www.ajlifesciences.com / ISSN: 2663-1040 *AJ Life Sci. 2022, 5 (1): 24-30* **Research Article**

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.5.1.3

In vitro bioassays of some plant extracts against Banana anthracnose caused by *Colletotrichum musae*

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Abstract

Anthracnose of banana caused by *Colletotrichum musae* is an invasive disease, which is difficult to control in the export process. Plant extracts used to control plant diseases are gradually becoming substitutes for traditional fungicides because they are safe for the human body and environmentally friendly. The study was conducted to record the prevalence of banana anthracnose in two locations and to determine the best medium for the growth of *C. musae* and botanical extracts for managing the fungus under *in-vitro* conditions. Six media *viz*., Ripen banana peels media (RBPM), Unripen banana peels media (URBPM), Ripen banana flash fruit media (RBFFM), Unripen banana flash fruit media (URBFFM), Banana leaf media (BLM) and Potato dextrose agar media (PDA), four botanical extracts including Garlic (GC), Mint (MT), Eucalyptus (EC) and Neem seed (NS) at 2, 4 and 6% concentrations were tested against the fungus and the control (CL) remain untreated. Potato dextrose agar media (PDA) was used as a standard semi-synthetic growth media. The prevailing frequency of the disease was high in the investigation area. The maximum linear mycelial growth of the *C. musae* was recorded on PDA (85 mm) followed by URBFFM (40 mm), RBFFM (26 mm) whereas the lowest mycelial growth was recorded on BLM (20 mm) and URBPM (8 mm). *In-vitro* experiments showed that PDA was the best medium for the mycelial growth of *C. musae*. Moreover, tested botanical extracts showed high variation in reducing the colony growth of the fungus. EC was found most effective in reducing the colony growth of the fungus followed by NS, GC and MT. These results suggest that all the botanical extracts may be potentially useful for controlling anthracnose of bananas, EC extract can be effectively used for the field experiment.

Keywords: Botanical extracts, Efficacy, Growth media, Incidence, Prevalence

Article Info:
Received:
February 12, 2022
Received Revised:
August 19, 2022
Accepted:
August 19, 2022
Available online:
September 17, 2022

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1 INTRODUCTION

Banana (*Musa* spp.) is one of the most valuable agricultural products in the world with commercial plantations accounting for nearly 15% of the global banana production [1]. It is one of the world's most consumed fruit, with an annual production of 115.7 million tons in 2018 and is grown in 130 countries [2]. In Pakistan, the planting area is over 34,800 hectares and the production is 154,800 tons. It is mainly planted

in Sindh province, where soil and climate conditions are favorable for the cultivation of fruits and vegetables. In addition, the province accounts for 87% of banana crop cultivation [3]. In some parts of the world, it is used globally as a dessert and as a staple meal. Among fruit crops, it is one of the most valuable foreign-exchange-earning crops in global trade in many countries in Africa [4]. The area harvested banana in Pakistan is about 29,735 ha with the production of 135,660 tons [5]. Pakistan has a low banana yield compared with many developed countries in the world. Diseases caused by fungi, viruses and other pathogenic organisms are one important factor.

Anthracnose caused by *Colletotrichum musae* is one of the main threat to banana production, which seriously affects the quality, market and consumption of banana [6]. It is a host-specific pathogen and a static infection caused by the pathogen during fruit ripening. It is the most harmful postharvest disease in the world [7]. The disease is a standout amongst the most essential and broadly circulated maladies of maturing and ready bananas and is especially connected with wastage following wounds such as scratches and different injuries supported by the fruits during handling and transport.

Thiabendazole fungicides are used to control postharvest anthracnose and prolong the shelf life of banana fruits [8]. However, these products entail risks to public health and are harmful to the environment [9]. Natural substitutes for traditional antifungal treatments can meet people's demand for healthy and non-toxic food [8]. Plant pathogen control uses extracts from plant species such as Eucalyptus, *Azadirachta indica*, garlic, mint, regret, yarrow, ginger, and basil. In addition, there are 237 species of plants from Brazilian vegetation, whose antibacterial potential has been tested by Brazilian scientists. Most of the work on the aggregation of pathogens is related to the diseases caused by those in plant reserves (30% of the work and isolation), such as *Alternaria, Bipolaris, Crinipellis, Corynespora* and *Colletotrichum* of which 15% work alone [10].

Keeping in view the losses that occur due to anthracnose disease and the economic importance of the banana crop, the present study of anthracnose in banana was carried out. The disease is usually controlled by various management approaches, including crop hygiene, plant materials, disease-free planting materials, crop rotation and so on. These methods have one or other drawbacks, and a single strategy is not successful. The use of chemicals is hindered by health hazards and their use. The use of botanical or plant extracts is the only feasible way to control the disease. As there is scanty information about the botanical extracts for this disease in Pakistan, therefore, the objective of the present study was to assess different botanical extracts against anthracnose of banana. Plant extracts can be an important part of the integrated disease management plan.

2 MATERIALS AND METHODS

2.1 Sample collection, isolation and identification

Samples of banana fruit showing typical symptoms of anthracnose were collected from two major banana growing areas of Tandojam and Tando Allahyar and were brought to the laboratory of the Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam for isolation of the fungus and further studies. Through the morphological characteristics of fungi as described by Zakaria et al. [11], associated fungi were isolated and identified with the help of keys.

2.2 Confirmation of pathogenic nature of the isolated fungus

The pathogenicity of fully mature unripen banana fruit was studied. Therefore, *C. musae* was inoculated on the crown of unripen banana fruit. Before inoculation, the fruit surface was disinfected with a 5% sodium hypochlorite solution. 5mm dia culture block of *C. musae* cut out of PDA plate was placed in the center of unripen banana fruit. These disks were then covered with a double layer of moistened and sterilized blotter paper. The inoculated fruits were kept in a moist room to ensure the best condition for disease occurrence. The uninoculated fruits were used as control. After 15 days of inoculation, the disease occurrence in all fruits was detected, and the fungus was re-isolated to confirm Koch's postulates.

2.3 Efficacy of different culture media

The effects of different media on the growth of *Colletotrichum musae* were studied by using Ripen banana peels media (RBPM), Unripen banana peels media (URBPM), Ripen banana flesh fruit media (RBFFM),

Unripen banana flesh fruit media (URBFFM), Banana leaf media (BLM) and Potato dextrose agar media (PDA). Different types of media were prepared according to the composition and placed in a 250 ml conical flask. Then the different types of media were evenly poured into the sterilization plate, each plate 20 ml, and coagulated. A 5 mm diameter block was cut from the 7-day-old pathogen culture and inoculated in the center of each plate. Each medium was kept for three replicates. Radial growth (colony diameter) was cross-recorded every 48 hours until the pathogen grew up to 90 mm on any plate.

2.4 *In vitro* effect of different botanical extracts against *Colletotrichum musae*

Garlic (GC), Mint (MT), Eucalyptus (EC) and Neem seeds (NS) were tested against *C. musae* in *in-vitro* conditions at the concentration of 2%, 4% and 6% of each plant extract. The effectiveness of plant extracts was assessed by the reduction of linear colony growth on PDA (e.g., banana leaves, banana ripen and unripen fruit peels and PDA).

3 RESULTS AND DISCUSSIONS

3.1 Disease incidence of the Anthracnose disease of banana at different localities

Anthracnose disease caused by *C. musae* was recorded in all surveyed fields with different intensities. The results showed that the maximum (65%) anthracnose disease incidence was recorded at Tando Jam followed by (55%) in Tando Allahyar (Table 1).

Table 1. Incidence of banana anthracnose in different areas.

Locality	No. of fields	Samples taken/ field	Samples studied			
			Disease	Healthy	Total	Incidence%
Tando Jam	04	25	65	35	100	**65.00**
Tando Allahyar	04	25	55	45	100	**55.00**

3.2 Isolation and identification of the fungi

Various pathogens such as *Colletotrichum musae, Fusarium* sp., and *Aspergillus* sp. were isolated from the infected parts of the banana showing the symptoms of anthracnose disease with a different number of colony frequencies (%). *C. musae* was isolated as a dominant fungus as compared to other fungi. *C. musae* was isolated at the highest frequency (72%) (Fig. 1).

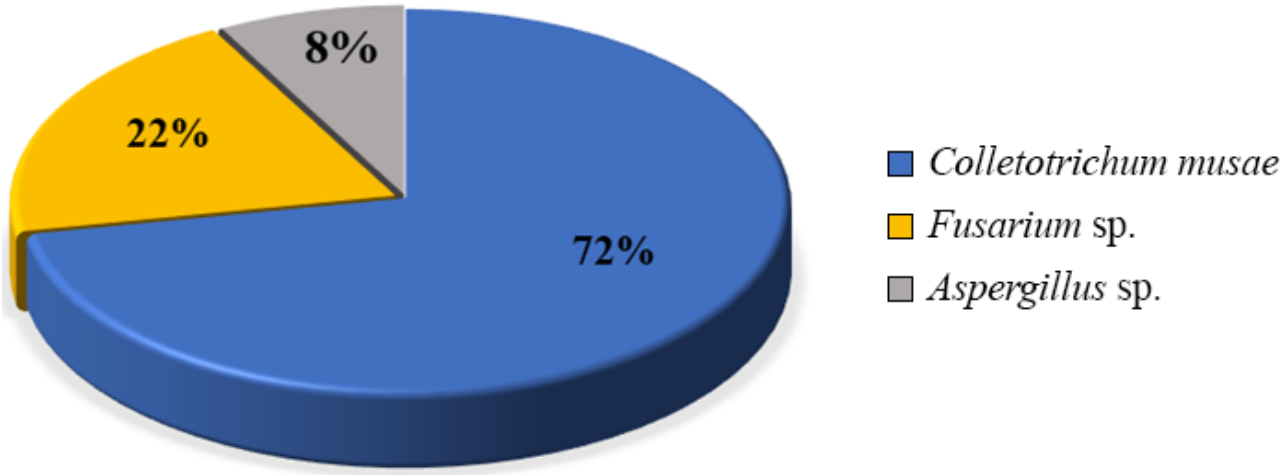


Fig. 1. Isolation frequency percent of isolated pathogens from banana tissue

3.3 Pathogenicity test

To determine the etiology of the disease, the pathogenicity test of the most frequently isolated pathogen *C. musae* was carried out by spore injection method. The results showed that the incidence rate of pathogen *C. musae* was 71% with the spore injection method and the incidence rate in the control was 0% (Table 2).

Table 2. Pathogenicity test of *Colletotrichum musae*

Inoculation Method	R1	R2	R3	Overall%
Spore injection method	70%	68%	75%	**71%**
Control	0%	0%	0%	**0%**

3.4 Effects of different media on the linear mycelial growth of *Colletotrichum musae*

The highest linear mycelial growth was recorded in PDA media that is (85 mm), followed by URBFFM (40 mm). However, the lowest linear mycelial growth was recorded in the case of RBFFM (8 mm) followed by BLM (20 mm), URBP (25.00), RBFFM (26 mm) (Fig. 2). From the results, it is observed that PDA is the best medium for the linear mycelial growth of *C. musae* as comparing other tested media.

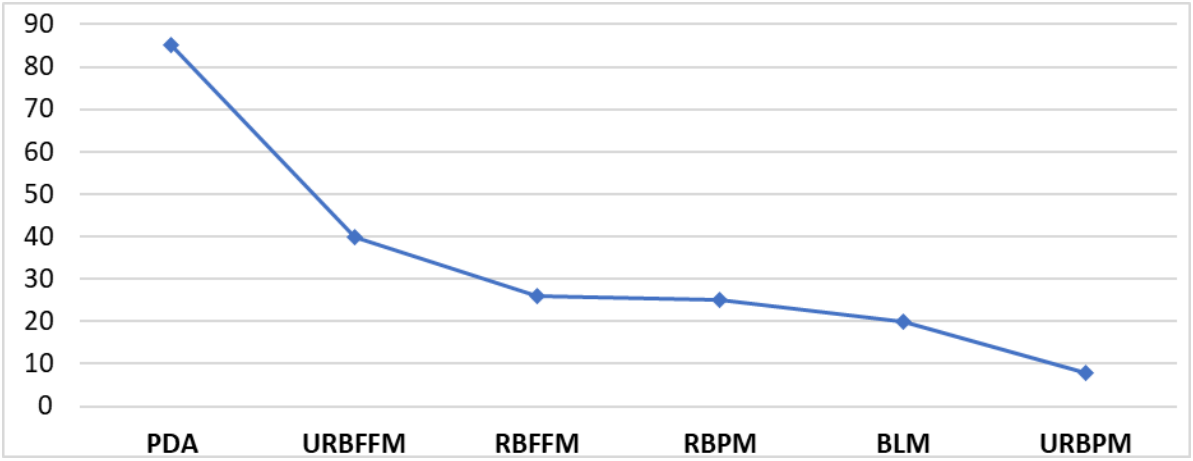
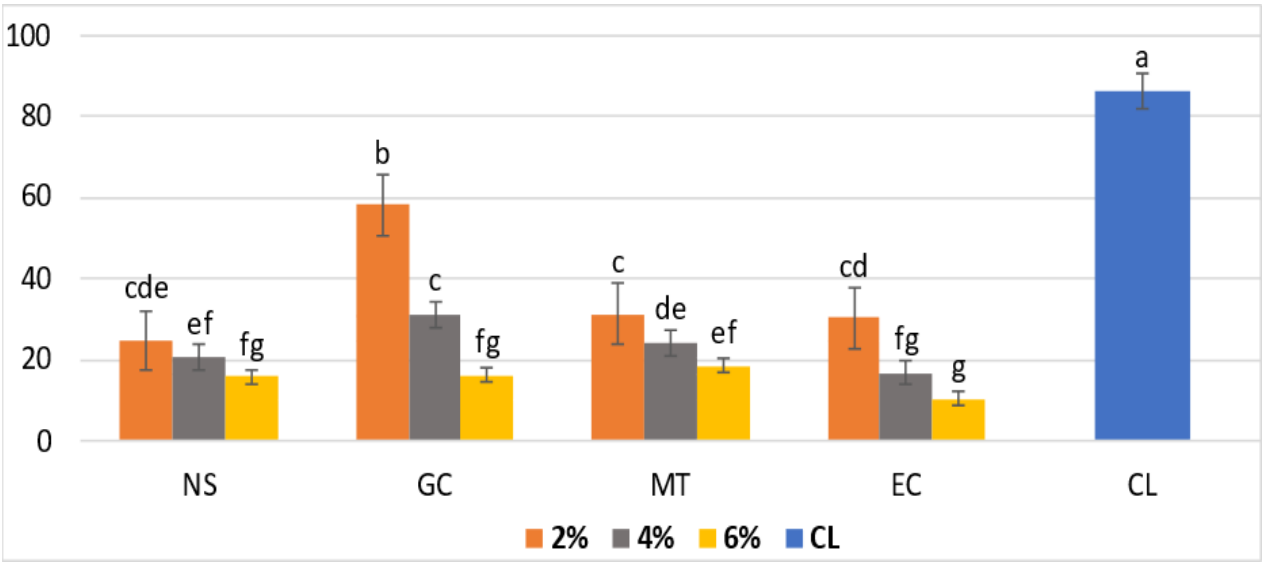


Fig. 2. Effects of tested media on the linear mycelial growth of *Colletotrichum musae*

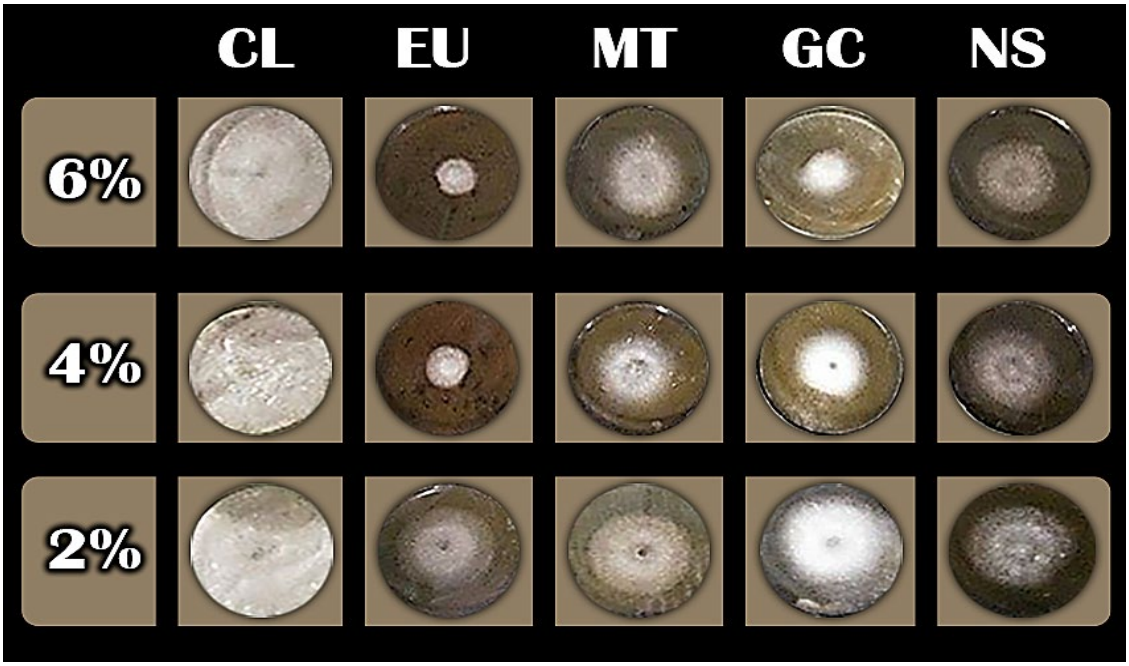
3.5 Effect of different botanical extracts on the linear mycelial growth of *Colletotrichum musae*

All the plant extracts significantly reduced the mycelial growth of the test fungus at ($P<0.05$). Among all the plant extracts EC and NS extracts were found most effective against the colony growth of the *C. musae* at their highest doses whereas, MT and GC were less effective at their highest doses.

Among all the plant extracts the EC was discovered most effective at all applied doses. The minimum colony mycelia growth of 10.33 and 16.66 mm was recorded when plant extracts were used at 6 and 4% respectively. Whereas at the lowest dose of 2%, the growth of the fungus was recorded 30.33 mm after seven days of the inoculation. The NS extracts were also found effective at their highest dose as compared to MT and GC extracts. The NS extracts supported 15.66, 20.66 and 24.66 mm growth at 6, 4 and 2% doses, respectively. The performances of the GC extract were not satisfactory in inhibition of mycelial growth in comparison to others. In a 2% concentration, the GC extract fails to inhibit the mycelial growth of the fungus (Fig. 3a and 3b).



(a)



(b)

Fig. 3. a & b showing effects of selected botanical extracts on the mycelial growth of *Colletotrichum musae*

Anthracnose is a common disease of several economic crops and the reason of the major losses. For growers around the world, this is a reemerging disease problem [12]. Keeping in view the misfortunes that happen because of Anthracnose wither illness the study was completed from the real banana developing ranges of different areas. Anthracnose disease incidence was recorded at Tandojam (65%) followed by (55%) in Tando Allahyar. The *C. musae* is a major and dominant fungus responsible to cause the Anthracnose of banana. During our research different fungi such as *Fusarium* sp. *Aspergillus* sp. and *C. musae* with different frequencies (%) were isolated from the banana tissue showing the Anthracnose disease symptoms. Laboratory studies were conducted to study the effect of six different culture media, on mycelial growth including RBPM, URBPM, RBFFM, URBFFM, BLM and PDA. These studies have indicated that PDA gave good growth and better sporulation of *C. musae*. Plant extracts are eco-friendly pesticides that can be used against different foliar as well as soil-borne diseases. Rout et al. [13] evaluated 13 different plant extracts and stated that Onion, Garlic, Sadabahar and Begunia extracts completely stopped the growth of the causal pathogen at 10% concentration. Neem, Karanja and custard apple leaf extracts

reduced more than 90% radial growth of *C. musae.* In our study, the efficacy of different treatments in controlling the post-harvest pathogen of banana was evaluated under *in-vitro* conditions. Based on the inhibition of four treatments on the growth of radial mycelium growth of pathogenic fungi, the experimental results were compiled viz., EU, MT, GC, NS and CL treatment remained untreated. Results relieved that as the dose of the plant extract increases, the fungal growth of the test fungus decreases. Among all the tested plant extracts EC was found mainly valuable during dropping the colony development of the *C. musae*. The minimum colony growth of 15.66, 16.00, 18.33 and 10.33 mm colony mycelia growth of the test fungus was recorded at the highest doses of NS, GC, MT and EC extracts respectively. The maximum colony mycelia growth of 24.66, 58.33, 31.33 and 39.33 mm was recorded at the lowest dose of NS, GC, MT and EC respectively except for control 86.33 mm. The moderate doses of the plant extract resulted from 20.66, 31.00, 24.00 and 16.66 mm colony mycelia growth of the test fungus when applied NS, GC, MT and EC extracts respectively.

4 CONCLUSIONS

PDA is proven to support the best growth of the banana anthracnose pathogen *Colletotrichum musae*. Under *in-vitro* Eucalyptus (EC) was found to be the best plant extract to inhibit the mycelial growth of C. musae. Field trials are needed before recommending the management of banana anthracnose by plant extract.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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