDA), were used to study the cultural characteristics, while to study the sporulation SNA (Spezieller and Nahrstoffarmer Agar) and V8 juice agar were used. Twenty ml of the medium was poured into the sterilized Petri plates. After solidification 5 mm mycelial disc from actively growing seven days old culture of *Fusarium* was placed at the centre of the medium and plates were incubated at 28 °C for seven days. The observations were recorded for colony colour, colony diameter, pigmentation, growth pattern on different media.

Results and Discussion

Collection of soil samples and isolation of different *Fusarium* spp.

The soil samples were collected from different areas of Chrysanthemum growing regions in southern districts of Karnataka namely Bengaluru, Chamarajanagar, Chikkaballapur, Kolar and Mysore. The Chrysanthemum fields of healthy crop stand were selected to collect the soil samples. The soil samples were collected from the rhizosphere regions of Chrysanthemum plants for the isolation of non-pathogenic *Fusarium* spp. Totally 54 soil samples were collected. *Fusarium* strains were isolated by serial dilution plating technique using Selective *Fusarium* Agar (SFA) and PCNB (Pentachloronitrobenzene) agar media. The colonies which were showing whitish to cream, curdy, raised and dense mycelia with purple to violet tinge were selected and purified for further studies. The isolated cultures of *Fusarium* spp. were confirmed by microscopic observations of morphological characteristics like micro and macroconidia by referring to *Fusarium* laboratory manual (Leslie and Summerell, 2006) [8]. Totally 67 cultures of *Fusarium* were isolated and they were assigned with respective code numbers. The details of rhizospheric soil samples collected from different areas and *Fusarium* isolates obtained with assigned code numbers is represented in Table 1.

The Chrysanthemum plant infected with *Fusarium* wilt was selected and used for the isolation of pathogenic *Fusarium* spp., infected root area was used for the isolation of pathogen by Tissue isolation technique. Thus, obtained isolates were confirmed by morphological characters namely micro and macroconidia through microscopic observations and they were used for pathogenicity tests. Based on the pathogenicity tests, one virulent isolate which showed prominent results was selected and assigned with code number UASB PF. This pathogenic *Fusarium* isolate was used as pathogen control in further studies (Plate 3).

Screening of isolated *Fusarium* cultures for non-pathogenicity

Different inoculation assays were followed for screening of *Fusarium* isolates for their non-pathogenicity behaviour. Firstly, test tube assay using spore suspension and drenching of spore suspension to crown region of chrysanthemum suckers were tested. All the isolates were screened by sucker dip in broth cultures and also drenching of broth to chrysanthemum suckers. (Table 2a and 2b).

In test tube method, the treated suckers were observed for symptoms of wilt and the per cent wilt incidence was recorded. The typical wilt symptoms were appeared in 5 days after inoculation. Observations were recorded up to 15 days after inoculation.

Table 1: Soil samples of chrysanthemum fields collected from southern parts of Karnataka and *Fusarium* cultures isolated

Sl. No.	District	Location	No. of soil samples	*Fusarium* cultures isolated
1.	Bengaluru	Attibele Doddaballapur Nelamangala	22	BAF1, BAF2, BAF3, BAF4, BAF5, BAF6, BAF7, BAF8, BAF9, BAF10, BAF11, BAF12 BDF1, BDF2, BDF3, BDF4, BDF5, BDF6, BDF7, BDF8 BNF1, BNF2, BNF3, BNF4, BNF5, BNF6, BNF7, BNF8, BNF9, BNF10, BNF11
2.	Chamarajanagar	Kollegal Yelandur	8	CKF1, CKF2, CKF3, CKF4 CYF1, CYF2, CYF3, CYF4, CYF5
3.	Chikkaballapur	Gauribidanur Chikkaballapur	10	CGF1, CGF2, CGF3, CGF4, CGF5 CCF1, CCF2, CCF3, CCF4, CCF5, CCF6, CCF7
4.	Kolar	Malur	5	KMF1, KMF2, KMF3, KMF4
5.	Mysore	Nanjangud T. Narasipura	9	MNF1, MNF2, MNF3, MNF4, MNF5, MNF6 MTF1, MTF2, MTF3, MTF4, MTF5
Total			54	67

Table 2a: Screening of *Fusarium* isolates for the non-pathogenicity by different methods

Isolates	Test tube assay (% wilt incidence)	Spore drench method (% wilt incidence)	Seedling drench with culture broth (% wilt incidence)	Sucker dip in culture broth (% wilt incidence)
BAF1	60	70	50	60
BAF2	50	60	40	50
BAF3	70	---	80	60
BAF4	50	70	50	70
BAF5	60	50	50	60
BAF6	---	---	---	---
BAF7	50	40	60	30
BAF8	70	80	50	40
BAF9	60	50	---	60
BAF10	50	40	60	50
BAF11	60	70	80	50
BAF12	---	60	50	50
BDF1	50	70	40	60
BDF2	60	80	60	70
BDF3	60	70	---	50
BDF4	---	40	60	70
BDF5	70	80	60	80
BDF6	50	40	---	80
BDF7	50	70	50	70
BDF8	70	80	60	80
BNF1	70	---	80	60
BNF2	70	80	50	40
BNF3	60	80	60	---
BNF4	50	70	50	70
BNF5	50	60	40	50
BNF6	70	80	50	40
BNF7	60	80	60	70
BNF8	70	---	80	60
BNF9	60	70	80	50
BNF10	---	---	---	---
BNF11	70	80	50	40
CKF1	70	80	50	40
CKF2	50	60	40	50
CKF3	70	80	50	40

Table 2b: Screening of *Fusarium* isolates for the non-pathogenicity by different methods

Isolates	Test tube assay (% wilt incidence)	Spore drench method (% wilt incidence)	Seedling drench with culture broth (% wilt incidence)	Sucker dip in culture broth (% wilt incidence)
CKF4	---	---	---	---
CYF1	60	70	80	50
CYF2	70	80	60	80
CYF3	50	60	40	50
CYF4	70	80	50	40
CYF5	70	80	50	40
CGF1	---	---	---	---
CGF2	60	70	80	50
CGF3	70	---	80	60
CGF4	70	80	50	40
CGF5	60	80	60	70
CCF1	50	60	40	50
CCF2	60	70	80	50
CCF3	---	---	---	---
CCF4	50	60	40	50
CCF5	70	80	50	40
CCF6	70	80	50	40
CCF7	50	60	40	50
KMF1	60	70	80	50
KMF2	50	60	40	50
KMF3	60	70	80	50
KMF4	70	---	80	60
MNF1	60	80	60	70
MNF2	50	60	40	50
MNF3	50	70	50	70
MNF4	50	---	80	60
MNF5	70	80	60	80

MNF6	50	60	40	50
MTF1	70	80	50	---
MTF2	50	70	50	70
MTF3	50	60	40	50
MTF4	50	60	40	50
MTF5	60	70	80	50
Control	0	0	0	0
Pathogen	50	60	80	100

The percentage of wilt disease appeared was varied between 50 to 70%. Among the *Fusarium* isolates screened using test tube assay, seven *Fusarium* cultures *viz.,* BAF6, BAF12, BDF4, BNF10, CKF4, CGF1 and CCF3 did not show any wilt symptoms. In the other method like drenching of spore suspension to the chrysanthemum suckers, eleven *Fusarium* cultures *viz.,* BAF3, BAF6, BNF1, BNF8, BNF10, CKF4, CGF1, CGF3, CCF3, KMF4 and MNF4 did not appeared to have any wilt symptoms. In such screening assays, the percentage of disease incidence was ranged from 40 to 80%.

In the culture broth screening methods, the method of drenching recorded the wilt disease incidence in the range of 40 to 80%. Eight *Fusarium* spp. *viz.,* BAF6, BAF9, BDF3, BDF6, BNF10, CKF4, CGF1 and CCF3 showed positive for no wilt symptoms and remaining 59 isolates were positive for wilt incidence. In sucker dip method, seven *Fusarium* cultures *viz.,* BAF6, BNF3, BNF10, CKF4, CGF1, CCF3 and MTF1 showed no wilt symptoms. The percentage of disease incidence observed in this assay was ranged between 50 to 80 %.

Among 67 isolates, the five *Fusarium* isolates namely, BAF6, BNF10, CKF4, CGF1 and CCF3 showed no wilt symptoms (Plate 1 and 2) in all the four screening assays, they were retested to completely confirm their non-pathogenicity and proved to be non-pathogenic in nature. The pathogenic isolate UASB PF showed typical wilt symptoms in all methods of screening and symptoms were observed on 2-3 days after inoculation. The five non-pathogenic *Fusarium* isolates were given with new code numbers *i.e.,* BAF6-UASB NPF-I, BNF10-UASB NPF-II, CKF4-UASB NPF-III, CGF1-UASB NPF-IV and CCF3-UASB NPF-V and were used in further studies (Plate 3).

Host range studies of selected *Fusarium* isolates on the non-target plants of Asteraceae family

The selected *Fusarium* cultures was tested for their non-pathogenicity on other crop plants. Seven different crop plants from Asteraceae family were tested and found to have no wilt symptom up to 30 days of inoculation. Rowe (1980) [16] have done the comparative studies on the pathogenicity and the host range studies of isolates of *Fusarium oxysporum* causing the crown and root rot of tomato. He has tested 17 different crop plants of four different families like Solanaceae, Cucurbitaceae, Leguminosae and Gramineae. Similarly, Zarafi *et al.,* (2014) [17] conducted the host range studies of *Fusarium oxysporum* f. sp. *strigae* which is used for the biocontrol of *Striga hermonthica* on maize and sorghum. They have used 26 different economically important crops in Nigeria in a screen house for host range studies using the two isolates in a granular formulation

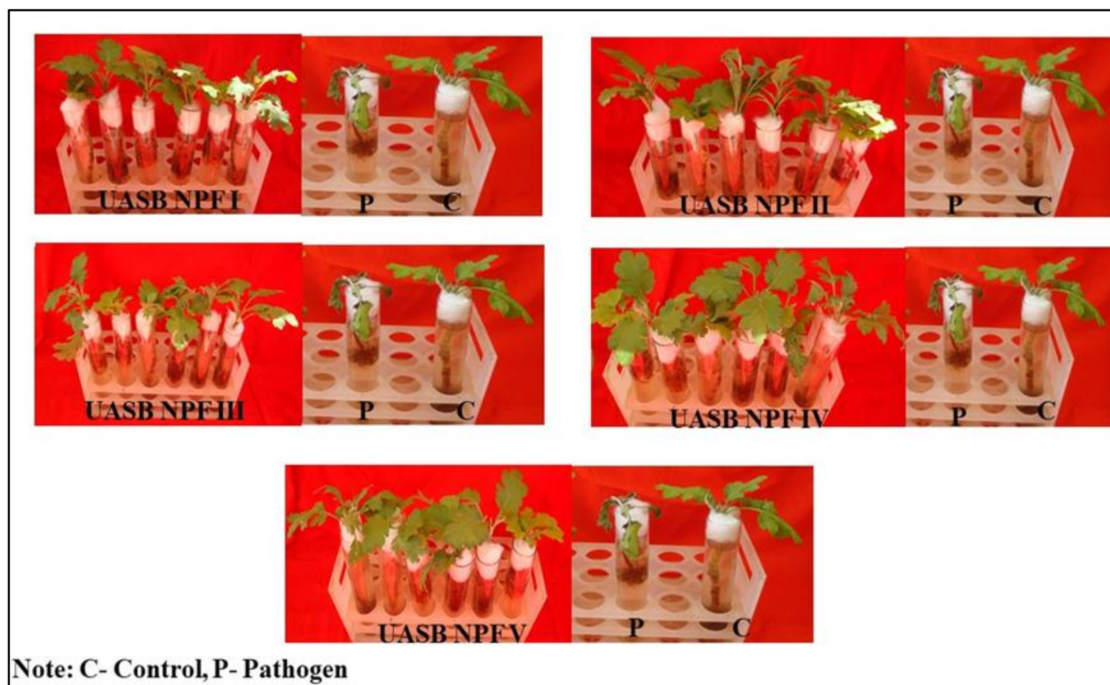

Plate 1: Test tube assay of screening non-pathogenic *Fusarium* isolates

Plate 2: Suckers dip in culture broth method of screening non-pathogenic *Fusarium* isolates

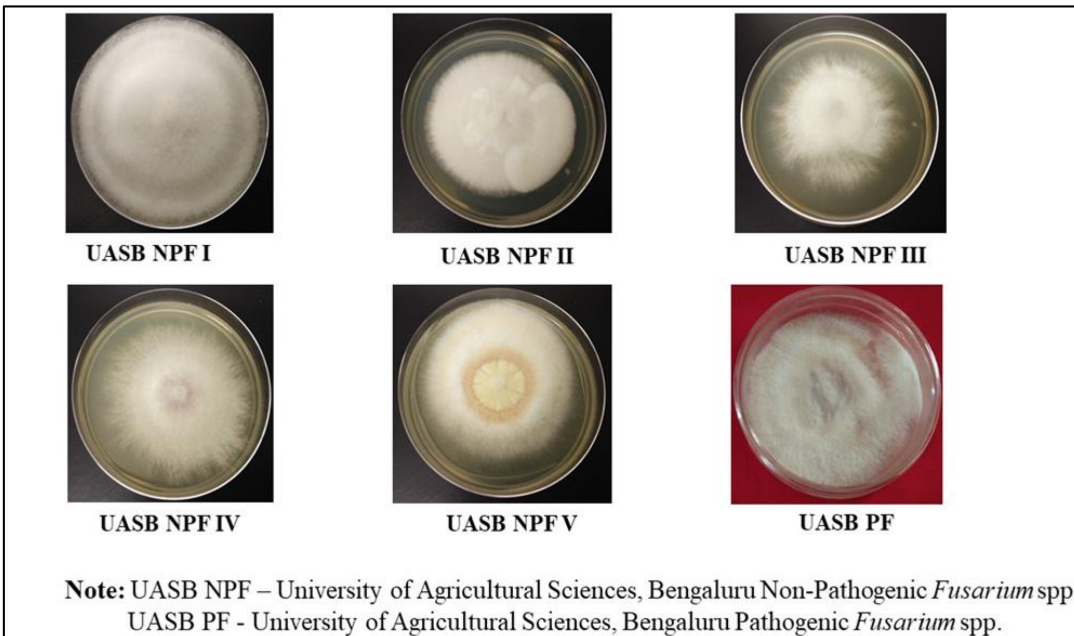

Plate 3: Pure cultures of non-pathogenic *Fusarium* isolates and the wilt pathogen

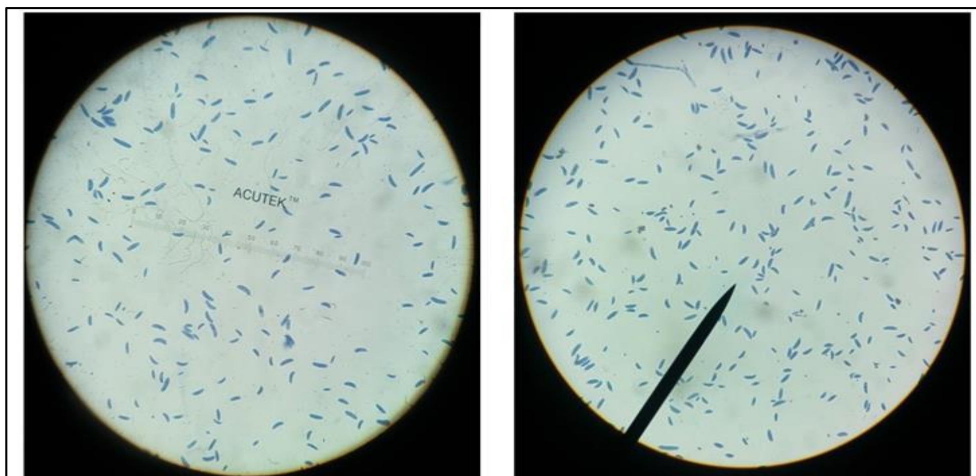

Plate 4: Microscopic observation of conidial structures of *Fusarium* isolates under 40x

Morphological and cultural characteristics of selected non-pathogenic *Fusarium* isolates

The morphological and cultural characteristics of five non-pathogenic *Fusarium* cultures were observed on eight different culture media. The microscopic observations on macroconidia, microconidia and chlamydospores with dimensions were observed for the isolates grown on potato dextrose agar (PDA) (Plate 4). Tapered, hook shaped, blunted shaped apical cells of macroconidia of the isolates were observed. Basal cells of microconidia were varied like notched type to slightly notched, foot shape and elongated typed cells were founded. The microconidial shapes of isolates were round to oval shaped with short to long mono or polyphialides were studied. Such observations were also made by Ciampi e*t al.,* (2009) [5] by using Scanning electron microscope to study two species of *Fusarium* causing wilting of coloured Callas (*Zantedeschia aethiopica* (L.) Spreng.) in Chile.

Chlamydospores were found to be in terminal and intercalary positions bearing rough to smooth, oval shaped spores which are in pairs or in clusters or in chains. The recorded microscopic observations are presented in Table 3. Nel *et al.*, (2006) [12] observed the morphological characteristics of non-pathogenic *Fusarium oxysporum* cultures isolated from the rhizosphere of healthy banana plantations and they observed, abundant oval to kidney shaped microconidia in false heads and abundant macroconidia which are slightly sickle-shaped, thin walled and delicate, with an attenuated apical and a foot-shaped basal cells. Short monophialides were found and chlamydospores were present and were formed singly or in pairs. Similar observations were made by Rodrigues and Menezes (2005) [15] on microconidia, macroconidia and chlamydospores in order to characterise the isolated endophytic *Fusarium* cultures of cowpea plants. Monuj *et al.,* (2017) [10] observed 8 isolates of *Fusarium solani* causing root rot of Patchouli in Assam and described the morphology of macroconidia which were sickle shaped and elongated with blunt ends and microconidia were round to oval shaped. The chlamydospores were oval, intercalary and terminal among the isolates.

The study on colony morphology and growth characteristics on different culture media such as Czapek's Dox Agar (CDA), Malt Extract Agar (MEA), Oat Meal Agar (OMA), Potato Carrot Agar (PCA), Richard's Agar (RCA), Sabouraud's Dextorse Agar (SDA), Spezieller and Nahrstoffarmer Agar (SNA) and V8 juice agar (Plate 5). Growth of non-pathogenic *Fusarium* isolates on different media showed differences in colony morphology, growth characteristics and pigmentation (Table 4a and 4b). Raised mycelial growth of cream to whitish network bearing different pigmentation was found on CDA media. On MEA plates, the *Fusarium* isolates were flat to dense, raised mycelia with pale yellow, purple pigmentations were observed. Slightly to densely raised white mycelia with concentric rings with regular to irregular periphery were seen in plates of RCA. Loose to flat network of dull white to light pinkish mycelial growth producing different pigments were found in V8 juice agar plates. Thin to dense growth of mycelia with irregular to round periphery on SDA and SNA. These differences in the growth of selected *Fusarium* isolates are in agreement with the findings of Chittem and Kulkarni (2008) who observed the growth of *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium oxysporum* f. sp. *gerberae* on different culture media.

Table 3: Morphological characterization of non-pathogenic *Fusarium* isolates

Isolates	Macroconidia			Microconidia			Chlamydospores	Colony colour
	Size	Apical cell	Basal cell	Size	Shape	Phialide		
UASB NPF I	26×7.2 µm	Tapered	Foot shaped	7×2.2 µm	Small, round shape	Short monophialide	Terminal, in chains, globose shape with rough surface, 4.3 µm diameter	Pale pink to white mycelia
UASB NPF II	29×7 µm	Hook shape	Notched	9.1×2.4 µm	Round/ oval shape	Short monophialide	Intercalary/ terminal, single / in chains, smooth to rough surface with oval shape, 4.2 µm diameter	Shiny milky white mycelia
UASB NPF III	30×7 µm	Slightly tapered	Elongated	7.8×2.5 µm	Oval shape	Long polyphialide	Terminal, in clusters, smooth, globose shape, 5.0 µm diameter	Dull violet to whitish mycelia
UASB NPF IV	32×7.2 µm	Blunted	Slightly notched	8×2.3 µm	Round shape	Medium monophialide	Intercalary, in pairs, rough surface with oval shape, 4.6 µm diameter	Medium violet to whitish mycelia
UASB NPF V	28×8 µm	Hook shape	Foot shaped	7.9×2.6 µm	Oval shape	Medium monophialide	Intercalary/ terminal, in clusters, rough surface with globose shape, 4.8 µm diameter	Pale yellow to orangish with whitish mycelia
UASB PF	29×7µm	Hook shape	Notched	8×2.5 µm	Oval shape	Short monophialide	Terminal/ intercalary, in clusters, oval shape with rough surface, 5.2 µm diameter	Pale purple with whitish mycelia

Table 4a: Colony morphology of non-pathogenic *Fusarium* isolates on different culture media

Isolates	Czapek's Dox Agar (CDA)	Malt Extract Agar (MEA)	Oat Meal Agar (OMA)	Potato Carrot Agar (PCA)
UASB NPF I	Raised fluffy dense whitish mycelia, flat at periphery with light yellowish tinge	Creamish white compact mycelia with raised colonies. Pale yellowish tinge in the centre	Grey to whitish fluffy mycelia. Dull growth with regular periphery	Densely raised very thick fluffy white mycelia at the centre. Very dense whitish network all over the regular periphery
UASB NPF II	Raised dense creamish mycelia. Light yellow to orange pigments in the centre and white coloured in the irregular periphery	Raised fluffy dense whitish mycelia. Light yellow to orange pigmentation	Slightly raised flat dull white mycelia with concentric rings. Flat network towards the irregular periphery	Densely raised thick white mycelia at the centre with concentric rings present. Flat and loose network towards the periphery
UASB NPF III	Loose network of mycelium. Raised mycelia in the centre. No clear periphery	Densely raised light purplish mycelia with violet colour tinge producing purple colour pigmentation.	Flat network of pale pinkish mycelia. Loose network of mycelia towards the regular	Pink colour tinge at the centre with pink pigment. Raised white mycelia around the centre.

		Concentric rings with raised whitish mycelia at the periphery	and round periphery	Transparent network of irregular periphery
UASB NPF IV	Flat loose network of mycelia. Circular raised whitish mycelia at the periphery. Yellowish tinge at the centre	Flat growth of mycelia. Ray type with concentric rings with pink to purplish pigmentation and with purple tinge.	Flat network of pinkish mycelia all over with concentric rings. Has regular and round periphery	Pink tinge at the centre covered by thick white mycelia. Flat and thin network towards the round periphery
UASB NPF V	Raised dense creamish mycelia in the centre, circular margin with concentric rings. Light yellow pigmentation.	Flat creamish white mycelia. Concentric rings, circularly raised light yellowish periphery and with orange pigmentation	Pale yellow to dull white mycelia with concentric rings at the centre. Has irregular periphery	White dense tinge at the centre surrounded by light orangish pigment. Has concentric rings. Dense network of mycelia towards the regular periphery

Table 4b: Colony morphology of non-pathogenic *Fusarium* isolates on different culture media

Isolates	**Richard's Agar (RCA)**	**Sabouraud's Dextrose Agar (SDA)**	**Speziller Nahrstoffarmer Agar (SNA)**	**V8 Juice Agar (V8JA)**
UASB NPF I	Thick densely raised fluffy whitish mycelia at the centre with concentric rings. Slightly raised and loose network of creamish mycelia towards the regular periphery	Raised whitish mycelia at the centre with dense network. Has round periphery. Light yellowish tinge present at the background	Loose network of white mycelial colonies with the irregular periphery.	Very thick and densely raised whitish mycelia at the centre. Slightly loose network over the irregular periphery.
UASB NPF II	Densely raised whitish mycelia at the centre with concentric rings. Slightly raised and thick network of whitish mycelia	Raised mycelia with loose network. Has irregular periphery with light yellowish tinge at the centre	Loose network of slightly raised whitish mycelia at the centre. Also appears with the concentric rings	Densely raised creamish to white mycelia all over. Concentric rings with flat network towards the periphery
UASB NPF III	Slightly raised white mycelia around the pinkish tinge at the centre with concentric rings. Thin and loose network towards the regular periphery	Raised creamish mycelia with dense network all over the periphery with concentric rings	Raised dull growth of loose network of mycelia with irregular periphery	Flat network of dull white to light pinkish mycelia present. Very thin transparent network towards the regular periphery
UASB NPF IV	Flat mycelia producing pink pigment with concentric rings. Periphery is covered by loose and very thin network of mycelia	Dense mycelia at the centre. Flat and loose network of light-yellow mycelia towards the periphery	Flat, very thin growth of mycelia with the round periphery	Has light pinkish tinge at the centre. Irregular network of mycelia all around producing pinkish pigment.
UASB NPF V	Raised creamish mycelia with concentric rings. Light yellowish to range pigments with irregular periphery	Slightly raised light yellowish mycelia at the centre with loose and flat network of round periphery. Has light yellow tinge	Slightly raised light yellowish mycelia. Loose network at the centre and slightly dense at the periphery	Thick and dense whitish mycelia with concentric rings producing light yellow to orangish pigment. Thick network over the irregular periphery

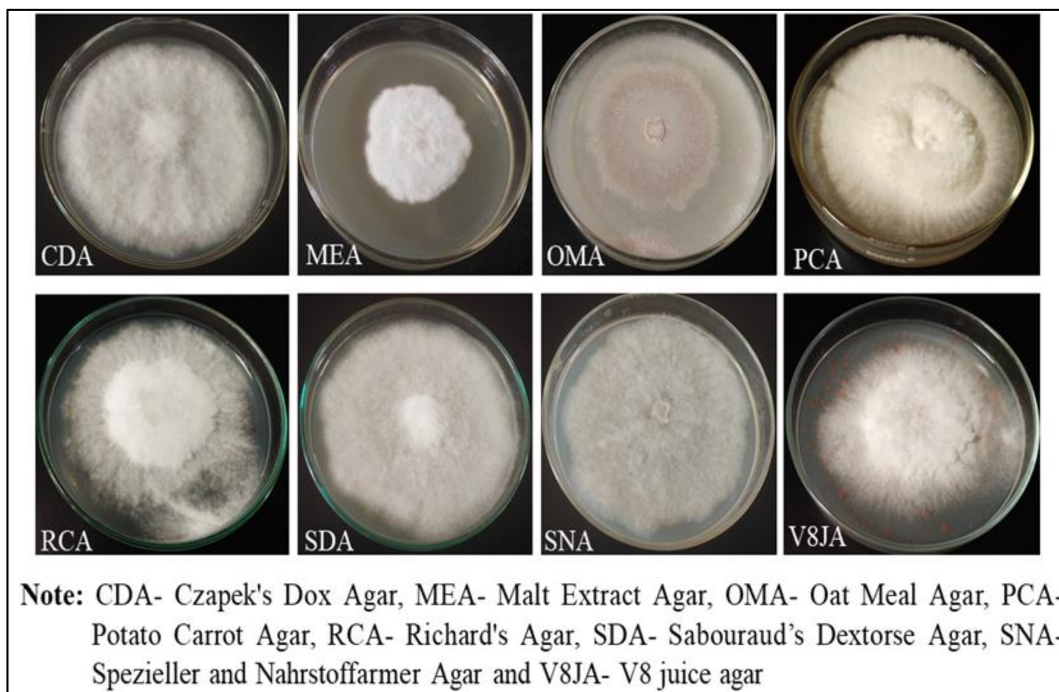

Plate 5: Colony morphology of non-pathogenic *Fusarium* isolate UASB NPFI on different culture media

Rodrigues and Menezes in 2005 [15], studied the endophytic *Fusarium* and also described the colony morphology of endophytic *Fusarium oxysporum* on Potato Dextrose Agar. The colony was found to be initially with white aerial mycelium, becoming salmon, with a tendency towards violet, and a purple back, whereas, the endophytic *Fusarium solani* isolate showed abundant aerial mycelium, cream to white surface or purple coloured with under surface showing a dark

violet to pink colour or colourless. Joseph *et al.,* (2017) [7] studied the influence of different culture media like PDA, malt extract agar, V8 juice and temperature on growth and sporulation of *Lasiodiplodia theobromae, Pestalotiopsis microspora* and *Fusarium oxysporum* isolated from Njangsa, an oily seed trees in Cameroon.

The observations made regarding the colony diameter of the five non-pathogenic *Fusarium* isolates on fourth day of inoculation were varied from 29 to 41.33 mm in CDA, 32 to 41.67 mm in MEA, 24 to 32.67 mm in OMA, 28.33 to 39 mm in PCA, 26.33 to 31.67 mm in RCA, 28.67 to 38.33 mm in SDA, 25.33 to 40.33 mm in SNA and 21.67 to 36.33 mm in V8 JA. Similarly, the observations on seventh day of inoculation were varied from 60.67 to 73 mm in CDA, 61 to 70.67 mm in MEA, 59.67 to 70.67 mm in OMA, 61.67 to 72.33 mm in PCA, 56.00 to 68.33 mm in RCA, 65.00 to 70.00 mm in SDA, 56.33 to 71.00 mm in SNA and 51.67 to 69.00 mm in V8 JA (Fig 1). These are in evidence with the work of Pradeep *et al.,* (2013) [14] who studied the colony diameter of *Fusarium moniliforme* KUMBF1201 which attained 54 mm of diameter after five days of incubation and also calculated the average growth rate, which was 10.8 mm/ day.

Future line of work

The five non-pathogenic *Fusarium* isolates were further studied for their bio control activities against the wilt pathogen of Chrysanthemum.

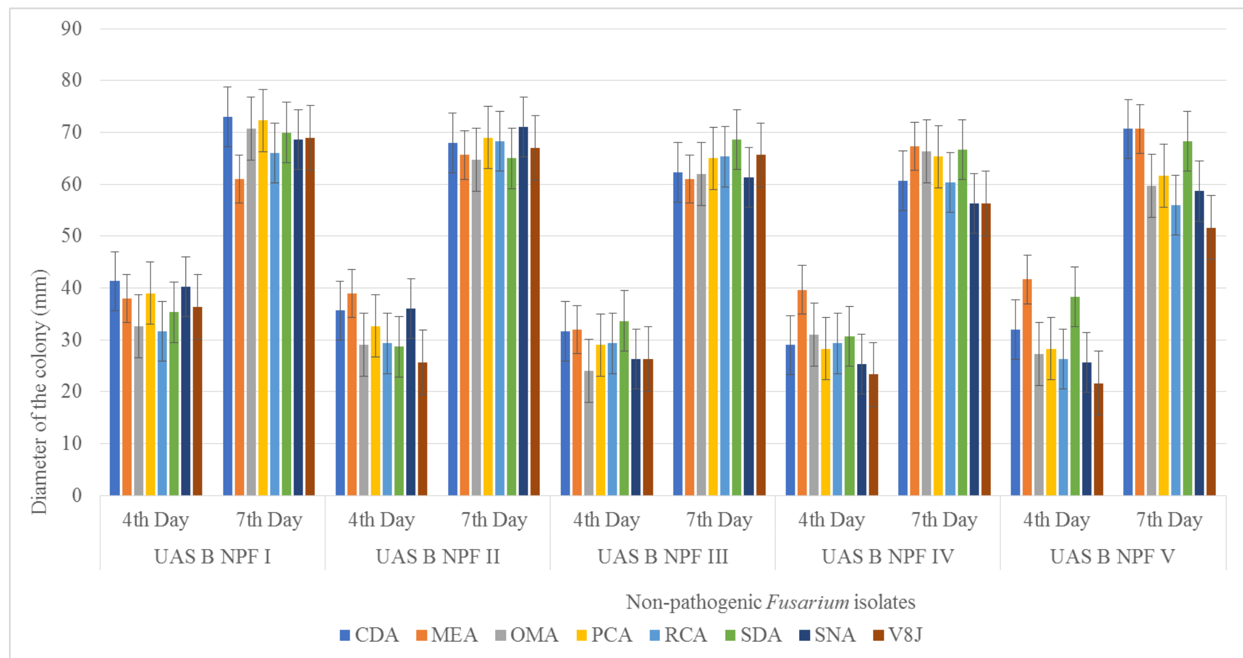

Fig 1: Colony diameter of the non-pathogenic *Fusarium* isolates on different culture media

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