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Beyond nourishment: The impact of micronutrients on *Magnaporthe grisea* sporulation infecting finger millet

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Abstract
Finger millet blast, caused by *Magnaporthe grisea* is a devastating and recurrent issue across all finger millet cultivation areas globally. The main challenge linked to cultivating the *M. grisea* culture involve achieving sporulation on culture plates. Therefore, to investigate the impact of amino acids (L-tyrosine, arginine and phenylalanine) and vitamins (riboflavin and ascorbic acid) in promoting sporulation, experiment was conducted at three concentrations (0.01g /l, 0.02 g/l and 0.03 g/l) using three different cultural media, rice straw extract agar (RSEA), oat meat agar (OMA) and a combination of RSEA and OMA. Among all the amendments, L-tyrosine at the concentration of 0.03 g/l recorded highest mean radial growth of 9.00 cm, which was highest among all the treatments. Whereas, RSEA+OMA amended with phenyl alanine at 0.03 g/l produced highest number of conidia (33.33 conidia/40x microscopic view).

Keywords: Beyond nourishment, micronutrients, *Magnaporthe grisea*, finger millet

Introduction
Finger millet is highly valued for its nutritional content, providing high levels of calcium, iron, protein and essential amino acids. Its gluten-free, making it a great choice for those with celiac disease. Its cultivation is also environmentally friendly due to its drought tolerance (Poonacha *et al.*, 2023) [11]. Despite its resilience, the productivity of the plant is susceptible to various biotic and abiotic factors. Notably, the blast disease caused by *Magnaporthe grisea* incurs substantial yield losses. According to Prajapathi (2013) [12] the loss of grain yield occurred due to the blast disease was 35.78 per cent along with loss of fodder yield of 43.72 per cent. Nevertheless, the pathogen exhibits high variability, characterized by a pathogenic lifestyle distinguished by remarkable morphogenetic and biochemical specialization, resulting in the emergence of novel pathotypes.

To develop resistant genotypes against these new pathotypes, it is imperative to evaluate a large number of accessions for blast disease resistance for which abundant sporulation of the pathogen being a crucial requirement. In experiments with three isolates of *Pyricularia grisea* originating from rice, finger millet and *Panicum* sp., prune agar and oatmeal agar were found to promote the highest levels of mycelial growth and sporulation for isolates from both rice and finger millet (Khadka *et al.*, 2012) [6]. Rajashekara *et al.*, 2016 [13] reported, the Rice straw extract oat meal agar media was suitable for inducing sporulation in *M. oryzae* cultures.

Newly isolated cultures demonstrate a greater capacity to produce conidia in petri plates, but over time or during extended storage periods, their sporulation ability diminishes. So, use of media with common compositions will not be able to stimulate the sporulation in the cultures. The common challenge of achieving sporulation in *Pyricularia* sp. cultures is well recognized in studies (Narayanarao *et al.*, 1972) [9]. Plant pathogens rely on amino acids as vital nutrients, crucial for regulating diverse biological functions within microorganisms. These functions encompass pivotal processes such as cell division, cell wall formation, cell growth, metabolism, inter-microbial communication (quorum sensing), and interactions with hosts (Idrees *et al.*, 2020) [5]. Additionally, vitamins serve indispensable roles in the spore production of fungal plant pathogens by acting as co-factors in numerous enzymatic reactions essential

for metabolism, growth, and development (Averianova *et al.*, 2020) [1]. Thus, both amino acids and vitamins are intricately involved in the growth and development of plant pathogens.

So, Keeping above problems in view, efforts have been undertaken to know the influence of micro-nutrients on the sporulation of *M. grisea* by supplementing the optimal media with micro-nutrients such as vitamins and amino acids.

Materials and Methods

Isolation of the pathogen and proving pathogenicity

Blast-infected finger millet tissues showing typical blast symptoms were cut into small bits. The standard spore drop technique was utilized for the isolation of mono-conidial isolate of *M. grisae* (Rajashekara *et al.*, 2016) [13]. The pathogenicity of each isolate was tested using disease-free finger millet seeds. To develop healthy seedlings, seeds were sowed in pots filled with sterile soil and these seedling were sprayed with conidial suspension of *M. grisae* 20 days after sowing and observed for the development of the disease symptoms. The pathogen was re-isolated from the symptoms so developed on the inoculated plants and compared with the original culture to prove pathogenicity.

Identification of suitable media/substrate for spore production

Three cultural media i.e., Rice straw extarct agar (RSEA), Oat meal agar (OMA) and Rice straw extract oat meal agar medium (RSEA+OMA) was prepared with the following compositions.

a) Rice Straw Extract Agar Medium (RSEA)

Hundred and fifty grams of rice straw was boiled in 800 mL of water for 30 minutes. After filtering the decoction through muslin cloth, 20 grams of sucrose was added, and the final volume was made up to 1000 mL by adding distilled water. The pH was adjusted to 6.5 and the broth was autoclaved at 1.1 kg cm^{-2} pressure for 15 minutes at 121 °C. Twenty grams of agar was added to the Rice Straw Extract (RSE) broth and the required quantity of the prepared medium was dispensed into required number of conical flasks, plugged with non-absorbent cotton and wrapped with paper. The flasks containing the medium were autoclaved at 1.1 kg cm^{-2} pressure for 15 minutes at 121 °C.

b) Oat meal agar (OMA)

Oat meal powder was dissolved in 500 mL of distilled water. Agar (20 g) was melted separately in 500 mL distilled water. Both the solutions were mixed thoroughly and the mixed solution was sterilized at 1.1 kg cm^{-2} pressure for 15 minutes and preserved for further use (Waller *et al.*, 2001).

c) Rice straw extract oat meal agar medium (RSEA+OMA)

Rice straw extract oat meal agar medium was prepared by adding 20 g oat meal agar powder and 10 g agar powder in 1000 ml RSE Broth. The medium was thoroughly mixed by warming in microwave oven before autoclaving at 15 lbs pressure for 15 min at 121 °C.

Three aminoacids (L-tyrosine, arginine and phenylalanine) and two vitamins (riboflavin and ascorbic acid) were added at three concentration *i.e.*, 0.01 g/l, 0.02 g/l and 0.03 g/l were incorporated and mixed well in conical flask containing three different media at about 50 °C and poured 20 ml in each Petri dish of 9 cm diameter. The freshly growing mycelium from the selected culture plate was cut 5 mm with cork borer and inoculated at the centre of the Petri dish under aseptic condition in an isolation chamber. The mycelial growth and amount of sporulation was recorded seven days post inoculation.

Results and Discussion

Isolation of the pathogen

The pathogen was isolated by following spore drop technique. Microscopic analysis of the culture revealed the presence of pyriform conidia that were two-septate, three-celled, hyaline to brown and bore basal appendage at the point of attachment to the conidiophore. On RSEA, colony was whitish to greyish with black colour pigmentation on reverse side of the Petri plate (Fig. 1)

Similar approach was adopted by Dhua (1986) [2] to isolate *M. oryzae* and Palanna *et al.* 2023 [10] used spore drop technique to isolate *M. grisea* infecting finger millet. The isolate was identified as *M. grisea* based on morphology of colony and conidial characters comparing with the original descriptions (Saccardo, 1880; Hebert, 1971) [14]. The morphological characters were found to be similar to the previous studies (Klaubauf *et al.* (2014) [7], Shanmugapackiam *et al.* (2019) [16] and Palanna *et al.* (2023) [10].

Pathogenicity test

The pathogenicity of the *M. grisea* isolate was confirmed by conducting experiments on finger millet susceptible variety (*Uduru mallige*) under greenhouse condition. Initial signs of infection appeared between 7 to 12 days post-inoculation, manifesting as small brown spots. These spots eventually developed into spindle-shaped lesions with pointed ends, measuring approximately 0.5 to 1.0 cm wide, characterized by a greyish center and surrounded by brownish margins. The pathogen was subsequently isolated from the infected leaves, and the morphology of the mycelium, conidia, and conidiophores closely matched the original descriptions (Fig. 1)

Similar kind of results proving pathogenicity was done by Urashima and Silva (2011) [18], Gowrisri *et al.* (2019) [3] and Shahriar *et al.* (2020) [15]. They observed symptoms ranging from tiny brown specks to spindle-shaped lesions measuring a few millimeters across. These lesions typically feature small grey or whitish centers bordered by brown margins upon inoculation with pathogen suspension.

Identification of suitable media/substrate for spore production

The impact of amino acids (L-tyrosine, arginine and phenylalanine) and vitamins (riboflavin and ascorbic acid) in promoting sporulation, experiment was conducted at three concentrations (0.01 g/l, 0.02 g/l and 0.03 g/l) using three different cultural media, Rice Straw Extract Agar (RSEA), Oat Meat Agar (OMA) and a combination of RSEA and OMA.

The highest mean radial growth was recorded in RSEA+OMA (7.03cm) followed by OMA (6.67cm). The lowest mean radial growth was recorded in RSEA (6.50cm). Among all the media amended with different compounds, the RSEA+OMA amended with L-tyrosine recorded highest

mean radial growth of 8.10cm followed by phenyl alanine amended RSEA media (8.03cm). Whereas, ascorbic acid amended RSEA media recorded least mean radial growth of 3.92cm (Table 1).

The sporulation in the culture media supplimented with amendments was recorded after seven days post inoculation. Among all the culture media, the highest mean sporulation was recorded in RSEA+OMA with 21.70 conidia/40x microscopic view. Whereas, the least mean sporulation was recorded in the OMA (6.67 conidia/40x microscopic field). Among all the media amended with different compounds, The RSEA+OMA amended with phenyl alanine recorded highest mean sporulation of 29.33 conidia/40x microscopic view. The lowest mean sporulation was recorded in OMA amended with arginine (12.00 conidia/40x microscopic field) (Table 2).

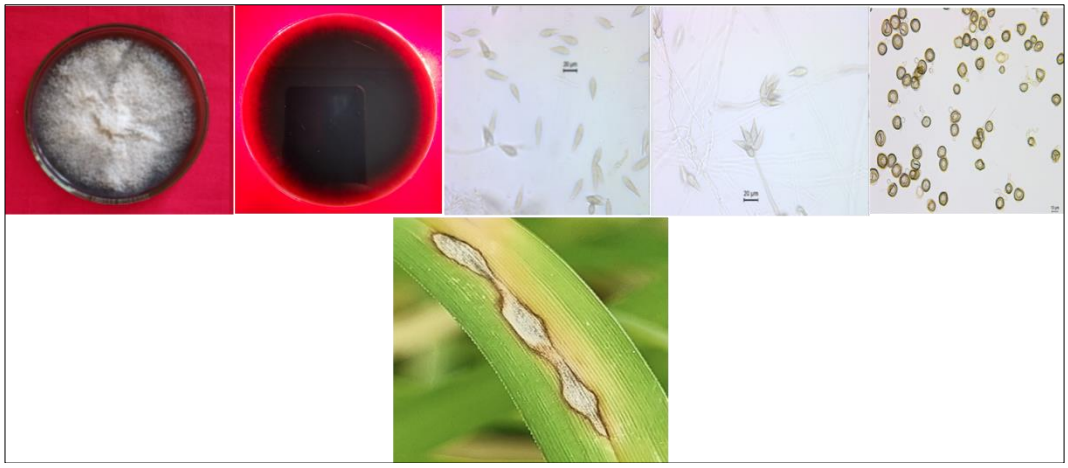

Fig 1: Morphological, cultural and virulence characteristics of *M. grisea* isolates from finger millet. A) Upper colony morphology, B) pigmentation at lower side of plate, C) Morphology of conidia, D) conidiophores bearing conidia, E) Appressoria and D) Symptoms on leaves 10 dpi

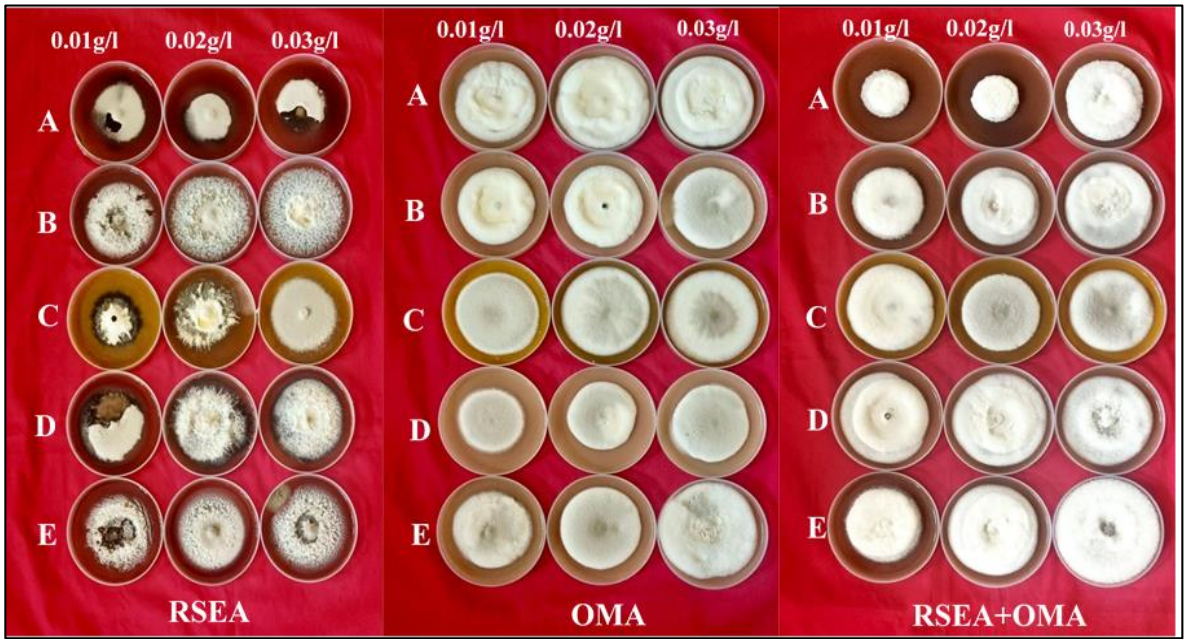

Fig 2: Effect of aminoacids and vitamins on sporulation of *M. grisae*

Table 1: Effect of aminoacid and vitamins on the radial growth (cm) of *M. grisea* culture

Sl. No	Culture media	RSEA			Mean	OMA			Mean	RSEA+OMA			Mean
	Amino acid/Vitamins concentration	0.01 g/l	0.02 g/l	0.03 g/l		0.01 g/l	0.02 g/l	0.03 g/l		0.01 g/l	0.02 g/l	0.03 g/l	
1	Ascorbic acid	5.00	5.30	5.00	3.92	4.50	5.00	8.80	6.10	6.00	7.00	7.70	6.90
2	Phenyl alanine	7.60	8.00	8.50	8.03	6.70	7.10	7.40	7.06	6.00	7.00	7.70	6.90
3	Riboflavin	5.50	6.40	7.00	6.30	7.50	7.50	7.60	7.53	5.00	7.00	7.70	6.56
4	Arginine	6.50	7.50	8.00	7.33	4.50	5.30	6.80	5.53	5.00	7.30	7.80	6.70
5	L- Tyrosine	6.70	7.00	7.10	6.93	6.70	7.30	7.40	7.13	8.00	7.30	9.00	8.10
	Mean	6.26	6.84	7.12	6.50	5.98	6.44	7.60	6.67	6.00	7.12	7.98	7.03
		Aminoacids/vitamins (A)			Media (B)			Concentration (C)		A X B X C			
	SE(m)	0.09			0.06			0.07		0.27			
	C.D @ 1%	0.25			0.18			0.20		0.76			

Table 2: Effect of aminoacid and vitamins on the sporulation of *M. grisea* culture

Sl. No	Culture media	RSEA			Mean	OMA			Mean	RSEA+OMA			Mean
	Amino acid/Vitamins concentration	0.01 g/l	0.02 g/l	0.03 g/l		0.01 g/l	0.02 g/l	0.03 g/l		0.01 g/l	0.02 g/l	0.03 g/l	
1	Ascorbic acid	13.33	13.66	14.66	13.88	12.66	13.33	14.33	13.44	14.33	15.66	16.00	15.33
2	Phenyl alanine	22.00	25.00	27.00	24.66	20.33	23.33	24.00	22.33	26.66	28.00	33.33	29.33
3	Riboflavin	18.66	19.66	22.00	20.10	16.33	17.66	21.66	18.55	22.00	23.33	25.33	23.55
4	Arginine	13.66	14.66	15.66	14.66	11.33	12.33	14.33	12.00	15.00	16.00	16.66	15.88
5	M- Tyrosine	22.00	23.00	25.66	23.55	18.00	21.33	22.66	20.66	23.00	24.66	25.66	24.44
	Mean	17.93	19.19	20.99	19.37	15.73	17.59	19.39	17.39	20.19	21.53	23.39	21.70
		Aminoacids/ vitamins (A)			Media (B)			Concentration (C)		A X B X C			
	SE(m)	0.22			0.17			0.16		0.65			
	C.D @ 1%	0.61			0.47			0.46		1.83			

The RSEA+OMA amended with L-tyrosine at the concentration of 0.03 g/l recorded highest mean radial growth of 9.00cm, which was highest among all the treatments. Whereas, RSEA+OMA amended with phenyl alanine at 0.03 g/l produced highest number of conidia (33.33 conidia/40x microscopic field). Whereas, least sporulation was recorded in arginine (0.01 g/l) amended OMA media with 11.33 conidia/40x microscopic view. Among all cultural media, the RSEA+OMA was found to be most suitable for culture growth and sporulation (Fig 2).

The Rajashekara *et al.* (2016) [13] documented that, after seven days of plating, a higher level of sporulation was observed in the rice straw extract oat meal agar medium compared to the other two media. Narayanarao *et al.*, 1972 [9] found that the isolates from *Eleusine coracana* and *Setaria italica* sporulated better with shikimic acid and phenylalanine than with tyrosine. A crucial aspect of plant-pathogen interactions involves the significant contribution of amino acids. Pathogens find it more energetically advantageous to obtain and metabolize amino acids directly from plants. Hence, various pathogens have the ability to specifically influence the activation of genes essential for amino acid transport (Sonawala *et al.*, 2018 and Li *et al.*, 2020). This strategic manipulation facilitates the direct acquisition of amino acids from the host plants. Which will enhance the ability of the plant pathogen proliferate. The phenyl alanine is an essential amino acid which has to be obtained from the food source. So amendment of Phenyl alanine helped in the increased sporulation of the pathogen.

Conclusion

This study provides insights into how micronutrients enhance the sporulation and mycelial growth of the pathogen. Among all the amendments, L-tyrosine at the concentration of 0.03 g/l recorded highest mean radial growth of 9.00 cm, which was highest among all the treatments. Whereas, RSEA+OMA amended with phenyl alanine at 0.03 g/l produced highest number of conidia (33.33 conidia/40x microscopic view). This study helps in screening genotypes through artificial inoculation in both greenhouse and field conditions, as the sporulation of the pathogen is a prerequisite for this screening process. Consequently, it contributes to the development of disease-resistant genotypes against blast disease in finger millet.

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