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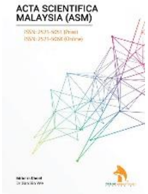

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RESEARCH ARTICLE

ANTIFUNGAL POTENTIAL OF EXO-METABOLITES PRODUCED BY *PENICILLIUM CAPSULATUM* AGAINST PHYTOPATHOGENIC FUNGI

Niharika Mohanty, Atmaja Elina Mishra and Nibha Gupta*

Plant Pathology and Microbiology Division, Regional Plant Resource Centre, Bhubaneswar, Odisha-751015
****Corresponding author: nguc2003@yahoo.co.in***

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ABSTRACT

Fungi are well-known producers of secondary metabolites, commonly referred to as exo-metabolites, which play crucial roles in ecological interactions, including plant protection. Among these metabolites, many exhibit potent antifungal properties, making them effective biocontrol agents against phytopathogenic fungi that cause significant agricultural losses. This study investigates the antifungal potential of solvent extracts derived from the extracellular secondary metabolites of *Penicillium capsulatum*, specifically targeting five phytopathogenic fungi: *Fusarium equseti*, *Fusarium acuminatum*, *Colletotrichum gloeosporioides*, *Fusarium javanicum*, and *Nigrospora sphaeria*. Using a solvent extraction method, partially purified fungal metabolites were tested for their antifungal activity via the pour plate method. The extracts were incorporated into agar medium in two concentrations: 100µl (lower dose) and 500µl (higher dose), and the growth reduction percentage was calculated based on the colony diameter measurements. The results indicated that the toluene extract of *P. capsulatum* exhibited the highest growth reduction against *F. equseti*, achieving 45.00 ± 3.54% at the higher dose and 37.50 ± 3.54% at the lower dose. The methanolic extract demonstrated complete inhibition (100.00 ± 0.00%) of *F. acuminatum* and *C. gloeosporioides* at the higher dose, while acetic acid, DMSO, and acetonitrile extracts showed varying levels of antifungal activity against other target pathogens. These findings highlight the potential of *P. capsulatum* metabolites as effective antifungal agents against diverse phytopathogenic fungi, presenting a promising alternative to chemical fungicides. Furthermore, the results support the need for further exploration of fungal metabolites in the development of sustainable biocontrol strategies for managing plant diseases.

KEYWORDS

Fungi, Antagonism, Metabolite, *Penicillium capsulatum*, Growth reduction.

1. INTRODUCTION

Fungi produce a wide range of secondary metabolites, commonly referred to as exo-metabolites, which play significant roles in their ecological interactions. Among these metabolites, many possess antifungal properties that can be particularly effective against phytopathogenic fungi i.e. organisms that cause diseases in plants (Chen et al., 2021). Phytopathogenic fungi are responsible for substantial agricultural losses worldwide, as they lead to plant diseases like rusts, blights, and wilts, which severely impact crop yields (Mahadevakumar and Sridhar 2021).

Exo-metabolites, including enzymes, organic acids, and antimicrobial compounds, can inhibit the growth of these harmful fungi, making them valuable tools in biocontrol strategies. These metabolites often interfere with the cell walls or metabolic processes of the target fungi, leading to their suppression or death (Bharadwaj et al., 2021). Utilizing such naturally derived antifungal compounds offers a more sustainable and environmentally friendly alternative to synthetic chemical fungicides, which are known to have adverse effects on ecosystems and can lead to resistance in pathogenic strains (Jimenez-Reyes et al., 2019).

Numerous endophytic fungi have been identified for their potent antifungal properties. For instance, ergot alkaloids produced by the fungus *Claviceps purpurea* have been found to exhibit strong antifungal activity (Dembitsky 2014). Additionally, acetylenic metabolites produced by *Penicillium* and *Aspergillus* species, along with other filamentous fungi, have emerged as a distinct chemical family of antifungal compounds. These metabolites have even inspired the development of synthetic fungicides due to their unique structure and bioactivity (Baldt *et al.,* 2013; Kuklev et al., 2013). In recent years, new discoveries continue to highlight the potential of fungi as biocontrol agents. For example, capsulactone, isolated from the endophytic fungus *Penicillium capsulatum* XL027, which was obtained from the leaves of *Panax notoginseng*, exhibited antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. Although its antimicrobial activity was modest, capsulactone represents another important example of the capacity of biocontrol fungi to produce bioactive secondary metabolites with potential applications in combating pathogens (Xian et al., 2021).

The ability of fungi to produce bioactive secondary metabolites has long been recognized, with griseofulvin being one of the earliest reported examples. This compound, classified as a benzofuran, was isolated from *Penicillium* species and demonstrated antifungal activity by inhibiting the growth of the plant pathogen *Botrytis cinerea*, leading to abnormal hyphal growth and disorientation (Rodrigo et al., 2021). This discovery significantly advanced the understanding of fungal secondary metabolites and their role as biocontrol agents against phytopathogens. These findings underscore the chemical diversity of fungal metabolites and their continued relevance as a resource for developing new biocontrol agents to manage both plant and microbial pathogens (Rush et al., 2021).

Thus, the exploration of exo-metabolites produced by fungi as antifungal agents holds promise in developing novel plant protection methods, which can mitigate the threats posed by phytopathogenic fungi while promoting

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sustainable agricultural practices.

2. Materials and Methods

2.1 Microorganisms

This experiment was conducted by selecting a biocontrol fungi *Penicillium capsulatum* and tested against five phytopathogenic fungi: *Fusarium equseti*, *Fusarium acuminatum*, *Colletotrichum gloeosporioides*, *Fusarium javanicum*, and *Nigrospora sphaeria*. These were collected from Microbiology and Plant Pathology Laboratory of Regional Plant Resource Centre, Bhubaneswar, Odisha.

2.2 Solvent Extraction Method

Extracellular secondary metabolites of *Penicillium capsulatum* were separated through solvent extraction and partially purification by column chromatography. Mass scale production of the fungal strain *Penicillium capsulatum* was done. This organism was inoculated in Sabouraud dextrose broth medium and incubated at 30˚C. After incubation period, filtration and solvent extraction was done. Partial purification was done through column chromatography process by passing the crude extract using polar to nonpolar solvents (Acetic acid, methanol, ethanol, isolpropanol, acetonitrile, acetone, chloroform, pyridine, DMSO, ethyl acetate, toluene and hexane) (Chapla et al., 2014). Prepared extracts were tested for antifungal activity against five numbers of phytopathogenic fungi by following pour plate method. Plates were prepared by adding different solvent extracts in molten agar medium in two doses i.e. 100µl (lower dose) and 500µl (higher dose). 6mm disc cuttings of the well grown fungal culture were placed at the middle of the plate. Colony diameter of the organisms was measured and percentage of growth reduction was calculated (Gu et al., 2022).

3. Results and Discussion

This study demonstrates the significant antifungal potential of various solvent extracts from *Penicillium capsulatum* against five key phytopathogenic fungi, including *Fusarium equseti*, *Fusarium acuminatum*, *Colletotrichum gloeosporioides*, *Fusarium javanicum*, and *Nigrospora sphaeria*. The results reveal that among the extracts tested, the toluene extract exhibited the highest growth inhibition against *F. equseti*, with a reduction percentage of 45.00 ± 3.54% at a higher dose and 37.50 ± 3.54% at a lower dose. This suggests that toluene extract may contain bioactive compounds that are highly effective against this particular pathogen. Interestingly, the chloroform extract showed no inhibitory effect at a lower dose but exhibited some activity at the higher dose, albeit with a lower reduction percentage of 14.03 ± 0.00% (figure 1). In contrast, *F. acuminatum* was completely inhibited by the methanolic extract at a higher dose (100.00 ± 0.00%), followed by notable inhibition from the acetic acid extract. Toluene (55.00 ± 1.77%) and DMSO (48.75±1.77%) extracts also displayed moderate antifungal activity at lower doses (figure 2). These results indicate the versatility of *P. capsulatum* extracts, particularly methanol which effectively inhibited multiple phytopathogens, including *Colletotrichum gloeosporioides* up to 100.00%, and *F. javanicum*, where the methanolic extract demonstrated high efficacy at both lower and higher doses (figure 3 and 4). Additionally, acetonitrile, acetone, and ethyl acetate extracts also exhibited antifungal activity, particularly against *F. javanicum* and *N. sphaeria*. Notably, 100% growth inhibition of *N. sphaeria* was observed with acetic acid extract at both dose levels, further highlighting its potential as a potent biocontrol agent (figure 5).

Complementing these findings, previous research has shown that ethyl acetate extracts from various fungal species exhibit similar biocontrol properties against a range of phytopathogens. For example, pretrichodermamide A, isolated from *Trichoderma harzianum* and *Epicoccum nigrum*, has demonstrated antimicrobial efficacy against *Ustilago maydis*, a common plant pathogen (Harwoko et al., 2019). Similarly, ethyl acetate extracts from *Fusarium oxysporum* endophytes have been shown to contain palmitic, stearic, and octadecenoic acids, which are known to act as potent fungicides (Killic et al., 2021).

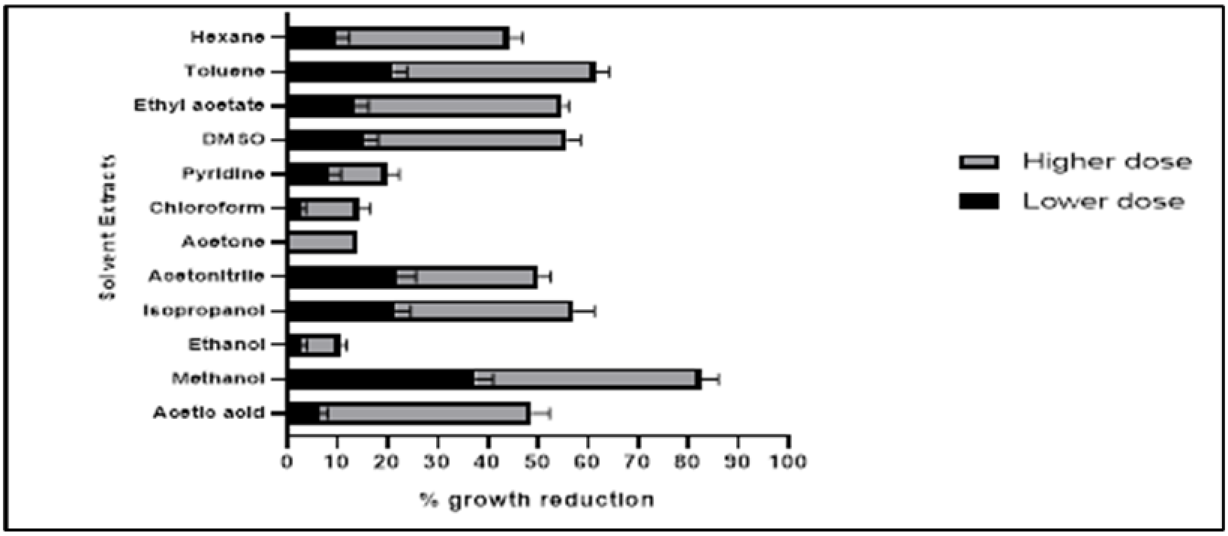

Figure 1: Effect of solvent extracts of *P. capsulatum* against *Fusarium equiseti*

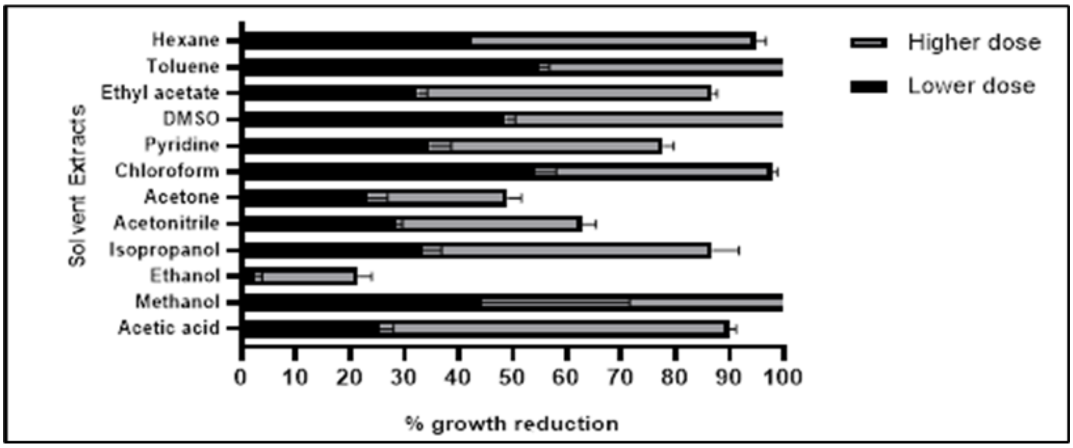

Figure 2: Effect of solvent extracts of *P. capsulatum* against *Fusarium acuminatum*

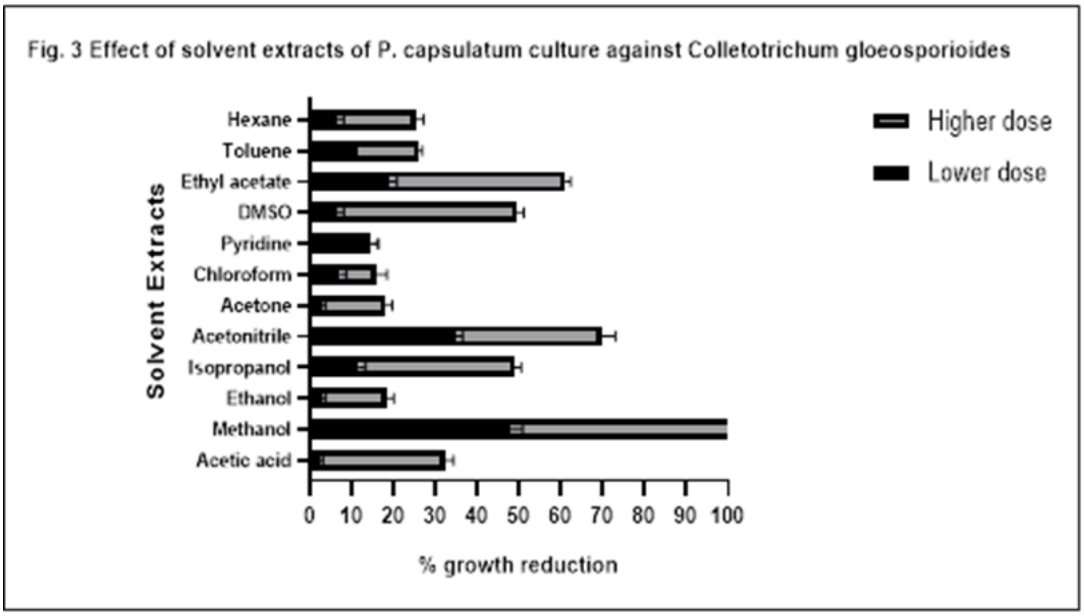

Figure 3: Effect of solvent extracts of *P. capsulatum* against *Colletotrichum gleosporioides*

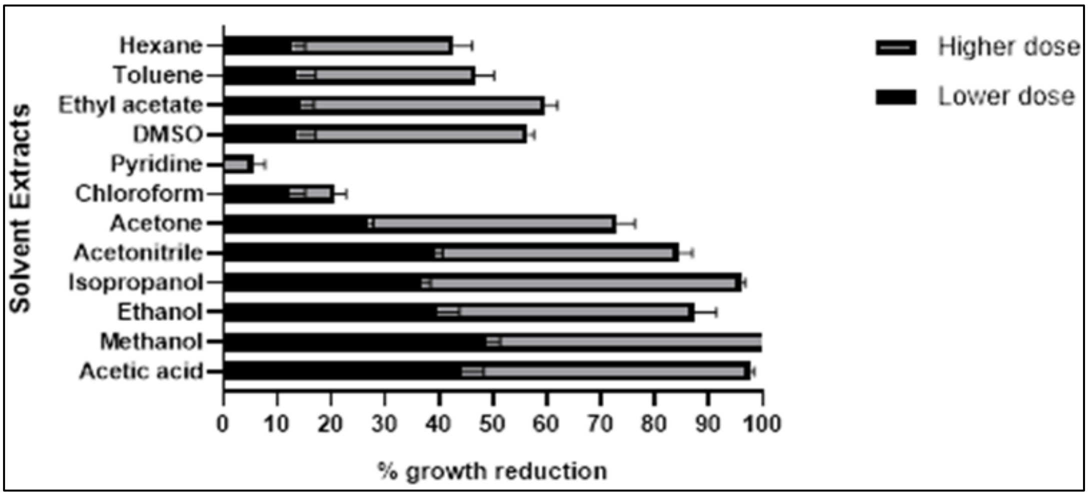

Figure 4: Effect of solvent extracts of *P. capsulatum* against *Fusarium javanicum*

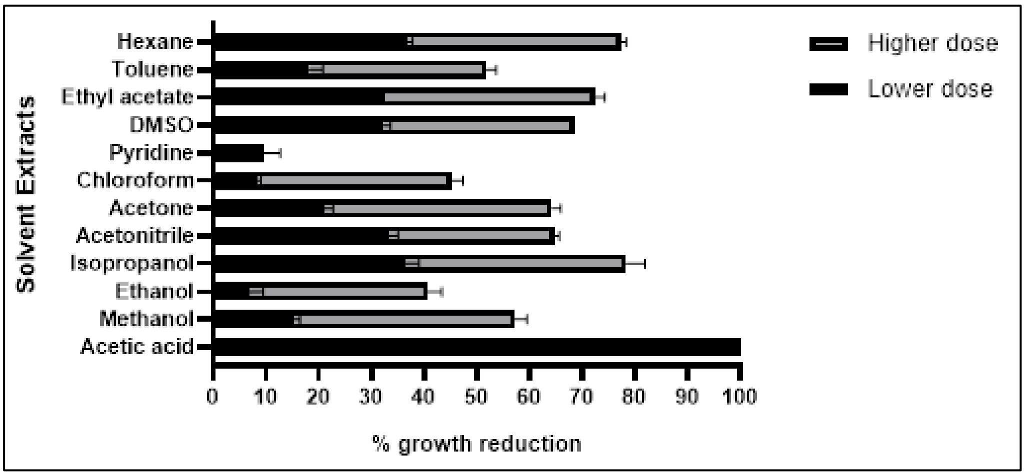

Figure 5: Effect of solvent extracts of *P. capsulatum* against *Nigrospora spherica*

These fatty acids, along with other metabolites such as leucinostatins produced by *Purpureocillium lilacinum*, exhibit broad-spectrum activity against both bacterial and fungal pathogens, further highlighting the role of fungal metabolites in plant protection (Wang et al., 2016). Moreover, *Metarhizium anisopliae* has emerged as an effective biocontrol agent, particularly against *Fusarium graminearum*, due to its ability to produce fungistatic secondary metabolites, enhance wheat growth, and trigger defense responses in plants (Hao et al., 2021). Additionally, the susceptibility of other plant pathogens such as *Alternaria solani*, *Rhizoctonia solani*, *Pythium ultimum*, and *Colletotrichum lagenarium* to fatty acids produced by fungal endophytes emphasizes the broad biocontrol potential of fungal metabolites (Raynor et al., 2004; Liu et al., 2008).

In summary, the findings from this study, coupled with the existing body of research, underscore the substantial role of fungal secondary metabolites as effective biocontrol agents. The use of solvent extracts, particularly those derived from *P. capsulatum* and other fungi, offers a promising alternative to chemical fungicides for managing plant diseases.

4. Conclusion

The findings from this study highlight the considerable antifungal potential of *Penicillium capsulatum* solvent extracts against a range of phytopathogenic fungi, including *Fusarium equseti*, *Fusarium acuminatum*, *Colletotrichum gloeosporioides*, *Fusarium javanicum*, and *Nigrospora sphaeria*. The highest level of inhibition was observed with the toluene and methanolic extracts, particularly in the case of *F. equseti* and *F. acuminatum*, where methanol demonstrated complete growth inhibition at a higher dose. The versatility of *P. capsulatum* extracts across different solvents, particularly methanol, acetic acid, and toluene, underscores the effectiveness of these natural compounds in managing various plant pathogens. The complete inhibition of *N. sphaeria* by acetic acid further reinforces the potential of these fungal secondary metabolites as potent biocontrol agents. These results demonstrate the value of exploring fungal exo-metabolites as an eco-friendly alternative to chemical fungicides, offering an avenue towards sustainable agricultural practices. The efficacy of these natural antifungal agents provides a promising strategy to reduce the harmful environmental impacts associated with synthetic fungicides while ensuring effective pathogen control. Continued research is needed to further optimize these extracts for practical applications, as well as to investigate their modes of action and broader spectrum of activity, contributing to innovative and sustainable plant protection solutions.

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