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Evaluation of Antimicrobial Activity of Deodar Substrate and Hemp Seeds against Fungal Pathogens Infecting Apple

Arunesh Kumar[1], Meenu Gupta[2], Satish Kumar Sharma[1], Surender Kumar[3*], Chidembra Bhardwaj[5], Ranjeet Singh Verma[4], Ankita[6] and Gokul Kumar[7]

[1]Dept. of Plant Pathology, [2]Dept. of Vegetable Science, [3]Dept. of Biotechnology, [4]Dept. of Silviculture and Agroforestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh (173 230), India

[5]Dept. of Agriculture, Khalsa College Amritsar, Punjab (143 002), India

[6]Dept. of Silviculture and Agroforestry, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala (680 656), India

[7]Dept. of Entomology, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh (176 062), India

Corresponding Author

Surender Kumar

e-mail: kr.surender@outlook.com

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Abstract

The experiment was conducted during May–June, 2024 at Dr Y S Parmar University of Horticulture and Foresrty, Nauni, Solan (H.P.) to study the antimicrobial activity of deodar substrate and hemp seeds against major soil-borne pathogens of apple. An *in vitro* investigation was performed to assess the effects of deodar sawdust (*Cedrus deodara*) and hempseed (*Cannabis sativa*) on the mycelial growth of three soil-borne (*Fusarium oxysporum*, *Sclerotium rolfsii* and *Dematophora necatrix*) and one foliar (*Colletotichum* spp) pathogen in apple trees, aiming to find sustainable alternatives to chemical fungicides. The most significant inhibitory impact of this organic amendment was seen on *Fusarium oxysporum*, showing 74.44% reduction in growth with 23 mm mycelial development one week post inoculation, followed by *Dematophora necatrix*, which demonstrated 70.00% inhibition. Conversely, the least inhibition was noted for *Sclerotium rolfsii* (16.67%) which had maximum mycelial growth (75.00 mm). Combining deodar sawdust and hempseed could have synergistic effect and antagonistic effects to certain soil-borne pathogens in apple orchards, while complementary enhancement of soil health and resilience, however, it is important to conduct field trials for tailored recommendations.

Keywords: Bio-products, deodar sawdust, hempseed, apple, pathogens, sustainable

1. Introduction

Apple (*Malus × domestica*) is a predominant fruit crop grown in Himachal Pradesh, contributing 60–65% of the state's total horticultural output, with 313,000 hectares dedicated to its cultivation and a total apple production of 2,276,000 mt (Anonymous, 2021). The state is renowned for producing high-quality apples and is often referred to as the "Apple State of India". China is the biggest producer of apple in the world which accounts for ~49% global Apple production followed by Turkey, the United States, Poland and India (Anonymous, 2021)). However, its cultivation is challenged by various soil-borne pathogens such as *Dematophora necatrix, Fusarium oxysporum, Phytophthora cactorum and Collectotricum spp.* which have significant impact on fruit yield and quality.

Phytophthora rot or collar rot caused by *Phytophthora cactorum*, is one of the economically most important soil-borne oomycete pathogens in the world, which infects more than 200 plant species spanning 54 families, the majority of which consist of herbaceous and woody plants (Chen et al., 2023). *Dematophora necatrix* is another prominent soil-borne filamentous ascomycete pathogen responsible for white root rot disease in apples, which has characteristic symptoms including rotting of roots, leaf yellowing and shedding, wilting and finally plant death andhas a broad host range (Watpade et al., 2022).

Fusarium oxysporum, responsible for root rot in apple trees and affecting a wide variety of hosts, is one of the most destructive pathogens infecting numerous crop species such as garlic, onion, and capsicum from diverse plant families across the globe (Liu et al., 2022). Once colonizedin the vascular bundle and producing conidia in the infected plants, it spread throughout the plant via waterresulting in stunted growth, wilting, chlorosis, discoloration of the vascular tissue, and early senescence (Fradinet al., 2009; Dita et al., 2018). The seedling blight disease in apples, caused by *Sclerotium rolfsii* (Sacc.), poses a significant risk to apple cultivation, particularly in the western Himalayas (Dong et al., 2022), and leads to approximately 40% losses in apple nurseries annually.

Currently, there are no resistant rootstocks available to combat these harmful pathogens and the commonly recommended fungicides like thiram and aureofungin (Bhardwaj and Agarwala, 1986) fail to effectively control the concerned diseases. The overuse of these chemicals disrupts the balance of plant and soil microflora, negatively impacting beneficial microorganisms in the rhizosphere and human health (Gupta et al., 2013; Sharma et al., 2012). Additionally, it contributes to the emergence of resistant strains of pathogens due to the continuous application of fungicides (Chaudhary et al., 2011).

Deodar (*Cedrus deodara*) and hempseed (*Cannabis sativa*) are recognized for their allelopathic characteristics and ability to suppress potential phytopathogens (Chaudhary et al., 2011; Kumar et al., 2015). Given this context, it was proposed that the application of deodar sawdust and hempseed as organic amendments might foster a more conducive environment for beneficial microorganisms while inhibiting harmful pathogens. Consequently, this study aimed to unravel the antagonistic potentials of deodar sawdust and hempseed in managing diseases caused by various soil-borne and foliar pathogens affecting apple trees, with the goal of developing sustainable alternatives to chemical fungicides.

2. Materials and Methods

2.1. Plant material collection

The experiment was conducted during May–June, 2024 at UHF Dr Y S Parmar University of Horticulture and Foresrty, Nauni, Solan (H.P.). The heartwood of *Cedrusdeodara* and hempseeds were collected in July, 2024 from the forest in Nauni, Solan (H.P.) located at an elevation of 1275 meters above sea level. They were cleanedwith water, dried, crushed into a fine powder, and subsequently kept in airtight containers (Bhardwaj, 2021).

2.2. Preparation of culture media

Dissolve 5 g each of hemp seeds and deodar sawdust in 50 ml of distilled water separately, boiled before combining them to reach a final volume of 100 ml, which was then filtered. Then 2 grams of dextrose was added to the extract as a carbohydrate source and continuously stirred with a glass rod to prevent clumping (Figure 1). The pH of the medium was adjusted to 6.5, and then 2 g of agar was added as a solidifying agent. The prepared medium was sterilized in an autoclave at 15 psi and 121°C for 15–20 minutes, and then poured into sterile Petri dishes under a laminar airflow chamber and left to solidify.

2.3. Fungal pathogen cultures

Four fungal strains including threes soil borne (*Fusarium oxysporum*., *Dematophora necatrix* and *Sclerotium rolfsii*) and one foliar pathogen (*Colletotrichum* spp.) were procured from the Department of Plant Pathology, Dr YSP UHF Nauni, Solan (HP) for the present study. They were maintained as pure cultures in the potato dextrose agar (PDA) slants (Himedia) at 25±3°C and 4°C.

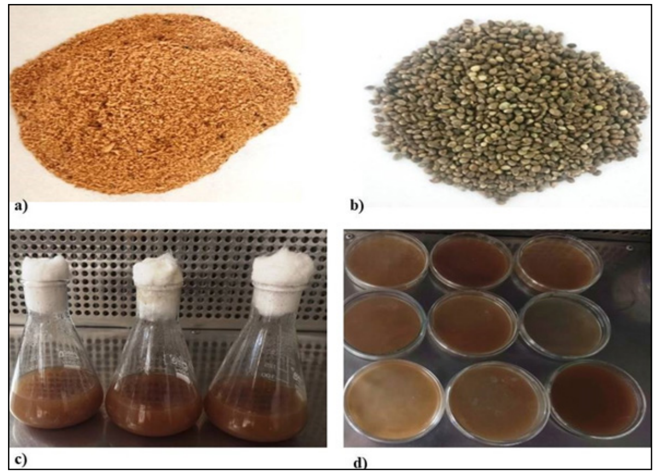

Figure 1: Preparation of culture media using a) Deodar Saw Dust and b) Cannabis Seeds, autoclaved in the flasks (c) and poured in the sterile Petri plates

2.4. Assay of antifungal activity

Fungal bits (3 mm) from each of the four fungal strains were inoculated onto the deodar saw dust-hempseed media separately alongwith the controls in the PDA plates for comparative analysis. The plates were incubated at 25±3°C for 3–7 days and observed for fungal growth patterns.The experiment was conducted in a completely randomized block design with three replicates.The zone of inhibition was observed for various treatments and size of these zones was measured using the Vernier Caliper.

2.5. Statistical analysis

The average size of the zone of inhibition for different treatments, including the control, was evaluated by employing Duncan's Multiple Range test with OpStat software (Sheoran, 2010).

3. Results and Discussion

Inhibition of *Fusarium oxysporum* growth as 23 mm profused mycelial growth with 74.44% growth inhibition was recorded seven days post-inoculation on deodar saw dust-hempseed media as compared to the negative controls having 90 mm mycelialgrowth on PDA during the same timeframe (Figure 2). In case of *Dematophora necatrix,* 27 mm growth with 70% growth inhibition was recorded one week after inoculation as compared to the negative controls (90 mm), and had floccose growth pattern on selective (deodar saw dust-hempseed) media (Figure 3). *Colletotrichum* spp. showed 29 mm sparse growth with a 67.78% growth inhibition on selective media (Figure 3). Minimum growth inhibition (16.67%) was observed in case of *Sclerotium rolfsii,* which has 75 mm mycelial growth

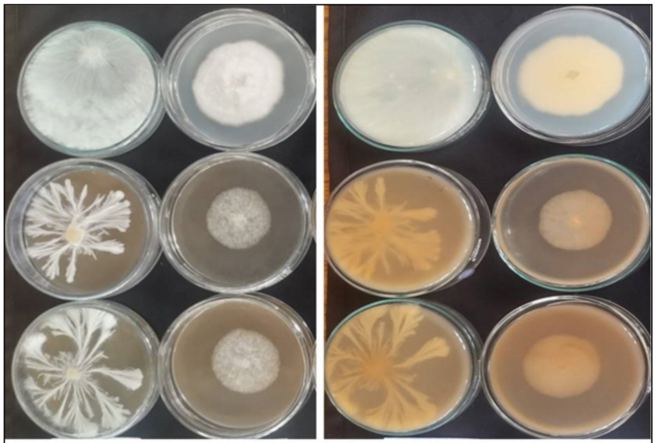

Figure 2: Growth patterns of *Sclerotium rolfsii* and *Fusarium oxysporum* in the dorsal (a) and ventral side (b) on selective (deodar saw dust-hempseed) media (second and third rows) and PDA (first row)

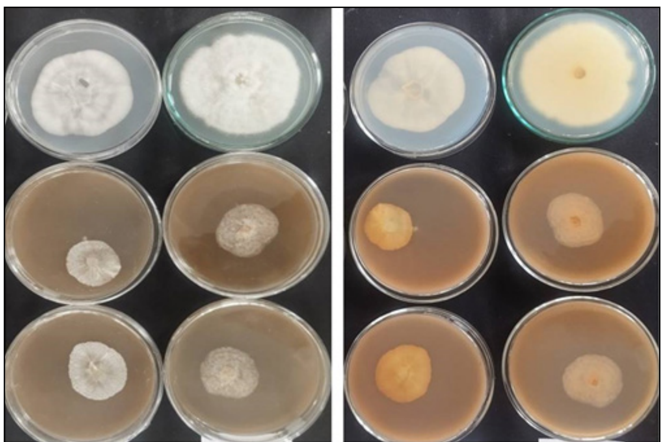

Figure 3: Growth patterns of *Dematophora necatrix* and *Colletotrichum* spp in the dorsal (a) and ventral side (b) on selective (deodar saw dust-hempseed) media (second and third rows) and PDA (first row)

on the selective media as compared to the negative control (90mm). It exhibited a fan-shaped and slightly cottony colony on deodar sawdust-hempseed media (Table 1 and Figure 2). Furthermore, Duncan's Multiple Range test showed significant mean differences in themycelial growth of different fungal pathogens on the selective media and PDA (Table 1).

Numerous initiatives have been undertaken to create eco-friendly alternatives to chemical fungicides and pesticides for an effective management of plant diseases, including the utilization of biological control agents like *Trichoderma* against a variety of fungal pathogens in plants (Tomah et al., 2023) (Figure 4). *Trichoderma* spp. inhibits the growth of pathogens through mechanisms such as nutrient competition, the production of hydrolytic enzymes and antibiotics, promotion of plant growth, and activation of defense responses (Pal and Gardener, 2006) under both *in vitro* and field conditions. Additionally, plants serve as a nearly limitless source of structurally diverse and biologically active compounds, also known as allelochemicals, which have insecticidal and antimicrobial abilities (Jacobson, 2019; Basim et al., 2006),

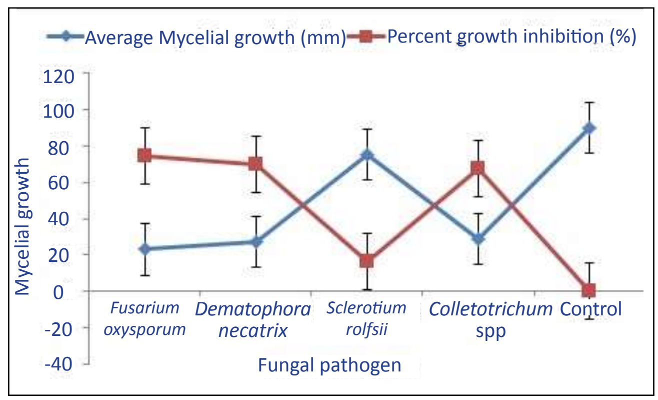

Figure 4: Line graph depicting the variation in rates of mycelial growth and zone of growth inhibition of *Fusarium oxysporum*, *Dematophora necatrix*, *Sclerotium rolfsii* and *Colletotrichum* spp. on the selective (deodar saw dust-hempseed) media and control (PDA)

Table 1: Effect of deodar substrate and hemp seed media on cultural growth and morphological characteristics of various plants pathogens on selective media and control during *in vitro* assays

Sl. No.	Name of disease	Pathogen associated	Colony color	Colony growth	Average mycelial growth (mm) LSD=5.87)	Per cent growth inhibition (%)
1.	Root rot	*Fusarium oxysporum*	Slightly White	Profuse	23d	74.44
2.	White rot	*Dematophora necatrix*	Slightly White	Floccose	27cd	70.00
3.	Seedling blight	*Sclerotium rolfsii*	White and slightly Cottony	Fan shaped	75b	16.67
4.	Anthracnose	*Colletotrichum* spp.	Slightly White	Sparse	29c	67.78
5.	Control (Potato Dextrose Agar)	Individual Pathogen	Slightly White	Sparse	90a	Nil

which demand comprehensive research to assess their potential antagonistic effects against significant agricultural insect pests and pathogens.

In the diverse ecosystem of the Himalayas, numerous plant species are esteemed for their distinctive natural products which have plant disease control activities such as insecticidal attributes (Tewary et al., 2005). Himalayan Cedar*, Cedrus deodara* grows plentifully across the western Himalayas at elevations ranging from 1200 to 3000 meters produces an essential oil exhibiting certain inhibitory effects on various pests and pathogens (Singh et al., 1984; Singh and Rao, 1985; Singh and Agrawal, 1988; Singh et al., 1989) such as insecticidal activities against moth, *Plutella xylostella* L. (Lepidoptera). The pentane fraction of cedar oil was identified as the most toxic to moth larvae, with an LC50 value of 287 g ml^{-1}, followed by himachalenes (LC50=362 g ml^{-1}), atlantones (LC50=365 g ml^{-1}), and acetonitrile at 425 µg ml^{-1}, which suggested the insecticidal potential of cedar oil and its applicability in pest management.

In the present study, the antimicrobial properties of the *Cedrus deodar*and Hempseeds were evaluated to determine the effectiveness of deodar substrate and hemp seed in fighting against soil-borne and foliar pathogens affecting apple. White to slightly off-white colony growth, characterized by abundant, fluffy, and sparse fan-shaped growth patterns was observed in the four primary fungal pathogens which infect apple treed in Himachal Pradesh. It was found white to slightly white colony growth with profuse, floccose, sparse, fan shaped growth patterns of four major fungal pathogens infecting apple in Himachal Pradesh.

4. Conclusion

This studydetermined that the integration of deodar sawdust and hempseed may produce a synergistic impact in combating to certain soil-borne and foliar pathogens in apple orchards, along with complementary enhancements of soil health and resilience, however, it is important to conduct field trials for tailored recommendations.

5. Acknowledgment

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