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Research Article

IMPACT OF NUTRIENT MEDIA ON GROWTH AND ANTAGONISTIC BEHAVIOUR OF SOME FUNGI

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A B S T R A C T

The present experiment was conducted to measure the effectiveness of different media on the growth and antagonistic effect of biocontrol agents against some fungi. Five numbers of fungal isolates *Fusarium equseti, Fusarium acuminatum, Colletotrichum gloeosporioides, Fusarium javanicum, Nigrospora sphaeria* were tested against five numbers of biocontrol fungi *Penicillium citrinum, Penicillium oxalicum, Aspergillus ochraceus, Penicillium capsulatum, Talaromyce cnidii* by dual culture technique in five different nutrition media such as Saboroud Dextrose (SD), Czapek's Dox (CZ), Potato Dextrose (PD), Malt Extract (ME), Oatmeal agar (OA). Observation was taken by measuring the zone of inhibition along with the radial growth of both of the fungal isolates. Results obtained that the highest inhibition zones, i.e., 1.6 cm, 1.2 cm, 1.2 cm, 3cm and 0.7 cm, observed in PD, SD, SD, and ME media, respectively, in the case of five biocontrol agents (*P. citrinum, P. oxalicum, A. ochraceus, P.capsulatum, T. cnidii*) were significantly exhibited the strongest antagonism against the fungus *F. equiseti*. In case of *Fusarium acuminatum*, highest inhibition zones, i.e., 1.8 cm, 1.5 cm, 1.5 cm, 1.7cm and 4.0 cm, observed in ME, SD, SD, PD and PD media, respectively, against five biocontrol agents (*P. citrinum, P. oxalicum, A. ochraceus, P.capsulatum, T. cnidii*). These agaents were significantly exhibited the strongest antagonism against the fungus *F. acuminatum.* In case of *C. gloeosporioides*, highest inhibition zones, i.e., 1 cm, 0.8 cm, 1 cm, 1.2cm and 1cm, observed in ME, SD, PD, ME, and PD media, respectively, against five biocontrol agents (*P. citrinum, P. oxalicum, A. ochraceus, P.capsulatum, T. cnidii*). These agaents were significantly exhibited the strongest antagonism against the fungus *C. gloeosporioides.* In case of *F. javanicum*, highest inhibition zones, i.e., 1.6 cm observed in ME media and other four 1.5 cm, 1 cm, 2.8cm and 0.5cm observed in SD media, respectively, against five biocontrol agents (*P. citrinum, P. oxalicum, A. ochraceus, P.capsulatum, T. cnidii*). These agaents were significantly exhibited the strongest antagonism against the fungus *F. javanicum.* . In case of *N. sphaeria*, highest inhibition zones, i.e., 2.3 cm, 1 cm, 1 cm, 2cm and 1.1cm, observed in PD, PD, ME, CZ, and PD media, respectively, against five biocontrol agents (*P. citrinum, P. oxalicum, A. ochraceus, P.capsulatum, T. cnidii*). These agaents were significantly exhibited the strongest antagonism against the fungus *N. sphaeria.* CZ and OMA media was having less impact on growth of fungi and also not giving any effect on the antagonistic behavior of biocontrol fungi as compared to other nutrient media.

INTRODUCTION

Fungi have a significant impact on different ecological processes, and their interactions with other microorganisms are essential components of microbial ecosystems. The antagonistic behavior of fungi refers to their ability to hinder the growth and development of other microorganisms. Some factors like nutrient media that influencing the antagonistic behavior of the biocontrol fungi against some fungi. The use of fungal biological control agents to combat plant pathogens has grown significantly due to several factors. Fungi possess a high reproductive rate, both sexually and asexually, which allows for rapid multiplication. Additionally, they have a short generation time, meaning they can reproduce quickly. Furthermore, fungi demonstrate target specificity, meaning they can effectively target particular pathogens without harming other organisms (Thambugala *et al*., 2020). This combination of characteristics makes fungi valuable allies in the *Fig.*ht against plant diseases.

In a study, the antagonistic activities of five biocontrol agents, namely *T. harzianum, G. roseum, B. subtilis, S. noursei*, and *S. natalensis*, were evaluated against *C. acutatum* and *C. gloeosporioides*. The biocontrol agents demonstrated inhibitory effects on mycelial growth and conidial germination of *Colletotrichum* isolates in vitro. Among them, *Streptomyces*

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noursei and *Streptomyces natalensis* exhibited the strongest antagonism (Zivkovic *et al*., 2010).

Several studies have investigated effective biocontrol agents to combat postharvest diseases globally. For instance, some researchers demonstrated demonstrated the potential of Trichoderma species in controlling postharvest crown rot complex in banana (Alvindia and Natsuaki, 2008; Sangeetha *et al*., 2009). This complex is caused by various fungal pathogens such as *C. musae*, *F. verticillioides*, *and L. theobromae*. Additionally, (Long *et al*., 2023) isolated a new strain, *T. parareesei* N4-3, which shows promise in controlling Fusarium wilt disease caused by *F. oxysporum* f. sp. in banana plants.

In 2009, Mohamed and Saad discovered that using Pichia anomala proved to be a successful method for controlling Diplodia postharvest rot in guava fruit, which is caused by Lasiodiplodia theobromae (Pat.). An in vitro dual culture experiment was conducted to study the antagonistic activity of *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp. against *Pythium debaryanum*. All the species of *Trichoderma* showed the ability to inhibit the pathogen (Gomathi and Ambikapathy, 2011). *T. trachyspermus* R-17 was a promising biocontrol agaent and identified as an antagonistic strains against *F. pseudograminearum (Fp),* which causing crown rot of wheat (Zhao *et al* ., 2022). This paper describes briefly the impact of different nutrient media on the biocontrol potentiality of microbial antagonists particularly against plant fungal pathogens.

MATERIALS AND METHODS

This experiment was conducted by selecting five numbers of fungal isolates *Fusarium equseti, Fusarium acuminatum, Colletotrichum gloeosporioides, Fusarium javanicum, Nigrospora sphaeria* and tested against five numbers of biocontrol fungi *Penicillium citrinum, Penicillium oxalicum, Aspergillus ochraceus, Penicillium capsulatum, Talaromyce cnidii.* These were collected from Microbiology and Plant Pathology Laboratory of Regional Plant Resource Centre, Bhubaneswar, Odisha. Radial growth of the fungi for determining the antagonistic behavior was evaluated by dual culture assay by placing the agar blocks of pure culture (3mm in diameter) of actively growing in both the sides of petri dishes containing five different types of media i.e. Sabouraud Dextrose (SD), Czapek's Dox (CZ), Potato Dextrose (PD), Malt Extract (ME), Oatmeal agar (OA) media. The plates were incubated. The radial growth of both of the fungi with inhibition zone in respectively medium was observed and recorded (Zhang *et al*., 2014).

RESULTS

Antagonistic Effect of bio control fungi against growth of some fungi on Culture media

Fusarium equseti was tested against 5 different biocontrol fungi by growing them on five different nutrient media. In case of *P. citrinum*, highest inhibition zone i.e.1.6 cm was observed in PD media while in CZ and OMA media there was no impact on the growth of the phytopathogenic fungi. In case of *P. oxalicum*, highest zone of inhibition i.e. 1.2 cm followed by 1.1c.m was observed in SD and ME media while in PD and OMA media there was no impact on the growth of the fungi. *P. oxalicum* showed antagonistic activity against *F. equseti* in SD and ME media respectively. In case of *A. ochraceus*, it was observed that *A. ochraceus* displayed antagonistic activity against *F. equseti*. The inhibition zones were measured *i.e.* 1.2cm, 0.5cm, 0.7cm, 0.9cm on SD, CZ, PD and ME media respectively. In case of *P. capsulatum*, highest zone of inhibition i.e. 3cm was observed in SD medium followed by ME, PD and CZ i.e.2.5cm,2.5cm and 0.2cm respectively while in OMA media there was no impact on the growth of the fungi. In case of *T. cnidii*, the zone of inhibition was observed i.e. 0.6cm, 0.5cm, 0.6cm, 0.1cm on SD, CZ, PD, OMA medium while in ME medium zone of inhibition was the highest and it is 0.7cm and having antagonistic activity against *F. equseti* (*Fig.*-1).

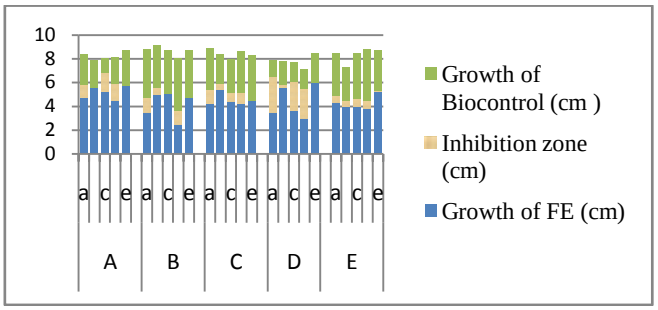

Fig.1 Comparative effectiveness of different fungi on the growth of *Fusarium equseti*

Fusarium acuminatum was tested against 5 different biocontrol fungi by growing them on five different nutrient media. In case of *P. citrinum*, highest inhibition zone i.e.1.8 cm was observed in ME media while in CZ and OMA media there was no impact on the growth of the phytopathogenic fungi. *P. citrinum* exhibited antagonistic activity against *F. acuminatum.* In case of *P. oxalicum*, highest zone of inhibition i.e. 1.5 cm followed by 0.5cm was observed in SD and PD media while in CZ, ME and OMA media there was no impact on the growth of the fungi. *P. oxalicum* showed antagonistic activity against *F. acuminatum* in SD and PD media respectively. In case of *A. ochraceus*, it was observed that *A. ochraceus* displayed antagonistic activity against *F. acuminatum.* The inhibition zones were measured *i.e.* 1.2cm, 0.5cm, 0.7cm, 0.9cm on SD, CZ, PD and ME media respectively. In case of *P. capsulatum*, highest zone of inhibition i.e. 1.7 cm was observed in PD medium followed by SD,CZ,ME and OMA i.e. 1.5cm,0.4cm,1.5cm and 1.2cm respectively. In case of *T. cnidii*, it was observed that *T. cnidii* was having antagonistic activity against *F. acuminatum* by observing the zone of inhibition i.e. 0.7cm, 0.1cm, 4cm, 1.1cm on SD, CZ, PD, ME medium while in OMA medium, there was no impact on the growth of the fungi (*Fig.*-2).

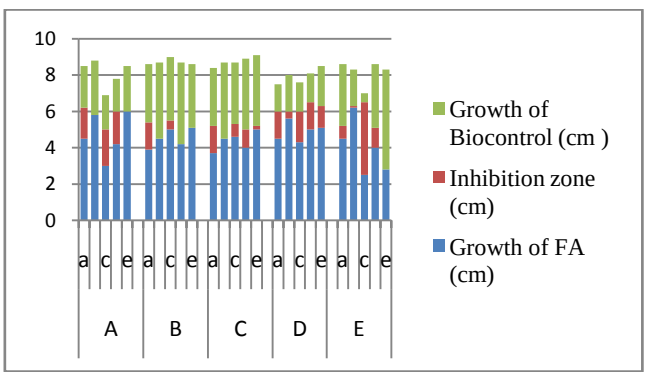

Fig. 2 Comparative effectiveness of different fungi on the growth of *Fusarium acuminatum*

Colletotrichum gloeosporioides was tested against 5 different biocontrol fungi by growing them on five different nutrient media. In case of *P. citrinum*, highest inhibition zone i.e.1cm was observed in ME media while in SD and OMA media there was no impact on the growth of the phytopathogenic fungi. *P. citrinum* exhibited antagonistic activity against *C. gloeosporioides*. In case of *P. oxalicum*, highest zone of inhibition was observed in SD medium i.e. 0.8cm followed by CZ,PD,ME media i.e. 0.2cm,0.4cm,0.5cm while in OMA media there was no impact on the growth of the fungi. *P. oxalicum* showed antagonistic effect against *C. gloeosporioides*. In case of *A. ochraceus*, it was observed that *A. ochraceus* displayed antagonistic activity against *C. gloeosporioides*. The inhibition zones were measured *i.e.* 0.6cm, 0.7cm, 1cm, 0.5cm and 0.1cm on SD, CZ, PD ME and OMA media respectively. In case of *P. capsulatum*, highest zone of inhibition i.e. 1.2 cm was observed in ME medium followed by SD,CZ,PD and OMA i.e. 1 cm,0.7cm,1cm and 0.2cm respectively. In case of *T. cnidii*, it was observed that *T. cnidii* was having antagonistic activity against *C. gloeosporioides* by observing the zone of inhibition i.e. 0.3cm, 0.1cm, 1cm, 0.1cm and 0.1cm on SD, CZ, PD, ME and OMA medium (*Fig.*-3).

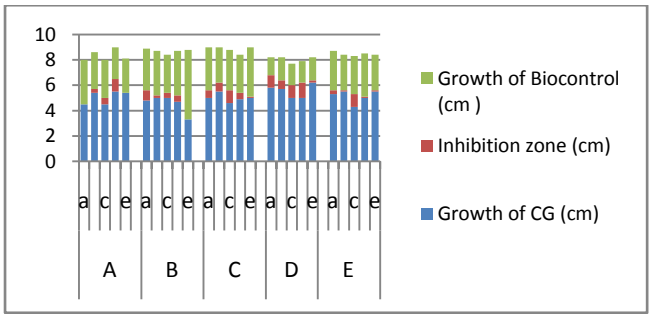

Fig. 3 Comparative effectiveness of different fungi on the growth of *Colletotrichum gloeosporioides*

Fusarium javanicum was tested against 5 different biocontrol fungi by growing them on five different nutrient media. In case of *P. citrinum*, highest inhibition zone i.e.1.6cm was observed in ME media and SD, CZ, PD and OMA media also inhibiting the growth of the phytopathogenic fungi. *P. citrinum* exhibited antagonistic activity against *F. javanicum.* In case of *P. oxalicum*, highest zone of inhibition i.e. 1.5cm followed by 0.1cm,1.2cm,0.2cm was observed in SD,CZ,PD,ME media while in OMA media there was no impact on the growth of the fungi. *P. oxalicum* showed antagonistic effect against *F. javanicum*. In case of *A. ochraceus*, it was observed that *A. ochraceus* displayed antagonistic activity against *F. javanicum by observing* zone of inhibition *i.e.* 1cm, 0.2cm, 0.6cm, 0.8cm on SD, CZ, PD and ME media. In case of *P. capsulatum*, highest zone of inhibition i.e. 2.8 cm was observed in SD medium followed by ME, PD, CZ, and OMA i.e. 2 cm,1.6cm,0.2cm and 0.1cm respectively. In case of *T. cnidii*, it was observed that *T. cnidii* was having antagonistic activity against *F. javanicum* by observing the zone of inhibition i.e. 0.5cm, 0.2cm, 0.4cm on SD, CZ, PD medium and *T. cnidii* was having antagonistic activity against *F. javanicum* by observing the zone of inhibition i.e. 0.3cm on ME medium (*Fig.*-4).

Nigrospora sphaeria was tested against 5 different biocontrol fungi by growing them on five different nutrient media. In case of *P. citrinum*, highest inhibition zone i.e.2.3cm was observed in PD media while in SD, CZ, ME and OMA media, it was also inhibiting the growth of the phytopathogenic fungi. *P. citrinum* exhibited antagonistic activity against *N. sphaeria*.

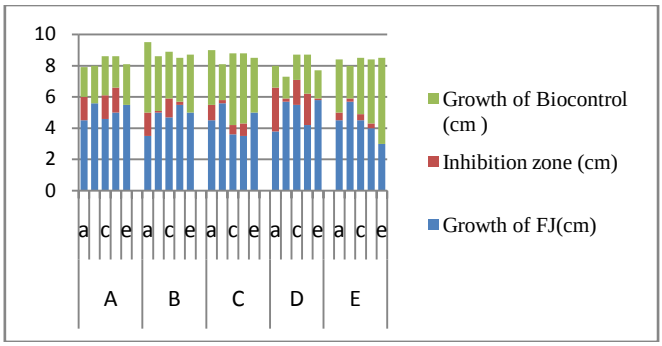

Fig. 4 Comparative effectiveness of different fungi on the growth of *Fusarium javanicum*

In case of *P. oxalicum*, it was observed that *N. sphaeria* was having antagonistic activity against *P. oxalicum* by observing the zone of inhibition i.e.0.5cm, 1cm,0.5cm on SD, PD,ME media while in CZ, the zone of inhibition was 0.5cm and *P. oxalicum* showed antagonistic effect against *N. sphaeria*. In case of *A. ochraceus*, highest zone of inhibition i.e. 1cm followed by 0.8cm,0.6cm,0.6cm was observed in ME, SD,CZ,PD, media while in OMA media there was no impact on the growth of the fungi. *A. ochraceus* was having antagonistic activity against *N. sphaeria.* In case of *P. capsulatum*, highest zone of inhibition i.e. 2 cm was observed in CZ medium followed by SD, PD, ME i.e. 1.7 cm, 1.5cm, 1cm respectively. *P. capsulatum* was having antagonistic activity against *N. sphaeria*. In case of *T. cnidii*, it was observed that *T. cnidii* was having antagonistic activity against *N. sphaeria* by observing the zone of inhibition i.e. 1cm, 0.7cm, 1.1cm, 0.2cm and 0.3cm on SD, CZ, PD, ME and OMA medium (*Fig.*-5).

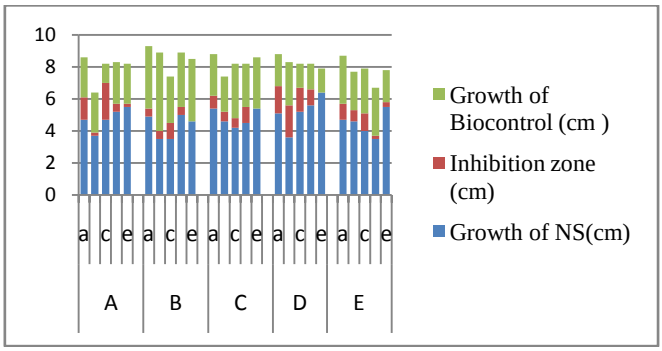

Fig. 5 Comparative effectiveness of different fungi on the growth of *Nigrospora sphaeria*

Abbreviation for Fig.1-5 – Comparative effectiveness of different Biocontrol fungi A = Penicillium citrinum , B= Penicillium oxalicum, C=Aspergillus ochraceus, D=Penicillium capsulatum, E= Talaromyce cnidii against five numbers of phytopathogenic fungal isolates *F. equseti, F. acuminatum, C. gloeosporioides, F. javanicum, N. sphaeria* grown in five different nutrition media such as a= Saboroud Dextrose (SD); b= Czapek's Dox (CZ); c= Potato Dextrose (PD); d= Malt Extract (ME); e= Oatmeal agar (OA)

DISCUSSION

In a series of studies by (McDougal *et al*., 2012; Rahman 2009), the effectiveness of various Trichoderma strains in controlling fungal pathogens was investigated using dual culture techniques. The research revealed significant fungicidal interactions between Trichoderma and target

pathogens. For instance, when assessing the impact of *Trichoderma virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433, and *T. harzianum* IMI-392434 on *Ceratocystis paradoxa*, the causative agent of pineapple disease in sugarcane, *T. harzianum* IMI-392432 demonstrated the highest percentage inhibition of radial growth (PIRG), reaching 63.80% in Method I and 80.82% in Method II. Similarly, in an in vitro assay, it was observed that *T. viride* effectively inhibited the growth of *Fusarium oxysporum* f.sp. lycopersici by 76.94%. These findings underscore the potential of *Trichoderma* strains, particularly *T. harzianum* and *T. viride*, as biocontrol agents against various pathogenic fungi, suggesting their promising role in agricultural disease management strategies (Ayele *et al*., 2021). Effective isolates of *Pseudomonas sp*. were screened for their antagonistic activity against *Colletotrichum capsici*. Two *Pseudomonas* sp. isolates were subjected to dual culture assays on PDA against *Colletotrichum capsici*. Both isolates demonstrated significant inhibition, with isolate P1 reducing radial growth by 78% and isolate P6 by 89%. These findings suggest the potential of these Pseudomonas sp. isolates as effective agents in controlling *Colletotrichum capsici* (Linu and Jisha, 2013). Detailed report on the zone of inhibition by inoculating both the biocontrol and pathogenic fungi on different types of growth medium was not available in most of the research work. In our study we focused on this aspect and we have resulted that, *P. capsulatum* was having antagonistic activity against *F. equseti* with the highest inhibition zone in both PDA and ME media and also having activity against *Fusarium javanicum, Nigrospora sphaeria* in SD and CZ medium. Biocontrol fungi *A. ochraceus, P. capsulatum, T. cnidii* were having antagonistic activity against *C. gloeosporioides* with higher inhibition zone in PD and SD media respectively.

CONCLUSION

Fungal antagonists are potential agents that can be explored to provide effective and safe management of plant diseases. Several fungal biocontrol agents have been tested and proven to possess antagonistic properties against plant pathogenic fungi. Our recent study showed that nutrient media was clearly affecting the growth and antagonistic behavior of completely all biocontrol agents against all five pathogenic fungi. The highest inhibition zone was observed in dual assay of both the fungi grown in SD, PD, CZ and ME media. When grown together, both fungi experienced significant impacts on their growth and exhibited antagonistic behavior.

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