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# Pectolytic enzymatic activity and phenolic response of leaf spot pathogen from sugarcane (*Saccharum officinarum*) under controlled nutritional condition

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**Abstract**
Sugarcane (*Saccharum officinarum*) is a major commercial crop affected by various biotic stresses, among which foliar pathogens like *Helminthosporium tetramera* play a significant role in yield reduction. This study aimed to evaluate the pectolytic enzyme activity and phenolic response of *H. tetramera* under different nutritional conditions using Czapek-Dox medium amended with varied carbon and nitrogen sources. The enzymatic assay, based on the maceration of potato discs, was conducted to determine the macerating enzyme (ME) activity, while total phenolic content in culture filtrates was estimated using the Folin–Ciocalteu method. Results demonstrated that among carbon sources, fructose supported the highest pectolytic activity (ME = 200), whereas lactose enhanced phenolic production (3.595 mg/L). Nitrogen source $Co(NO_3)_2$ significantly induced both macerating enzyme activity (ME = 166.66) and phenol production (3.225 mg/L). The differential enzymatic expression and phenolic secretion suggest an adaptive metabolic response of *H. tetramera* influenced by nutritional composition.

**Keywords:** Sugarcane, *Helminthosporium tetramera*, macerating enzyme, phenolic content, carbon and nitrogen sources, pectolytic activity

## Introduction

Sugarcane (*Saccharum officinarum* L.), a critical source of sugar and bioethanol, is extensively cultivated in tropical and subtropical regions. In India, it represents a vital agricultural commodity contributing to both economy and employment. However, its production is severely challenged by biotic stresses, especially fungal pathogens, which are responsible for significant yield losses and reduced crop quality [1, 2]. Among foliar diseases, leaf spot caused by *Helminthosporium tetramera* is particularly destructive, inducing necrotic lesions that impair photosynthesis and accelerate senescence [3].

The success of phytopathogens largely depends on their enzymatic arsenal, particularly pectolytic enzymes such as polygalacturonases, pectin lyases, and pectate lyases, which degrade the plant cell wall's pectin matrix [4]. These enzymes facilitate pathogen entry and colonization by weakening plant tissues, thus enhancing virulence. Macerating enzyme (ME) assays serve as a standard tool to quantify such pectolytic activities [5]. These assays provide insights into how pathogens adapt their enzymatic expression in response to available nutrients, especially carbon and nitrogen sources. Carbon sources not only act as energy substrates but also as signaling molecules that regulate fungal metabolism and pathogenicity [6, 7]. For example, fructose and lactose are known to induce differential expression of hydrolases in fungi like Alternaria and Fusarium [8]. Similarly, nitrogen availability has been shown to modulate the synthesis of secondary metabolites and enzymes through mechanisms involving nitrogen catabolite repression [9, 10]. Such modulation is often strain-specific and influenced by the chemical nature of nitrogen, whether organic or inorganic [11]. Phenolic compounds, another important component in plant–pathogen interactions, serve as both antimicrobial agents and signaling molecules [12]. Their presence in culture filtrates reflects the oxidative stress response of pathogens and potential host-derived resistance mechanisms [13]. Fungal species often manipulate host phenolics either by degradation or by inducing their accumulation, which can lead to host hypersensitivity [14]. Despite the known importance of nutritional modulation in plant–pathogen dynamics, limited studies have evaluated the enzymatic and phenolic responses of *Helminthosporium tetramera* under controlled nutritional conditions. Most previous studies have focused on pathogens like Colletotrichum, Fusarium, and Alternaria [15, 17], with little information available on *H. tetramera* affecting sugarcane. Thus, this study addresses a critical gap by evaluating the macerating enzyme activity and phenolic response of *H. tetramera* grown in Czapek-Dox medium supplemented with different carbon (dextrose, glucose, lactose, fructose) and nitrogen sources ($KNO_3$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $Ba(NO_3)_2$).

The objectives of the present study were: (i) to assess the influence of selected carbon and nitrogen sources on the pectolytic enzyme activity of H. tetramera, (ii) to quantify the phenolic compounds in the culture filtrates under these conditions, and (iii) to interpret the nutritional modulation of pathogenic virulence factors. This research provides a foundation for future biocontrol strategies and offers biochemical markers for pathogen aggressiveness under varied environmental contexts.

## Materials and Methods

### Fungal Isolation and Culture

Infected leaf samples of Sugarcane (*Saccharum officinarum*) were collected from Sinnar and Niphad regions of Nashik district. The *Helminthosporium tetramera* leaf

spot disease were Isolated from Sugarcane plant and identified based on standard morphological characteristics (Barnett & Hunter, 1998) and were sub-cultured on Czapek-Dox agar.

**Activity of Pectolytic enzyme- (Macerating enzyme)**

Cylindrical plugs, 8 mm in diameter, are cut from healthy potato tubers with a No. 4 cork borer. The plugs are injected with distilled water under vacuum for one hour. Disc of 0.4 mm thickness are cut with sliding hand microtome from these plugs. They are washed quickly with distilled water and stored in a petridish.

Ten discs are placed in 5 ml of an enzyme solution (culture filtrate) in a watch glass. At interval of 5 min. they were subjected to slight tension by hand or pulled apart. As soon as the first disc has lost coherence, the mean time for loss of coherence in all discs were noted and taken as the reaction time (R.T.) in minute.

$$\text{Mean time} = \frac{\text{Sum of time when discs macerated}}{\text{No of attempts at which disc macerated}}$$

Macerating activity (ME) is expressed as

$$ME = \frac{1000}{R.T.}$$

The tests were carried out at room temperature and at 5 pH.

**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. Prepare the standard curve with different concentrations of catecoal. Read the unknowns from known curve and calculate the amount of total phenol.

**Result and Discussion**

**Pectolytic Enzyme Activity (Table No.1 and 2):** The macerating enzyme (ME) activity was highest in cultures amended with fructose (ME = 200), followed by lactose (ME = 138.88), and glucose (ME = 131.57). Control cultures without supplemental carbon showed comparatively lower activity (ME = 156.25). Fructose appeared to be the most effective inducer of pectolytic activity, possibly due to its rapid metabolization and signaling potential, which aligns with findings from Fusarium oxysporum studies [18,19]. Among nitrogen sources, $Ni(NO_3)_2$ demonstrated the highest ME value (200), followed by $Co(NO_3)_2$ and $Ba(NO_3)_2$ (both 166.66), indicating a strong role of nitrate-derived compounds in enhancing pectolytic enzyme production. This result parallels earlier observations in Botrytis cinerea and Sclerotinia sclerotiorum, where nitrate forms significantly induced hydrolase production [20, 21].

**Phenolic Response (Table No.3 and 4):** Culture filtrates showed varied total phenol concentrations under different nutrient conditions. Lactose yielded the highest phenol level (3.595 mg/L), followed by $Co(NO_3)_2$ (3.225 mg/L). These results indicate that phenolic induction may be associated with increased metabolic activity or oxidative stress within the pathogen, as observed in similar studies with *Alternaria brassicicola* [22]. The relationship between ME and phenolic content suggests a nutritional co-regulation of virulence and stress responses in *H. tetramera*. While carbon sources modulated energy metabolism and enzyme secretion, nitrogen sources appeared to impact stress-related pathways, possibly via redox signaling and secondary metabolite pathways [23, 24].

**Table 1:** Determination of pectolytic activity by Macerating enzyme method of *Helminthosporium tetramera* grown on Czapek-Dox liquid medium containing different carbon sources at 8th day incubation period

| Time Carbon sources | 2 | 4 | 6 | 8 | 10 | 12 | 14 | No of time discs macerated | Total Time | Mean time | M.E. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 3 | 2 | 1 | 2 | - | 2 | - | 5 | 32 | 6.4 | 156.25 |
| Dextrose | 2 | - | 1 | 1 | 3 | 2 | 1 | 6 | 52 | 8.6 | 115.38 |
| Glucose | 1 | - | 2 | 3 | 3 | 1 | - | 5 | 38 | 7.6 | 131.57 |
| Lactose | 1 | 2 | - | 3 | 3 | 1 | - | 5 | 36 | 7.2 | 138.88 |
| Fructose | 2 | 3 | 3 | 2 | - | - | - | 4 | 20 | 5 | 200 |

**Table 2:** Determination of pectolytic activity by Macerating enzyme method of *Helminthosporium tetramera* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period.

| Time Nitrogen sources | 2 | 4 | 6 | 8 | 10 | 12 | 14 | No of time discs Macerated | Total Time | Mean time | M.E. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 3 | 1 | 2 | 2 | 1 | 1 | - | 6 | 42 | 7 | 142.85 |
| $KNO_3$ | - | 3 | 2 | 3 | 1 | 1 | - | 5 | 40 | 8 | 125 |
| $Ni(No_3)_2$ | 2 | 2 | 3 | 3 | - | - | - | 4 | 20 | 5 | 200 |
| $Co(NO_3)_2$ | 2 | 3 | 2 | 1 | 2 | - | - | 5 | 30 | 6 | 166.66 |
| $Ba(NO_3)_2$ | 3 | 2 | 2 | 2 | 1 | - | - | 5 | 30 | 6 | 166.66 |

**Table 3:** Production of total phenol (mg/I) in culture filtrate by *Helminthosporium tetramera* grown on Czapex-Dox liquid medium containing different carbon sources at 8th day incubation period.

| Carbon Sources | Phenol ( mg/ I ) |
|---|---|
| Control | 3.045 |
| Dextrose | 3.331 |
| Glucose | 2.865 |
| Lactose | 3.595 |
| Fructose | 3.399 |

**Table 4:** Production of total phenol (mg/l) in culture filtrate by *Helminthosporium tetramera* grown on Czapek-Dox liquid medium containing different nitrogen sources at 8th day incubation period

| Nitrogen Sources | Phenol ( mg/ I ) |
|---|---|
| Control | 2.675 |
| $KNO_3$ | 2.733 |
| $Ni(No_3)_2$ | 3.066 |
| $Co(NO_3)_2$ | 3.225 |
| $Ba(NO_3)_2$ | 2.865 |

## Conclusion

This study clearly establishes that the pectolytic activity and phenolic response of *Helminthosporium tetramera*, a foliar pathogen of sugarcane, are significantly influenced by the nature of nutritional inputs. Fructose and $Ni(NO_3)_2$ emerged as potent inducers of macerating enzyme activity, while lactose and $Co(NO_3)_2$ stimulated higher phenolic production. These findings indicate that specific nutritional environments enhance enzymatic and biochemical responses associated with fungal virulence. Understanding the role of such nutritional factors in pathogen development provides deeper insights into host–pathogen interactions and opens potential avenues for disease management. For example, regulating the availability of certain nutrients in the rhizosphere or foliar environment may suppress pathogen aggressiveness by limiting the induction of key virulence factors. The data also hint at possible metabolic trade-offs between growth, enzyme secretion, and stress adaptation mechanisms, which may be exploited in integrated pest management systems.

Furthermore, the study adds to the growing body of literature emphasizing the plasticity of fungal metabolism under changing environmental conditions. It underlines the necessity of considering nutritional variables in both in *vitro* and in *vivo* pathogenicity assessments.

## 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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