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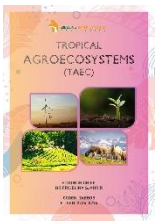

RESEARCH ARTICLE

PROFILING OF GROWTH INHIBITION OF *FUSARIUM SPP*. TREATED WITH FUNGAL CULTURE OF DIFFERENT GROWTH PERIOD

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ABSTRACT

Globally, biotic stress caused by many microorganisms has been a significant problem. The fungal infections by phytopathogenic fungi cause various diseases in crops resulting loss of productivity and quality. The present work highlights that the fungal culture, *Penicillium oxalicum* can be used as a biocontrol agent against several strains of *Fusarium spp.*, and it involves the bioactivity by fungi, i.e., formation of different secondary metabolites, and enzymes as well as suppressiveness that can control the pathogenic fungi. The bioactivity was observed in fungal cultures though differed as per incubation period. The bioactive potential of fungal culture has been shown in terms of percentage (%) of growth inhibition of *Fusarium spp* and calculated based on morphological growth on plate culture techniques. Highest percentage of growth inhibition was observed in the 12-day-old culture of *Fusarium spp.* followed by 5-day-old culture. Focusing on different incubation periods on bioactive potential of *Penicillium oxalicum* maybe useful for the formation of biologically active metabolites and also effective for pharmaceutical research and show antagonistic behavior against plant pathogens.

KEYWORDS

Bioactivity, Secondary metabolite, Incubation period, Pathogens

1. INTRODUCTION

For the management of plant diseases, biocontrol agents are widely used against fungal pathogens to eradicate the negative impacts on the environment (Chowdhury et al., 2024). Biocontrol agents play an important role in management of disease and increasing food production. However, the researchers should thoroughly examine the range of biocontrol agents to reduce the use of synthetic chemicals (Tyagi et al., 2024). Chemical fungicides can harm, and kill the microorganisms to some extent, and can also cause drug resistance to pathogenic fungi. For example, citrus postharvest infections have evolved resistance to routinely used fungicides such as thiamethoxam and imidazoline (Sánchez-Torres and Tuset, 2011). Exposure to pesticides, whether direct or indirect, poses a significant risk to human health. Therefore, the use of chemical fungicides should be limited. Some European nations prohibit or restrict the use of postharvest fungicides to specific licensed compounds (Wisniewski et al., 2016). Green environmental protection fungicides are becoming increasingly popular, and new safe fungicides are always being researched to replace chemical fungicides to prevent the development of postharvest illnesses in fruits and vegetables (Ling et al., 2024). In recent years, biological control has shown great promise for research and development. Biological management is a popular method for effectively preventing disease attacks (Leneveu-Jenvrin et al., 2020). Antagonistic microorganisms can suppress or destroy pathogenic bacteria, minimizing the need for chemical fungicides. Endophytic fungus utilize many techniques to manage diseases, including producing antifungal chemicals, altering their appearance, and competing for resources and space. Using fungal endophytes as microbial biological control agents is an effective and environmentally friendly technique to prevent postharvest illness (Wen et al., 2024). New constraints on chemical use and environmental concerns have sparked interest in biocontrol agents. *Penicillium oxalicum*, a fungal agent, effectively reduces illness caused by *Fusarium spp.* The biocontrol potential of *P. oxalicum* warrants the development of an efficient manufacturing procedure. In addition, aerially generated *P. oxalicum* conidia were more efficient against Fusarium wilt than submerged conidia (Larena et al., 2002). We aimed to determine the percentage of growth inhibition of *Fusarium spp*. treated with the culture of *P.oxalicum* of different incubation period.

Mycotoxin contamination and disease outbreaks threaten economic return and form implications to human, animal, and food security. Furthermore, Fusarium is a genus that has significant phytopathogens and records associated with host-plant interaction, synthesis of secondary metabolites is both basic and applied research in pathological systems. Fumonisin is a mycotoxin formed by *Fusarium spp.,* it serves several animal diseases, human diseases and also include phytopathogenicity (Blacutt et al., 2018). There is a lot of information regarding *Fusarium spp*. as a plant pathogenic fungi (Tian et al., 2021). Plant-pathogenic Fusarium species include *Fusarium oxysporum*, *F. solani*, *F. verticillioides*, *F. proliferatum* etc.

F. oxysporum is a typical soil-borne pathogen. It lives in the soil for a long period as chlamydospores, enters the roots, spreads throughout the tissues, and cause death in plants (Arie, 2019). *F. proliferatum*, a widespread and diverse fungal pathogen, affects a variety of plants, including maize, wheat, and pine (Proctor *et al.,* 2010). *Fusarium sp.* causes a variety of illnesses, including vascular wilt disease, bakanae disease in rice, dry rot in plants, tracheomycosis, and so on (Flood, 2006; Raghu et al., 2018; Galvez and Palmero, 2022; Fravel et al., 2003). One report revealed that *F. equiseti* is the causal organism for chilly wilt in Kashmir along with *F. oxysporom* (Hami et al., 2021). Fusarium head blight (FHB) in wheat (*Triticum aestivum L.)* is caused by a group of interconnected

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Fusarium species. In the outdoors, the mildly pathogenic *F. poae* frequently colonizes the infection sites of the virulent *F. graminearum* (Tan et al., 2021). *F. verticillioides,* another plant pathogen cause diseases in humans, plant, and animals, and *F.solani* caused disease in bean plants by the production of mycotoxin (Blacutt et al., 2018; Sasan and Bidochka, 2013). By limiting the growth of the aforementioned infections, we can produce different antifungal metabolites that will be employed in future pharmacological research. Further, various bioactive potential of the fungal extracts were evaluated and determined the percentage of growth reduction of various *Fusarium spp.* like *F. equiseti, F.poae, F.oxysporom, F.javunicum, F. proliferatum, F. verticillioides,* and*. solani* treated with fungal culture of different incubation periods.

2. Materials and Methods

2.1 Source of Fungi

Penicillium oxalicum, the test fungi, and seven strains of *Fusarium spp.* such as *F. equiseti, F.poae, F.oxysporom, F.javunicum, F. proliferatum, F. verticillioides,* and *F. solani* used as pathogens in antimicrobial activity test were obtained from the culture collection of Microbiology Laboratory, Plant Pathology and Microbiology division, Regional Plant Resource Centre, Bhubaneswar.

2.2 Evaluation of Bioactive Potential Against *Fusarium Spp.* Treated with Test Fungi of Different Incubation Period

Master plate preparation of test fungi and phytopathogenic fungi was done. During the screening phase, the sabouraud dextrose medium was discovered to be the most effective medium for increasing antibacterial activity. As a result, the sabouraud dextrose agar medium served as the foundation for modifying the culture conditions. The cultures were cultured in sabouraud dextrose broth medium to identify the ideal time for maximum development. Test fungi were prepared in liquid broth at 30^0C with a pH of 5.8. Observations were performed after harvesting the culture from the 5th to the 30th day. The culture filtrate was filtered and concentrated using the Soxhlet equipment, after which ethyl acetate was added to the concentrated filtrate for 72 hours. The top layer was separated and evaporated using Soxhlet. Evaporated samples were dissolved in methanol, and a methanolic extract was produced. Methanolic extracts of chosen fungi were evaluated for antifungal activity (Patro and Gupta, 2022; Lahouar et al., 2016).

2.2.1 Antifungal Activity Test

Solvent extract of test fungi, *P. oxalicum* grown in different incubation periods were screened for antimicrobial activity against *F. equiseti, F.poae, F.oxysporom, F.javunicum, F. proliferatum, F. verticillioides,* and *F. solani*. Three different concentrations (100 μl, 300 μl, and 500 μl) of extract were taken. Percentage (%) of growth inhibition was calculated on the basis of external growth of plate culture techniques.

- Zone of Diameter of negative control- Zone of Diameter of Test/ Zone of Diameter of negative control×100
- Zone of Diameter of negative control- Zone of Diameter of Positive control/ Zone of Diameter of negative control×100

Percentage of growth reduction (1-2)

3. Results and Discussions

The present study revealed that Percentage of growth inhibition of *Fusarium spp.* treated with *Penicilium oxalicum* of different incubation period of 5days, 7days, 9days, 12days, 15days, 18days, 21days, 25days and 30days. Fusarium is a large genus with an estimated 1,500 members, out of which seven species were taken for our experiment (Arie, 2019). The seven strains of *Fusarium spp.* (*F. equiseti, F.poae, F.oxysporom, F. javunicum, F. proliferatum, F. verticillioides,* and *F. solani*) denoted as T1, T2, T3, T4, T5, T6, T7 respectively. The antifungal activity test has shown some fine results to elucidate its preference for culture and environmental conditions. In one report, a strategy was developed to portray *Penicillium oxalicum* as a biocontrol agent by combining PCR and a selective medium (Larena and Melgarejo, 2009). The solvent extracts of fungal metabolites grown in 5 days of incubation period were evaluated for antifungal properties are represented in **figure 1**. *F. proliferatum* is a harmful phytopathogen causes various plant diseases like garlic rot (Tonti et al., 2012; Galvez and Palmero, 2022). Our investigation revealed Highest percentage of growth inhibition was shown in the case of concentration of 300 μl of *F. proliferatum* (57.24%), and *F. verticillioides* (56.98%). Growth of *F. solani* (500 μl) were inhibited by extracts of (54.54%) 5days olds of fungal culture. Lowest percentage of growth inhibition was shown in case of *F.oxysporom* followed by *F.poae*. The antifungal potential of *Penicillium oxalicum* was shown excellent results against four strains of *Fusarium spp.* in case of 5 days of incubation.

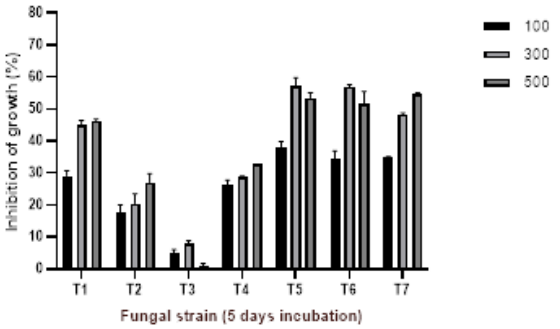

Figure 1: Effect of 5days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

Mycotoxins produced by several fungal cultures like *Aspergillus spp., Fusarium spp.,* and *Penicillium spp.* which is harmful to plants, animals, and humans. Noteworthy mycotoxins are aflatoxin, fumonisin, ocratoxins, etc (Bennett and Klich, 2003). Solvent extracts of test fungi, i.e., *P. oxalicum* produced in 7 days of incubation period were examined for antifungal activity against seven strains of *Fusarium spp.* Observations recorded for percent growth inhibition of test *Fusarium spp*. by *P. oxalicum* are shown below. Bioactivity have shown some good results to explain its preferences for culture and environmental conditions. The solvent extracts of fungal metabolites grown in 7 days of incubation period were evaluated for antifungal properties are represented in **figure 2.** The highest percentage of growth inhibition was shown in the case of a concentration of 500 μl of *F. proliferatum* (55.3%), and 300 μl of *F. verticillioides* (51.69%). Growth of *F. solani* (500 μl) was inhibited by extracts of (49.65%) 7 days olds of fungal culture. Lowest percentage of growth inhibition was shown in case of *F.oxysporom* followed by *F.poae*. The bioactive potential of *Penicillium oxalicum* showed excellent results mainly against four strains of *Fusarium spp.* in case of 7 days of incubation

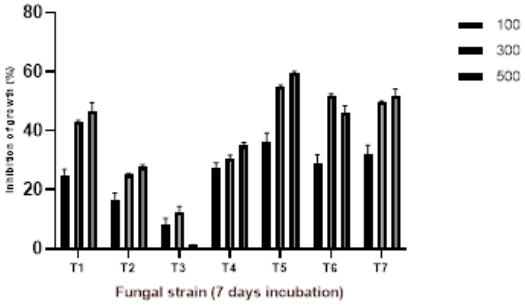

Figure 2: Effect of 7 days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

The results of antimicrobial activity test of 9 days of incubation exhibited that the solvent extracts of test fungi were activated mostly against *F.proliferatum* shown in **Figure 3.** The best amount of concentration was 500 μl in the case of the above strain of *Fusarium spp.* The highest antifungal activity was shown in *F.proliferatum* in almost all concentrations comparatively. The percentage of growth inhibition of *F.proliferatum* is 33.59%, 52%, and 57.6% respectively. Previously the percentage of growth reduction was quite good in case of *F. verticillioides.* But in this 9 days of incubation, the percentage was less in all three sort of concentration (6.10%, 11.46%, 22.23%). Lowest percentage of growth inhibition was shown in the case of *F.oxysporom*. The bioactive potential of *Penicillium oxalicum* was shown excellent results mainly against three strains of *Fusarium spp.* in the case of 9 days of incubation.

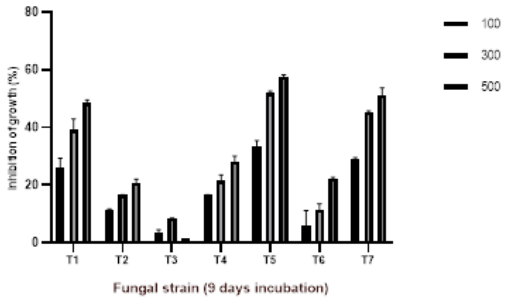

Figure 3: Effect of 9days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

The present investigation was focused on studying the antifungal potential of test fungi against seven different *Fusarium spp.* by using different incubation period. Liu et al., 2024 reported on the antifungal potential of *Weissella cibaria* KM14 isolated from traditional Korean food kimchi against three spoilage fungi. In 12 days of incubation, extracts of fungal culture shown excellent bioactive potential against almost all *Fusarium spp*. represented in Figure 4. The highest percentage of growth reduction was shown in *F. equiiseti* followed by *F. verticillioides.* Previously, extracts of fungal culture was shown less bioactivity against *F.oxysporom*. But in this case, the growth reduction of *F. oxysporom* was 39.55%, 41.79%, and 33.58% in 100μl , 300μl, and 500μl respectively. *Fusarium equiseti* is having pathogenicity and causes fruit rot disease in watermelon (Rahman et al., 2022). The lowest percentage of growth inhibition was shown in the case of *F.proliferatum* in almost all concentrations. The antifungal potential of *Penicillium oxalicum* was shown excellent results against six strains of *Fusarium spp.* in case of 12 days of incubation.

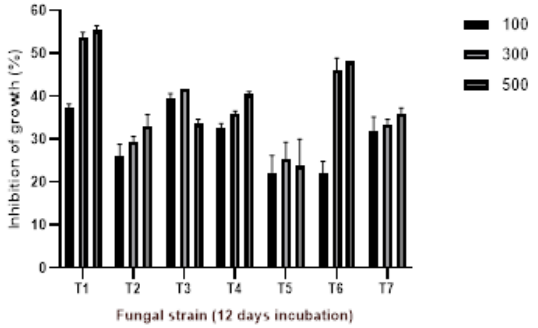

Figure 4: Effect of 12days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

Solvent extracts of test fungi, i.e., *P. oxalicum* produced in 15 days of incubation period were examined for antifungal activity against seven strains of *Fusarium spp.* observations recorded for percent growth inhibition of test *Fusarium spp*. by *P. oxalicum* are shown in below. Generally, fungicides are used to treat fusarial diseases but frequent applications of fungicides resulting resistance to plants, and environmental pollution, hazardous to humans and animals. Biological control is a viable option, with several antagonists already accessible. Several species of fungi, for example, Trichoderma, are used as biocontrol agent of plant rot disease (Grosch et al., 2006; Rojo et al., 2006; El-Kassas and Khairy, 2009). The solvent extracts of fungal metabolites grown in 15 days of incubation period were evaluated for antifungal properties are represented in **figure 5**. The highest percentage of growth inhibition was shown in case of concentration of 300 μl of *F. equiiseti* (58.41%), and 100 μl of *F. poae* (39.48%). Growth of *F. solani* (500 μl) was inhibited by extracts of (40.31%) 15 days olds of fungal culture. The extremely low percentage of growth inhibition was shown in case of *F.proliferatum*. The antifungal potential of *Penicillium oxalicum* was shown excellent result mainly against three strains of *Fusarium spp.* in case of 15 days of incubation.

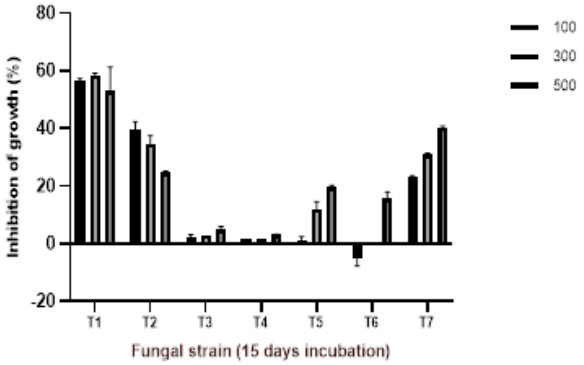

Figure 5: Effect of 15 days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

The results of antimicrobial activity test of 18 days of incubation exhibited that the solvent extracts of test fungi was activated mostly against *F. equiiseti* shown in **Figure 6**. The best amount of concentration was 500 μl in the case of the above strain of *Fusarium spp.* The highest antifungal activity was shown in *F. equiiseti* almost all concentrations comparatively. The percentage of growth inhibition of *F. equiiseti* is 26.43%, 32.22%, and 33.88% respectively and the % of growth inhibition of *F.poae* is 7.95%, 13.63%, 32.95%. Previously the percentage of growth reduction was quite good in case of *F. verticillioides.* But in these 18 days of incubation, the percentage was less comparatively (1.61%, 6.5%, 9.84%). Lowest percentage of growth inhibition was shown in the case of *F. verticillioides*. The antifungal potential of *Penicillium oxalicum* was shown excellent result mainly against three strains of *Fusarium spp.* in case of 18 days of incubation.

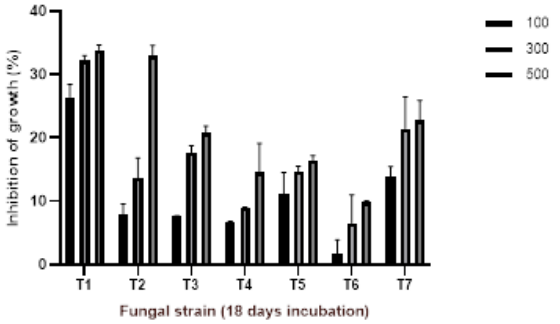

Figure 6: Effect of 18 days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

The data of bioactive potential of 21 days of incubation revealed that four strains of *Fusarium spp.,* (*F. equiseti, F.poae, F.oxysporom, F.javunicum)* showed a good percentage of growth reduction in 500 μl of concentration represented in **Figure 7**. *F.oxysporom* is reported as an airborne fungi as a pathogen in humans and a soilborne pathogen in plants (Dignani and E. Anaissie, 2004). The lowest percentage of growth inhibition was shown in the case of *F. verticillioides*. The antifungal potential of *Penicillium oxalicum* was shown excellent results mainly against three strains of *Fusarium spp.* in the case of 21 days of incubation.

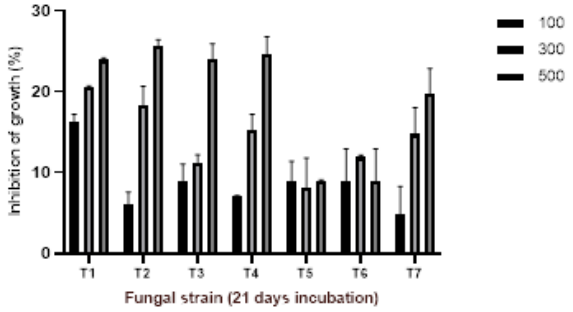

Figure 7: Effect of 21 days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

The solvent extracts of fungal metabolites grown in 25 days of incubation period were evaluated for antifungal properties are represented in **Figure 8**. The highest percentage of growth inhibition was shown in case of concentration of 300 μl , and 500 μl of *F.equiseti* (51.19%, 58.4%) and *F. poae* (25.32%, 30.35%). Growth of *F. solani* (500 μl) was inhibited by extracts of (26.79%) 25days olds of fungal culture. The lowest percentage of growth inhibition was shown in the case of *F.oxysporom* followed by *F. verticillioides*. The antifungal potential of *Penicillium oxalicum* was shown good results against two strain of *Fusarium spp.* in the case of 25days of incubation.

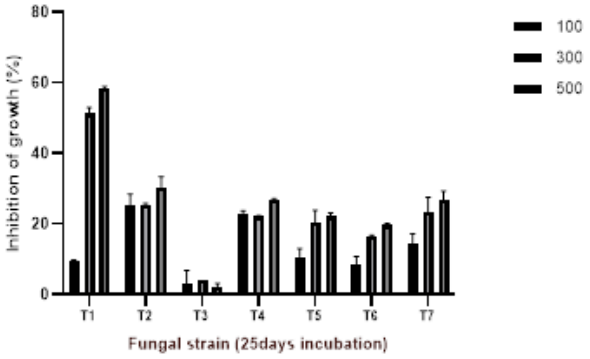

Figure 8: Effect of 25 days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

Lastly, the results of the antimicrobial activity test of 30 days of incubation

exhibited that the solvent extracts of test fungi were activated mostly against *F.equiseti* shown in **figure 9**. The best amount of concentration was 500 *µl* in the case of the above strain of *Fusarium spp*. The percentage of growth inhibition potential of *F.equiseti* was drastically increased and the inhibition is 10.75%, 47.94%, and 59.51%. Previously the percentage of growth reduction was quite good in case of *F. proliferatum.* But in these 30 days of incubation, the percentage was less in all three sorts of concentration. The lowest percentage of growth inhibition was shown in the case of *F.oxysporom*. The bioactive potential of *Penicillium oxalicum* showed excellent results mainly against three strains of *Fusarium spp.* in case of 30 days of incubation.

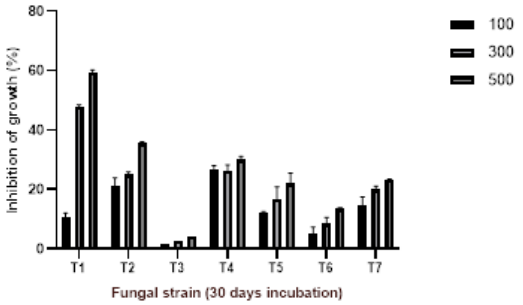

Figure 9: Effect of 30 days of incubation period on growth inhibition of different *Fusarium spp.* (%) by *P.oxalicum*

Antifungal activity was assessed in solvent extracts of fungi generated over different culture conditions like different incubation period. From the above observations, the highest antifungal activity was observed in 12days old culture of *Penicillium oxalicum* against almost all strains of *Fusarium spp.* followed by a 5-day-old culture of fungi against four strains of *Fusarium spp.* reported.

4. Conclusion

Biocontrol agents are being considered as a safer and more environmentally friendly alternative to synthetic chemicals in sustainable agriculture to manage plant diseases and pests. To be effective in the field, adjustments such as refining formulation and distribution systems, scaling up manufacturing, conforming to regulatory requirements, and increasing cost-effectiveness are necessary. Enhancement of new and modified media encourages the production of bioactive chemicals that might be further defined, and it is considered that researching natural products from endophytic fungus may be a strategy to eliminate the problem of phytopathogenic diseases. In this regard, our finding may have the potential to act against different strains of plant pathogens and secondly by modifying the incubation period is the essential factor to find the desired product and give us a clue to develop biocontrol agents against fusarial disease of plants. This putative antifungal agent may be beneficial for other infections; more investigation is required.

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