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Evaluation of fungicides and oil cakes for the management of Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) in banana

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Abstract
The Panama wilt is highly devastating fungal diseases of banana plantains and it is caused by *Fusarium oxysporum* f. sp. *cubense* (FOC). It causes heavy yield losses in several cultivars of banana. Six FOC isolates were collected from different areas of Tirunelveli and Thoothukudi districts of Tamil Nadu and the pathogen was identified as FOC based on cultural characters. This pathogen has different races which causes greater yield loss in most of the banana varieties. The seven fungicides and five oil cakes were evaluated against *Fusarium oxysporum* f. sp. *cubense* (FOC). The virulent isolate was identified by the pathogenicity test and also evaluated the fungicides and oilcakes under laboratory condition. Among the seven fungicides used, the complete inhibition of mycelial growth was observed in Carbendazim at all concentrations followed by Tebuconazole + Trifloxystrobin at 500 and 1000 ppm. The other fungicides such as Propiconazole, Tebuconazole recorded 100 percent inhibition at 1000 ppm concentration followed by Hexaconazole (88.88%) and Azoxystrobin (72.22%). Copper oxy chloride at 1000 ppm recorded least mycelial reduction. The oilcakes of Neem, Mahua, Gingelly, Groundnut and Coconut were evaluated against FOC. Among the five oil cakes tested, Neem cake recorded the inhibition of 18.66 and 55.55 percent at 5 and 10 percent concentrations followed by mahua cake (16.22 and 51.33 %). Coconut cake recorded least mycelial inhibition of 3.33 and 5.77 percent at 5 and 10 percent concentrations respectively.

Keywords: Banana, FOC, *in vitro*, Fungicides, Oilcakes

Introduction
Banana is the most important crop after rice, wheat, and maize based on the gross value production in world. It is the oldest fruit crop of the world as well as in India and belonging to the Musaceae family. It is being cultivated by more than 130 countries over the world and highly staple food for more than 400 millions of people in developing countries (Molina and Valmayor, 1999). In Tamil Nadu, it is cultivated in an area of 94.61 million hectares with an annual production of 4331.65 million tonnes (Indiastat, 2019) [1]. In Tirunelveli district, Bananas is cultivated in an area of 8854 million hectares with an annual production of 211954 million tonnes and productivity of 23.94 MTha-1 during 2012- 2013 and In Thoothukudi district, cultivated in an area of is 9586 hectares with an annual production of 621672 million tonnes and productivity of 64.85 MTha-1 during 2012- 2013.

In India, Panama disease caused by FOC race 1 strain, resulted the highest yield losses of 50-70% and several varieties such as Rasthali, Amirtapani, Karpooravalli, Monthan, Ney Poovan, and Virupakshi are being affected by this race (Ghag, 2019) [5]. The fungus enters the plant system via roots and colonize the vascular tissues, blocking the water and nutrients transport that leads to yellowing of older leaves followed by breaking of petioles and hanging down of leaves around the pseudostem. In severe cases longitudinal splitting can also be observed on the pseudostem. Distinguishing internal symptom observed in corm is light yellow to dark brown color vascular discoloration. (Yin *et al*., 2011) [16]. Once the fungus enters in field, it can reside in soil for indefinite period. The chlamydospores survivals up to 30 years in soil or in infected planting materials and in alternate host roots (Gnanasekara *et al.*, 2015) [4]. Nowadays, the fungicides are being used for the management of plant diseases in an effective mannered as these compounds have direct effect on the pathogen (Jamil and Kumar, 2010) [7]. Likewise, organic amendments also play an important in the control of the plant pathogens. Apart from pathogen control it enhances the plant growth, soil fertility and increases the beneficial soil microorganisms (Lazarovits *et al*., 2001) [9]. With this background, the present study was conducted to know the effectiveness of fungicides and organic amendments against FOC.

Materials and Methods

Collection and isolation of Fusarium wilt infected rhizomes

The Fusarium wilt infected rhizome samples were collected from several banana growing areas of Agaram, Pakkapatty, Kongarayakurichi, Thiruchendur located in Tirunelveli and Thoothukudi districts of Tamil Nadu by tissue segment method. Fusarium wilt infected banana rhizome samples were cleaned to remove the attached soil particles. Infected rhizome portion were cut approximately at 50 mm × 0.5 - 1mm size and washed with sterile distilled water for 5 min then surface sterilization was carried out at 1% Sodium hypochlorite for 30 sec. After washing with sterile distilled water the infected portion dried with sterile tissue paper and plated five bits per plate and incubated at 25±2 ^{0}C for 7 days. The plates were observed for the different types of mycelial growth on the medium, various FOC isolates were isolated from various infected rhizome samples. The cultures were maintained and observed for morphological characters Ingle *et al*, (2013) [6].

Morphological characters of FOC

Six isolates of FOC were grown on PDA medium to study the growth, cultural variability and conidial characters. Five mm culture disc was cut from the seven days old culture plate using a sterilized cork borer and placed at the centre of each Petri plate containing 20 ml of solidified PDA medium. The plates were incubated at room temperature (28±2^{0}C) for seven days. The growth and morphological characters of the isolates *viz.*, colony morphology, colour of mycelium and shape of conidia were observed under microscope.

Preparation of fungicides at different concentrations

The fungicides carbendazim 50% WP (Bavistin), propiconazole 25% EC (Tilt), hexaconazole 5% EC (Contaf), azoxystrobin 23% EC (Amister), copper oxychloride 50 %WP (Coprus), tebuconazole 50 % EC (Folicur) and Tebuconazole 50% + Trifloxystrobin 25% WG (Nativo) were evaluated against the FOC. The fungicidal solutions were prepared at recommended concentrations (250, 500 and 1000 ppm) and added into hundred ml of sterilized PDA medium and thoroughly mixed.

Effect of fungicides on FOC by poison food technique

The efficacy of fungicides was evaluated against FOC using poisoned food technique. The sterilized PDA medium along with fungicide solution were distributed into the sterilized Petri plates @ 20 ml per plate. Seven day old mycelial disc was (9mm) cut from FOC pathogen by using sterilized Cork borer and placed in centre of the each Petri plate. The PDA medium without fungicidal solution served as control and were incubated at room temperature (28^0± 2 ^{0}C) for 3 days. Three replications were observed for each treatment. Percent inhibition over control was calculated by using the following formula by (Yadav *et al*., 2014) [17].

$$I = \frac{C - T}{T} \times 100$$

Where,
I = Percent inhibition over control
C = Mycelial growth of pathogen in control (cm)
T = Mycelial growth of the pathogen in treatments (cm)

Preparation of aqueous oil cake extracts

The different oil cakes namely neem cake, mahua cake, sesame cake, ground nut cake and coconut cake were made into powder and taken hundred gram of each oil cakes. The oil cakes @1gram per 1.25 ml soaked in sterile distilled water and kept overnight. After soaking, different oil cakes were grounded separately in sterile Pestle and Mortar. Each oil cake extract was filtered via 2 layers of sterile muslin cloth and centrifuged at 10000 rpm for 15 min. The supernatant of each oil cake extract serves as a standard solution (100%) (Dubey and Patel, 2000) [2]. It was further diluted to 5 and 10% concentration for *in vitro* studies. Each oil cake extract was taken at the rate of 5ml and 10 ml were mixed with 95 and 90 ml of sterilized PDA medium to obtain 5 and 10 percent concentrations.

Effect of oil cakes on FOC by poisoned food technique

The efficacy of each oil cake extract was evaluated against FOC using poisoned food technique. The sterilized PDA medium along with freshly prepared oil cake extract was sterilized and distributed in each Petri plate @ 15ml per plate and allowed to solidify. Seven day old actively growing mycelial disc (9mm) was taken from pure culture and inoculated at the centre of the each Petri plate and incubated at room temperature. Medium without oil extracts served as control. The mycelial growth of FOC was measured in all different treatments after incubation of 7 days.

Statistical analysis

Lab experiment were carried out under Completely Randomized Block Design (CRD). The data were recorded in Microsoft Excel spread sheet and analyses were done by the AGRES standard error and significant difference between values were determined using Duncan's Multiple Range Test (p=0.05).

Results and Discussion

Collection and isolation of FOC

Six isolates of FOC were isolated from the infected rhizome sample collected from various banana growing areas of Tamil Nadu. The isolates of FOC were named as FOC1, FOC2, FOC3, FOC4, FOC5 and FOC6. (Table 1 and Plate 1)

Morphological characters of FOC

Six FOC isolates produced different mycelial growth characters *viz*., colony morphology, colour of mycelium, shape of conidia were recorded separately. Among six isolates, FOC1 isolate produced vigorous growth and the mycelium was creamy white in colour. The mycelium of FOC 2 isolate produced profuse type of mycelium and fast growth and centre of the mycelium shrinkage. FOC3 isolate cottony growth, centre of the mycelium pinkish colour and sclerotia formed. FOC4 isolate vigorous growth and white colour mycelium. FOC5 mycelium pinkish in colour in later stage violet colour pigmentation. FOC6 isolate produced cottony growth with violet colour mycelium. Among the six isolates produced oval shaped micro conidia and sickle shaped macro conidia were recorded separately (Table 1and Plate 1).

Table 1: Morphological characters of FOC

S. No	Isolates	Village	District	Morphological characters	Shape of conidia
1	FOC1	Agaram	Tirunelveli	Cottony, vigorous growth and creamy white colour mycelium	Micro conidia -Oval to kidney shaped Macro conidia- Sickle shaped
2	FOC2	Pakkapatty1	Tirunelveli	White colour and profusetype mycelium, fast growth, center of themycelium shrinkage	Micro conidia -Oval to kidney shaped Macro conidia- Sickle shaped
3	FOC3	Pakkapatty2	Tirunelveli	Cottony growth, center of the colony light pinkish colour and fast growth, sclerotia formed.	Micro conidia -Oval to kidney shaped Macro conidia- Sickle shaped
4	FOC4	Kongarayakurichi1	Tirunelveli	White colour, thin but vigorous growth	Micro conidia -Oval to kidney shaped Macro conidia- Sickle shaped
5	FOC5	Kongarayakurichi2	Tirunelveli	Vigorous growth, mycelium pink in colour in later stages violet colour pigmentation	Micro conidia -Oval to kidney shaped Macro conidia- Sickle shaped
6	FOC6	Thiruchendur	Thoothukudi	Violetcolour mycelium and cottony growth	Micro conidia -Oval to kidney shaped Macro conidia- Sickle shaped

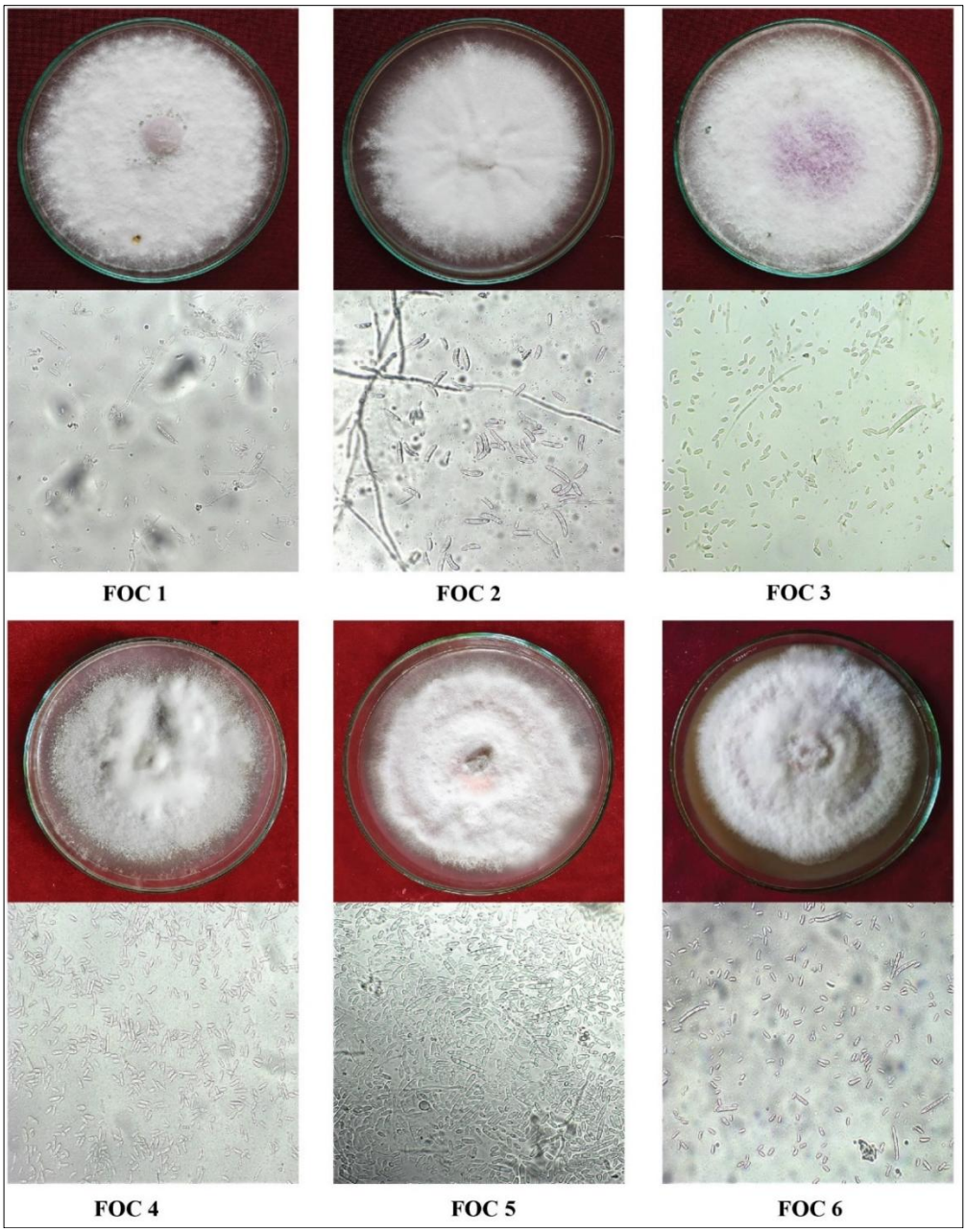

Plate 1: Morphological characters of FOC

***In vitro* evaluation of fungicides against the FOC**

Seven fungicides namely such as Carbendazim (50% WP), Propiconazole (25% EC), Tebuconazole (50%EC), Copper oxychloride (50%WP), Hexaconazole (5% EC) Azoxystrobin (23%EC) and Tebuconazole 50% + Trifloxystrobin 25% WG were assessed for antifungal activity against FOC at 250, 500 and 1000 ppm concentrations by poisoned food technique. Carbendazim ranked first and completely reduced the mycelial growth at all concentrations by recording 100 percent inhibition. Tebuconazole + Trifloxystrobin ranked second inhibiting the mycelial growth at 500 ppm and 1000 ppm concentrations. Other fungicides like Propiconazole, Tebuconazole exhibited 100 percent reduction of the pathogen at 1000 ppm followed by Hexaconazole (85.55%),

Azoxystrobin (72.22%). Copper oxy chloride recorded less reduction of pathogen (44.44%) at the above mentioned all concentrations (Table 2 and Plate 2).

The present results are in agreement with Priya *et al*., (2019) [12]. They tested ten different fungicides and found that the combination product of Tebuconazole + Trifloxystrobin, which completely inhibited radial growth of *Fusarium* spp. effectively at 500 and 1000 ppm concentrations. Maitlo *et al*., (2014) [10] and Kumar and Mane (2017) [8] reported the effectiveness of Carbendazim against *Fusarium oxysporum* f. sp. *ciceri*. Mailem *et al*., (2015) [11] and Somu *et al*., (2014) [13] reported the effectiveness of fungicides Tebuconazole and Propiconazole which had 100 percent inhibition of Fusarium wilt in chick pea and banana at 1000 ppm concentration respectively.

Table 2: *In vitro* evaluation of fungicides against the FOC

Treatment No.	Fungicides	*mycelial growth (in cm) at different conc.			* Per cent mycelium inhibition over control at different conc.		
		250ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
T_1	Carbendazim 50% WP	0.00	0.00	0.00	100 (89.71)[a]	100 (89.71)[a]	100 (89.71)[a]
T_2	Propiconazole 25% EC	1.20	0.50	0.00	86.66 (68.58)[c]	94.44 (76.31)[b]	100 (89.71)[a]
T_3	Tebuconazole 25% EC	1.53	1.00	0.00	83.00 (65.64)[d]	88.88 (70.52)[c]	100 (89.71)[a]
T_4	Hexaconazole 5% EC	3.70	2.40	1.00	58.88 (59.09)[e]	73.33 (58.90)[d]	88.88 (70.52)[b]
T_5	Azoxystrobin 23 % SC	4.60	3.80	2.50	48.88 (44.36)[f]	57.77 (49.47)[e]	72.22 (58.16)[c]
T_6	Copper oxychloride 50% WP	7.30	6.20	5.00	18.88 (25.74)[g]	31.11 (33.89)[f]	44.44 (41.80)[d]
T_7	Tebuconazole50%+Trifloxystrobin 25% WG	1.00	0.00	0.00	88.88 (70.52)[b]	100 (89.71)[a]	100 (89.71)[a]
T_8	Control	9.00	9.00	9.00	-	-	-
CD(p=0.05)		0.16	0.13	0.11	1.80	1.48	1.23

*****Mean of three replications

Values in parentheses are arcsine transformed

The treatment means are compared using Duncan multiple range test (DMRT)

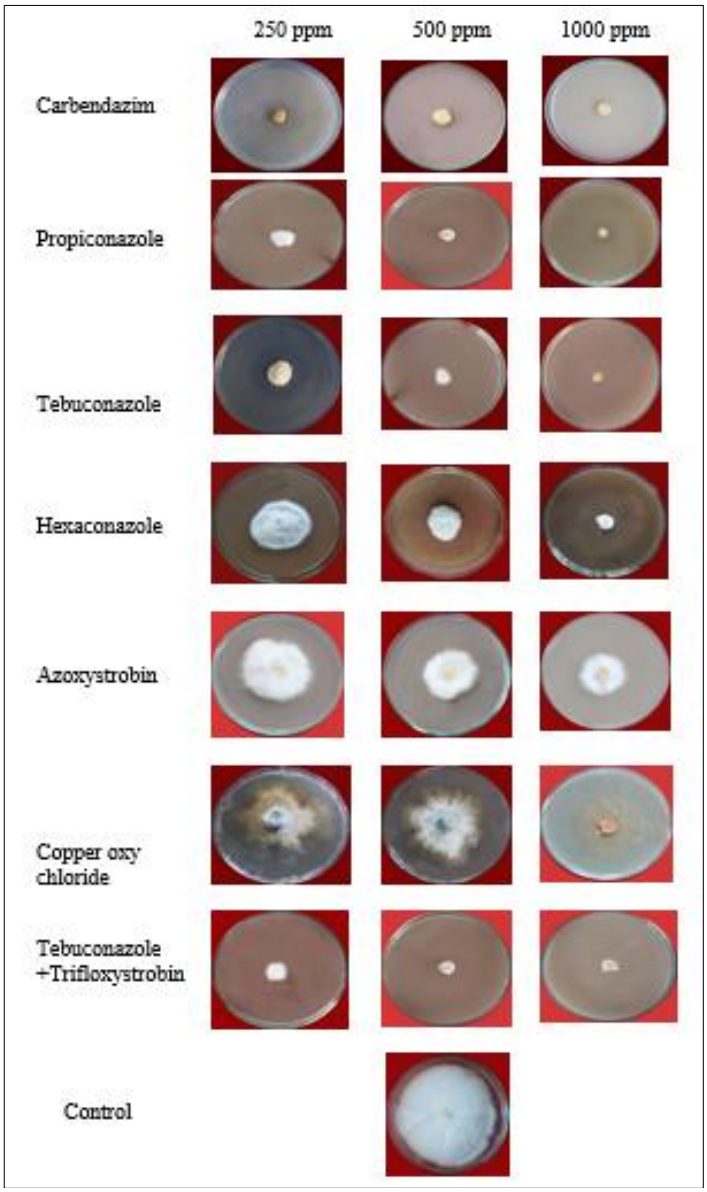

Plate 2: *In vitro* evaluation of fungicides against the FOC

***In vitro* evaluation of oilcakes against the FOC**

Five oil cake extracts namely Neem cake, Mahua cake, Gingelly cake, Groundnut cake and Coconut cake were assessed for antifungal activity against FOC at 5 and 10 percent concentrations. The neem cake extract recorded the maximum inhibition of the pathogen 18.66 and 55.55 % at 5 and 10 percent concentrations followed by mahua cake extract (16.22 and 51.33%) respectively. Other oil cakes Gingelly cake (8.33 and 15.33%), ground nut cake (5.11 and 8.00%) and coconut cake was observed with least mycelial reduction of 3.33 and 5.77 percent at all concentrations respectively (Table 3 and Plate 3).

In the present study, five oil cake extracts were tested for their effectiveness against FOC. Among the oil cake extracts screened, Neem cake was found to be the most effective against FOC at all concentrations followed by mahua cake. The result was in accordance with findings of Yelmame *et al*., (2010) [15] who reported that, the neem cake extract highly reduced the growth of the *Fusarium solani* at 10 percent concentration. Theradimani *et al*., (2018) [14] and Dhivya *et al*., also (2017) [3] revealed that the neem cake had significant effect on inhibition of mycelial growth of *F. oxysporum* f. sp. *Ciceri* at 5 and 10 percent concentrations respectively.

Table 3: *In vitro* evaluation of oilcakes against the FOC

Treatment No.	Organic amendments	*Mycelial growth(in cm) At different conc.		Per cent mycelium inhibition over control at different conc.	
		At 5%	10%	At 5%	10%
T_1	Neem cake	7.32	4.00	18.66 (25.56)[a]	55.55 (47.86)[a]
T_2	Mahua cake	7.54	4.38	16.22 (23.74)[b]	51.33 (45.76)[b]
T_3	Gingelly cake	8.25	7.62	8.33 (16.74)[c]	15.33 (23.05)[c]
T_4	Groundnut cake	8.54	8.28	5.11 (13.09)[d]	8.00 (16.36)[d]
T_5	Coconut cake	8.70	8.48	3.33 (10.46)[d]	5.77 (13.82)[d]
T_6	Control	9.00	9.00	-	-
CD (p=0.05)		0.21 0.26		2.43	2.98

*Mean of three replications

*Values in parentheses are arcsine transformed

*The treatment means are compared using Duncan multiple range test (DMRT)

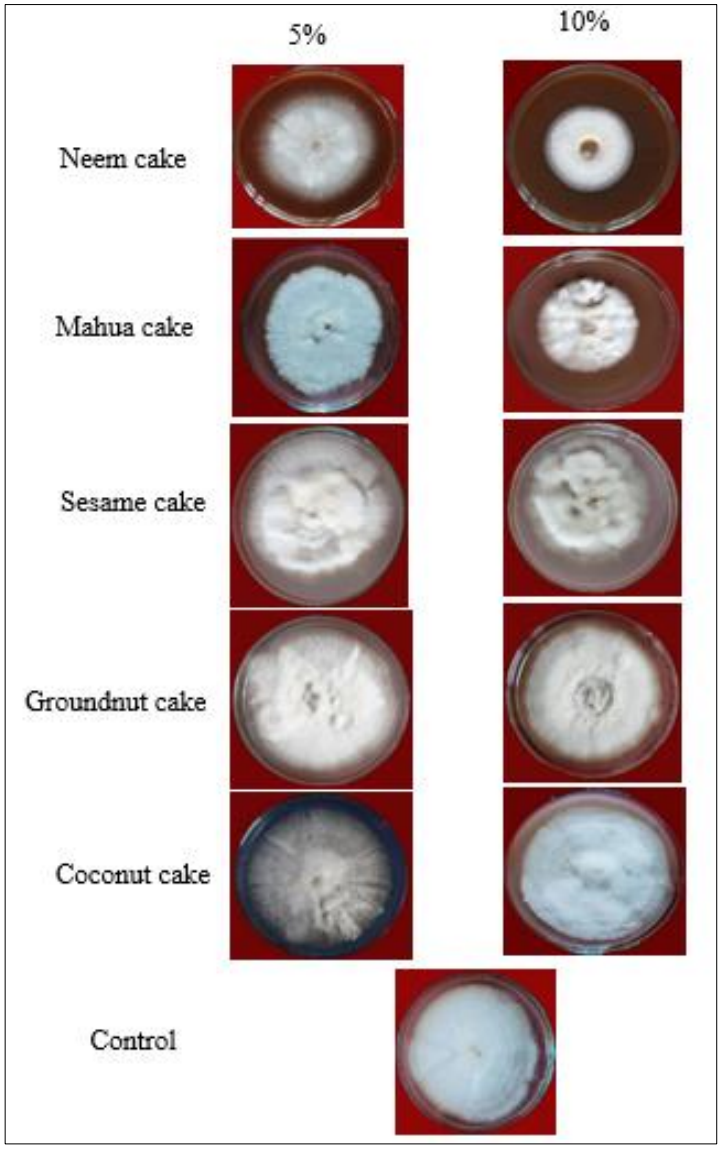

Plate 3: *In vitro* evaluation of oilcakes against the FOC

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