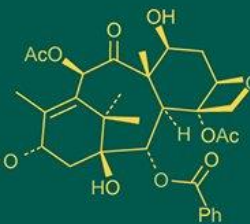
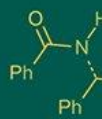


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Effect of temperature on the performance of Arbuscular Mycorrhizal Fungal (AMF) consortia isolated from diverse terrestrial regions of Chhattisgarh, India

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Abstract

Arbuscular mycorrhizal fungi (AMF) form obligate symbioses with plant roots and enhance host nutrient and water uptake, and their establishment and functioning are influenced by temperature among other environmental and biological factors. In this study, eight indigenous AMF consortia isolated from diverse terrestrial regions of Chhattisgarh differing in their component genera and species, including *Glomus*, *Rhizophagus irregularis*, *Acaulospora*, *Funneliformis*, *Ambispora callosa*, *Archaeospora*, *Scutellospora* and *Paraglomus occultum* — were evaluated for their response to four temperature regimes (10 °C, 20 °C, 30 °C and 40 °C) using maize (*Zea mays* L.) as the host plant under controlled BOD incubator conditions for 20 days after sowing. AMF consortia, temperature and their interaction significantly influenced total root colonization, vesicle colonization, vesicle density, shoot length and root length. Maize seedlings failed to survive to the observation stage at 40 °C; this outcome is best interpreted as reflecting the combined stress of the 40 °C regime together with the confined, static-air pot-culture conditions of the BOD incubator, rather than temperature acting as the sole determinant of seedling survival. Among the regimes that permitted seedling survival, 30 °C was associated with the highest mean total root colonization (67.16%), vesicle colonization (62.72%) and shoot length (114.59 mm), followed by 20 °C and 10 °C. Consortium AMF12, a mixture dominated by *Paraglomus occultum* and *Glomus corymbiforme*, together with AMF7 and AMF8, consistently ranked among the better-performing consortia across temperatures, with AMF12 recording complete (100%) root and vesicle colonization at 30 °C and the highest overall shoot length. AMF8 additionally performed comparatively well at the cooler 10 °C and 20 °C regimes, indicating that consortium identity, not temperature in isolation, shaped the observed pattern of response. Shoot and root length followed broadly parallel, though not strictly linear, trends alongside colonization, indicating that colonization percentage alone is an incomplete predictor of plant benefit and that consortium-specific efficiency, host physiological condition, and the incubator microenvironment together with temperature all contribute to the outcome. The results suggest that 20-30 °C represents a favourable thermal window for AMF establishment and maize seedling elongation under these controlled conditions, and that indigenous consortia from Chhattisgarh, particularly AMF12, AMF7 and AMF8, merit further evaluation as candidate bioinoculants for maize, alongside consideration of the other agronomic and environmental factors that will co-determine their field performance.

Keywords: Arbuscular mycorrhizal fungi, AMF consortia, temperature stress, maize, root colonization, Chhattisgarh

Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms that establish mutualistic associations with the roots of the majority of terrestrial plant species. Through the development of intraradical structures such as arbuscules and vesicles, and an extensive extraradical hyphal network, AMF improve host plant acquisition of phosphorus, nitrogen and water, and confer tolerance to a range of abiotic stresses. The functional efficiency of this symbiosis, however, is not fixed; it is shaped by the identity of the fungal partner, host physiological status, substrate and moisture conditions, and prevailing environmental factors, among which temperature is one important, but not exclusive, determinant. Temperature can influence several stages of the AMF life cycle, from spore germination and hyphal extension to root penetration, arbuscule and vesicle formation, and sporulation,

though its effects are rarely independent of other interacting factors such as substrate moisture, aeration, light and host nutritional status. Moderate soil temperatures are generally reported to favour colonization and nutrient exchange, whereas temperatures outside this range, in combination with other stresses, can delay germination, reduce hyphal growth, or, at the seedling level, contribute to compromised host survival before symbiosis can be established. Because AMF communities are highly diverse and often locally adapted, consortia isolated from different agro-climatic zones can differ in their tolerance to temperature and other stresses, and in the growth benefits they confer to a common host.

Chhattisgarh, with its diverse terrestrial landscapes spanning plains, plateaus and forested tracts, harbours a rich diversity of native AMF populations that remain comparatively underexplored for their functional performance under controlled temperature regimes. Identifying consortia that perform well across a range of temperatures, or that are specifically adapted to particular thermal windows, is of direct relevance to developing region-appropriate bioinoculants for maize (*Zea mays* L.), a crop of major importance in the state and one known to respond strongly to AMF inoculation.

The present study was therefore undertaken to evaluate the effect of four temperature regimes (10 °C, 20 °C, 30 °C and 40 °C) on the colonization behaviour and plant-growth-promoting performance of eight indigenous AMF consortia isolated from diverse terrestrial regions of Chhattisgarh, using maize as the host plant under controlled BOD incubator conditions.

Materials and Methods

The effect of temperature on arbuscular mycorrhizal fungal (AMF) colonization and establishment was studied under controlled conditions using a BOD incubator during the winter season at the College of Agriculture, Raipur. The experiment was laid out in a Factorial Completely Randomized Design with two factors comprising four temperature regimes viz., 10 °C, 20 °C, 30 °C and 40 °C, along with thirteen AMF treatments, including twelve AMF isolates and one uninoculated control. Chemically sterilized disposable glass containers were used for the experiment. Sterilized sand-soil mixture (1:1) served as the growth medium and was sterilized by autoclaving at 121 °C and 15 psi pressure for 1 hour on two consecutive days. AMF inoculum obtained from trap culture was applied @ 50 g per container with a spore load of 500-1000 spores. Maize seeds were surface sterilized with 1% sodium hypochlorite solution before sowing and plant population was maintained at three plants per container. All treatments were maintained under their respective temperature regimes throughout the experimental period in the BOD incubator. Observations on mycorrhizal parameters, including percent root colonization and the number of vesicles, as well as shoot, root length were recorded after 20 days of crop growth. Root colonization was assessed following the method described by Giovannetti and Mosse (1980)

Statistical Analysis

Data were analysed by two-factor analysis of variance (AMF consortia × temperature) appropriate to the factorial design, and treatment means were compared using Duncan's

Multiple Range Test (DMRT) at the 5% level of significance.

Results and Discussion

Both AMF consortia and temperature, and their interaction, exerted a significant influence on all mycorrhizal and growth parameters recorded in maize. A consistent pattern emerged across virtually every parameter measured: performance increased from 10 °C to a peak at 30 °C, while no seedlings survived to the observation stage at 40 °C, precluding any colonization or growth response at this temperature.

Mycorrhizal Colonization Parameters

Total root colonization varied significantly across the temperature regimes, rising from a mean of 36.42% at 10 °C to 45.31% at 20 °C and reaching 67.16% at 30 °C, before falling to zero at 40 °C, coinciding with complete seedling mortality at that regime. At 10 °C, AMF8 recorded the highest colonization (83.33%), clearly ahead of the other consortia. At 20 °C, AMF3 took the lead (71.11%), followed closely by AMF8 (60%). At 30 °C, AMF12 achieved complete colonization (100%), statistically on par with AMF7 (98.89%) and closely followed by AMF8 (97.78%); this AMF12 × 30 °C combination represented the single highest colonization response recorded across the entire experiment. Averaged across temperatures, AMF8 recorded the highest overall mean colonization (60.28%), while AMF11 recorded the lowest (27.78%).

Vesicle colonization followed a closely similar trend, with means of 34.19%, 40.49% and 62.72% at 10 °C, 20 °C and 30 °C, respectively, and none recorded at 40 °C. AMF8 again led at 10 °C (75.56%), AMF3 at 20 °C (64.44%), and AMF12 achieved complete vesicle colonization (100%) at 30 °C, ahead of AMF8 (96.67%) and AMF7 (94.44%). The density of vesicles per 100 root segments mirrored these colonization trends, with the highest counts consistently associated with AMF8, AMF12 and AMF7 at their respective optimal temperatures, and negligible or nil vesicle formation under the 40 °C regime.

These patterns are broadly consistent with reports that moderate temperatures around 24-32 °C favour AMF spore germination, hyphal proliferation and root penetration, while temperatures at the cooler or hotter extremes are associated with reduced fungal development, as noted by Schenck and Schroder (1974) ^[8] and Smith and Roncadori (1986) ^[9]; however, temperature was evaluated here alongside the fixed conditions of BOD incubator pot culture, and factors such as substrate volume, aeration and moisture retention at 40 °C cannot be ruled out as contributing to the outcome. The absence of colonization at 40 °C in the present study coincided with seedling mortality, and while high temperature is the most obvious candidate stressor, the incubator microenvironment at that setting may also have played a role, so the finding should not be read as establishing temperature as the sole cause. This is worth noting because AMF have been reported to alleviate heat stress in already-established maize plants exposed to temperatures of 40-44 °C (Zhu *et al.*, 2011; Mathur *et al.*, 2018) ^[12, 7], suggesting that seedling-stage vulnerability, possibly compounded by incubator conditions, is a more complete explanation than temperature acting in isolation.

Plant Growth Parameters

Shoot length showed a marked increase with rising temperature up to 30 °C, with means of 69.56 mm, 106.70 mm and 114.59 mm at 10 °C, 20 °C and 30 °C, respectively, and no shoot growth recorded at 40 °C. At 10 °C, AMF11 produced the tallest shoots (88.33 mm); at 20 °C, AMF8 was the best performer (120 mm); and at 30 °C, AMF12 recorded the greatest shoot length (125 mm), statistically at par with AMF2, AMF11 and AMF8. Averaged across temperatures, AMF12 (78.83 mm) and AMF11 (78.25 mm) recorded the highest overall mean shoot length, both markedly exceeding the uninoculated control (61.50 mm).

Root length, shoot and root fresh weight, and shoot and root dry weight all followed a broadly similar pattern, increasing from 10 °C to a peak around 20-30 °C and remaining nil at 40 °C. Total dry weight, integrating shoot and root biomass, was highest at 30 °C overall, but notably, the single highest total dry weight was recorded not at 30 °C but with AMF8 at 20 °C (338.67 mg), statistically on par with AMF7 and AMF8 at 30 °C (approximately 330-334 mg). This indicates that while 30 °C was the generally optimal temperature, AMF8 achieved its peak biomass response at a comparatively cooler regime, reflecting a temperature-dependent shift in biomass partitioning between shoot and root tissue rather than a simple scaling of colonization intensity.

Growth responses paralleled mycorrhizal colonization closely but not in strict linear proportion: several of the highest-biomass treatment combinations did not correspond to the single highest colonization values, indicating that colonization percentage alone is an incomplete predictor of the growth benefit conferred to the host, consistent with Smith and Roncadori (1986) [9] and Hayman (1974). The consistently strong performance of AMF8, AMF7 and AMF12 across multiple growth parameters suggests that these consortia possess superior functional efficiency in nutrient and water transfer, rather than simply achieving higher root occupancy. The improved growth recorded at 20-30 °C is consistent with more efficient AMF-mediated phosphorus and nitrogen acquisition at moderate temperatures (Liu *et al.*, 2016) [6] and with better host water

status and photosynthetic activity under favourable thermal conditions (Zhu *et al.*, 2010) [10]. At 10 °C, both colonization and growth declined together, indicating that cold stress restricted plant metabolism and fungal establishment in parallel (Chen *et al.*, 2014; Gavito *et al.*, 2005) [3, 4], a pattern also reported for AMF × temperature interactions in maize (Turudu *et al.*, 2025) [10]. The independent behaviour of root biomass, which remained comparably high at both 20 °C and 30 °C despite peak colonization occurring only at 30 °C, further suggests that temperature can shape root architecture and carbon allocation independently of fungal occupancy (Gavito *et al.*, 2005; Carvalho *et al.*, 2015) [4, 2].

Consortium-Specific Thermal Response

Taken together, the results point to differences among the eight consortia in their response across temperatures, though this response was shaped jointly by temperature, consortium composition, and the shared incubator environment rather than by temperature in isolation. AMF12, dominated by *Paraglomus occultum* and *Glomus corymbiforme*, and AMF7, comprising *Funneliformis*, *Rhizophagus* and *Glomus mosseae*, performed best under the warmer 30 °C regime, achieving near-complete or complete colonization and the greatest shoot growth. AMF8, comprising *Acaulospora*, *Glomus mosseae*, *Funneliformis* and *Ambispora callosa*, distinguished itself by comparatively strong performance across all three surviving temperature regimes, including the coolest (10 °C), and by producing the single highest biomass response at 20 °C, suggesting a degree of adaptability that is not explained by temperature alone. In contrast, AMF11, despite composing *Archaeospora*, *Scutellospora* and *Glomus multicaulis* and performing reasonably in shoot length, generally recorded the lowest mean colonization values, while AMF3, the mixed consortium, showed intermediate but comparatively more stable performance across temperatures. These differences underscore that consortium composition and its interaction with the growing environment, and not temperature considered on its own, determine the practical utility of a given inoculant for a given setting.

Table 1: Effect of different temperature regimes and AMF consortia on total root colonization (%) in maize

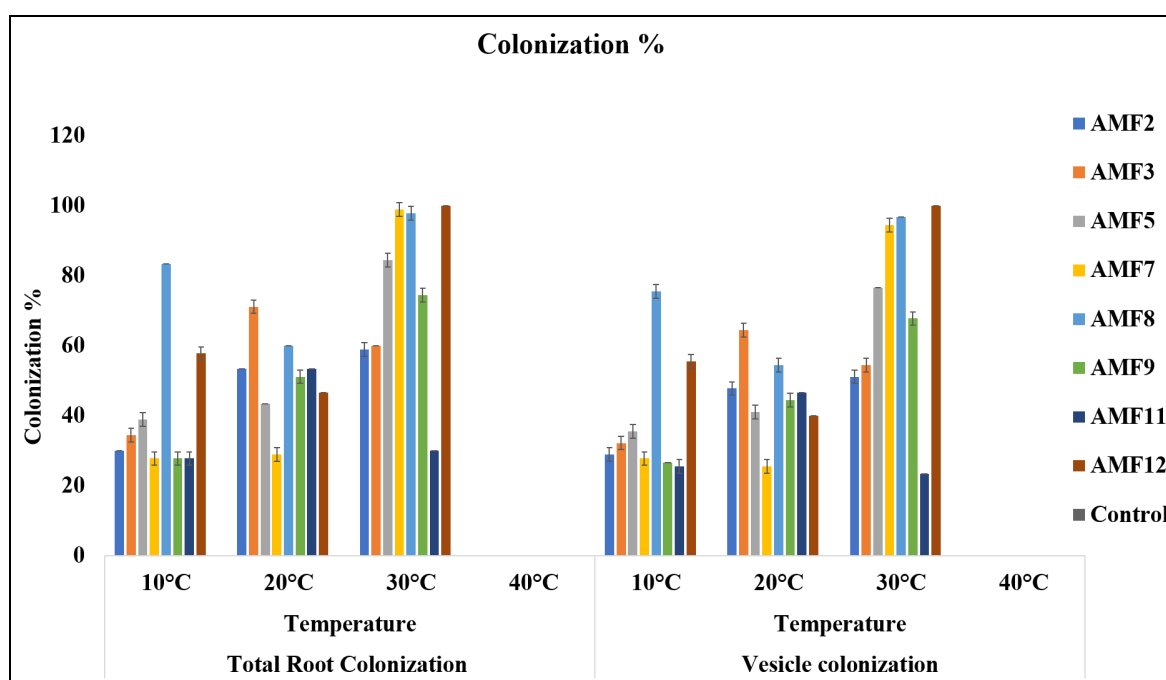
	Total Root Colonization%				
AMF consortia	Temperature				Mean (AMF consortia)
	10 °C	20 °C	30 °C	40 °C	
AMF2	30 ⁿ (33.21)	53.33 ^h (46.91)	58.89 ^{fg} (50.12)	0 ^p (0)	35.56 ^e (32.56)
AMF3	34.44 ^m (35.93)	71.11 ^e (57.49)	60 ^f (50.77)	0 ^p (0)	41.39 ^c (36.05)
AMF5	38.89 ^l (38.58)	43.33 ^k (41.17)	84.44 ^c (66.79)	0 ^p (0)	41.67 ^c (36.64)
AMF7	27.78 ^o (31.79)	28.89 ^{no} (32.50)	98.89 ^{ab} (86.49)	0 ^p (0)	38.89 ^d (37.69)
AMF8	83.33 ^c (65.91)	60 ^f (50.78)	97.78 ^b (82.99)	0 ^p (0)	60.28 ^a (49.92)
AMF9	27.78 ^o (31.79)	51.11 ⁱ (45.64)	74.44 ^d (59.64)	0 ^p (0)	38.33 ^d (34.27)
AMF11	27.78 ^o (31.79)	53.33 ^h (46.91)	30 ⁿ (33.21)	0 ^p (0)	27.78 ^f (27.98)
AMF12	57.78 ^g (49.48)	46.67 ^j (43.09)	100 ^a (90)	0 ^p (0)	51.11 ^b (45.64)
Control	0 ^p (0)	0 ^p (0)	0 ^p (0)	0 ^p (0)	0 ^g (0)
Mean	36.42 ^c (35.39)	45.31 ^b (40.49)	67.16 ^a (57.78)	0 ^d (0)	
Statistic	AMF Inocula	Temperature	Interaction		
SEm	0.3036	0.2024	0.6071		
CD 5%	0.8558	0.05705	1.7116		
C.V.%	3.1344				

Note: Values in parentheses are arcsine-transformed values. Mean comparison was performed using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Table 2: Effect of different temperature regimes and AMF consortia on vesicle colonization (%) in maize roots

	Vesicle Colonization%				Mean (AMF Consortia)
AMF Consortia	Temperature (°C)				
	10 °C	20 °C	30 °C	40 °C	
AMF2	28.89 ⁿ (32.50)	47.78 ⁱ (43.73)	51.11 ^h (45.64)	0 ^q (0)	31.94 ^f (30.47)
AMF3	32.22 ^m (34.58)	64.44 ^f (53.40)	54.44 ^g (47.55)	0 ^q (0)	37.78 ^{cd} (33.88)
AMF5	35.56 ^l (36.59)	41.11 ^k (39.88)	76.67 ^d (61.12)	0 ^q (0)	38.33 ^c (34.39)
AMF7	27.78 ^{no} (31.79)	25.56 ^{op} (30.36)	94.44 ^c (76.52)	0 ^q (0)	36.94 ^d (34.67)
AMF8	75.56 ^d (60.38)	54.44 ^g (47.55)	96.67 ^b (79.48)	0 ^q (0)	56.67 ^a (46.85)
AMF9	26.67 ^{no} (31.09)	44.44 ^j (41.81)	67.78 ^e (55.42)	0 ^q (0)	34.72 ^e (32.08)
AMF11	25.56 ^{op} (30.36)	46.67 ⁱ (43.09)	23.33 ^p (28.88)	0 ^q (0)	23.89 ^g (25.58)
AMF12	55.56 ^g (48.19)	40 ^k (39.23)	100 ^a (90)	0 ^q (0)	48.89 ^b (44.36)
Control	0 ^q (0)	0 ^q	0 ^q	0 ^q (0)	0 ^h (0)
Mean (Temperature)	34.198 ^c (33.94)	40.494 ^b (37.35)	62.716 ^a (53.85)	0 ^d (0)	
Statistic	AMF consortia	Temperature	Interaction		
SEm	0.2570	0.1713	0.5140		
CD 5%	0.7245	0.4830	1.4490		
C.V.%	2.8145				

Note: Values in parentheses are arcsine-transformed values. Mean comparison was performed using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

**Fig 1:** Temperature-mediated variation in vesicle colonization of maize roots inoculated with different AMF consortia**Table 3:** Effect of different temperature regimes and AMF consortia on shoot and root length (mm) of maize

	Shoot Length (mm)					Root Length (mm)				
AMF consortia	Temperature				Mean (AMF consortia)	Temperature				Mean (AMF consortia)
	10 °C	20 °C	30 °C	40 °C		10 °C	20 °C	30 °C	40 °C	
AMF2	77 ⁱ	105.67 ^{defg}	122 ^{ab}	0 ^l	76.17 ^a	127.33 ^{lm}	133.33 ^{kl}	193.33 ^{ef}	0 ^p	113.5 ^f
AMF3	58.33 ^k	110.67 ^{cdef}	105 ^{defg}	0 ^l	68.50 ^{bc}	92 ^o	153.33 ^j	180 ^{gh}	0 ^p	106.33 ^g
AMF5	66.00 ^j	104.33 ^{fg}	116.67 ^{bc}	0 ^l	71.75 ^b	96.33 ^o	186.67 ^{fg}	194.33 ^e	0 ^p	119.33 ^e
AMF7	72.67 ⁱ	112 ^{cde}	117.67 ^{abc}	0 ^l	75.58 ^a	124 ^m	176 ^h	193.33 ^{ef}	0 ^p	123.33 ^d
AMF8	65.00 ^{jk}	120 ^{ab}	120 ^{ab}	0 ^l	76.25 ^a	133.33 ^{kl}	196.67 ^e	239.33 ^b	0 ^p	142.33 ^b
AMF9	61.67 ^{jk}	104.33 ^{fg}	104.33 ^{efg}	0 ^l	67.58 ^c	96.67 ^o	283.33 ^a	233.33 ^{bc}	0 ^p	153.33 ^a
AMF11	88.33 ^h	104.33 ^{fg}	120.33 ^{ab}	0 ^l	78.25 ^a	115 ⁿ	183.33 ^g	231.33 ^c	0 ^p	132.42 ^c
AMF12	78 ⁱ	112.33 ^{cd}	125.00 ^a	0 ^l	78.83 ^a	110 ⁿ	200 ^e	217 ^d	0 ^p	131.75 ^c
Control	59 ^{jk}	86.67 ^h	100.33 ^g	0 ^l	61.50 ^d	94 ^o	165.67 ⁱ	136.67 ^k	0 ^p	99.08 ^h
Mean (temperature)	69.56 ^c	106.70 ^b	114.59 ^a	0 ^d		109.85 ^c	186.482 ^b	202.07 ^a	0 ^d	
Statistic	AMF Consortia	Temperature	Interaction			AMF Consortia	Temperature	Interaction		
SEm	1.18	0.79	2.36			1.22	0.82	2.45		
CD 5%	3.32	2.22	6.65			3.45	2.30	6.91		
C.V.%	5.62					3.41				

Note: Mean comparison was performed using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

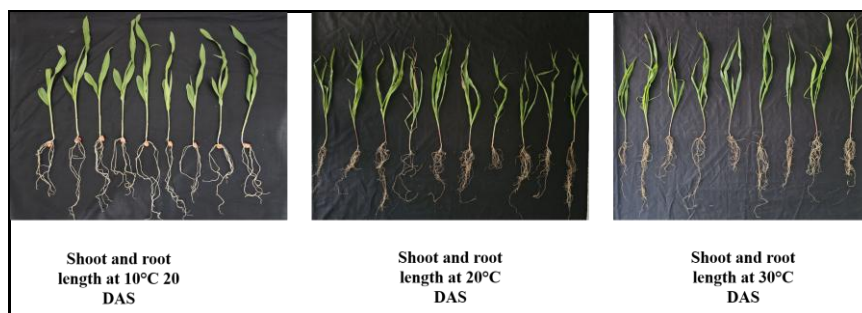


Fig 2: Pictorial representation of shoot and root growth of maize inoculated with different AMF consortia under varying temperature regimes

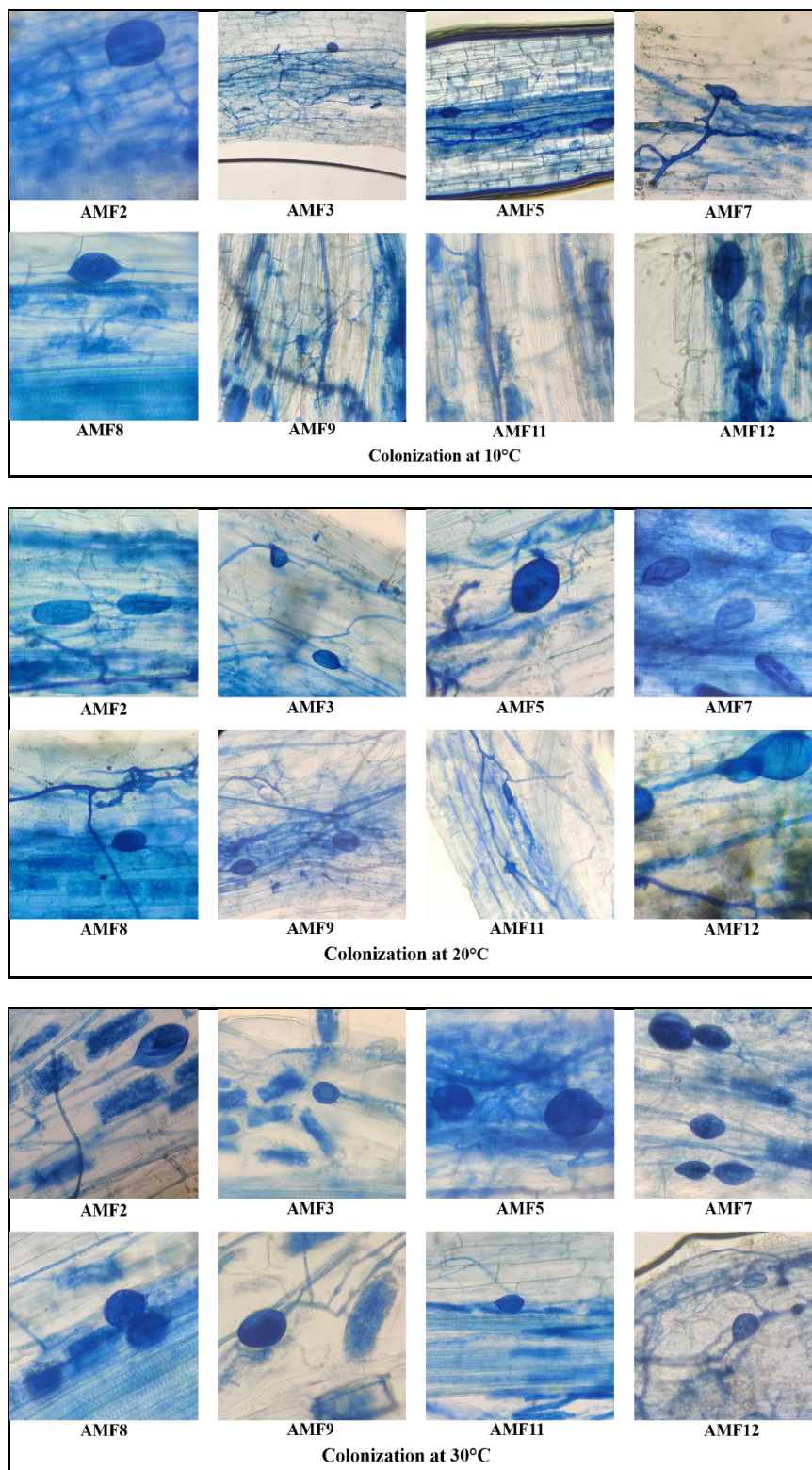


Fig 3: Pictorial representation of AMF colonisation in maize roots under different temperature regimes

Conclusion

Temperature was an important, but not the sole, factor shaping both the colonization behaviour of indigenous AMF consortia from Chhattisgarh and the growth of their maize host under the conditions of this study. The range of 20-30 °C was associated with the most favourable colonization and growth outcomes, while at 40 °C seedlings did not survive to the observation stage an outcome likely arising from the combined effect of high temperature and the constrained BOD incubator pot-culture environment, rather than from heat stress alone. Among the eight consortia evaluated, AMF12, AMF7 and AMF8 consistently emerged as comparatively strong performers, with AMF12 achieving complete root and vesicle colonization and the greatest shoot elongation at 30 °C, and AMF8 additionally demonstrating good performance and peak biomass accumulation at the cooler 20 °C regime. These findings indicate that colonization intensity alone does not fully account for host growth benefit, and that consortium-specific functional efficiency, together with the broader growing environment, should guide inoculant selection. The consortia identified here particularly AMF12, AMF7 and AMF8 represent promising candidates for further evaluation as region-appropriate bioinoculants, with future work under field or more variable environmental conditions needed to disentangle the specific contribution of temperature from other co-occurring factors.

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