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Synergistic effect of oil cakes and *Trichoderma asperellum* in the suppression of *Sclerotium rolfsii*

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Abstract

Root and tuber crops are important as principal staple and nutritive foods for human beings. They are mainly used as food, animal feed and in industries. The tropical tuber crops are susceptible to many pathogens in field as well as in storage which lead to significant economic loss. Elephant foot yam (EFY) (*Amorphophallus paeoniifolius*) is a highly preferred crop in tropical and sub-tropical regions due to its high production potential (50–80 t ha^{-1}), market acceptability and lucrative economic returns. In organic cultivation of EFY, many growers practice incorporation of oil cakes to soil and use bio-agent, *Trichoderma* to enhance crop health and to reduce collar rot incidence. Collar rot caused by *Sclerotium rolfsii* is the most destructive and predominant disease, which causes significant crop loss. In the present study, oil cakes of coconut, groundnut, neem, mustard and sesame were evaluated in sterile and unsterile conditions for their ability to suppress *S. rolfsii.* The ability of oil cakes to support the growth and sporulation of *Trichoderma asperellum* was also studied. All the oil cakes completely inhibited the growth of *S. rolfsii.* When the concentration of oil cakes was reduced to 50% by diluting it with soil, sesame and groundnut oil cakes could not arrest the mycelia growth. Except neem oil cake, pathogen suppression potential of all other cakes was reduced upon sterilization. It varied from 39.53% (mustard oil cake) to 87.44% (sesame oil cake). Under unsterile condition, mycelial growth of *Trichoderma* completely covered the substrates, neem and groundnut oil cakes, which turned to green due to the sporulation. No growth was observed in coconut oil cake while scanty growth without sporulation was noticed with mustard and sesame oil cakes. Upon sterilization, mycelial growth of *Trichoderma* completely covered all the substrates except coconut oil cake. Thus, neem oil cake is ideal for the organic cultivation of elephant foot yam since it suppresses the pathogen as well as promotes growth and proliferation of *Trichoderma*.

Keywords: Elephant foot yam, Neem oil cake, Trichoderma, *Sclerotium rolfsii*, Mustard oil cake

Introduction

Tubers play an important role in world food security and are mostly used as energy supply in different countries. Tropical tuber crops include cassava, yams, taro, sweet potato, elephant foot yam, arrowroot etc and are rich in nutrients. In India, they are sources of sustenance and livelihood security of about 200 million people across more than 20 different states (Liu et al., 2014). Current crop production systems target the exploitation of sustainable techniques by maintaining a balance with the environment. High input conventional agriculture that advocates large scale chemical inputs and mutely result in irrevocable environmental catastrophes. The necessity for environmental conservation coupled with the desire for safe foods has made organic farming one

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of the fastest-growing agricultural enterprises (Katsvairo et al., 2007). Organic cultivation allows production of profitable and high-quality food with less human and environmental exposure to dangerous agrichemicals as compared to conventional agricultural systems.

Elephant foot yam (EFY) (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) responds well to organic manures and can be grown with lesser chemical inputs, using the organic wastes available in-home gardens. It offers ample scope for organic production as well as export of EFY to the Middle East, Europe and USA, since there is a great demand for organically produced tuberous vegetables, among Asians and Africans living in Europe, USA and Middle East (Suja et al., 2010). The corms of EFY are popularly used as a vegetable in various delicious cuisines and preparation of indigenous ayurvedic medicines (Misra et al., 2005; Leen et al., 2021). The tubers are rich in carbohydrates, starch, calcium potassium, phosphorous and vitamin C. In India, EFY is mostly cultivated in the states of Andhra Pradesh, Tamil Nadu, West Bengal and Kerala.

Like all other crops, EFY is also susceptible to many pathogens causing heavy loss to the crop (Misra et al., 2005). Among the field diseases, collar rot disease caused by *Sclerotium rolfsii* is the most destructive and prevalent disease in all EFY growing areas causing yield loss up to 100% (Misra, 1997; Gogoi et al., 2002; Kumar et al., 2017). The disease is distributed in tropical and sub-tropical regions of the world where high temperatures prevail (Mahato et al., 2017). Collar rot is a fast spreading and destructive disease and is becoming more serious at seedling stage causing rot at collar region especially in area where paddy based cropping system is followed (Vishruta et al., 2021). Several methods are employed to mitigate the disease including the use of chemical fungicides (Theertha et al., 2017). Heavy and widespread application of chemical pesticides has created the public's growing concern for the human health conditions and the environmental pollution associated with pesticide usage. In addition, reasons such as the development of pesticide-resistant strains of pathogens and the lack of continuous approval of some of the most effective fungicides have motivated the search for alternative approaches (Aswathy et al., 2019).

Soil borne pathogens can be controlled or suppressed by using organic amendments such as soil application of composts, green manure, FYM (farmyard manure) and oilcakes (Suja and Sreekumar, 2014). The pathogen suppressing potential of oil cakes is seen with many oil cakes against soil borne pathogens. Many beneficial microbes have been located and have showed remarkable results in the management of diseases (Lakshmipriya et al., 2016). Application of *Trichoderma* is recommended to manage collar rot in elephant foot yam (Linet et al., 2018). Among the investigated biological control agents, *Trichoderma* species have attracted special position due to its peculiar biological characteristics since the early 1930s (You et al., 2016). Considering the above facts, the present study aimed to assess the antifungal properties of five commonly available oil cakes and to evaluate their efficiency to support the growth and proliferation of *T. asperellum*.

Materials and Methods

Microbial cultures

The cultures of *S. rolfsii* and *T. asperellum* were obtained from Microbial repository, Division of Crop Protection, ICAR- Central Tuber Crops Research Institute. The cultures were subcultured on PDA slants and maintained at 28±2°C in a BOD incubator.

Oil cakes

Oil cakes of coconut, ground nut, neem, mustard and sesame were purchased from the local market and used for the study.

Testing of antifungal properties of oil cakes

Two hundred grams of oil cakes of coconut (T_1), ground nut (T_2), neem (T_3), mustard (T_4) and sesame (T_5) were taken in a container separately and added 50 ml sterile distilled water. The cakes were then mixed well and filled up to 2/3 length (10cm) of the test tubes [15cm (L) × 2.5cm (dia)] using a spatula. The upper portion of the filled oil cake was gently pressed to make a firm surface to keep the mycelial discs. The experiment was conducted under two conditions:

1. Using sterile oil cake: the filled tubes were plugged with absorbent cotton and sterilized at 15 lbs pressure and 121°C for 15 min.
2. Without sterilization: the filled tubes were plugged with cotton and used without sterilization. Triplicates were maintained in all cases.

The culture of *S. rolfsii* obtained from the repository was grown on Potato Dextrose Agar (PDA) medium (HIMEDIA). The mycelial discs of 5 mm diameter were aseptically cut from the growing edges of 48 h old culture using a corkborer. Four discs were transferred and placed on the top of the oil cakes in each tube (Fig. 1). The mycelia discs were placed in a way that each disc forms 90° with the adjacent disc. The tubes filled with sterile and non-sterile oil cakes were arranged in stands and incubated in BOD incubator at 28±2°C. Tubes containing potting mixture (1:1:1 sand, soil and farmyard manure, respectively) in sterile and unsterile condition served as the control.

Fig. 1. Mycelial discs kept on the surface of oil cakes

Antifungal properties of diluted oil cakes (50%)

The oil cakes, which showed inhibitory action in the first experiment was diluted to 50% strength by adding potting mixture. Hundred grams of oil cakes of coconut, ground nut, neem, mustard and sesame and 100 g of potting mixture were weighed and mixed in 1:1 ratio. Fifty ml of sterile distilled water was added to the mixture and mixed well. The diluted oil cakes were tested as the way described above.

Growth and sporulation of Trichoderma asperellum on oil cakes

Culture of *T. asperellum* was obtained from microbial repository of ICAR-CTCRI. The culture was sub-cultured on PDA. The inoculated plates were allowed to grow and sporulate in a BOD incubator. Culture discs of 1cm (diameter) were cut from the sporulated culture using a cork borer. Eight discs each were transferred to a vial containing 10 ml sterile distilled water. The vials containing culture discs were mixed in a cyclomixer to hasten the process of dislodging of conidia into water. One ml of conidial suspension was dispensed into sterile and unsterile oil cakes in conical flasks, which contained 100 g moistened oil cakes. The inoculated flasks were plugged well and gently mixed for the distribution of conidial suspension. Each treatment was replicated thrice. The inoculated flasks were incubated at 28±2˚C for 10 days.

Results and Discussion

Antifungal properties of oil cakes

A new method, test tube-based assessment was standardized for the precise quantification of mycelial growth of *S. rolfsii* on various substrates. *S. rolfsii* started growing in potting mixture within 24 h of inoculation. Under natural condition (unsterile), all the oil cakes could completely inhibit the mycelial growth of the pathogen (Table 1). When the oil cakes were sterilized, sesame oil cake lost antifungal property and enhanced mycelia growth of *S. rolfsii* (Fig. 2). The improved growth ranged from 25.9% (72 h) to 38.0% (48 h) over the growth in potting mixture.

Table 1. Mycelial growth of S. rolfsii on five oil cakes under natural and sterile condition

Type of oil cake	Mycelial growth in cm (unsterile)			Mycelial growth in cm (sterile)		
	24h	48h	72h	24h	48h	72h
Coconut cake (T_1)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Ground nut cake (T_2)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Neem cake (T_3)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Mustard cake (T_4)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Sesame cake (T_5)	0.00^{b}	0.00^{b}	0.00^{b}	1.20^{a}	2.10^{a}	2.70^{a}
Potting mixture (T_6)	0.90^{a}	1.50^{a}	1.90^{a}	0.80^{a}	1.30^{a}	2.00^{a}

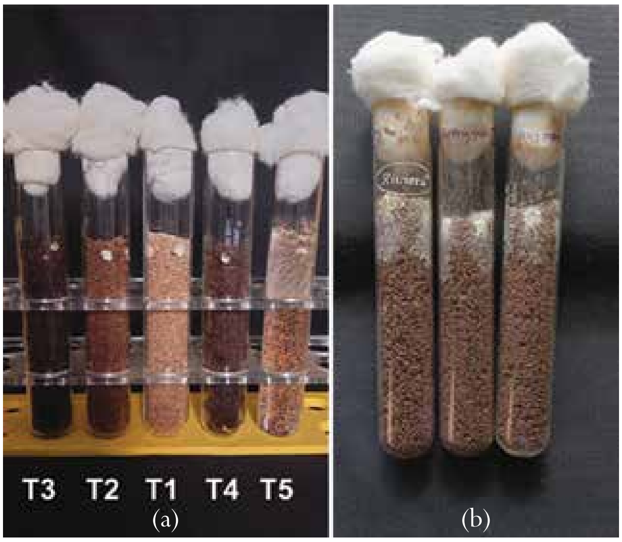

Fig. 2. (a) Mycelial growth of *S. rolfsii* on sterile oil cakes of coconut, groundnut, neem, mustard and sesame (b) Growth on potting mixture

Antifungal properties of diluted oil cakes

When the strength of oil cake was diluted to half (50%), the antifungal activity of groundnut oil cake and sesame oil cake was reduced (Table 2). The mycelial growth inhibition was 63.3% and 69.5% by ground nut cake and sesame oil cake respectively over the growth in potting mixture (72 h). Sterilization of the substrate leads to reduction of antifungal activity in four oil cakes. Neem cake was the only substrate, which could completely arrest the mycelial growth even at 50% strength (Fig. 3). After 24 h of incubation, mycelial growth inhibition by the oil cakes ranged from 16.6% (sesame oil cake) to 100.0% (neem cake) over the growth on potting mixture. Same trend continued after 72 h of incubation also. It ranged from 12.6% (sesame oil cake) to 100.0% (neem cake).

Table 2. Mycelial growth of S. *rolfsii* on five oil cakes (50% strength) under natural and sterile condition

Treatment	Mycelial growth in cm (natural)			Mycelial growth in cm (sterile)		
	24h	48h	72h	24h	48h	72h
Coconut cake (T_1)	0.00^{b}	0.00^{b}	0.00^{b}	0.73^{bc}	1.10^{b}	1.90^{bc}
Ground nut cake (T_2)	0.83^{a}	1.00^{ab}	1.00^{b}	0.83^{b}	1.06^{b}	1.86^{bc}
Neem cake (T_3)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}
Mustard cake (T_4)	0.00^{b}	0.00^{b}	0.00^{b}	0.63^{bc}	1.16^{b}	1.70^{c}
Sesame cake (T_5)	0.60^{ab}	0.70^{b}	0.83^{b}	1.36^{ab}	2.76^{a}	3.76^{ab}
Potting mixture (T_6)	1.00^{a}	2.03^{a}	2.73^{a}	1.63^{a}	3.26^{a}	4.30^{a}

Oilseed cakes are essential organic amendments after getting from the oilseed extraction and then successfully used in an agricultural ecosystem for various purposes (Ayyandurai, 2022). The addition of the oil cake into the soil increases the cation exchange capacity water holding capacity and makes a good soil structure for plant growth and effectively reduces harmful soil-borne pathogens. In the plant protection aspect, oil cake produced high antifungal principles against soil-borne pathogens like bacteria, fungi and nematodes (Dar et al., 2018; Mahato et al., 2018).

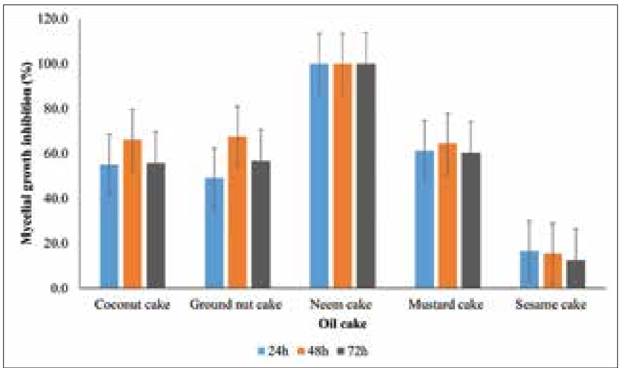

Fig. 3. Mycelial growth inhibition (%) over potting mixture shown by sterilized diluted (50%) oil cakes

The antifungal property containing oil cakes play a significant role in reducing plant disease in a wide range of soil-borne pathogens. The disease suppression of oil cakes was reported against many pathogens by various workers. Oilcakes of groundnut, neem, mahua and mustard at 10% concentration were evaluated for their antifungal action against *R. solani*, the incitant of collar rot and web blight of cowpea by poisoned food technique and highest inhibition (100%) was obtained with mustard oil cake, this was followed by groundnut oil cake and neem cake with a suppression of 79.62% and 76.29%, respectively (Aparna and Girija, 2018). Mahua cake and neem cake highly inhibited *S.rolfsii*, *Rhizoctonia solani*, *Pythium*, *Fusarium* and other soil-borne pathogens (Latha and Rajeswari, 2019). Neem cake extract was effective against *Macrophomina phaseolina* with maximum mycelial inhibition of 52.90% followed by coconut cake extract with 50.33% against control at 10 % conc. At 15% concentration, groundnut cake extract was proved effective against *Macrophomina phaseolina* with maximum mycelial inhibition of 65.0 % followed by neem cake with 51.10 % (Kanmani et al., 2020). *Fusarium oxysporum* and *Colletotrichum capsici* were sensitive to oil seed cake extracts of madhuca and simarouba cake extracts followed by neem cake extract. The *Aspergillus flavus* was highly sensitive to neem followed by madhuca and simarouba extracts (Udupa et al., 2021). Maximum mycelial inhibition of 71.95 per cent with no sclerotia production was observed with mustard cake, when tested against *S. rolfsii* causing Southern blight of China aster (Kashyap and Chandel, 2022).

The high efficiency of neem oil cake or the extract of neem oil cake over other cakes had seen in studies. Aqueous extracts of neem cake and karanj cake were tested against some important phytopathogenic fungi and neem cake extract was most effective with GI50 value of 0.27% and 0.11% against *Curvularia lunata* and *Helminthosporium pennisetti* respectively (Kumar et al., 2013). Undiluted neem cake extraction effectively inhibited the growth of the four phytopathogenic fungi, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Collectotrichum* spp. and *Phytophthora capsici* (Duong et al., 2014). Highest reduction against *S. rolfsii* causing root rot complex in chilli was recorded with neem seed cake (79.54%), followed by mustard cake (75.04%), castor cake (68.22%) and groundnut cake (48.55%) (Bhattacharjee et al., 2015). Application of neem cake in soil reduced 72.70 % disease incidence in tomato caused by *S. rolfsii* followed by sesame cake with 66.83 % suppression (Mahato et al., 2018). Antifungal activity of various elicitors like ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, coconut oil cake and neem oil cake were evaluated and neem oil cake was the best elicitor (Meena et al., 2022). However, none of the studies reported about the loss of antifungal activity of oil cakes on sterilization.

Growth and sporulation of *Trichoderma asperellum* on oil cakes

The growth and sporulation of *T. asperellum* on five oil cakes were observed for 7 days. Mycelial growth, sporulation and the color of the substrate on mycelial colonization were recorded at an interval of 24 h. Under natural condition, good mycelial growth was observed on groundnut cake and neem cake (Table 3). There was no growth on coconut oil cake and scanty growth observed on mustard cake and sesame oil cake. Sporulation was best on neem cake and the substrate completely turned green due to sporulation (Fig. 4). The mycelial growth and sporulation commendably improved consequent to sterilization of the substrate. The poorest growth and sporulation were on coconut oil cake. The color of the substrate turned to dark green on other four substrates. *Trichoderma* successfully grew well and profusely sporulated on sterile as well as unsterile neem cake.

The use of microorganisms as biocontrol agents has provided a very promising alternative and less hazardous method for plant disease control (Cook, 1985). *Trichoderma* spp is the most widely used bio-control agent, which plays an important role in the environment by promoting plant growth and protects the plant from soil borne pathogens. They are environmentally safe, readily available, cheaper and with minimal negative effects (Adhikari et al., 2022). In India alone, more than 250 *Trichoderma* based formulations are sold commercially (Lee et al., 2016). The combined application of oil cakes and bio-control agent such as *Trichoderma sp,* is a biological approach to control the soil borne pathogens. *T. harzianum* along with mustard cake showed higher antagonistic activity against the causal agent of *Fusarium* wilt as compared to *T. harzianum* alone (Jangir et al., 2020). Soil application of mustard oil cake (MOC) @ 1 kg per 2×2 m^2 plot fortified with *T. harzianum* @ 5 ml per m^2 was very effective in managing basal rot of *Pippali* (*Piper longum* Linn.). Catkin caused by *S. rolfsii* with reduced disease incidence (10.65%) and increased plant growth parameters (Dutta et al., 2022).

Fig. 4. Mycelial growth and sporulation of *T. asperellum* on five oil cakes

The oil content in the oil cakes, coconut, sesame, groundnut and mustard uniformly inhibited the growth of *S. rolfsii* and *T. asperellum*. The antifungal property of these cakes decreased on sterilization, and allowed the

Table 3. Mycelial growth and sporulation of *Trichoderma* on sterile and unsterile oil cakes

Type of the oil cake	Properties	Unsterile	Sterile
Coconut cake (T_1)	Mycelial growth	No growth	Scanty growth
	Sporulation	-	Light sporulation +
	Colour on sporulation	-	Light green
	Mycelial growth	Fully covered	Fully covered
Ground nut cake (T_2)	Sporulation	Sporulated+	Moderately sporulated ++
	Colour on sporulation	Light green	Green
	Mycelial growth	Fully covered	Fully covered
Neem cake (T_3)	Sporulation	Sporulated+++	Sporulated+++
	Colour on sporulation	Green	Green
Mustard cake (T_4)	Mycelial growth	Scanty growth	Fully covered
	Sporulation	No sporulation	Sporulated +
	Colour on sporulation	-	Green
	Mycelial growth	Very scanty	Fully covered
Sesame cake (T_5)	Sporulation	No sporulation	Sporulated +
	Colour on sporulation	-	Green

*+ mild sporulation, ++ good sporulation and +++ very good sporulation

growth of *S.rolfsii* and *T. asperellum* in sterilized oil cakes. But, neem cake had differential action on *S. rolfsii* and *T. asperellum.* Irrespective of sterile/ unsterile condition, neem cake completely suppressed the growth of *S. rolfsiii* and supported the growth and sporulation of *Trichoderma*. In an integrated system, the desired trait is inhibition of the pathogens and promotion of growth and proliferation of beneficial agents. Thus, neem cake is the ideal oil cake for the organic cultivation of elephant foot yam since it suppresses the pathogen as well as promotes the proliferation of bio-agent.

Conclusion

One of the major challenges in organic cultivation is the management of diseases and pests. The growers have very limited options to save the crop. Application of organic amendments, oil cakes and bio-agents play a crucial role in organic management of diseases. The crop, elephant foot yam responds well to organic manures. Collar rot caused by *S. rolfsii* is the most destructive pathogen in elephant foot yam. Application of *Trichoderma* is being recommended for management of collar rot incidence. In this context, few oil cakes were explored for their selective potential to inhibit the pathogen as well as to promote the growth of *T. asperellum*. The oil cakes of coconut, groundnut, mustard and sesame inhibited both the organisms under natural condition. Because of their activity towards *S. rolfsii*, they can be applied for the pathogen suppression in field. These four oil cakes cannot be combined with *T. asperellum* for field application, since they inhibit the growth and sporulation of the latter. They lost antifungal activity on sterilization; hence these four oil cakes can be used for multiplication of *Trichoderma* in labs. Irrespective of sterile/unsterile condition, neem cake exhibited differential activity by completely suppressing the growth of *S. rolfsii* and supporting the growth and sporulation of *Trichoderma*. Thus, neem cake is the ideal oil cake for the organic cultivation of elephant foot yam since it suppresses the growth of *S. rolfsii* and promotes the growth and proliferation of *T. asperellum*.

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