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Research Article

Assessing the *in vitro* efficacy of biocontrol agents and oil cakes against basal rot of onion incited by *Fusarium oxysporum* f.sp. *cepae*

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Abstract
Onions are an important vegetable crop, which is infected by many soils and foliar pathogens. Among them, *Fusarium* Basal Rot (FBR) causes yield losses of up to 50 per cent in the field and 30 to 40 per cent during post-harvest storage of bulbs. For management of basal rot of onion, the efficacy of native antagonists such as six different *Trichoderma* sp. (T_1-T_6), five different *Bacillus* sp. (B_1-B_5) and five different oil cakes was assessed against the *Fusarium oxysporum* f.sp. *cepae* under *in vitro* condition. Among them, T_3 collected from Kulithalai recorded maximum virulence as well as dark green sporulation with conidia length of 2.68–3.25 and breadth of 2.54-3.46µ. Among the tested isolates, In the case of *Bacillus sp.,* isolate B_4 recorded the maximum inhibition zone (66.16%), followed by *B. subtilis* (B_5), which recorded a (59.03%) inhibition on the mycelial growth. Among the five different oil cakes, the filtrates of neem cake showed a maximum inhibition zone against *F. oxysporum* f.sp. *cepae* of 1.29 cm @ 15% concentration, followed by groundnut cake at 1.36 cm @ 30% concentration. Hence the different control measures, *Trichoderma* sp. showed critically acclaimed performance under *in vitro* than others. The combined application of *Trichoderma* sp, *Bacillus* sp and neem oilcake significantly inhibited the growth of basal rot of onion due to the presence of the antimicrobial property.

Keywords: Biocontrol, *In vitro* evaluation, Onion, Organic amendments, *Trichoderma* sp.

INTRODUCTION

Fusarium basal rot (FBR) is an economically significant onion disease that causes yield losses of up to 50 percent in the field and 30 to 40 percent during post-harvest storage of bulbs (Sintayehu *et al.* 2011; Gupta and Gupta, 2014). Onion basal rot results in a yield loss of more than 50% (Mishra *et al*. 2014). According to Ben Kalman *et al*. (2020), *Fusarium oxyspoum* f.sp. *cepae* (FOC) growth rate was 0.83–0.87 cm per day. The losses due to this disease range from 30 % to 100 % (Priya *et al. 2016).* The optimum temperature for its development is 25 - 32°C (Howard *et al.* 2007). Infection occurs at soil temperatures of 15°C to 32°C, with an optimum at 28°C-32°C, because infection and disease development is favoured by high soil temperatures (Mishra *et al.* 2014). The agent of basal rot infects the onion root epidermis and then extends

into the vascular tissue at the basal region. The characteristic symptoms of the rot disease include onion leaves curving, drooping, turning pale, and discolouring, and then the contaminated tissue gets brown and hydrated. As a result of these serious infections, the plant eventually dies (Idris Bektas *et al.* 2019). Due to their rapid growth, ease of handling, and aggressive colonisation of the rhizosphere, antagonistic bacteria such as *Bacillus subtilis* are considered ideal biological control agents (Gnanamanickam *et al*. 2002). Mardanova *et al*. (2017) mentioned that, *B. subtilis* could inhibit *Fusarium* infection by producing hydrolytic enzymes and cyclic lipopeptides. But interestingly majority of *Trichoderma* species are plant growth-promoting fungi that generate phytohormones and the 1 -aminocyclopropane-1-carboxylate (ACC) deaminase enzyme as well as *Trichoderma* appears to be the best candidate among nonpathogenic fungal microorganisms for use in green technologies due to its broad bio-fertilization and bio-stimulatory potential (Tyskiewicz *et al*., 2022). Ayyandurai *et al.* (2021) found that *Trichoderma longibrachiatum* induce growth promotion activities in groundnut plants and successfully inhibits the growth of soil-borne pathogens. Deshmukh *et al*. (2016) reported that soil amendments containing various organic matter types are crucial for disease control and soil quality improvement. Also, organic amendments are one of the most effective methods for controlling soil-borne diseases . This study mainly focused on the isolation and biochemical characterization of *Trichoderma* sp., *Bacillus* sp., and this antagonistic activity was assessed against the basal rot pathogen *Fusarium oxysporum* f.sp. *cepae* of onion. In addition, oilcakes' extracts were also tested against the pathogen under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of the pathogen

Onion samples infected with basal rot were collected from ten different locations *viz.* Tiruchirappalli (Manapparai), Perambalur (Padalur), Salem (Thalaivasal), Karur (Thogaimalai) and Madurai (Usilampatty) districts of Tamil Nadu. The infected bulbs were isolated using the tissue segment technique described by Rangaswami (1958). With a sterile scalpel, the infected areas were cut into small pieces (1 -2 cm) and washed three times in 1 percent sodium hypochlorite for one minute, 70% ethanol for one minute, and sterile distilled water. Using sterile dried tissue paper, excess moisture in the sample was removed. The sample was placed in a Petri dish containing Potato Dextrose Agar (PDA) medium at equal distances. The pure (axenic) culture of FOC was obtained using the single hyphal tip method (Tutte 1969), then transferred to agar slants and stored at 4°C for future research. Ten total isolates of FOC were designated as FOC_1, FOC_2, FOC_3, FOC_4, FOC_5, FOC_6, FOC_7, FOC_8, FOC_9, FOC_{10} from different locations. The study has already found that among the ten isolates of basal rot pathogen, FOC_7 is a highly virulent isolate (Muthukumar *et al.,* 2022)

Isolation of a native fungal antagonist

Trichoderma was isolated using the serial dilution technique (Koch 1883). The healthy onion rhizosphere soil was collected from Manapparai, Salem, Kulithalai, Musiri, Perambalur and Usilamapatty places of Tamil Nadu (Table 1). For isolation of antagonist, each ten gram soil is suspended in 100 ml of sterile, deionized water and thoroughly mixed. The particles of soil were allowed to settle. From this suspension, 1 ml of the clear supernatant was removed, added to 9 ml of distilled water in a test tube, and vigorously shaken (10^{-1}). Subsequent dilutions were then performed. One ml of a 10^{-3} dilution was withdrawn and spread over the *Trichoderma* selective medium-containing Petri plate. These plates were incubated at 28±2 °C and the development of colonies was monitored frequently. After three days of incubation, *Trichoderma* cultures were purified on PDA-poured Petri plates using the single hyphal tip method, and test tube slants were stored at 4°C for further research. These isolate designations were denoted as T_1, T_2, T_3, T_4, T_5 and T_6.

Isolation of native bacterial antagonists

Using a serial dilution technique and nutrient agar media, the native bacterial antagonists of *B. subtilis* were isolated (Allen, 1953). Aneja (1993) reported that 1 g of rhizosphere soil was collected from five distinct location- Manapparai, Tharagampatti, Salem, Thalaivasal and Perambalur of Tamil Nadu (Table 2). The antagonist was isolated by using the technique of serial dilution. One ml of dilution 10^{-5} and 10^{-6} was added to the Nutrient agar (NA) medium. On NA medium-containing plates, representative colonies were isolated, purified, and preserved in NA slants. These cultures were kept on refrigerated agar slants and were periodically sub-cultured. The isolates were labelled as B_1, B_2, B_3, B_4 and B_5.

Biochemical tests for *B. subtilis*

Gram staining

A loopful of bacterial culture was transferred to a clean slide and a smear was made, which was air-dried and heat-fixed. The smear was flooded for 1 min with ammonium oxylate crystal violet. Excess stain was poured off and the slide was washed in a gentle steam of water. Lugol's iodine solution was applied and allowed to remain for one minute, decolorized with ethyl alcohol. The smear was washed in a gentle steam of water and was counterstained with safranin for 30

seconds. The gram-negative cells appeared red in colour and the gram-positive cells appeared purple in colour (Cyrabree and Hindshill, 1975).

Starch hydrolysis

The filter paper was dipped in a day-old culture suspension, placed on Petri dishes containing starch agar medium, and incubated for two days. The plates were flooded with a one-percent iodine solution. A colourless halo around the growth and a blue colour in the rest of the plates showed the utilisation of starch by the microorganism (Stolpe and Godkeri, 1981).

Methyl-red test

Five millilitres of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100 ml distilled water) were sterilised in clean test tubes. The tubes were then inoculated with the test organisms and incubated at 37°C for 48 hours. At the end of incubation, a red colour indicated a positive reaction (Olutiola *et al*. 2000).

Citrate utilisation evaluation

Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old and which incubates at 35ºC to 37ºC for 18 to 24 hours. In the case of some organisms, they may require up to 7 days of incubation due to their limited rate of growth in a citrate medium. The development of the blue colour denoting alkalinization indicates positive utilization.

Hydrogen sulphide (H_2S) production

Pre-sterilized tubes containing SIM agar were streaked with the test cultures all along the walls of the test tubes. Inoculated tubes were incubated for 48 h at 28±2°C. After incubation, the development of black colour along the line of the stab was noted as positive for the test (Cappuccino and Sherman, 1992).

Antagonistic bioassays

Efficacy of *Trichoderma* sp. against the *F. oxysporum* f.sp. *cepae* by Dual culture assay

Using the dual culture approach outlined by Dennis and Webster (1971) was used to test the efficacy of *Trichoderma* sp. isolates against FOC. A 9 mm culture disc obtained from the periphery of a seven-day-old *F. oxysporum* f.sp. *cepae* culture was inoculated 75 mm from the edge of a Petri dish containing 15 ml of sterile and solidified PDA medium. A FOC disc was positioned on the PDA medium as a control treatment. The inoculum discs were harvested from the edges of 7-day -old fungal cultures that were still actively developing. For each treatment, four replicas were kept. For six days, plates were incubated at 25°C in the dark. Three Petri dishes were utilised for each isolate in the entirely random experimental setup. At a magnification of 100x, microscopic examinations were performed to look for mycoparasitism at the interface between the two fungi cultures. The inhibition of mycelial growth of *F. oxysporum* f.sp. *cepae* were determined. The effectiveness of antagonists was determined by the pathogen's growth inhibition. The percentage of mycelial growth inhibition was calculated using a formula reported by Vincent (1927).

Percent inhibition (I) = C – T/C ×100 …..Eq. 1

C = average diameter of *F. oxysporum* f.sp. *cepae* fungal growth (cm) in control

T = average diameter of *F. oxysporum* f.sp. *cepae* fungal growth (cm) in treatment

Efficacy of *Bacillus* sp. against the *F. oxysporum* f. sp. *cepae* by Dual culture assay

In vitro efficacy of *B. subtilis* against *F. oxysporum* f.sp. *cepae***.** A nine mm mycelial disc of the onion basal rot pathogen *F. oxysoporum* f. sp. *cepae* was placed 75 mm from the edge of a Petri dish containing 15 ml of sterile and solidified PDA medium. The *Bacillus* sp isolates streaked 1cm away from the edge of Petri plate. The plates were incubated at room temperature (28±2ºC). The observations were taken after five days for the presence of the inhibition zone over the pathogen was calculated using formulae already described. Control was maintained with Petri plate only streaked with *Bacillus* isolates

Collection of different oil cakes and their extract preparation

Different oilcakes were collected, such as neem (*Azadirachta indica*), pungam(*Pongamia pinnata*), groundnut (*Arachis hypogaea*), mustard (*Brassica juncea)*, and sesame (*Sesamum indicum*) from local modern oil mills in Chidambaram/Cuddalore of Tamil Nadu in India . For preparing the oilcake extract, the required amount of oilcake was sterilized in an autoclave at 121.6^{o}C for 15-20 min and chopped into small pieces, then used an equal amount of sterilised distilled water. The mixture was squeezed through a double-layered muslin cloth. The required amount of stock solution was added to PDA medium to get the desired concentration of 5, 10 and 15%. The oilcake extract obtained was considered to be of 100% concentration (Ayyandurai *et al*., 2022).

Efficacy of oilcake extracts against *F. oxysporum* f.sp. *cepae*

Using the agar well diffusion assay, the efficacy of oilcake extract against *F. oxysporum* f.sp. *cepae* was evaluated.. The five oil cake extract with three different concentrations *viz.,* 5 %, 10%, and 15%, were tested

against the *F. oxysporum* f.sp. *cepae* individually in same Petri plate and sterile distilled water used as control. Each plate was inoculated with a 9mm disc of 7-day-old FOC culture using a sterile cork borer and the oil cake extract is poured in 1cm away from the periphery of Petri plate. The inoculated Petri dishes were incubated for seven days at 28 ±2ºC. The inhibition zone of *F. oxysporum* f.sp. *cepae* was measured after seven days of incubation.

RESULTS AND DISCUSSION

Morphological and cultural characteristics of *T. harzianum* isolates

A total of six *Trichoderma* isolates *viz.,* T_1, T_2, T_3, T_4, T_5 and T_6 were isolated from different locations of Tamil Nadu (Table 1). Among them, T_3 collected from Kulithalai recorded maximum virulence and showed dark green sporulation with conidia length of 2.68-3.25µ and breadth of 2.54-3.46µ followed by T_5 collected from Perambalur showed cottony fluffy green sporulation with conidia length 2.64-3.72µ and breadth of 2.43-3.29. The comparable findings were also observed by Erayya (2014); the morphological characters of *Trichoderma* sp. included concentric rings, surface topography, and pigmentation on the reverse side of the plates used to characterize the *Trichoderma* isolates isolated from Pantnagar of Uttarakhand.

Cultural and biochemical characterization of the native bacterial antagonist *B. subtilis*

Six isolates of *Bacillus sp.,* such as B_1, B_2, B_3, B_4 and B_5 were isolated from the onion rhizosphere in different localities of Tamil Nadu Table. 2. Among the six isolates of *B*acillus sp *viz.,* B_1, B_4 and B_5, whitish grey to whitish brown colonies with serrated margins were produced. Various biochemical tests were carried out to confirm whether the selected isolates belonged to *B. subtilis* or not, and the results were evaluated in comparison with the previous observations. As per gram-positive and gram-negative reactions (Gram staining), cell shape, spore formation, starch hydrolysis, methyl red test, citrate utilisation test, and hydrogen sulphide tests performed for the identification of the effective native isolates of *Bacillus* showed that all the isolates produced similar results with regard to gram staining (positive), starch hydrolysis (positive), methyl red test (negative), citrate utilisation test (positive), and H_2S production (negative). With the help of this biochemical characterization, the isolated bacterial species was confirmed as *B. subtilis* (Table 2). Vignesh *et al*. (2020) observed similar biochemical and cultural characteristics. Similarly, Sundaramoorthy and Balabaskar (2013) mentioned that the different isolates of *Bacillus* produced different colony colours, like white to whitish grey. Parvathi *et al*. (2009) identified eighty-two isolates as *Bacillus* spp. by biochemical tests was supported by the findings of the present results.

Efficacy of *Trichoderma* sp. isolates against *F. oxysporum* f.sp*. cepae*

The antagonistic activity of six different *Trichoderma sp. was* collected from different regions of Tamil Nadu, which was tested for its antagonistic activity against *F. oxysporum* f.sp. *cepae* by dual culture method (Table 3; Fig. 1). Among the six tested isolates, *T. harzianum* (T_3) recorded the maximum inhibition zone (78.04%), followed by *T. harzianum* (T2), which recorded (48.91%) inhibition on the mycelial growth, and the isolate *T. harzianum* (T_1) recorded the minimum inhibition (17.67%). Similarly, Taha Yassin *et al*. (2020) reported that *T. viride* strain exerted the highest antagonistic potency against *A. alternata* (75.04%), followed by *F. proliferatum* (64.23%), *B. sorokiniana* (62.65%) and *D. halodes* (51.54%) which support findings of present results. Jagraj Singh *et al*. (2018) found that *T. harzianum* was very effective in controlling *F. oxysporum* f. sp. *lycopersici* where inhibition zone formation was highest (75.9%), followed by *T. viride and T. koningii* with inhibition zones of 67.7 and 55.6%, respectively and also the present results accorded with the findings of Nwankiti and Gwa (2018), who confirmed the antagonistic potency of *T. harzianum* strain against *F. oxysporum*, with a mycelial inhibition rate of 45.69%. In addition, Abhiram and Masih (2018) reported that *T. viride* suppressed the mycelial growth of *F. oxysporum* strains, demonstrating inhibition rates of 62.50% - 71.00%. Ayyandurai *et al.* (2022) reported that 25 isolates of *Trichoderma* sp. efficacy were investigated against the *S. rolfsii*. Among them, *T. longibrachiatum* and *T. asperellum* had a more inhibitory effect on the growth of *S. rolfsii*. The isolate T (SP)-20 of *T. longibrachiatum* showed 84.44% inhibition of mycelial growth of pathogen followed by T(AR)-10 of *T. asperellum* (75.55%), thus clearly stated that the *Trichoderma* sp. was highly effective against the soil-borne pathogens.

Efficacy of *B. subtilis* isolates against *F. oxysporum* f.sp*. cepae*

The antagonistic activity of *B. subtilis* collected from different regions of Tamil Nadu was tested for its antagonistic activity against *F. oxysporum* f.sp. *cepae* by dual culture (Table 4; Fig 1). Among the tested isolates, *B. subtilis* (B_4) recorded the maximum inhibition zone (66.16%) followed by *B. subtilis* (B_5), which recorded a (59.03%) inhibition on the mycelial growth, and the isolate *B. subtilis* (B_3) recorded the minimum inhibition (21.72%). Mardanova *et al*. (2017)

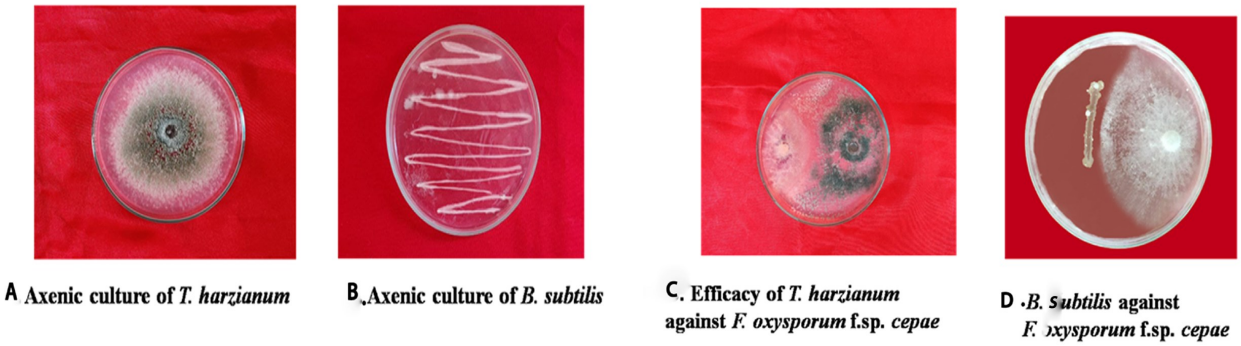


Fig. 1. *Showing effect of biocontrol against the F.oxysporum f.sp. cepae. A) Axenic or pure culture of Trichoderma harzianum T_3, B) Axenic or pure culture of Bacillus subtilis B_5). C) Efficacy of T. harzianum T_3 against F. oxysporum f.sp. cepae FOC_7 (dual culture assay) .D) Efficacy of Bacillus subtilis B_5 against F. oxysporum f.sp. cepae FOC_7 (dual culture assay)*

Table 1. Morphological and cultural characters of *T. harzianum* isolates

S. No	Iso-late	Location	Latitude/ longitude	Colony characters	Length	Breadth	Con-centric rings	Sporulation initiate after (hrs)
1	T_1	Manapparai	10.937347, 78.421188	Dark green sporulation	2.34-3.53	2.0-3.65	+	48-56
2	T_2	Salem	10.622817, 78.447189	White mycelium withdark green sporulation	2.45-3.40	2.36-3.56	+	48-58
3	T_3	Kulithalai	39.57178 , 75.467142.	Green to bright greensporulation	2.68-3.25	2.54-3.46	+	48
4	T_4	Musiri	10.954884, 78.443932.	Deep green sporulation	2.18-3.26	1.75-3.46	+	52
5	T_5	Perambalur	9.9651242. 77.7885004	Cottony fluffy greensporulation	2.64-3.72	2.43-3.29	+	55-56
6	T_6	Usilamapatty	11.230000, 78.879997	Dark green sporulation	2.32-3.36	2.85-3.02	+	48-50

also reported that *B. subtilis* was able to suppress *Fusarium* infection through the production of hydrolytic enzymes and cyclic lipopeptides. In addition to their effect on *Fusarium*, *Bacillus* isolates showed antagonistic activities against numerous fungal and bacterial pathogens (Wu, 2015). Zalila-Kolsi *et al.* (2016) showed that antagonistic *B. subtilis* strains that inhibit fungal growth *in vitro* also reduce the production of mycotoxins as well as the occurrence and frequency of Fusarium-caused diseases in greenhouse and field conditions

Efficacy of different oilcakes against *F. oxysporum* f.sp. *cepae*

The efficacy of culture filtrates of different oil-cake against *F. oxysporum* f.sp. *cepae by the* agar well diffusion method is depicted in Table 5; Fig 2. The result showed that the filtrates of neem cake showed a maximum inhibition zone of *F. oxysporum* f.sp. *cepae* of 1.29 cm @ 15% concentration, followed by groundnut cake at 1.36 cm @ 30% concentration, 2.28 cm was recorded as the least inhibition zone in this Pungam cake at 15% concentration. Similarly, Ayyandurai *et al.* (2021) tested the different oilcake extracts against the *Sclerotium rolfsii* by poisoned food technique. Among them, mahua cake (10%) recorded the maximum inhibition zone of 1.29cm, followed by neem cake (10%), which recorded a 1.93cm inhibition zone. The minimum inhibition zone that was observed in coconut cake (10%) was 7.92cm, and also who

Table 2. Biochemical and culture characters of *B. subtilis* isolates on nutrient agar medium

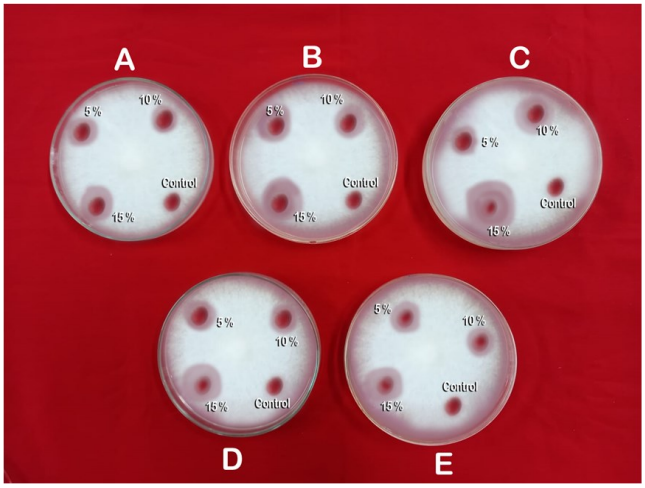
S. No.	Isolate	Locality	Latitude/ longitude	Colony morphology	GR	SH	MRT	CUT	H2S
1	B_1	Manapparai	10.622817/ 78.447189	Whitish grey with serrated margin	+	+	-	+	-
2	B_2	Tharagampatti	39.57178/ 75.467142.	Fuzzy white with serrated margin	+	+	-	+	-
3	B_3	Salem	11.230000/ 78.879997	Opaque with serrated margin	+	+	-	+	-
4	B_4	Thalaivasal	10.937347/ 78.421188	Whitish grey with serrated margin	+	+	-	+	-
5	B_5	Perambalur	39.57178 / 75.467142	Rough with serrated margin	+	+	-	+	-

*GR- Gram Reaction; SH- Starch Hydrolysis; MRT- Methyl Red Test; CUT- Citrus Utilization Test; H_2S- Hydrogen Sulphide

Table 3. Efficacy of *T. harzianum* isolates against *F. oxysporum* f.sp. *cepae* (FOC_6)

S. No.	Isolate	Radial mycelial growth (mm)*	PROC* (%)
1.	T_1	74.09^f	17.67
2.	T_2	45.98^b	48.91
3.	T_3	19.76^a	78.04
4.	T_4	59.76^d	33.60
5.	T_5	61.23^e	31.96
6.	T_6	59.76^d	33.60
7.	T7 (Control)	90.00	-

*Mean of three replication, All the letters show the significant effect as per Duncan's Multiple Range Test (DMRT); "a" : highly significant



A. Mustard cake, B. Groundnut cake, C. Neem cake, D. Pungam cake, E. Gingelly cake

Fig. 2. *Showing efficacy of different oil cakes extract against F. oxysporum f.sp. Cepae A)5 %, 10 % and 15% concentration of mustard oil cake extract against F. oxysporum f.sp. cepae , Control- Sterile distilled water. B)5 %, 10 % and 15% concentration of Groundnut oil cake extract against F. oxysporum f.sp. cepae. C)5 %, 10 % and 15% concentration of Neem oil cake extract against F. oxysporum f.sp. cepae. D)5 %, 10 % and 15% concentration of Pungam oil cake extract against F. oxysporum f.sp. cepae. E)5 %, 10 % and 15% concentration of Gingelly oil cake extract against F. oxysporum f.sp. cepae*

Table 4. Screening of *B. subtilis* isolates against *F. oxysporum* f.sp. *cepae* (FOC_6) (Dual culture technique)

S. No.	Isolate	Radial mycelial growth (mm)*	PROC*
1.	B_1	56.89^c	36.78
2.	B_2	67.98^d	24.46
3.	B_3	70.45^e	21.72
4.	B_4	30.45^a	66.16
5.	B_5	36.87^b	59.03
6.	B6 (Control)	90.00	-

*Mean of three replication, All the letters show significant effect as per Duncan's Multiple Range Test (DMRT) ; PROC – Per cent Reduction Over Control

mentioned the antifungal activity of mahua oilcakes due to the presence of terpenoid, phenolic compounds and bio active compounds *viz.,* Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z, Z, Octadecanoic acid (33.59) and a1-Octyn-3-ol during GC-MS analysis .

Conclusion

The present study concluded that *Trichoderma* sp. and *Bacillus* sp. could be used as effective biocontrol agents to control the Fusarium wilt disease of onions. These antagonists exhibited a wide mode of action against the pathogens, offering an alternative strategy

Table 5. Efficacy of different oilcakes against *F. oxysporum* f.sp. *Cepae* (Agar well diffusion method)

S. No.	Different cake	Inhibition zone (cm)		
		5%	10%	15%
1.	Mustard cake	2.80[e]	2.35[e]	2.10[d]
2.	Groundnut cake	1.70[b]	1.53[b]	1.36[b]
3.	Neem cake	1.57[a]	1.39[a]	1.29[a]
4.	Pungam cake	2.50[d]	2.37[d]	2.28[e]
5.	Gingelly cake	2.3[c]	2.09[c]	2.01[c]
6.	Control	9.00[f]	9.00[f]	9.00[f]

*Mean of three replication, Values in the column followed by common letters did not differsignificantly at 5% level by Duncan's Multiple Range Test (DMRT)

for disease management. Furthermore, oil cakes are a rich source of bioactive antifungal compounds, especially mahua cake and neem cake had secondary metabolites like phenolic and flavonoids compounds that exhibited antimicrobial activity . Further studies are recommended to test the effectiveness of using the two antagonists like *Trichoderma* sp. and *Bacillus* sp., either separately or in combination with the oilcake in open fields to develop long-term *Fusarium* wilt disease management strategies for onion.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abhiram, P. & Masih, H. (2018). *In vitro* Antagonism of *Trichoderma viride* against *Fusarium* oxysporum strains. *Journal of Pharmacognosy and Phytochemistry* 7 (2), 2816–2819. https://doi.org/10.1016/j.jksus.2021.101363
2. Allen, E. K. (1953). Experiments in soil microbiology. Burgess Publishers. C Minnepolis 107p
3. Aneja, K. R.(1993). Experiments in Microbiology, Plant pathology and tissue culture .Wishwaprakasham, New Delhi 117-195.
4. Ayyandurai, M., R. Akila, K. Manonmani, M. Theradimani & S. Vellaikumar (2021). Phytostimulation and growth promotion activities of *Trichoderma* spp. on groundnut (*Arachis hypogaea* L.) crop. *Journal of Applied and Natural Science* 13 (4), 1172-1179.
5. Ayyandurai, M., R. Akila, M. L. Mini & K. Manonmani (2022). Gas chromatography–mass spectrometry (GC-MS) analysis of antimicrobial compounds produced by mahua oil cake against the stem rot pathogen-*Sclerotium rolfsii*. *Journal of Applied and Natural Science* 14 (2), 600-606.
6. Ayyandurai, M., R. Akila, K. Manonmani, M. L. Mini, S. Vellaikumar, S. Brindhadevi & M. Theradimani (2022) Combined Application of *Trichoderma longibrachiatumT* (SP)-20 and *Trichoderma asperellum* T (AR)-10 in the Management of Stem Rot of Groundnut.*Legume Research-An International Journal,* 1, 7.
7. Ben Kalman, Dekel Abraham, Shaul Graph, Rafael Perl-Treves, Yael Meller Harel & Ofir Degani (2020) Isolation and Identification of *Fusarium* spp., the Causal Agents of Onion (*Allium cepa)* Basal Rot in Northeastern Israel. *Biology* 9(4), 69 https://doi.org/10.3390/biology9040069
8. Cappuccino, J. G. & Sherman, N. (1992). Microbiology: A Laboratory Manual. The Benjamin/Cummings Publishing Company, California. https://doi.org/10.12691/ajmr-1-4-6
9. Cyrabree, T. & Hindshill, G. (1975). Fundamental Experiments in Microbiology, W.B. Saunders Company, London 61-66. https://doi.org/10.32622/ijrat.742019233
10. Dennis, C. & Webster, J. (1971). Antagonistic properties of species-groups of *Trichoderma* Production of volatile antibiotics. *Transactions of the British Mycological Society* 57(1), Pages 41-48, IN4. https://doi.org/10.1016/S0007-1536(71)80078-5
11. Deshmukh. M. A., Gade, R. M., Belkar, Y. R. & Koche, M. D. (2016). Efficacy of Bio agents, bio-fertilizer and soil amendments to manage root rot in green gram. *Legume Research* 39(1),140-141.
12. Erayya, (2014). Designing a triple combination of copper, *Trichoderma* and chitosan and its evaluation against late blight caused by *Phytophthora infestans* (Mont.) de Bary. Thesis, Ph.D. G.B. Pant University of Agriculture and Technology, Pantanagar. Pp.65-90.
13. Gnanamanickam, S. S., Vasudevan, P, Reddy, M. S., Defago & G, Kloepper, J. W. (2002). *Biological control of crop diseases*. New York (NY): Marcel Dekker. Pp.1-9.
14. Gupta, R. & Gupta, R. (2014). Effect of Integrated Disease Management Packages on Diseases Incidence and Bulb Yield of Onion (*Allium cepa* L.). *SAARC Journal of Agriculture*. 11, 2 (Mar. 2014), 49–59. DOI:https://doi.org/10.3329/sja.v11i2.18401.
15. Howard, F. (2007). Interactions between *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne* spp. in *Vigna unguiculata* pathogenesis by *F. o. tracheiphilum* as affected by *M. javanica* and host cultivar. April 2007. *Plant Pathology* 40(3),465 – 475. DOI:10.1111/j.1365-3059.1991.tb02405.
16. Idris, Bektas & Mustafa, Kusek. (2019). Phylogenetic and Morphological characterization of *F. oxysporum* f.sp. *cepae* the causal agent of Basal rot on onion isolated from Turkey. *Fresenius Environmental Bulletin* 28(3), 1733-1742. . https://doi.org:10.1080/09583157.2020.1839381
17. Jagraj, Singh, Vipul, Kumar, Seweta, Srivastava & Adesh kumar. (2018). *In vitro* evaluation of *Trichoderma* sp against *Fusarium oxysporum* f.sp. *lycopersici* causing Tomato wilt. *Journal of Plant Pathology* 17(2), 59-64. DOI:

10.3923/ppj.2018.59.64
18. Koch, R. (1883). Bericht uber die Thatigkeit der deutschen Cholerakommisionen in Aegypten und Ostindien. *Wien Med Wochenschr* 33, 1548-1551. DOI: 10.25646/5107
19. Mardanova, A. M., Hadieva, G. F., Lutfullin, M. T., Khilyas, I. V., Minnullina, L. F., Gilyazeva, A. G., Bogomolnaya, L. M. & Sharipova, M. R, (2017). *Bacillus subtilis* strains with antifungal activity against the phytopathogenic fungi. *Agricultural Sciences* 8, 1–20. DOI : 10.4236/as.2017.81001
20. Mishra, R. K., Jaiswal, R. K., Kumar, D., Saabale, P. R. & Singh, A. (2014). Management of major diseases and insect pests of onion and garlic: a comprehensive review. *Journal of Plant Breeding and Crop Science* 6,160–170. DOI:10.5897/JPBCS2014.0467
21. Muthukumar, G., R. Udhayakumar, A. Muthukumar, N. Muthukumaran & M. Ayyandurai (2022) Survey on disease incidence of basal rot of onion incited by *Fusarium oxysporum* f. sp. *cepae* in major onion growing tracts of Tamil Nadu. *Journal of Pharmacognosy and Phytochemistry,* 11(8S): 1445-1454.
22. Nwankiti, A. O. & Gwa, V. I. (2018). Evaluation of antagonistic effect of *Trichoderma harzianum* against *Fusa rium oxysporum* causal agent of white yam (*Dioscorea rotundata* poir) tuber rot. *Trends in Technical & Scientific Research;* 1(1),0012-8. DOI:10.19080/TTSR.2018.01.555554
23. Olutiola, P. O., Famurewa, O. & Sonntag, H. G. (2000). An introduction to General Microbiology: A Practical Approach. Bolabay Publications 157-75. DOI: 10.12691/ajmr-6-1-4
24. Parvathi, A, Krishna, K, Jose, J, Joseph, N. & Nair, S. (2009). Biochemical and molecular characterization of *Bacillus pumilus* isolated from coastal environment in Cochin, India. *Brazilian Journal of Microbiology,* 40(2), 269-275. do 10.1590/S1517-838220090002000012.
25. Priya, R. U., Arun, sataraddi & Darshan, S. (2016) Survey of purple blotch of onion (*Alternaria porri* (Ellis) Cif.) in northern parts of Karnataka. *International Journal of Agriculture, Environment and Biotechnology* 9(3), 367- 373. DOI : 10.5958/2230-732X.2016.00048.6
26. Rangaswami, G. (1958). An agar block technique for isolating soil microorganism with special reference to Phythiacous fungi. *Science and Culture* 24, 85.
27. Sintayehu, A, Sakhuja, P. K., Fininsa, C. & Ahmed, S. (2011). Management of *Fusarium* basal rot *Fusarium oxysporum* f. sp. *cepae* on shallot through fungicidal bulb treatment. *Crop Protection* 30,560–565. https://dx.doi.org/10.1016/j.cropro.2010.12.027
28. Stolpe, S. & Godkeri, K. (1981). Non Pathogenic members of *Pseudomonas*. In: Marthiner (ed). The Prokaryotes, Springer-Verlag, New York 719-741. DOI:10.32622/ijrat.742019233
29. Sundaramoorthy, S. & Balabaskar, P. (2013). Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici. Journal of Applied Biology and Biotechnology Appl Biol Biotech*, 1, 36–40. DOI: 10.12691/wjar-5-1-2
30. Taha, Yassin M, Abdel-Fattah, Mostafa, A. A, Al-Askar, A. A, Sayed, S. R. M. & Mostafa, Rady (2020). Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* starains against some fusarial pathogens causing stalk rot of maize, *in-vitro*. *Journal of King Sand University- Science*. https://doi.org/10.1186/s41938-021-00463-w
31. Tutte, J. (1969). Plant pathological methods fungi and bacteria. Burgress publishing company, U.S.A. pp. 229.
32. Tyskiewicz, R, Nowak, A., Ozimek, E. & Jaroszuk-Sciseł, J. (2022). *Trichoderma*: The Current Status of Its Application in Agriculture for the Biocontrol of Fungal Phytopathogens and Stimulation of Plant Growth. *International Journal of Molecular Sciences* 23, 2329. https://doi.org/10.3390/ijms23042329
33. Vignesh, K. (2020). Combinational effect of AM fungi and PGPR for the management of wilt disease in tomato. Thesis.
34. Vincent, J. M. (1927). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 59: 850. doi: 10.1038/159850b0. PMID: 20343980.
35. Wu, (2015). Bacilysin overproduction in *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. *Scientific Reports* 5:1297510.1038/srep12975.
36. Zalila-Kolsi, I, Mahmoud, A. B, Ali, H, Sellami, S, Nasfifi, Z, Tounsi, S. & Jamoussi, K. (2016). Antagonist effects of *Bacillus* spp. strains against *Fusarium graminearum* for protection of durum wheat (*Triticum turgidum* L. subsp. *durum*). *Microbiological Research* 192 148–158. doi: 10.1016/j.micres.2016.06.012. Epub 2016 Jul 6. PMID: 27664733.