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Effect of Different Carbon, Nitrogen Sources and Trace Elements on Mycelial Biomass Production of *Colletotrichum gloeosporioides* Causing Anthracnose in Citrus

Deepika Sharma, Kumud Jarial* and R. S. Jarial

Dept. of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh (177 001), India

Corresponding Author

Kumud Jarial
e-mail: kumudvjarial@rediffmail.com

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Abstract

The research was conducted during October, 2022 to March, 2023 at Research Laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India to study the effect of different carbon, nitrogen sources and trace elements on the growth of *Colletotrichum gloeosporioides* using Czapek's Dox broth as basal medium. In all, nine carbon sources viz., arabinose, dextrose, fructose, galactose, lactose, mannitol, starch, sucrose and xylose, six nitrogen sources viz., ammonium sulphate, asparagine, aspartic acid, potassium nitrate, sodium nitrate and urea and five different trace elements viz., ammonium molybdate, cupric sulphate, ferrous sulphate, zinc sulphate, magnesium sulphate were evaluated to see their effect on mycelial biomass production of test pathogen. All the carbon, nitrogen sources and trace elements under evaluation resulted in efficient biomass production that was compared with the mycelial biomass recorded in control devoid of any carbon, nitrogen source and trace element. Among carbon sources, maximum dry mycelial weight was recorded in arabinose (871.97 mg) followed by that in starch (575.38 mg) and sucrose (529.83 mg), among nitrogen sources in aspartic acid (643.23 mg) followed by that recorded in sodium nitrate (390.87 mg) and ammonium sulphate (479.97 mg) and among trace elements in magnesium sulphate (1432.80 mg) followed by that in zinc sulphate (600.17 mg) and ammonium molybdate (504.20 mg) each after 14 days of incubation.

Keywords: Anthracnose, citrus, *Colletotrichum gloeosporioides*, carbon, nitrogen, trace elements

1. Introduction

Citrus (*Citrus* spp., Rutaceae) is one of the most significant crop produced for human consumption in the world and is grown in many countries with tropical and subtropical climates (Martinez et al., 2020). Several species of *Colletotrichum* have been identified causing anthracnose in citrus (Wang et al., 2021). Crop infected by *Colletotrichum gloeosporioides* lead to development of anthracnose symptoms mainly on the fruits leading to huge losses. Anthracnose restricts production worldwide. Anthracnose impacts fruit quality after harvest, which has a detrimental impact on fruit export and marketability (Bordoh et al., 2012). Pre- harvest anthracnose reduces yield and post-harvest anthracnose impairs quality of produce that result in huge financial losses (Smilanick et al., 2020). During present investigation, the pathogen was isolated from citrus crop infected with anthracnose.

Nutrition is the primary key component for growth, multiplication and survival of any biotic agent (Tripathi et al., 2022). Basic requirement for growth of all the phytopathogenic fungi is nutrition but their requirement differs among themselves (Kumar et al., 2012). Composition of nutrient medium determine rate of production, type of products produced, biomass yield and products and for desirable biomass production elements like carbon, nitrogen, oxygen, sulphur and phosphorous must be provided in suitable form (Chisti and Young, 2003). Carbon source plays a key role in mycelial growth and biomass production by providing essential nutrient source. Various carbon sources affect growth rate, morphology and metabolic activity of fungi, as fungi utilize these sources for energy and structural development. Assimilation of carbon sources contribute to fitness and pathogenicity of pathogen (Lok et al., 2021). Most common source of carbon are carbohydrates, which are necessary for biosynthesis, energy production and microbial fermentation (Duree and Eikmanns, 2015). The type and concentration of carbon source determines the efficiency of nutrient assimilation and impacts metabolic activity of

fungi leading to variations in mycelial growth (Pappagiani, 2004). Fruit colonization by pathogens is host dependent mechanism which is affected by sugar content involving carbon metabolism via environmental pH modulation (Bi et al., 2016). Mycelial biomass production requires carbon sources like fructose, maltose and saccharose etc. and nitrogen sources as building blocks (Kirsh et al., 2016). Nitrogen sources are essential for regulating the mycelial development, protein synthesis and enzymatic activity of fungus. Organic as well as inorganic form of nitrogen can be utilized by the fungus. Nitrogen not only promotes mycelial biomass accumulation but also enhances sporulation in fungi. The constituents of a nutrient medium supply the nitrogen for biosynthesis and cell maintenance to meet the needs for cell biomass and metabolite production. It is possible to use nitrogen in both organic and inorganic forms (Costa et al., 2002). Mycelial growth can be significantly affected by trace elements present in the nutrient medium. Although, trace elements are required in minute quantities, these play a crucial role in the various biochemical and physiological processes essential for the mycelial development (Robinson et al., 2021). Addition of trace elements in nutrient media enhances the mycelial growth of fungi (Falandysz, 2021). Different trace elements added to the basal media may cause the fungus to become more metabolically active, which would lead to greater growth (Bills et al., 2008). Keeping in view the importance of carbon and nitrogen sources as well as trace elements in the fungal growth and metabolism, present studies were conducted with an objective to find out best carbon, nitrogen source and trace element for the growth of *C. gloeosporioides* causing citrus anthracnose.

2. Materials and Methods

The research was conducted in Research Laboratory, Department of Plant Pathology, College of Horticulture and Forestry Neri (1189 m above mean sea level), Hamirpur, Himachal Pradesh, India during October, 2022 to March, 2023.

2.1. Effect of different carbon and nitrogen sources on average biomass of Colletotrichum gloeosporioides

To determine the effect of different carbon sources viz., arabinose, dextrose, fructose, galactose, lactose, mannitol, starch, sucrose, xylose and different nitrogen sources viz., ammonium sulphate, asparagine, aspartic acid, potassium nitrate, sodium nitrate and urea on the growth of test pathogen, Czapek's Dox broth was used as basal medium. Carbon sources were substituted with sucrose on the basis of their respective molecular weight, so as to provide equal amount of carbon in medium. Fifty ml of broth with replaced carbon source each replicated thrice was put in the Erlenmeyer flasks of 100 ml capacity and inoculated with a culture bit (5 mm dia.) of test fungus. Nutrient broth without any carbon source served as control. Similarly, in case of nitrogen source estimation different nitrogen sources were substituted with sodium nitrate in the basal medium on the basis of their respective molecular weight, so as to provide equal amount of nitrogen. Fifty ml of broth was put in the Erlenmeyer flasks of 100 ml capacity with replaced nitrogen source each replicated four times and inoculated with a culture bit (5 mm dia.) of test fungus. Nutrient broth without any nitrogen source served as control and the broths were then incubated at best temperature 28°C in BOD incubator up to 14 days. The data were recorded in terms of average dry weight (mg) of mycelium after 7 and 14 days of inoculation.

2.2. Effect of different trace elements on average biomass of Colletotrichum gloeosporioides

To determine the effect of various trace elements viz., ammonium molybdate, cupric sulphate, ferrous sulphate, zinc sulphate, magnesium sulphate for the growth of test pathogen, Czapek's Dox broth was used as basal medium. Ferrous sulphate in the basal medium was replaced with respective quantity of trace element on the basis of molecular weight, so as to provide the desirable concentration of trace element in the medium. Fifty ml of broth was put in the Erlenmeyer flasks of 100 ml capacity with replaced trace element each replicated four times and inoculated with a culture bit (5 mm dia.) of test fungus and the broths were then incubated at best temperature 28°C in BOD incubator up to 14 days. Nutrient broth without any trace element served as control. The data were recorded in terms of average dry weight (mg) of mycelium after 7 and 14 days of inoculation.

3. Results and Discussion

3.1. Effect of different carbon and nitrogen sources

3.1.1. Effect of different carbon sources

Data presented in Table 1 clearly depict that significantly mean maximum biomass (657.30 mg) of the test fungus was recorded when arabinose was used as a carbon source followed by that in starch (575.38 mg) while, mean minimum biomass (76.58 mg) was recorded when no carbon source was used significantly followed by mycelial biomass (127.05 mg) produced when galactose was used as a carbon source. Irrespective of different carbon sources under investigation, significantly mean minimum biomass (211.25 mg) was recorded after 7 days of incubation which increased drastically in next 7 days to reach its maximum (359.65 mg) after 14 days of incubation.

The body of the table reveals that, significantly maximum biomass (871.97 mg) was recorded with arabinose after 14 days of incubation followed by that recorded with starch (637.23 mg) after same duration of incubation. However, minimum biomass (26.23 mg) was recorded with control having no carbon source significantly followed by that on galactose (65.47 mg) after 7 days of incubation that differ significantly from the biomass produced by using rest all carbon sources. An intermediate level of mycelial biomass production was recorded with rest of the carbon sources at 7 and 14 days of incubation period (Figure 1).

Table 1: Effect of different carbon and nitrogen sources on the growth of *Colletotrichum gloeosporioides*

Carbon source	Dry weight (mg) of the fungus after incubation duration (days)		Overall mean	Nitrogen source	Dry weight (mg) of the fungus after incubation duration (days)		Overall Mean
	7	14			7	14	
Arabinose	442.63	871.97	657.30	Ammonium sulphate	236.37	479.97	358.17
Dextrose	223.50	290.73	257.12	Asparagine	180.77	366.43	273.60
Lactose	145.37	236.60	190.98	Aspartic acid	320.60	643.23	481.92
Starch	513.53	637.23	575.38	Potassium nitrate	201.87	410.10	305.98
Galactose	65.47	188.63	127.05	Sodium nitrate	251.83	529.90	390.87
Mannitol	166.80	225.77	196.28	Urea	145.33	318.20	231.77
Fructose	185.63	286.93	236.28	Control	104.43	227.57	166.00
Xylose	170.70	201.87	186.28	Overall mean	205.89	425.06	
Sucrose	172.63	529.83	351.23				
Control	26.23	126.93	76.58				
Overall mean	211.25	359.65					

	CD $p \geq 0.05$	SE(d)		CD $p \geq 0.05$	SE(d)
Carbon source	2.88	1.42	Nitrogen source	2.36	1.15
duration	1.29	0.64	duration	1.26	0.61
interaction	4.08	2.01	interaction	3.34	1.62

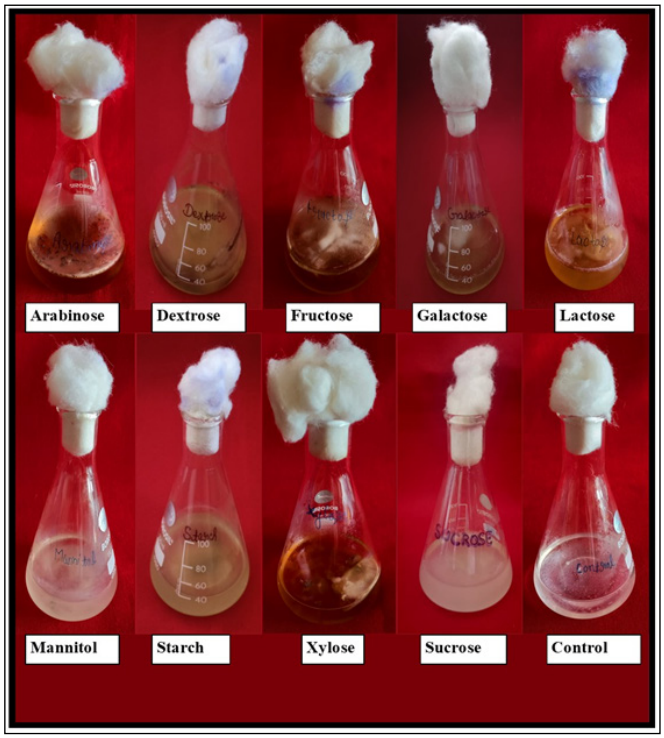

Figure 1: Effect of different carbon sources on mycelial biomass production of *Colletotrichum gloeosporioides*

Carbon source is a primary nutrient for the fungal growth and development. The type and availability of carbon sources can affect the fungal growth rate, biomass production, sporulation and enzymatic activity (Adnan et al., 2017). In present studies, arabinose followed by starch and sucrose proved to be good carbon sources as they supported good mycelial biomass of test pathogen. The results are somewhat in line with the findings of Diaz et al. (2012) who reported starch and xylose followed by glucose and sucrose to be best supportive for the growth of *C. gloeosporioides*. The results are further supported by Li et al. (2020) who reported glucose sucrose and fructose to be supportive for the growth of *C. gloeosporioides.*

3.1.2. Effect of different nitrogen sources

It is evident from the Table 1 that, irrespective of different durations of incubation, significantly mean maximum biomass of the test pathogen was recorded in aspartic acid (481.92 mg) followed by that recorded in sodium nitrate (390.87 mg) while, mean minimum biomass (166.00 mg) was recorded when no nitrogen source was used, significantly followed by that recorded in urea (231.77 mg). Irrespective of different nitrogen sources under investigation, significantly mean minimum fungal biomass was recorded after 7 days of incubation (205.89 mg) while maximum was recorded after 14 days of incubation (425.06 mg). The body of the table reveals that, significantly maximum biomass (643.23 mg) was recorded with aspartic acid after 14 days of incubation followed by that recorded with sodium nitrate (529.90 mg) after same duration of incubation. However, minimum biomass (104.43 mg) was recorded with control having no nitrogen source significantly followed by that on urea (145.33 mg) after 7 days of incubation that differ significantly from the biomass produced by using rest all nitrogen sources (Figure 2). An intermediate level of mycelial biomass production was

Figure 2: Effect of different nitrogen sources on mycelial biomass production of *Colletotrichum gloeosporioides*

recorded with rest of the nitrogen sources at 7 and 14 days of incubation period.

Nitrogen is an essential nutrient for the mycelial growth influencing various physiological processes from protein synthesis to enzyme activity. The type and amount of nitrogen significantly affects the overall biomass production. During present studies, aspartic acid produced maximum biomass of the pathogen which is in conformity with Chaturvedi (1965) who reported aspartic acid to be best nitrogen source for the growth of *C. gloeosporioides*. Critical role of nitrogen sources in fungal development, conidia production, regulation of nitrogen metabolism and virulence in *Colletotrichum gloeosporioides*.was proved by Bi et al. (2017).

3.2. Effect of different trace elements on mycelial biomass of Colletotrichum gloeosporioides

Data presented in the Table 2 clearly depict that significantly mean maximum biomass (861.33 mg) of the test fungus was recorded when magnesium sulphate (Figure 3) was used as a trace element followed by that recorded with zinc sulphate (600.17 mg). However, mean minimum biomass of test fungus (74.18 mg) was recorded when no trace element was used significantly followed by mycelial biomass (252.96 mg) produced when copper sulphate was used as a trace element.

Irrespective of different trace elements used, a drastic significant increase in fungal biomass was recorded after

Table 2: Effect of different trace elements on mycelial biomass of *Colletotrichum gloeosporioides*

Trace element	Dry weight (mg) of the fungus after incubation duration (days)		Overall Mean
	7	14	
Ammonium molybdate	246.57	504.20	375.38
Magnesium sulphate	289.87	1432.80	861.33
Zinc sulphate	393.30	807.03	600.17
Ferrous sulphate	251.83	523.17	387.50
Copper sulphate	169.28	336.63	252.96
Control	48.17	100.03	74.10
Overall mean	233.17	617.31	
	CD $p \geq 0.05$		SE(d)
Trace element	12.66		6.10
duration	7.31		3.52
interaction	17.91		8.63

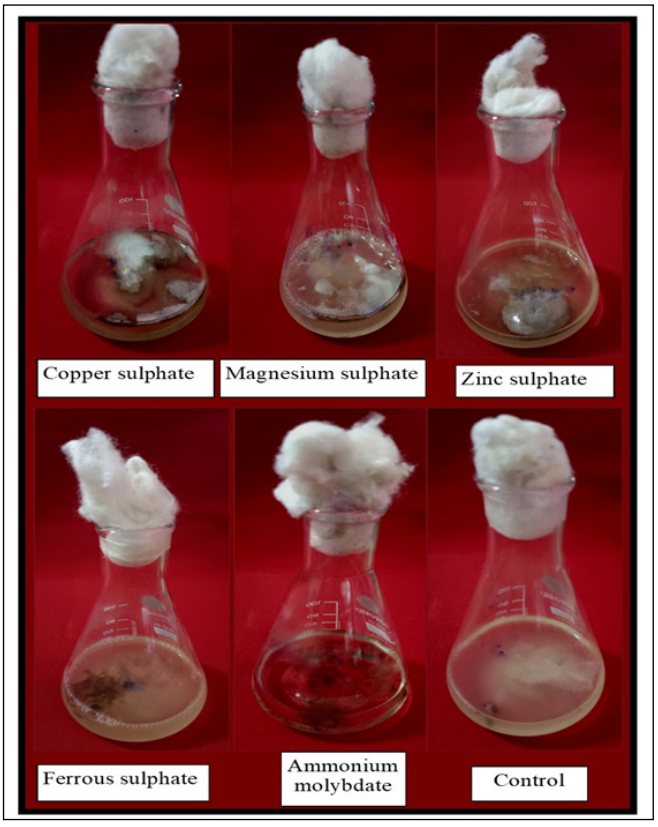

Figure 3: Effect of different trace elements on mycelial biomass production of *Colletotrichum gloeosporioides*

14 days of incubation (617.31 mg) in comparison to 7 days of incubation (233.17 mg). Body of the table reveals that, significantly mean maximum biomass (1432.80 mg) was recorded when magnesium sulphate was used as a

trace element after 14 days of incubation followed by that recorded with zinc sulphate (807.03 mg) after same duration of incubation. However, minimum biomass (48.17 mg) was recorded with control devoid of any trace element after 7 days of incubation followed significantly by the biomass (100.03 mg) produced by the same after 14 days of incubation. An intermediate level of mycelial biomass production was recorded with rest of the trace elements after 7 and 14 days of incubation.

Trace elements influence growth and sporulation of fungi (Karaffa et al., 2021). These are essential for the growth and development of the fungi and required in small quantity. If present in excess may suppress the growth and affect the physiology (Baldrian, 2003). But, no reports regarding use of trace elements in case of *C. gloeosporioides* have been found in the literature, so these results cannot be compared with available data.

4. Conclusion

Among carbon sources, arabinose followed by starch proved to be the best while, among nitrogen sources evaluated, aspartic acid was found to be the best supportive for the growth of *C. gloeospioides.* However, out of five different trace elements tested, maximum biomass of test fungus was recorded with magnesium sulphate.

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