The Pharma Innovation Journal 2021; 10(10): 2463-2466

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2463-2466

www.thepharmajournal.com
Received: 03-07-2021
Accepted: 09-08-2021

Mina Kumari
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

PK Jha
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Corresponding Author:
Mina Kumari
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Effect of culture filtrate of bio control agents of phylloplane and rhizosphere of *Aloe vera* on spore germination and growth of *Colletotrichum gloeosporioides*

Mina Kumari and PK Jha

Abstract
Aloe vera is an important medicinal plant. Black spot in *Aloe vera* is most serious fungal disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. In the present investigation culture filtrate of four native biocontrol agents, (*Trichoderma viride, T. harzianum, T. asperellum* and *Aspergillus niger*) isolated from phylloplane and rhizosphere of *Aloe vera* were tested *in-vitro* for its efficacy against spore germination and growth of pathogen. All the treatments were found effective and recorded significant reduction in spore germination and growth of the test pathogen over untreated control. Out of four biocontrol agents, *Aspergillus niger* was the most effective and exhibited a spore germination inhibition of 80.66% followed by *T. harzianum (*76.48%), *T. viride* (75.10 %) and *T. asperellum* (73.93 %) at 15% concentration. The highest growth suppression was recorded in *Trichoderma harzianum* (86.61%) followed by *T. viride* (85.18%), *A. niger* (84.78%) and *T. asperellum* (82.91%) at 15 % concentration. The results of this study revealed that all the culture filtrate of all biocontrol agents have the potential to inhibit the spore germination and growth of *C. gloeosporioides*.

Keywords: *Aloe vera*, black spot, culture filtrate, biocontrol agents, spore germination, mycelia growth

Introduction
Aloe barbadensis is commonly called as *Aloe vera* is an important medicinal plant, also known as Ghikwar, Gwarpatha or Ghrit Kumari, belongs to Asphodelaceae or Liliaceae family. It is an evergreen, perennial, succulent drought tolerant plant with a height of 60-100 cm. Its leaves are green, thick, fleshy and have jagged, thorny edges. It is best known for its healing *Aloe* gel which is the most important constituent of the plant and has great medicinal value. *Aloe vera* contains amino acids, anthraquinones, enzymes, lignin, minerals, mono and polysaccharides, salicylic acid, saponins, sterols and vitamins. With the onset of commercialism and recently boom in all natural products, *Aloe* has become the ingredients of selection in many skin care and health products.

Despite the high medicinal, nutritive and cosmetic value, *Aloe vera* yield are low due to many diseases. Among them anthracnose caused by *C. gloeosporioides*, leaf spots by *Alternaria* sp, *Curvularia* sp, *Fusarium* sp, bacterial rots are the major limiting factors in the *Aloe* production. Black spots disease is a major threat to *Aloe vera* cultivation throughout the world and is reported from the areas wherever it is cultivated for commercial purpose. The symptoms appeared on the leaves in the form of small dark brown necrotic spots on both sides which gradually enlarge to form big necrotic area. The infected area transforms from dark brown to black. Gradually the leaf surface was covered with numerous such lesions which become rotten and dried within 4-7 days with depression.

Chemical control had been regarded as the most effective measure to control the spread of plant diseases and results in combating disease appears in the short period of time. Chemical application is one of the easiest and most effective methods to control various plant diseases, but it has number of drawbacks like development of resistance strains of pathogen, more expensive and polluting environment. In recent years, use of antagonistic mycoflora for management of plant pathogen is gaining importance as they are ecofriendly and cost effective. Hence, in the present investigation, an effective management strategy, the efficacy of culture filtrate of native biocontrol agent was extracted to test against the spore germination and growth of *C. gloeosporioides* (Penz.) Penz. & Sacc., *in vitro* conditions as it causes huge yield losses of *Aloe* gel.

Materials and Methods

The present study was conducted in the Department of Plant Pathology, DRPCAU, Pusa, Samastipur. The experiment was laid out in Completely Randomized Design (CRD) with three replications. The materials and the methods used during the experiments are described below.

Isolation and purification of pathogen

For the isolation of pathogen black spot infected *Aloe vera* leaves were collected from Herbal Garden of DRPCAU, Pusa, Samastipur. The collected leaves were thoroughly washed in running tap water to remove the surface impurities. After that the leaf samples were cut into small pieces (2-3 mm) from the diseased portion along with adjoining healthy tissues with the help of sterile razor blade. Bits of infected tissues were surface-sterilized with 0.1% mercuric chloride solution for one minute followed by three washings with sterilized distilled water, so as to make them free from any traces of mercuric chloride. The disinfected tissue pieces were blotted between sterile Whatman No. 1 filter papers and aseptically plated randomly in 9 cm diameter Petri plates containing Potato dextrose agar (PDA) medium (3 pieces per plate). These were incubated in Biological Oxygen Demand (BOD) incubator at 28 ± 2ºC for seven days. The mycelia growing from the inoculated tissues were transferred onto fresh PDA medium amended with 1.0 mg/ml streptomycin sulfate and sub-cultured repeatedly until pure cultures of the isolates were obtained.

The purified isolate of the pathogen was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. by cultural and morphological characteristics by referring to the literature [1, 2]. The entire procedure for isolation of the pathogen was done under laminar air flow. Pathogenicity test was done following the Koch's postulate under greenhouse condition.

Preparation of culture filtrates of biocontrol agent

Each selected efficient native biocontrol agents were grown on PDA Petri plates, mycelial disc of each biocontrol agents were individually inoculated in 100 ml of sterilized potato dextrose broth medium in 250 ml Erlenmeyer conical flasks and incubated at 28 ± 2 ºC for 15 days. The culture filtrates were obtained after 15 days of incubation by filtering through Whatman No. 1 filter paper. The supernatant served as stock solution of 100% concentration and was further diluted to desired concentration of 5, 10 and 15% and then evaluated for spore germination and growth of test pathogen. Agar disc (5 mm in diameter) containing actively growing mycelium from 7-10 days old culture of *C. gloeosporioides*, that was maintained on PDA plate were used as the pathogen inoculum.

In vitro evaluation of culture filtrates on spore germination

The culture filtrate of four effective biocontrol agents (*Trichoderma viride, T. harzianum, T. asperellum* and *Aspergillus niger*) were tested for their efficacy against spore germination of *C. gloeosporioides* by cavity slide method [3]. Spore suspension of pathogen was made from 8-10 days old colonies in sterilized distilled water. One drop culture filtrate of bio control agents at different concentration (5, 10 and 15%) were added in depression of cavity slides and mixed with one drop spore suspension of pathogen using micropipette on different slides. The cavity slides were then placed on two pieces of glass rods in sterilized Petri plates lined with moistened Whatman No. 1 filter paper (to provide humid condition for spore germination) and placed in an incubator maintained at temperature 28±2 ºC. A control set was also run concurrently in which spores were mixed in sterilized distilled water. After four days of incubation germination percentage was determined by microscopic observation of 50-150 spores for evidence of germ tube emergence. Spores are scored as germinated when germ tube exceeded their lateral radius. The germination per cent of spores with different concentration of culture filtrate of biocontrol agents was calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{Number of spores germinated}}{\text{Total number of spores}} \times 100$$

Further inhibition of spore germination was calculated by the formula given by Vincent [4].

$$I = \frac{C - T}{C} \times 100$$

Where

I = Per cent inhibition of spore germination
C = Spore germination in control
T = Spore germination in treatment

In vitro evaluations of culture filtrate on mycelia growth of pathogen

Poisoned food technique [3] was used to evaluate the effect of culture filtrate of biocontrol agents on pathogen growth. Culture filtrate at different concentration (5, 10 and 15 %) was added to luke warm sterilized PDA media. Molten PDA media was poured in Petri plates and allowed to solidify. After solidification mycelial disc of 5 mm was cut by using a core borer from 7-8 days old pathogen culture and placed in the centre of each Petri plate. Plates inoculated with mycelial disc at same condition without any culture filtrate were served as control. Three replications were maintained for each treatment. Inoculated plates were then incubated in BOD at 28±2ºC for seven days. The colony diameter was measured by using scale and per cent inhibition of growth in each treatment was calculated by using the following formula given by Vincent [4]:

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition over control
C = Mean maximum colony diameter in control
T = Mean maximum colony diameter in treatment

Results and Discussion

Effect of culture filtrate on spore germination of pathogen

Culture filtrate of each biocontrol agent (*Trichoderma harzianum, T. viride, T. asperellum* and *Aspergillus niger*) at three concentrations (5, 10 and 15%) were used to study its efficacy against the spore germination of pathogen. Inhibition of spore germination of test pathogen increased with increase

in the concentrations of culture filtrate from 5 to 15% (Table 1). The interaction effect of culture filtrate and their different concentrations were non-significant on spore germination inhibition and reduced the spore germination in the range of 57.46 to 62.71 % at 5% concentration.

Table 1: Effect of culture filtrate of native biocontrol agents against spore germination of *C. gloeosporioides* at different concentrations

S. No.	Biocontrol agent	5%		10%		15%	
		Spore germination (%)*	Inhibition of spore germination (%)	Spore germination (%)	Inhibition of spore germination (%)	Spore germination (%)	Inhibition of spore germination (%)
1.	*Trichoderma harzianum*	30.54	61.33 (51.59)	21.26	73.17 (58.87)	18.56	76.48 (61.03)
2.	*Trichoderma viride*	32.41	58.93 (50.19)	26.45	66.43 (54.64)	19.61	75.10 (60.13)
3.	*Trichoderma asperellum*	33.61	57.46 (49.33)	29.45	62.62 (52.36)	20.64	73.93 (59.34)
4.	*Aspergillus niger*	29.40	62.71 (52.41)	20.54	73.91 (59.36)	15.35	80.66 (64.01)
5.	Control	82.00	0.00	80.00	0.00	75.00	0.00
		Culture filtrate (A)	Concentration of culture filtrate (B)			Interactions (A x B)	
C.D.		2.40	2.07			NS	
SE(d)		1.51	1.00			1.99	
SE(m)		0.81	0.70			1.41	

*Mean of three replications, Value given in parenthesis is after angular transformation.

The culture filtrates of bioagents were tested against spore germination and results are presented in Table-1. Of the four bioagents tested, lowest spore germination (15.35%) coupled with highest inhibition of spore germination (80.66%) was occurred in *Aspergillus niger* culture filtrates and was significantly superior over *Trichoderma harzianum* (18.56% and 76.48 %) at 15 % concentration. Whereas *Trichoderma viride* was moderately effective against spore germination (19.61%) and inhibited the spore germination (75.10 %) and statistically at par with spore germination and spore germination inhibition of *Aspergillus niger* at 15% concentration. Least spore germination (20.64%) and inhibition in spore germination (73.93 %) was recorded in culture filtrate of *Trichoderma asperellum* at 15% concentration. Philip *et al.* [5] evaluated the effect of culture filtrate of *Trichoderma* isolates and *Gliocladium virens* isolate on mycelial growth, spore production and spore germination. They observed that these biocontrol agents have inhibited the mycelial growth of pathogen (*Colletotrichum gloeosporioides*, *Pestalotiopsis disseminata*) by about 84% and the spore production and spore germination by above 70%. The mechanisms of antagonism by these antagonists were found to be due to hyperparasitism and antibiosis.

Effect of culture filtrate on mycelia growth of pathogen

Effect of culture filtrate on pathogen growth was determined by mycelial growth inhibition of the pathogen by using the Poisoned food technique. Shrinkage mycelium was observed on pathogen mycelial disc in culture filtrate treated plate. Faster growth rate of biocontrol agents suppressed the growth of *C. gloeosporioides* which is evident from the data presented in Table 2. However, the interaction effect of culture filtrate and their different concentrations were non-significant on mycelia growth inhibition of pathogen and exhibited growth inhibition in the range of 78.58 to 81.31 % at 5% concentration.

Table 2: Effect of culture filtrate of native biocontrol agents against mycelial growth of *C. gloeosporioides* at different concentrations

S. No.	Biocontrol agent	5%		10%		15%	
		Colony growth* (mm)	Per cent inhibition over control	Colony growth (mm)	Per cent inhibition over control	Colony growth (mm)	Per cent inhibition over control
1.	*Trichoderma harzianum*	14.39	81.31 (64.43)	12.02	84.19 (66.63)	10.04	86.61 (68.59)
2.	*Trichoderma viride*	15.34	80.08 (63.54)	13.19	82.64 (65.43)	11.11	85.18 (67.44)
3.	*Trichoderma asperellum*	16.49	78.58 (62.49)	14.36	81.10 (64.28)	12.81	82.91 (65.64)
4.	*Aspergillus niger*	14.98	80.55 (63.88)	13.68	81.99 (64.94)	11.42	84.78 (67.10)
5.	Control	77.00	0.00	76.00	0.00	75.00	0.00
		Culture filtrate (A)		Concentration of culture filtrate (B)		Interactions (A x B)	
C.D.		0.94		0.81		NS	
SE(d)		0.45		0.39		0.78	
SE(m)		0.32		0.28		0.55	

*Mean of three replications, Value given in parenthesis is after angular transformation.

Among the four tested biocontrol agents *T. harzianum* was found most effective and shown highest suppression of pathogen growth (86.61 %) followed by *T. viride* (85.18 %), *A. niger* (84.78 %) and minimum suppression was recorded in culture filtrate of *T. asperellum* (82.91%) at 15 % concentration. Suppression of pathogen growth increased with increase in concentration of culture filtrate. This may be due to the presence of higher concentration of secondary metabolites at higher concentration of culture filtrate that resulted into highest inhibition of pathogen growth.

Jeyalakshmi and Seetharaman [6] reported that *T. viride* reduced the mycelial growth of *Colletotrichum* spp. and grew over the pathogen and caused hyphal coiling, hyphal abnormalities, reduction in sclerotia production, lysis of hyphae and sclerotia. Vinale *et al.* [7] reported that the secondary metabolites of *T. harzianum* strain T22 and T39 have antifungal activity against *Leptosphaera muculans*, *Phytophthora cinnamomi*, and *Botrytis cinerea* by reducing its

growth. In the present investigation, *T. harzianum* and *T. viride* have shown better inhibiting ability against the growth of *C. gloeosporioides* than *T. asperellum*. This is due to production of different types of secondary metabolites by different species or isolates in the same species of *Trichoderma* [8, 9] so that they have different abilities in inhibiting the growth of pathogenic fungi. Nurbailis *et al*. [10] studied the antifungal activity of the culture filtrate of *Trichoderma harzianum, T. viride, T. koningii, Trichoderma* PP2, *Trichoderma* PP3 against *C. gloeosporioides* and observed that culture filtrate of *Trichoderma* spp. isolated from chili rhizosphere inhibited the growth of pathogen by the mechanism of competition, hyperparasites, and antibiosis.

Conclusions

It is concluded from the present investigation that culture filtrate of biocontrol agents like *T. harzianum, T. viride, T. asperellum* and *Aspergillus niger* have the potential of preventing the spore germination and mycelial growth of *C. gloeosporioides* in *in-vitro* conditions. This study demonstrated the possibility of using culture filtrate from efficient biocontrol agents to control mycelial growth as well as spore germination of pathogen for plant disease management.

Acknowledgement

The authors are thankful to Head, Department of Plant Pathology, DRPCAU, Pusa, Samastipur for providing essential laboratory facilities and support to conduct this research work successfully.

References

1. Alexopoulos CJ, Mims CW, Blackwell M. Introductory Mycology. Fourth Edition. John Wiley and Sons, Inc. New York 1996.
2. Agrios GN. Plant Pathology. 5th ed. Elsevier Academic Press Inc., New York 2005.
3. Sinclair JB, Dhingra OD. Basics plant pathology methods. Published by CRC Press. In.c Corporate. Buld, M W Boca Raton, Florida 1985.
4. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors, Nature 1947;159:1239-1241.
5. Philip T, Sharma DD, Shivapratap HR. Antagonistic effect of *Trichoderma* and *Gliocladium virens* on two foliar pathogens of mulberry (*Colletotrichum gloeosporioides*, *Pestalotiopsis disseminata*). Sericologia (France) 2000;40(1):109-121.
6. Jeyalakshmi C, Seetharaman K. Biological control of fruit rot and die-back of chilli with plant products and antagonistic microorganisms. Pl. Dis. Res. 1998;13(1):46-48.
7. Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M, Sivasithamparam K. Major secondary metabolites produced by two commercial Trichoderma strains active against different phytopathogens. Lett App Microbiol. 2006;43:143-8.
8. Vinale FK, Sivasithamparam LE, Ghisalberti R. Marra LS, Lorito M. Trichoderma-plant-pathogen interactions. Soil Biol Biochem 2008;40:1-10.
9. Wu Q, Sun R, Ni M, Yu J, Li Y, Yu C *et al*. Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. PloS ONE 2017;12(6):e0179957. DOI: 10.1371/journal.pone.0179957
10. Nurbailis N, Djamaan A, Rahma H, Liswarni Y. Potential of culture filtrate from Trichoderma spp. as biofungicide to *Colletotrichum gloeosporioides* causing anthracnose disease in chili. Biodiversitas Journal of Biological Diversity 2019;20(10).