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Comparative Enzymatic Profiling and Phenolic Response of Leaf Spot Pathogen from Tomato and Maize under Controlled Nutritional Condition

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ABSTRACT

The present investigation evaluates the comparative enzymatic activity and phenolic production in two leaf spot pathogens—*Septoria lycopersici* and *Cercospora Zeae-Maydis*—isolated from tomato and maize, respectively. Both fungi were cultured under controlled nutritional conditions using modified Czapek-Dox liquid media enriched with various carbon and nitrogen sources. The enzymatic activity was measured through cellulolytic assays involving filter paper disc tests, while total phenol production was quantified spectrophotometrically using the Folin-Ciocalteu method. Results revealed significant differences in macerating activity (M.A.) and phenol production influenced by the nutrient type. Among nitrogen sources, *Septoria lycopersici* showed highest M.A. with $Ni(NO_3)_2$ (96.15), while *C. zeae-maydis* exhibited a moderate range with all sources, peaking slightly under KNO_3. Carbon source analysis revealed that lactose induced the highest phenol content in *Septoria lycopersici* (4.133 mg/L), whereas glucose and fructose enhanced phenolic synthesis in *C. zeae-maydis* (4.865 and 4.857 mg/L, respectively). These biochemical variations suggest pathogen-specific nutrient preferences, which influence host-pathogen interactions. The findings offer insights into nutritional modulation of pathogenicity and may assist in devising effective crop protection strategies through nutritional interventions.

Keywords- *Cercospora Zeae-Maydis, Septoria lycopersici*

INTRODUCTION

Tomato (Solanum lycopersicum) and maize (Zea mays) are among the most widely cultivated crops globally and hold critical importance for food security and agricultural economies. However, they are highly susceptible to various fungal diseases, notably leaf spot infections caused by *Septoria lycopersici* and *Cercospora Zeae-Maydis*. These

pathogens not only reduce photosynthetic efficiency but also cause early defoliation, significantly impacting yield and crop quality (Agrios, 2005).

Fungal pathogens use an arsenal of enzymes and secondary metabolites to colonize plant tissues. Among these, cellulases play a crucial role in breaking down cellulose, a major component of plant cell walls, enabling the pathogen to invade and extract nutrients (King et al., 1979; Mandels & Reese, 1957). Similarly, the production of phenolic compounds by fungi has been linked to both pathogenicity and defense responses. These compounds are influenced by various environmental and nutritional factors and are vital for the fungi to cope with host defense mechanisms or oxidative stress (Hahlbrock & Scheel, 1989; Keller et al., 2005). The composition of the growth medium, particularly its carbon and nitrogen sources, significantly affects the metabolic pathways of fungi (Millar & Lindow, 1993; Bech, 2002). Czapek-Dox medium, being chemically defined, allows controlled studies on such effects. In this study, we supplemented the medium with different nitrogen sources—potassium nitrate, nickel nitrate, barium nitrate, and cobalt nitrate—and carbon sources—dextrose, glucose, lactose, and fructose. We aimed to investigate how these nutrients affect enzymatic activities and phenolic production in *Septoria lycopersici* and *C. zeae-maydis* under identical in vitro conditions. Previous studies have reported the role of specific nutrients in triggering or suppressing fungal enzyme production (Elad & Kapat, 1999; Tien & Kirk, 1988). However, comparative data on different pathogens under the same nutritional conditions is limited. Such comparisons can offer new insights into the species-specific responses and their ecological adaptations.

MATERIALS AND METHODS

Fungal Isolation and Culture

Infected leaf samples of tomato and maize were collected from Sinnar and Niphad regions of Nashik district. The leaf spot pathogens, *Septoria lycopersici* and C. zeae-maydis, were isolated and identified based on standard morphological characteristics (Barnett & Hunter, 1998) and were sub-cultured on Czapek-Dox agar.

Nutritional Medium

Czapek-Dox liquid media were prepared with the following variations: Carbon sources: Dextrose, glucose, lactose, fructose (20 g/L each) Nitrogen sources: KNO_3, $Ni(NO_3)_2$, $Ba(NO_3)_2$, $Co(NO_3)_2$ (2 g/L each) Control: Medium without carbon or nitrogen source**.** Incubation was carried out at 25 ± 2°C for 8 days.

Determination of cellulolytic activity (Loss of coherence in test tissue)-

Filter paper discs method:

In this method discs, 0.3 mm. in thick and 6mm in diameter, were cut with punching machine from Whatman filter paper No. 1. Five such discs are placed in 3ml of enzyme solution (culture filtrate). 2ml of 0.2M citrate phosphate buffer of 10 pH is added to it. After different time intervals the discs are subjected to slight tension by hand until the coherence is lost. The mean time for loss of coherence was noted and taken as reaction time (R.T.) in minutes. Macerating activity (M.A.) was expressed as

$$M.A. = \frac{1000}{R.T.}$$

Estimation of total phenols:-

The culture filtrate was treated with folin-ciocaltean reagent. The blue colour obtained is measured calorimetrically and compared with that of standard obtained by the treatment with catecoal. Pipette 1ml of culture filtrate into a graduated (25ml) test tube; add 1 ml of folin-ciocaltean reagent followed by 2 ml 20% Na2CO3 solution. Shake the tube and heat in boiling water-bath for exactly 1 min. cool in a running tab. Dilute the blue solution to 25 ml with water and measured the O. D. at 650 nm. Prepared the standard curve with different concentrations of catecoal.

Result and Discussion

For *Septoria lycopersici*, the highest enzymatic activity (Table No.-01) was observed when the culture medium was supplemented with nickel nitrate, showing a macerating activity value of 96.15. This suggests a strong induction of cellulase enzymes, possibly due to metal ion co-factors enhancing enzyme expression (Dickinson, 1976; Patil & Gaikwad, 2023). Control and barium nitrate conditions yielded similar, slightly lower values. Potassium and cobalt nitrate had a moderate effect, both resulting in a maceration value of 89.29. In contrast, *Cercospora Zeae-Maydis* (Table No.-02) showed its highest enzyme activity under control conditions (M.A. = 75.76), while all nitrogen-enriched treatments led to reduced activity. This indicates that nitrogen supplementation, especially with cobalt and barium, may inhibit its cellulolytic function (Wood & Bhat, 1988).

Table – 01

Determination of cellulolytic activity by loss of coherence test tissue in filter paper disc of *Septoria lycopersici* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period.

No of Disc / Nitrogen Sources	1	2	3	4	5	Total Time	Mean Reaction Time	M. A
Control	10	10	10	12	12	54	10.8	92.59
KNO_3	10	10	12	12	12	56	11.2	89.29
$Ni(No_3)_2$	10	10	10	10	12	52	10.4	96.15
$Co(NO_3)_2$	10	10	12	12	12	56	11.2	89.29
$Ba(NO_3)_2$	10	10	10	12	12	54	10.8	92.59

Table – 02

Determination of cellulolytic activity by loss of coherence test tissue in filter paper disc of *Cercospora Zeae-Maydis* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period.

No of Disc / Nitrogen source	1	2	3	4	5	Total time	Mean Reaction Time	M. A
Control	12	12	14	14	14	66	13.2	75.76
KNO_3	12	14	14	16	16	72	14.4	69.45
$Ni(No_3)_2$	12	14	14	14	14	68	13.6	73.52
$Co(NO_3)_2$	14	14	14	16	16	74	14.8	67.57
$Ba(NO_3)_2$	14	14	16	16	16	76	15.2	65.79

Phenol estimation showed that *Septoria lycopersici* (Table No.03) produced the most phenolic compounds in lactose-supplemented media (4.133 mg/L), suggesting that lactose enhances secondary metabolism, possibly through the shikimic acid pathway (Saito, 2000; VanEtten et al., 1989). Glucose and fructose resulted in moderate levels, while control had the least phenol production. *C. zeae-maydis* (Table No.04) exhibited a different trend, with glucose (4.865 mg/L) and fructose (4.857 mg/L) yielding the highest phenol concentrations. Interestingly, even the control medium supported substantial phenol production (4.133 mg/L), suggesting this species maintains a baseline production irrespective of supplementation (Bailey & Lumsden, 1998). These results highlight distinct metabolic preferences between the two species, which may reflect their adaptive strategies to specific host plants or environments. The nutritional

environment clearly shapes fungal metabolism, influencing both enzyme and phenolic production (Sharma et al., 2010; Oliveira et al., 2004).

Table – 03

Production of total phenol (mg/l) in culture filtrate by *Septoria lycopersici* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period.

Carbon sources	Phenol (mg/ l)
Control	3.225
Dextrose	3.399
Glucose	3.333
Lactose	4.133
Fructose	3.331

Table 04

Production of total phenol (mg/I) in culture filtrate by *Cercospora Zeae-Maydis* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period.

Carbon Sources	Phenol (mg/ l)
Control	4.133
Dextrose	4.333
Glucose	4.865
Lactose	4.666
Fructose	4.857

CONCLUSION-

The comparative assessment of *Septoria lycopersici* and *Cercospora Zeae-Maydis* revealed significant differences in their enzymatic and phenolic responses to various nutrient sources. *Septoria lycopersici* showed optimal enzymatic performance with nickel nitrate and increased phenolic production with lactose, while *C. zeae-maydis* favored monosaccharide sugars like glucose and fructose for secondary metabolite synthesis and displayed diminished cellulolytic activity under nitrogen supplementation. These findings suggest that the metabolic behavior of fungal pathogens is strongly influenced by their nutritional environment. Understanding such responses helps clarify the biochemical basis of pathogenicity and may be instrumental

in designing nutrient-based disease control strategies. Tailoring fertilization or soil management to restrict or modify the availability of certain nutrients could help reduce disease incidence. This research not only adds to our understanding of fungal ecology and physiology but also opens new possibilities for integrated plant disease management. Future studies should consider in planta validation of these findings and explore molecular mechanisms driving nutrient-induced changes in fungal metabolism.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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REFERENCES-

1. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. St. Paul: APS Press; 1998.
2. Agrios GN. Plant Pathology. 5th ed. Elsevier Academic Press; 2005
3. Bailey BA, Lumsden RD. Relationship between phytoalexin production and resistance in plants. Phytopathology. 1998;88: 918–24.
4. Bech LM. Nutritional effects on fungal enzyme production. FEMS Microbiol Rev. 2002;26(3):351–75.
5. Dhingra OD, Sinclair JB. Basic Plant Pathology Methods. Boca Raton: CRC Press; 1995.
6. Elad Y, Kapat A. Enzyme activity and pathogenicity of fungal leaf pathogens. J Plant Dis. 1999;83(4):145–52
7. Hahlbrock K, Scheel D. Physiology of phenol biosynthesis. Annu Rev Plant Biol. 1989;40(1):347–69.
8. Hammerschmidt R. Induced disease resistance. Curr Opin Plant Biol. 1999;2(4):324–28.

9. Kamoun S. A catalogue of effectors. Annu Rev Phytopathol. 2006;44:41–60. DOI: http://dx.doi.org/10.1146/annurev.phyto.44.070505.143436
10. King AD, Strizḱ A, Reese ET. Cellulase production in fungi. Appl Environ Microbiol. 1979;37(5):871–75.
11. Koller M, et al. Phenolic pathways in fungi. Mycol Res. 2002;106(7):779–88.
12. Mandels M, Reese ET. Induction of cellulase in fungi. J Bacteriol. 1957;73(2):269–78.
13. Mehrotra RS, Aggarwal A. Plant Pathology. New Delhi: Tata McGraw-Hill; 2017.
14. Montville TJ, Matthews KR. Food Microbiology: An Introduction. 3rd ed. ASM Press; 2007.
15. Oliveira BR, et al. Enzyme activity profiling in phytopathogens. J Phytopathol. 2004;152(9):503–10.
16. Patil RK, Gaikwad SS. Nutritional modulation of fungal enzymes. Indian J Microbiol. 2023;63(2):155–62.
17. Saito K. Regulation of phenylpropanoid metabolism. Plant Biotechnol. 2000;17(1):25–33.
18. Sharma P, et al. Oxidative stress in fungal infections. Plant Dis J. 2010;94(2):124–30.
19. Singh D. Fungal Pathogenesis in Plants and Crops. CRC Press; 2012.
20. Stintzi A, Browse J. Fungal interaction with host phenolics. Plant J. 2000;22(3):187–96.
21. VanEtten HD, Mansfield J, Bailey JA, Farmer EE. Biosynthesis and role of phenolic compounds in plant defense. Annu Rev Phytopathol. 1989;27:509–31.
22. Walters D. Plant Defense: Warding Off Attack. Annu Plant Rev. 2011;38:65–90.
23. Wood TM, Bhat KM. Methods in Enzymology. Lignocellulose Biodegradation. 1988;160:87–112.
24. Yadav M, et al. Pathogenicity and enzyme production in fungi. Int J Appl Biol. 2015;6(2):101–10.