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Influence of endophytes on seed yield in heat resilient maize hybrid/ RCRMH-2 seed production

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Abstract

The field experiment was conducted to study the influence of endophytes on seed yield and yield parameters in heat-resilient maize hybrid RCRMH-2 seed production at Agriculture Research Station and College of Agriculture, Bheemaranagudi, during Rabi 2024-25. The experiment was laid out in two factorial randomized block design with three replications and twelve treatments. The first factor consists Endophytes (P) viz., *Beauveria bassiana* (P₁), *Metarhizium anisopliae* (P₂), *Bacillus subtilis* (P₃) and second factor consists methods of application (M) viz., M₁: Seed treatment, M₂: Seed priming, M₃: Foliar spray and M₄: Soil drenching to both male (CML 451) and female (CAL1514) the parental lines. Among all treatments, P₂M₁ (*Metarhizium anisopliae* via seed treatment) recorded significantly the highest cob length (14.35 cm), cob diameter (3.49 cm), cob weight (101.52 g), number of seeds per cob (234.20), hundred seed weight (35.61 g), seed yield per hectare (30.77 q) and shelling percentage (85.98) over the remaining treatments and was followed by P₂M₂ (*Metarhizium anisopliae* via priming). The study indicated that seed treatment with *Metarhizium anisopliae* (@ 20 g kg⁻¹) can be used to get maximum seed yield, which is beneficial to the seed growers and producers.

Keywords: Endophytes, *Metarhizium anisopliae*, heat resilient maize hybrid, foliar spray, seed treatment

Introduction

Maize (*Zea mays* L.) is the world's leading crop and is widely cultivated as a cereal grain that was domesticated in Central America. It is one of the most versatile emerging crops, having wider adaptability. Globally, maize is known as the queen of cereals because of its highest genetic yield potential. Maize has high nutritional profile and is composed of starch (72%), protein (10%), fiber (8.5%), oil (4.8%), sugar (3.0%) and ash (1.7%), underscoring its nutritional significance in agricultural and dietary terms (Hokmalipour *et al.*, 2010)^[6].

Maize is the only food cereal that can be grown in diverse seasons, ecologies and uses, because of potential market and lower risk involved in the production. To meet the demand of the seed requirement the private and public sectors have introduced their hybrids.

As a public sector organization, the University of Agricultural Sciences, Raichur has developed heat stress resilient and multiple disease resistant maize hybrid - RCRMH-2.

Currently, 1235 billion metric tonnes of maize is being produced together by over 170 countries from an area of 235 million ha with an average productivity of 9.94 tonnes ha⁻¹ (Anon., 2024 a)^[1]. Among the maize-growing countries, India ranks 4th in area and 7th in production, representing around 4 per cent of the world's maize area and 2 per cent of total production. (Anon., 2024 b)^[2].

Recent studies predicted that world population may reach 8.6 billion by 2030 (www. India budget. gov.in) such an increasing human population places pressure on agriculture. There is a need to always meet the increasing demand. To feed this population and to run industries, an increase in production is needed. On the other side, today's agriculture is becoming input-intensive with more reliance on synthetic fertilizers and agrochemicals to fulfill the feed demand of the growing numbers. Use of synthetic fertilizer since last few years is impacting the soil quality (Lin *et al.*, 2019)^[9]. In this scenario, the use of beneficial endophytic microbes as an enchanting strategy to overcome the use of synthetic and chemical products (Ghulam *et al.*,

2018)^[5].

Endophytes are the endosymbionts that live and colonize the intercellular or intracellular locations of plants (Pimentel *et al.*, 2011)^[11] and complete their life cycle within the host without causing harm to host plants (Carroll, 1986; Petrini *et al.*, 1991)^[3, 10]. The interaction witnessed in both endophytes and plants is mutualistic interaction as they form a symbiotic connection with their host, living either between or within plant cells (Pimentel *et al.*, 2011; Holliday, 1989)^[11, 7]. Endophytes produces various plant growth hormones viz., auxin, abscisic acid, ethylene, GA₃, IAA, enzymes and biologically active metabolites which help augment the host betterment and increase the host tolerance to both biotic and abiotic stresses (Kirchhorf *et al.*, 1997)^[8] and plants provide food, shelter and offer numerous benefits to endophytes.

The present study aims to explore the potential ability of a few selected endophytic fungi in modulating plant growth and yield under field conditions. The reason behind the use of endophytes is to maintain a safe environment. These are the promising agents that promote their host growth even in stressed environments, like in phytoremediation. Today these endophytic bacteria grab the attention, because these are the potential source of sustainable agriculture. Instead of using agrochemicals which are the main cause of soil health and environmental depletion, farmers should start using these endophytes as bio control agents, bio fertilizers, bio fungicides, in seed treatment and in the reclamation of soil contaminated with heavy metals (phytoremediation) (Fiore *et al.*, 1995)^[4]. This application promotes sustainable agriculture and prevents the growing population of the world from future hazards in agriculture.

Material and Methods

A field experiment was conducted during rabi 2024-25 at CADA block, Agricultural Research Station, Bheemarayanagudi, UAS, Raichur. It is situated in the North-Eastern dry zone (Zone 2) of Karnataka state at latitude of 16.730 N, a longitude of 76.790 E, and an altitude of 379.40 m above mean sea level. The experiment was laid out in two factorial Randomized Complete Block Design (FRCBD) with three replications. The first factor consists Endophytes (P) viz., *Beauveria bassiana* at 5% (P₁), *Metarhizium anisopliae* at 5% (P₂), *Bacillus subtilis* at 8% (P₃) and second factor consists methods of application (M) viz., M₁: Seed treatment 20 g kg⁻¹ of seed, M₂: Seed priming for 12 hrs duration with a solution ratio of 1:1, M₃: Foliar spray at 30 DAS and 45 DAS and M₄: Soil drenching at 30 DAS and 45 DAS.

The freshly harvested seeds of the female parent (CAL 1514) and male parent (CML 451) of the maize hybrid RCRMH-2 was used for the sowing in 4:1 of female to male planting ratio followed by spacing of 60 cm x 20 cm. The soil of the experimental site was black loamy in texture, moderately alkaline in reaction, low in available nitrogen and medium in available phosphorus and high in available potassium. Detasseling was done as and when tassels emerged from the female parental line (CAL 1514).

The treatments were imposed to both male (CML 451) and female (CAL 1514) parental lines. Maize seeds were treated with endophytes *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus subtilis* at 20 g/kg of seed using jaggery solution as an adhesive, followed by shade drying for 30 minutes before sowing. For seed priming, surface-sterilized seeds were soaked in an endophytic solution (*B. bassiana* and *M. anisopliae* @ 5%, *B. subtilis* @ 8%) for 12 hours at a 1:1 ratio and later dried to restore original moisture content. Foliar application of the same

endophytes was carried out at 30 and 45 days after sowing (DAS) at a concentration of 20 g/liter. Additionally, soil drenching with the endophytes at 20 g/kg was done manually at 30 and 45 DAS. (Poovarasan *et al.*, 2022)^[12]. For synchronization of flowering of the parental lines foliar spray of amino acid @ 2 ml L⁻¹ + GA₃ @ 250 ppm + potash @ 5 per cent + urea @ 2 per cent to male parent was given at 30 and 45 days as the male parent was late flowering by 4-5 days.

The cobs were harvested after attaining physiological maturity based on the visual observations of the plant drying and the husk turning white. Cobs from male parent were harvested first, then from females (F₁) and kept separately. The cobs were sun dried in the threshing yard and yield parameters viz., cob length, cob diameter, cob weight, number of seeds per cob, hundred seed weight seed yield per hectare and shelling percentage were recorded. The data collected from the experiment were analyzed statistically by the procedure prescribed by Sundararajan *et al.* (1972)^[16]. Critical difference was calculated at 5 per cent level wherever 'F' test was significant.

Results and Discussion

The results pertaining to yield and yield parameters viz., cob length, cob diameter, cob weight, number of seeds per cob, hundred seed weight, seed yield per hectare and shelling percentage of the maize hybrid (RCRMH-2) were found to be significant due to the effect of endophytes and its application methods (Table 1 and 2). Among the different treatments imposed application of *Metarhizium anisopliae* via as seed treatment (P₂M₁) recorded significantly highest cob length (14.35 cm), cob diameter (3.49 cm), cob weight (101.52 g), number of seeds per cob (234.20), hundred seed weight (35.61 g), seed yield per hectare (30.77 q) and shelling percentage (85.98) followed by *Metarhizium anisopliae* applied as priming (P₂M₂). In contrast *Bacillus subtilis* applied as foliar spray (P₃M₃) recorded minimum cob length (11.35 cm), cob diameter (2.95 cm), cob weight (70.72 g), number of seeds per cob (100.72), hundred seed weight (29.70 g), seed yield per hectare (19.67 q) and shelling percentage (74.09). The enhancement in yield contributing parameters was attributed to increased nutrient bioavailability and optimized translocation of photosynthates during the flowering stages, facilitating the timely provision of yield promoting hormones through *Metarhizium* seed treatment. *Metarhizium anisopliae*, an endophytic microorganism, enhances maize yield by establishing a mutualistic association within plant tissues, which facilitates improved nutrient uptake particularly nitrogen and phosphorus through mineral solubilization and enhanced root surface interactions. This supported more effective assimilate partitioning towards reproductive structures, thereby increasing seed weight. This fungus produces and regulates phytohormones such as indole-3-acetic acid (IAA), cytokinins, and gibberellins, which are crucial for promoting cell division, elongation, and differentiation, thereby stimulating increased cob length, diameter, and seed development. Similar result was obtained in (Russo *et al.*, 2019)^[14] soybean. The improved plant vigour and root health also contribute to better seed or pod development, indirectly increasing shelling percentage by ensuring better kernel fill and quality. Taken together, these effects make *Metarhizium anisopliae* an effective, eco-friendly seed treatment that boosts yield and crop quality sustainably (Rivas-Franco *et al.*, 2020)^[13]. Similar result found in maize by (Poovarasan *et al.*, 2022)^[12]. In tomato plants, *M. robertsii* and *M. humeri* inoculation improved fruit weight (Siqueira *et al.*, 2020)^[15].

Table 1: Effect of endophytes and method of application on cob length, cob diameter cob weight and number of seeds per cob of maize hybrid RCRMH-2

Treatments	Cob length (cm)				Cob diameter (cm)				Cob weight (g)				Number of seeds per cob			
	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean
M ₁	13.16	14.35	12.91	13.47	3.24	3.49	3.18	3.30	97.81	101.52	92.23	97.18	186.60	234.20	179.48	200.09
M ₂	13.14	13.30	13.10	13.18	3.22	3.34	3.19	3.25	96.38	98.91	93.26	96.18	181.71	190.49	180.97	184.39
M ₃	12.88	12.87	11.35	12.36	3.17	3.08	2.95	3.07	89.97	89.85	70.72	83.51	178.40	175.18	100.72	151.43
M ₄	11.36	11.69	12.25	11.77	2.98	3.05	3.06	3.03	74.59	84.92	87.02	82.17	107.33	127.31	161.33	131.99
Mean	12.63	13.05	12.40		3.15	3.24	3.10		89.68	93.80	85.81		163.51	181.79	155.62	
	S.E.m. ±		CD @ 5%		S.E.m. ±		CD @ 5%		S.E.m. ±		CD @ 5%		S.E.m. ±		CD @ 5%	
P	0.17		0.52		0.02		0.08		0.28		0.84		5.33		15.74	
M	0.20		0.60		0.03		0.10		0.33		0.97		6.15		18.17	
P x M	0.35		1.05		0.05		0.17		0.57		1.68		10.66		31.48	

Legend:

Factor -I Endophytes (P)				Factor-II Method of application (M)			
P ₁ :	<i>Beauveria bassiana</i> @ 5%			M ₁ :	Seed treatment		
P ₂ :	<i>Metarhizium anisopliae</i> @ 5%			M ₂ :	Seed priming		
P ₃ :	<i>Bacillus subtilis</i> @ 8%			M ₃ :	Foliar spray		
				M ₄ :	Soil drenching		

Table 2: Effect of endophytes and method of application on hundred seed weight, seed yield per hectare and shelling percentage of maize hybrid RCRMH-2

Treatments	Hundred seed weight (g)				Seed yield per hectare (q)				Shelling percentage			
	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean	P ₁	P ₂	P ₃	Mean
M ₁	33.93	28.56	30.77	28.56	28.56	30.77	25.45	28.26	82.07	85.98	81.28	83.11
M ₂	33.69	26.82	28.89	26.82	26.82	28.89	25.76	27.16	81.96	85.88	81.35	83.06
M ₃	32.95	25.37	25.22	25.37	25.37	25.22	19.67	23.42	80.81	80.42	74.09	78.44
M ₄	30.82	20.26	23.31	20.26	20.26	23.31	25.12	22.90	75.94	78.20	78.52	77.55
Mean	32.82	25.25	27.05	25.25	25.25	27.05	24.00		80.19	82.62	78.81	
	S.E.m. ±		S.E.m. ±		S.E.m. ±		CD @ 5%		S.E.m. ±		CD @ 5%	
P	0.26		0.29		0.29		0.87		0.66		1.97	
M	0.30		0.34		0.34		1.01		0.77		2.27	
P x M	0.53		0.59		0.59		1.75		1.33		3.94	

Legend

Factor -I Endophytes (P)				Factor-II Method of application (M)			
P ₁ :	<i>Beauveria bassiana</i> @ 5%			M ₁ :	Seed treatment		
P ₂ :	<i>Metarhizium anisopliae</i> @ 5%			M ₂ :	Seed priming		
P ₃ :	<i>Bacillus subtilis</i> @ 8%			M ₃ :	Foliar spray		
				M ₄ :	Soil drenching		

Conclusion

Based on experimental results, it was concluded that application of 20 g /kg *Metarhizium anisopliae* via as seed treatment recorded higher seed yield and yield attributing parameters of hybrid maize. The results affirm that endophytes enhances seed early seedling establishment through improved phytohormone activity, nutrient mobilisation and endophytic root colonization so that seed producers may get around 17 per cent more seed yield than other treatments. It can be recommended as a practical, eco-friendly technology for augmenting productivity and sustainability in crop production.

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