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Short Communication: Fungicidal effect of flax seed oil against several fungal strains

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Abstract. *Mir RA, Ahmad MK. 2023. Short Communication: Fungicidal effect of flax seed oil against several fungal strains. Asian J Nat Prod Biochem 21: 1-5.* The plant world is provided with innate biologically active secondary metabolites, which regulate its defense mechanism. Chemical fungicides pose a great threat to the plant kingdom regarding disease resistance and phytotoxicity. Furthermore, chemical fungicides provide a short spectrum and poor control of plant pathogens. Seed oils have a great potential to act as a natural fungicide against several plant pathogens. The aim of this study was to evaluate the *in vitro* antifungal activity against eight fungal strains, *Mucor mucedo, Penicillium expansum, Trichoderma viride, Trichoderma harzianum, Phytophthora crocatum, Aspergillus niger, Phytophthora infestans,* and *Venturia inaequalis* by the poisoned food method. Antifungal activity was assessed regarding the inhibition percentage of radial growth on a solid medium (Potatoes Dextrose Agar/PDA). Flaxseed oil showed the highest (100%) antifungal activity against *M. mucedo* at a concentration of 5 µL/mL, while moderate (53% and 60%) antifungal activity was recorded for *P. expansum*, and *P. crocatum*. However, linseed oil showed negative results against *A. niger*, *T. viride,* and *T. harzianum.* Thus, the results of the present study revealed that flaxseed oil has tremendous fungicidal activity and could become an alternative to synthetic fungicides for controlling certain dreadful plant fungal diseases.

Keywords: Antifungal activity, flax seed oil, phytopathogenic fungi, secondary metabolites

INTRODUCTION

Flax has been grown by early civilizations for its tremendous health benefits and is mainly cultivated for seed and fiber production. However, plant extracts have various allopathic properties and are therefore explored for their biological use. Moreover, its seed oil is a rich source of fatty acids and secondary metabolites, which possess several biological activities. Therefore, the search for new natural products, including plant extracts, seems to be the need of the hour.

Increased fungicidal resistance of pathogenic fungi against chemical fungicides has led to an urgent need to identify alternative strategies for managing fungal pathogenicity. Agro-based industries are looking for novel natural organic compounds to be used as non-synthetic antimicrobial agents to eradicate the most notorious phytopathogens. The agriculture industry has undergone huge losses due to many fungal pathogens, namely *Mucor mucedo, Penicillium expansum, Phytophthora crocatum, Aspergillus niger, Phytophthora infestans,* and *Venturia inaequalis.* These phytopathogens are responsible for many dreadful plant diseases, such as root rots, mucor rots, citrus fruit rots, and post-harvest fungal infestations (Amiri 2019; Savary et al. 2012).

Phytopathogens have slowly developed resistance against several chemical pesticides, which led to excess chemicals with higher concentrations, proving fatal for soil and human health. Therefore, an alternative, safe, eco-friendly biopesticidal formulation is urgently needed. Much research has been done to find novel essential oils to replace chemical pesticides. Essential oils from *Artemisia annua* have been used to control the *Glyphodes pyloalis* pathogen of mulberry leaves (Oftadeh et al. 2021). Post-harvest storage is a serious problem faced by the agriculture and food industries. Chemical fungicides are used at a high concentration to reduce the losses. Essential oils can be used as an alternative to stored products and have been used against *Sitophilus granarius* (Demeter et al. 2021)

Essential oils have been continuously explored for their potential as antimicrobial agents. Several essential oils from *Allium sativum, Citrus sinensis* has been found effective against several phytopathogens (De Clerck 2020). Essential oils are used as spices, remedies, or for their pleasant odor; rich in terpenoids and non-terpenoid compounds, they possess various interesting allelopathic properties. Due to technological advancements, modern techniques are applied to distill volatile compounds (Baser and Buchbauer 2010; Baser and Demirci 2011). Those compounds are important in food, medicine, or cosmetics and are becoming increasingly popular as antibacterial, antioxidant, antifungal, and insecticide agents. (Mezzoug et al. 2007; M'barek et al. 2007; Rozman et al. 2007).

Natural oils are also explored for their insecticidal properties (Isman 2000). According to a recent study, seed oil has innate defense abilities (Halloin 1983; Harman 1983; Kremer et al. 1984; Ceballos et al. 1998; Ozer et al. 1999). Seed oils have been used as antimicrobial agents for rhizospheric soil (Helsper et al. 1994). Plant health is mainly affected by early fungal infections, which affect its growth and development processes (Alvarez-Castellano et al. 2001; Skaltsa et al. 2000). Flax is an ancient Egyptian crop cultivated worldwide as an edible oil crop (Kaithwas and Majumdar 2013). The antimicrobial effect of various plant extracts was studied and documented (Paiva et al. 2010). Incorporating essential oils from *Thymus vulgaris* in milk for cheese production provides an additional defense against several exogenous detrimental pathogens of dairy products (Licon et al. 2020)

Canada is the world's biggest producer of flax, with 38%, while the annual worldwide is 3.06 million tons (Fadzir et al. 2018). The antimicrobial properties of flaxseeds were studied for their therapeutic properties (Adolphe et al. 2010). Flax seed oil has antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties (Sharil et al. 2022). *Linum usitatissimum* oil is an antimicrobial agent in bovine mastitis caused by microbial infection (Kaithwas et al. 2011). In addition, the presence of polyphenols in flax seed has been considered an antimicrobial agent (Barbary et al. 2010; Pag et al. 2014). Flax seed cake was extensively studied and reported as an antimicrobial food additive (Freese et al. 1973; Zheng et al. 2005; Zuk et al. 2014). Antifungal activities of flax seeds were previously studied against many pathogenic fungal strains, including human pathogenic fungi such as *Candida albicans, Alternaria solani, Alternaria alternata, Penicillium chrysogenum,* and *Fusarium graminearum* (Guilloux et al. 2009). The *C. albicans* were highly susceptible to fixed oil than cefoperazone, with an inhibition zone of 10.33 mm and 4.33 mm, respectively (Kaithwas et al. 2011). Flax seeds also control the growth of food-borne fungal pathogens (Xu et al. 2007). Another study reported that flaxseed lignans extract demonstrated moderate (70% to 90%) antifungal activities at 2.5 to 3.0 mg/mL for both *Aspergillus flavus* and *A. niger* (Barbary et al. 2010). The antifungal nature of flax seed was reported due to the presence of α linolenic acid and linoleic acid, considered fungal growth suppressors (Abdelillah et al. 2013)

The bioactive compounds from flaxseeds were effective against *Escherichia coli, Salmonella paratyphi, Lactobacillus, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae,* and *Saccharomyces cerevisiae* (Narender et al. 2016). In addition, flax seed oil extracts exhibited antimicrobial properties against the growth of *A. alternata* and *A. solani* in in-vitro conditions (Guilloux et al. 2009). Recently it was reported that genetically modified flax-type (GT) seedcake extract possesses antimicrobial activity against *S. aureus* and *E. coli* (Czemplik et al. 2012). Seedcake extract was also reported as an alternative to inhibit microbial growth of a wide range and with partial selectivity (Zuk et al. 2014). The lignans from the flaxseed interfere with the bacterial cell wall and inhibit their growth (Cowan 1999; Barbary et al. 2010).

The present work aimed to investigate the antimicrobial effects of flax seed oil against many phytopathogens in-vitro so that an eco-friendly bio-sustainable organic formulation can be developed to eradicate the pathogenicity of tested fungi.

MATERIALS AND METHODS

Samples and materials

Commercially available flax seed oil was purchased from a local distributor in New Delhi, India. Peptone dextrose agar powder and peptone dextrose broth powder were purchased from Hi-Media, Mumbai. The antibiotic chloramphenicol was purchased from BM Scientific company Srinagar Kashmir.

Culture and maintenance of the fungal isolates

The antimicrobial activity of flax seed fixed oil was evaluated against different microbial strains. A total of eight plant fungal strains, namely *M. mucedo, P. expansum, Trichoderma viride, Trichoderma harzianum, P. crocatum, A. niger, P. infestans,* and *V. inaequalis* were tested in this study. First, the pure cultures were obtained from Globils Agri and Food Enterprises Igc Lassipora Pulwama Jammu and Kashmir, India. Next, the fungal cultures were identified based on the colony characteristics of the respective fungi. The stock culture was maintained on a Potato Dextrose Agar (PDA) medium. Next, the poison food method of antifungal activity was used for antimicrobial testing of flax seed oil.

Preparation of media

Fungal media

Thirty-nine grams of PDA Powder (high media) was dissolved in 1 liter of distilled water and then sterilized in an autoclave at 121°C (15 lbs pressure) for 15 minutes.

Determination of the antifungal activity of flax seed essential oil

The antifungal assay was performed in two parts: Inhibition Concentration (IC50) and Minimum Inhibitory Concentration (MIC).

Inhibition Concentration (IC50)

The pure cultures of fungal test strains were maintained on a PDA medium for seven days. The assessment of fungitoxicity was performed by the poisoned food method (Grover and Moore1962; Adjou et al. 2012). Specific initial concentrations (1, 2, 3, 4, and 5 μL/mL) were prepared by adding the appropriate amount of flax seed essential oil containing 0.5% Tween 80 (Tween-80: 500mg/100mL). Next, 100 microlitres of dissolved flax oil were aseptically poured into the Petri plate, and 20 mL of melted PDA was added at 45°C. Petri plates were gently shaken to avoid entrapment of air bubbles in the oil. The medium was allowed to solidify at room temperature for about one hour. 6 mm in diameter fungal discs were cut from the periphery of seven-day-old pure cultures using a sterile cork borer and aseptically placed in the center of Petri plates. Finally, 100mg/1000mL chloramphenicol was used as an antibiotic to avoid bacterial growth. Flax oil was not mixed with the media in control plates. Three replicates were maintained for each treatment. All the plates were incubated at 28°C for five days. The statistical data was collected after three and five days of incubation, and mean data was used to calculate the inhibition percentage. The percentage inhibition of the mycelia growth of the test fungi was calculated using the formula by (Rao and Srivastava 1994). The inhibition percentage was compared with the control plates.

Mycelial growth inhibition $I\ (\%) = C\text{-}T/C \times 100$

Where:
I : Inhibition percentage
C : Colony diameter in control (cm)
T : Colony diameter in treatment (cm)

Data analysis

All data were presented as mean ± Standard Error (SE) of replicates and were analyzed using Data Processing Software (DPS, version 7.05) following one-way Analysis of Variance (ANOVA). Significant differences ($P < 0.05$) among treatment means after controlling for multiple comparisons were determined from a Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Antifungal activity

The present study showed that flax seed oil exhibited a strong action against the mycelia growth of eight test fungi; *M. mucedo, P. expansum, T. viride, T. harzianum, P. crocatum, A. niger, P. infestans,* and *V. inaequalis* (Figure 1). A complete mycelia growth inhibition was observed at 5µL/mL concentration. In addition, flax seed oil had a significant ($p \leq 0.05$) higher inhibitory effect on *M. mucedo* and *P. crocatum* (Figure 2, Table 1). The range of mycelia growth inhibition was between 18% to 100% at a concentration of 5 µL/mL. However, the minimum inhibitory effect was observed in *T. viride*, *T. harzianum,* and *A. niger* (Table 2).

Minimum inhibitory concentration

Inhibition of the mycelial growth of test fungi by flax seed essential oil after five days of incubation ranged from 7.33 % to 100% (Table 3). The highest and lowest inhibition of mycelial growth at a concentration of 1 µL/mL were observed in *M. mucedo* (75.47%) and *T. viride* (7.33%), respectively. The inhibition of mycelial growth in all the test fungi at an EO concentration of 1 µL/mL was significantly ($p \leq 0.05$) different.

Table 1. Mycelial growth inhibition (in percentage) by flax oil on the radial growth of different fungi

Fungus species	Growth inhibition (%)	
	Control	100 µL conc.
Mucor mucedo	0	100
Penicillium expansum	0	53
Aspergillus niger	0	20
Phytophthora infestans	0	45
Venturia inaequalis	0	40
Phytophthora crocatum	0	60
Trichoderma harzianum	0	20
Trichoderma viride	0	18

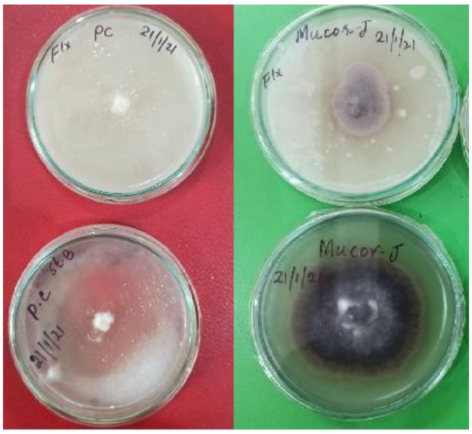

Figure 2. Effect of flax oil on *Phytophthora crocatum* and *Mucor mucedo*

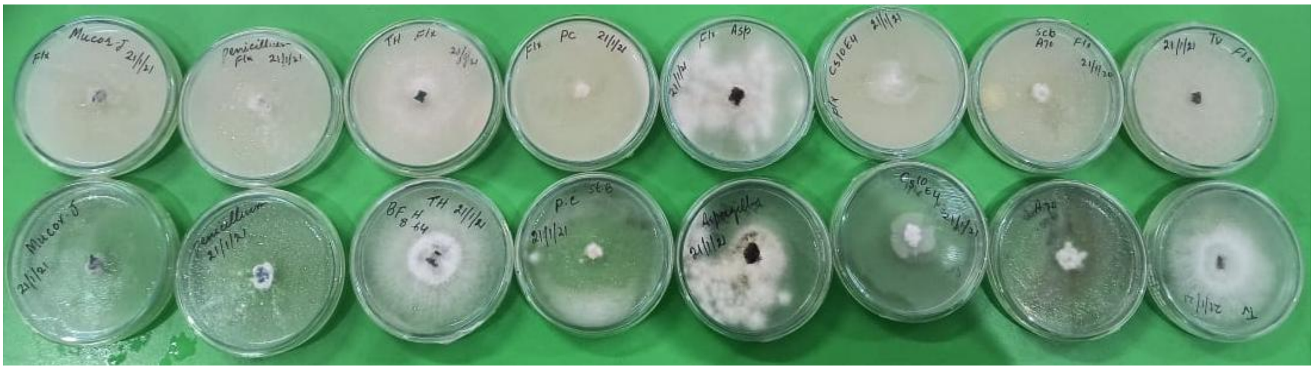

Figure 1. Antifungal activity of flax seed oil against eight different fungi

Table 2. Mycelial growth inhibition (%) of eight fungal strains after three days of incubation

Essential oil concentrations (µL/mL)	Mycelial growth inhibition (%)							
	Mucor mucedo	*Penicillium expansum*	*spergillus niger*	*Phytophthora infestans*	*Venturia inaequalis*	*Phytophthora crocatum*	*Trichoderma harzianum*	*Trichoderma viride*
1	39±0.57	21.66±0.33	8.5±0.28	17.16±0.16	17.16±0.72	25.16±0.60	8.16±0.44	7.33±0.33
2	49.33±0.44	26.5±0.28	10.16±0.33	21.5±0.28	21.33±0.88	31.5±0.76	10.16±0.44	9±0.28
3	59.33±0.66	31.66±0.33	12.66±0.33	25.66±0.33	25.66±1.20	34.33±3.28	12±0.57	10.33±0.33
4	79±1.15	42.33±0.88	15.66±0.33	32.66±1.45	33.33±0.88	50±1	16±0.57	14±0.57
5	100±0	53±0.57	20.33±0.66	43±0.57	42.66±1.76	63±1.52	20.5±0.86	18±0.57

Note: Data: means ±SE statistically significant differences between treatments ($P<0.05$)

Table 3. Mycelial growth inhibition e (%) of eight fungal strains after five days of incuba

Essential oil concentrations (µL/mL)	Mycelial growth inhibition (%)							
	Mucor mucedo	*Penicillium expansum*	*Aspergillus niger*	*Phytophthora infestans*	*Venturia inaequalis*	*Phytophthora crocatum*	*Trichoderma harzianum*	*Trichoderma viride*
1	75.47±2.18	39±0.57	12±0.57	25.66±0.33	20.33±0.66	31.66±0.33	12.66±0.33	7.33±0.33
2	78.62±1.66	40±1	12.66±0.33	26.5±0.28	25.16±0.60	42.33±0.88	15.66±0.33	10.33±0.33
3	84.28±1.66	44.65±2.27	15.66±0.33	31.5±0.76	33.33±0.88	54±2.30	17.16±0.72	16±0.57
4	93.71±1.66	64.33±1.85	17.16±0.72	34.33±3.28	39±0.57	56.66±1.20	18±0.57	17.16±0.72
5	100±00	50.35±0.54	19.31±0.63	40.85±0.54	40.53±1.67	59.85±1.45	19.47±0.82	17.1±0.54

Note: Data: means ±SE statistically significant differences between treatments ($P<0.05$)

The Minimum Inhibitory Concentrations (MICs) of flax seed oil on the test pathogens were within 1-5 µL/mL (Table 3). At a concentration of 5µL/mL, the mycelia growth of *M. mucedo* was completely inhibited. The essential oil of flax seed oil showed a fungicidal effect on three of the eight studied fungi: *P. expansum*, *P. crocatum,* and *M. mucedo*. Whereas linseed oil showed negative results against *A. niger, T. viride,* and *T. harzianum.*

The traditional use of medicinal plants to manufacture commercial drugs and their substitutes has been predominant since ancient times. These drugs help in the treatment of different forms of ailments caused by bacterial and fungal pathogens and in other important health-related activities (Cowan 1999).

The antimicrobial efficacy of flax seed oil has been carried out against many fungal pathogens, including *M. mucedo, P. expansum, P. crocatum, A. niger, P. infestans,* and *V. inaequalis.* The flax seed oil behaves differently in controlling the fungal growth *in vitro*. The *M. mucedo* and *Phytophthora* growth were completely inhibited at 100 microlitres per mL concentration. The other test fungal growth was also inhibited but not completely. The effect of antimicrobial inhibition was detected by observing growth patterns, sporulation, and mycelia growth inhibition. The present result follows Kreander et al. (2006), who reported that ethanol extract of *L. usitatissimum* showed antibacterial activity against *K. pneumonia* and *Pseudomonas aeruginosa*. The most common pathogen which causes serious problems in fruit trees is *P. crocatum*, which causes root rot in the apple, causing considerable losses to the apple industry during fruit growth.

The *M. mucedo* is a fungal plant pathogen that causes many fungal diseases, including mucor rot. Bukar et al. (2009) conducted a pathogen survey in Nigeria's sweet orange (*C. sinensis*) and found that 25% of decayed fruit were associated with Mucor spp. Mucor species has been found to cause fingernail infection among workers who squeezed cull orange fruit with bare hands (Sutherland-Campbell and Plunkett 1934). The *L. usitatissimum* extracts have antibacterial activity against three types of Gram-negative bacteria (*Shigella flexneri, Salmonella typhimurium,* and *E. coli*), and the maximum zone of inhibition was observed in *S. flexneri*. This may be considered promising natural-based antibiotics.

Recently, many studies have been conducted to explore the potential antimicrobial agents from natural resources. Plant extracts, especially essential oils, have been extensively studied for their natural phytochemicals. For example, genetically modified flax seed has many antimicrobial agents, including flavonoids (Zuk et al. 2011). Therefore, further studies should be done to know flax seed oil's exact mechanism.

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