The Andhra Agric. J 72 (3): 338-344, 2025
doi :10.61657/aaj.2025.216

Evaluation of various media for growth and sporulation of *Ampelomyces quisqualis*

J Vasanthi, P Kishore Varma, P Anil Kumar and N Kamakshi
Department of Plant Pathology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla-522101, Andhra Pradesh, India

ABSTRACT

An *in vitro* experiment was conducted to evaluate different liquid and solid culture media for the growth and sporulation of *Ampelomyces quisqualis*. The media tested included Potato Dextrose Broth (PDB), Czapek Dox Broth (CDB), and Oat Meal Broth (OMB), both with and without supplements *viz.,* Arabitol (A), Mannitol (M), and Malt Extract (Ma). Similarly, solid media variants were also prepared by adding agar-agar to the liquid media. Among the liquid media, OMB supported the highest pycnidiospore production, while CDB supplemented with Malt Extract (CDB+Ma) yielded the highest dry biomass. Among solid media tested, Czapek Dox Agar with Malt Extract (CDA+Ma) supported the maximum radial growth, followed by PDA+Ma. Oat Meal Agar (OMA) was found to support the highest sporulation. Overall, malt extract was identified as the most effective supplement in enhancing both radial growth and sporulation of *Ampelomyces quisqualis*.

Keywords: *Ampelomyces quisqualis, Biomass yield, Radial growth, Sporulation Supplements*

Ampelomyces quisqualis is a naturally occurring mycoparasite of powdery mildew fungi, widely distributed across various agroecosystems (Kiss *et al*., 2004). It parasitizes powdery mildew by developing intracellular pycnidia, which suppress host mycelial growth, sporulation, and conidial germination (Sztejnberg *et al*., 1989). While the fungus forms pycnidia both in nature and under *in vitro* conditions, its sporulation efficiency is highly influenced by the nutrient composition of the growth medium.

Although several studies have explored cultural requirements of *A. quisqualis*, no consensus exists on the optimal media composition. Additionally, data on the role of specific supplements in enhancing rapid conidial production remains limited. The present study aimed to evaluate different media, alone and in combination with nutritional supplements, to determine their efficacy in promoting sporulation, biomass accumulation and radial growth of *A. quisqualis*.

MATERIAL AND METHODS

Preparation of Liquid media

A. quisqualis cultures were initially maintained on Potato Dextrose Agar (PDA) for 15 days. Pycnidiospores suspension (Fig.1.)was prepared using 5 mL of sterile distilled water, and 1 mL of this inoculum was introduced into 50 ml of sterilized broth media in 250 ml flasks under aseptic conditions. Cultures were incubated at 25/ ±/ 1°C with constant shaking (120 rpm) for 27 days. Dry mycelial weight was determined after 27 days by filtering 50 ml of the liquid media with Whatman No.1 filter paper, oven-dried at 70°C for 48 hours, and weighed. Pycnidiospore counts were determined every alternate day using a Neubauer haemocytometer. Each treatment was replicated thrice.

Preparation of Solid Media

Each solid medium was autoclaved, poured into Petri dishes (20 ml/plate), and inoculated using 5 mm mycelial discs from 15-day-old cultures. Plates were incubated at 28/ ±/ 2°C. Observations on radial growth were recorded every 10 days up to 60 days after inoculation (DAI). Radial growth of the colonies was measured periodically and the colony morphology, including shape and colour of the colony was documented for each treatment. At 60 DAI, three agar plugs (0.5 mm) per plate were vortexed in sterile distilled water of 5mL and the released pycnidiospores were quantified using Neubauer haemocytometer.

Table 1. List of different liquid media

TREATMENTS	MEDIA
T1	Potato dextrose broth
T2	Potato dextrose broth with 2% malt extract
T3	Czapek Dox broth
T4	Czapek Dox broth with 2% malt extract
T5	Potato dextrose broth with 1% arabitol
T6	Czapek dox broth with 1% arabitol
T7	Potato dextrose broth with 1% mannitol
T8	Czapek Dox broth with 1% mannitol
T9	Oat meal broth
T10	Oat meal broth with 2% malt extract
T11	Oat meal broth with 1% arabitol
T12	Oat meal broth with 1% mannitol

Table 2. List of different solid media

TREATMENTS	MEDIA
T1	Potato dextrose agar
T2	Potato dextrose agar with 2% malt extract
T3	Czapek Dox agar
T4	Czapek Dox agar with 2% malt extract
T5	Potato dextrose agar with 1% arabitol
T6	Czapek dox agar with 1% arabitol
T7	Potato dextrose agar with 1% mannitol
T8	Czapek Dox agar with 1% mannitol
T9	Oat meal agar
T10	Oat meal agar with 2% malt extract
T11	Oat meal agar with 1% arabitol
T12	Oat meal agar with 1% mannitol

Area Under Mycelial Growth Curve (AUMGC) = AUMGC= $\sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2] [t_{i+1} - t_i]$

X_i = colony diameter (cm) at i^{th} observation

t_i = time (days after inoculation) at the i^{th} observation

n= total number of observations.

Area Under Mycelial Growth Curve (AUMGC) was calculated using standard formula (Tang *et al*.,2023).

RESULTS AND DISCUSSION

Effect of liquid media on the sporulation and dry biomass of *A. quisqualis*

The results pertaining to the effect of liquid media on the pycnidiospores (Conidia) production in *A. quisqualis* are presented in Table 3. The pycnidiospores production was observed from 15 DAI in liquid media. Therefore, the pycnidiospores count in different liquid media were recorded from 15DAI to 27DAI.

Among all treatments, OMB without supplementation recorded the highest pycnidiospores production (151.43 × 10t /ml) and moderate biomass (0.46 g). CDB+Ma gave the maximum biomass yield (0.63 g) and moderate sporulation (68.53 × 10t /ml), confirming malt extract's role in biomass enhancement.

Arabitol supplementation yielded inconsistent results; CDB+A and OMB+A performed poorly, while PDB+A showed moderate improvement in pycnidiospore production. Mannitol showed inhibitory effects in CDB+M (no growth), whereas PDB+M supported both sporulation (94.03 × 10t /ml) and biomass (0.47 g). Malt extract supplementation

Table 3: Evaluation of different liquid media for biomass yield and pycnidiospores production of *Ampelomyces quisqualis*

S.No	Media	Pycnidiospores production of *Ampelomyces* in liquid media at different intervals($\times 10^4 ml^{-1}$)							Weight of *Ampelomyces*	
		15DAI	17DAI	19DAI	21DAI	23DAI	25DAI	27DAI	Wet weight (g)	Dry weight (g)
1	CDB	43.03 (5.63) ^{a*}	55.60 (5.74) ^{b}	70.87 (5.87) ^{a}	80.07 (5.90) ^{b}	81.33 (5.91) ^{c}	95.40 (5.98) ^{d}	116.90 (6.06) ^{c}	2.43(1.85) ^{f**}	0.13(1.06) ^{de}
2	CDB+A	0.00 (0.00) ^{f}	0.00 (0.00) ^{g}	7.77 (4.89) ^{e}	13.74 (5.1) ^{g}	16.37 (5.21) ^{h}	20.43 (5.31) ^{h}	21.87 (5.34) ^{h}	1.45(1.56) ^{i}	0.04(1.02) ^{f}
3	CDB+M	0.00 (0.00) ^{f}	0.00 (0.00) ^{g}	0.00 (0.00) ^{g}	0.00 (0.00) ^{h}	0.00 (0.00) ^{j}	0.00 (0.00) ^{j}	0.00 (0.00) ^{j}	0.00(1.00) ^{j}	0.00(1.00) ^{f}
4	CDB+Ma	14.30 (5.15) ^{e}	19.33 (5.28) ^{e}	33.80 (5.52) ^{d}	43.07 (5.63) ^{e}	55.53 (5.74) ^{f}	63.20 (5.80) ^{f}	68.53 (5.83) ^{f}	3.30(2.07)	0.63(1.27) ^{a}
5	PDB	0.00 (0.00) ^{f}	0.00 (0.00) ^{g}	6.99 (4.84) ^{f}	14.90 (5.17) ^{f}	26.17 (5.41) ^{g}	55.83 (5.74) ^{g}	64.87 (5.81) ^{g}	1.90(1.70) ^{h}	0.28(1.13) ^{c}
6	PDB+A	23.20 (5.36) ^{c}	36.37 (5.56) ^{d}	52.27 (5.71) ^{b}	73.83 (5.86) ^{c}	88.77 (5.94) ^{b}	95.27 (5.97) ^{d}	105.47 (6.02) ^{d}	4.96(2.44) ^{a}	0.40(1.18) ^{b}
7	PDB+M	20.87 (5.31) ^{d}	42.83 (5.63) ^{c}	47.13 (5.67) ^{c}	58.83 (5.77) ^{d}	65.93 (5.81) ^{d}	77.07 (5.88) ^{e}	94.03 (5.97) ^{e}	4.84(2.41) ^{a}	0.47((1.21) ^{b}
8	PDB+Ma	41.73 (5.62) ^{a}	67.13 (5.82) ^{a}	70.30 (5.84) ^{a}	75.43 (5.87) ^{bc}	85.43 (5.93) ^{b}	98.30 (5.99) ^{c}	102.40 (6.01) ^{d}	4.60(2.33) ^{b}	0.44(1.20) ^{b}
9	OMB	26.30 (5.42) ^{b}	43.70 (5.64) ^{c}	73.50 (5.86) ^{a}	93.40 (5.97) ^{a}	120.07 (6.07) ^{a}	126.53 (6.10) ^{a}	151.43 (6.18) ^{a}	3.02(2.00) ^{d}	0.46 (1.20) ^{b}
10	OMB+A	0.00 (0.00) ^{f}	0.00 (0.00) ^{g}	0.00 (0.00) ^{g}	0.00 (0.00) ^{h}	3.78 (4.57) ^{i}	4.23 (4.62) ^{i}	5.40 (4.73)i	2.14(1.77) ^{g}	0.07(1.03) ^{ef}
11	OMB+M	0.00 (0.00) ^{f}	27.12 (1.43) ^{f}	54.13 (5.73) ^{b}	57.53 (5.76) ^{d}	59.23 (5.77) ^{e}	63.23 (5.80) ^{f}	67.10 (5.82) ^{fg}	2.79(1.94) ^{e}	0.19(1.09) ^{d}
12	OMB+Ma	40.17 (5.60) ^{a}	44.17 (5.64) ^{c}	72.47 (5.86) ^{a}	76.73 (5.88) ^{bc}	78.30 (5.89) ^{c}	102.23 (6.01) ^{b}	129.60 (6.11) ^{b}	2.90(1.97) ^{de}	0.31(1.14) ^{c}
	SEm(±)	0.014	0.005	0.006	0.011	0.007	0.003	0.005		
	C.D.(P<0.05)	0.041	0.014	0.019	0.033	0.021	0.009	0.016		
	CV(%)	0.755	0.245	0.237	0.406	0.239	0.097	0.172		

Data presented was the mean of three replications, *Ampelomyces quisqualis* growth in 50ml broth
CDB-Czapek Dox Broth, CDB+A-Czapek Dox Broth+Arabitol, CDB+M-Czapek Dox Broth+ Mannitol, CDB+Ma- Czpek Dox Broth+ Malt extract, PDB-Potato Dextrose Broth, PDB+A- Potato Dextrose Broth+Arabitol, PDB+M- Potato Dextrose Broth + Mannitol, PDB+Ma- Potato Dextrose Broth+ Malt extract, OMB- Oat Meal Broth, OMB+A-Oat Meal Broth+Arabitol, OMB+M-Oat Meal Broth +Mannitol, OMB+Ma- Oat Meal Broth +Malt extract

Figures with similar alphabets do n't differ significantly. '*'-Values in the parenthesis are log transformed values. '**'- Values in the parenthesis are square root transformed values.

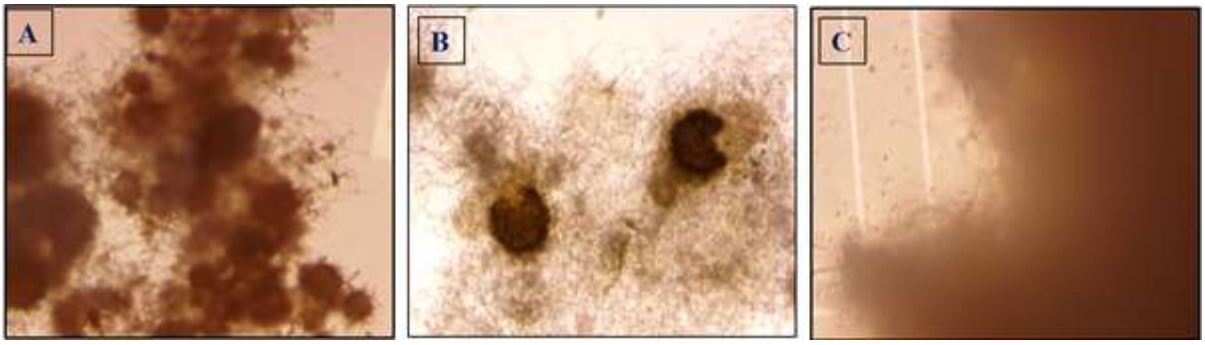

Fig.1. Microscopic view of pycnidia and pycnidiospores of *Ampelomyces quisqualis* A-Pycnidia observed (100X) B- Breaking of pycnidia observed (100X) C- Releasing of pycnidiospores from pycnidia (400X)

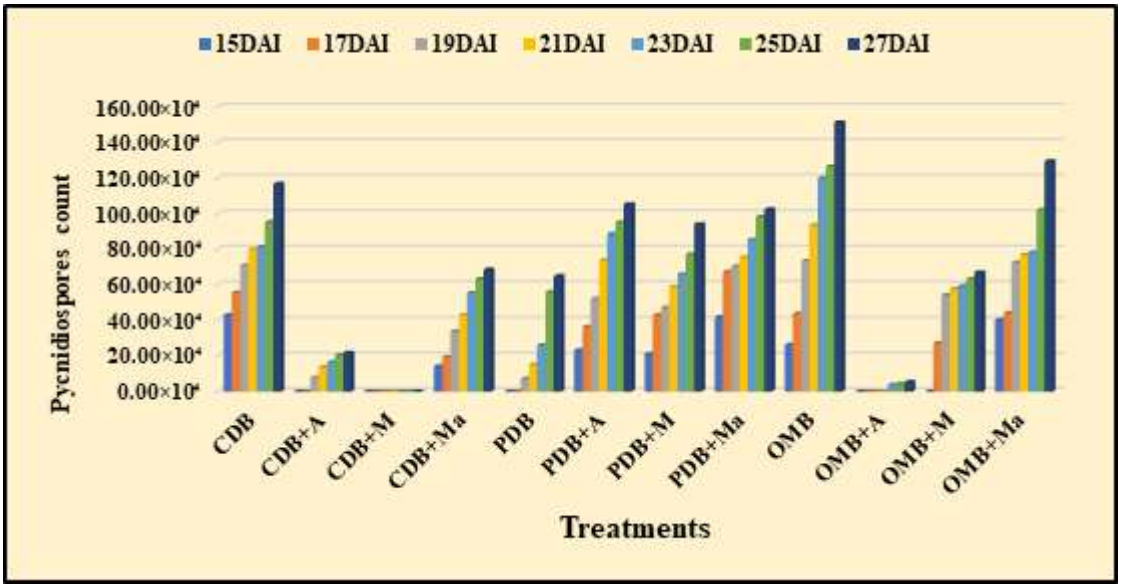

Fig. 2.Effect of different liquid media on pycnidiospores production of *Ampelomyces quisqualis* at different intervals

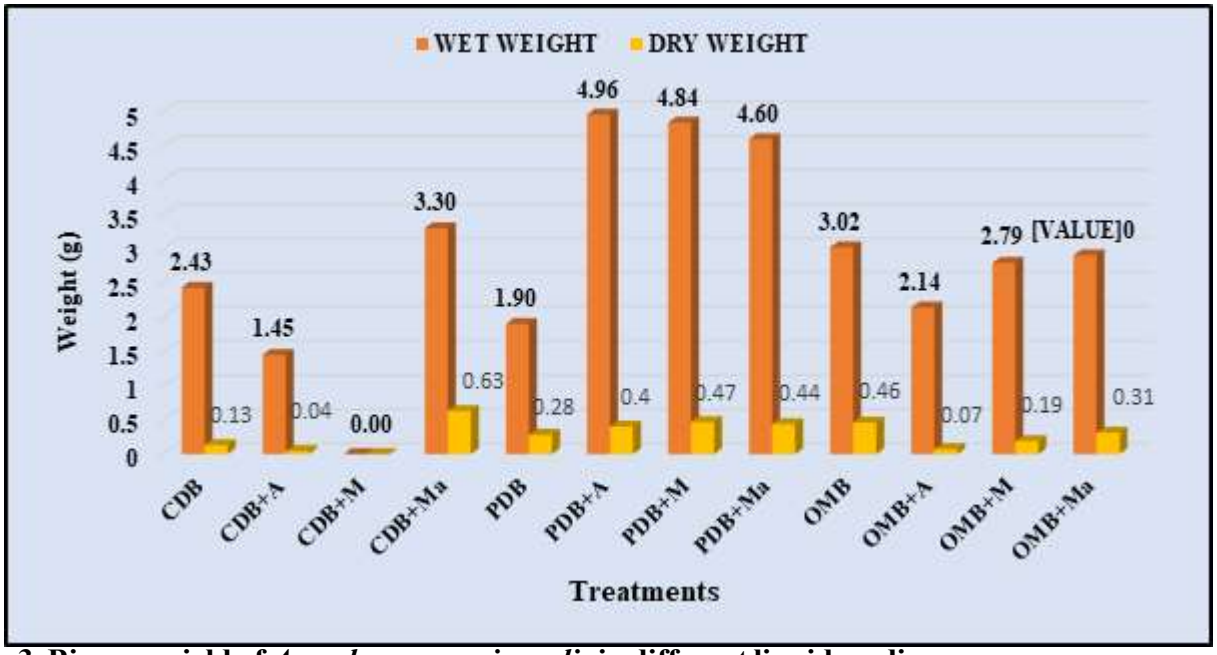

Fig.3. Biomass yield of *Ampelomyces quisqualis* in different liquid media

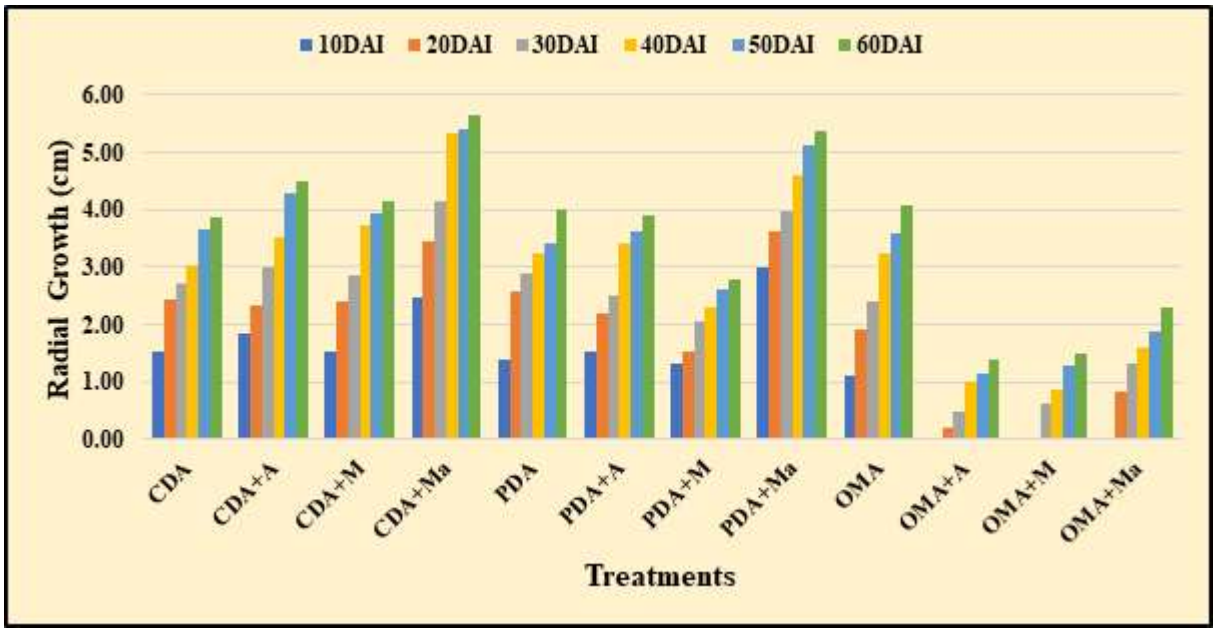

Fig.4. Effect of different solid media on radial growth of *Ampelomyces quisqualis*

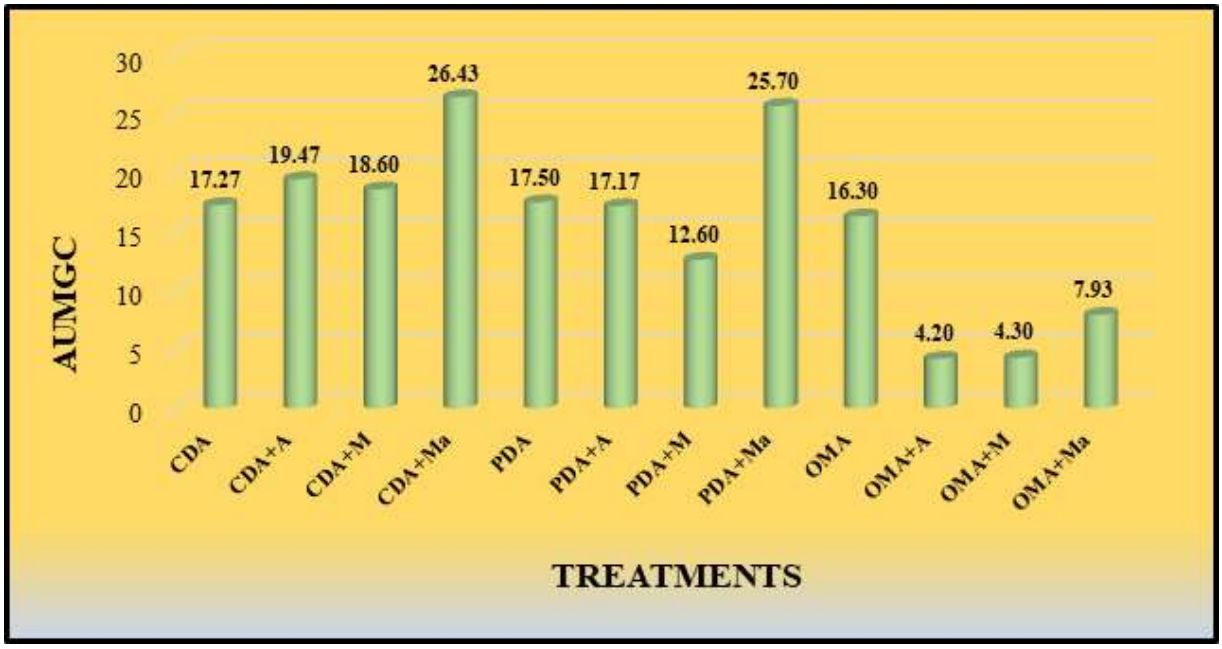

Fig. 5. Area Under Mycelial Growth (AUMGC) of *Ampelomyces quisqualis* on different solid media

universally improved sporulation and biomass across media.

Effect of solid media on the radial growth and sporulation of *A. quisqualis*

The results of the study on effect of solid media on radial growth and sporulation of *A.quisqualis* were presented in Table 4. In solid media tested, considerable radial growth was observed from 10 DAI and the *A.quisqualis* was found to be slow growing. Hence the data on radial growth was recorded at 10 days interval starting from 10 DAI up to 60 DAI and the sporulation was observed on 60 DAI . In solid media, radial growth was significantly enhanced in CDA+Ma (5.67 cm) and PDA+Ma (5.37 cm), indicating their suitability for colony expansion. Media containing OMA+M and OMA+A showed the least growth (<2 cm) and low AUMGC values. OMA and PDA+Ma recorded the highest pycnidiospore count of (800.60 × 10t /5 ml, 779.60 × 10t /5 ml) respectively. Colony shapes varied from round to irregular, while colony colors ranged from light brown to black.

Table 4. Evaluation of different solid media for morpho-cultural characteristics, radial growth and pycnidiospores production of *Ampelomyces quisqualis*

S.No	MEDIA	Shape	Colour	Pycnidiospores Count	Diameter of the colony (cm)						AUMGC
					10DAI	20DAI	30DAI	40DAI	50DAI	60DAI	
1	CDA	Round	Light Brown	202.83×10^4 (6.30)^{e*}	1.53(1.59)^{d**}	2.43(1.85)^{d}	2.73(1.93)^{e}	3.03(2.00)^{g}	3.67(2.16)^{e}	3.87(2.20)^{c}	17.27
2	CDA+A	Round	Brown	155.33×10^4 (6.19)^{f}	1.83(1.68)^{c}	2.33(1.82)^{e}	3.00(2.00)^{c}	3.53(2.12)^{d}	4.27(2.29)^{c}	4.50(2.34)^{b}	19.47
3	CDA+M	Round	Brown	73.06×10^4 (5.86)^{j}	1.53(1.59)^{d}	2.40(1.84)^{de}	2.87(1.96)^{d}	3.73(2.17)^{c}	3.93(2.22)^{d}	4.13(2.26)^{bc}	18.6
4	CDA+Ma	Irregular	Brown	334.76×10^4 (6.52)^{c}	2.46(1.86)^{b}	3.46(2.11)^{b}	4.13(2.26)^{a}	5.33(2.51)^{a}	5.40(2.53)^{a}	5.63(2.57)^{a}	26.43
5	PDA	Irregular	Black	301.93×10^4 (6.48)^{d}	1.40(1.54)^{e}	2.56(1.88)^{c}	2.90(1.97)^{d}	3.23(2.05)^{f}	3.40(2.09)^{f}	4.00(2.23)^{bc}	17.5
6	PDA+A	Irregular	Black	444.33×10^4 (6.64)^{b}	1.53(1.59)^{d}	2.20(1.78)^{f}	2.50(1.87)^{f}	3.40(2.09)^{e}	3.63(2.15)^{e}	3.90(2.21)^{bc}	17.17
7	PDA+M	Irregular	Black	102.66×10^4 (6.01)^{h}	1.33(1.52)^{f}	1.53(1.59)^{h}	2.07(1.75)^{h}	2.30(1.81)^{i}	2.60(1.89)^{g}	2.77(1.94)^{d}	12.6
8	PDA+Ma	Irregular	Black	779.60×10^4 (6.89)^{a}	3.00(2.00)^{a}	3.63(2.15)^{a}	3.97(2.22)^{b}	4.60(2.36)^{b}	5.13(2.47)^{b}	5.37(2.52)^{a}	25.7
9	OMA	Irregular	Black	800.60×10^4 (6.90)^{a}	1.10(1.44)^{g}	1.93(1.71)^{g}	2.40(1.84)^{g}	3.23(2.05)^{f}	3.57(2.13)^{ef}	4.07(2.24)^{bc}	16.3
10..	OMA+A	Round	Black	95.00×10^4 (5.97)^{i}	0(1.00)^{h}	0.20(1.09)^{j}	0.47(1.21)^{k}	1.00(1.41)^{j}	1.13(1.46)^{j}	1.40(1.54)^{f}	4.2
11	OMA+M	Round	Black	44.33×10^4 (5.64)^{k}	0(1.00)^{h}	0(1.00)^{k}	0.63(1.27)^{j}	0.87(1.36)^{k}	1.30(1.51)^{i}	1.50(1.58)^{f}	4.3
12	OMA+Ma	Irregular	Black	110.86×10^4 (6.04)^{g}	0(1.00)^{h}	0.83(1.35)^{i}	1.33(1.52)^{i}	1.60(1.61)^{i}	1.87(1.69)^{h}	2.30(1.81)^{e}	7.93
	SEm(±)			0.009	0.006	0.007	0.008	0.008	0.015	0.04	
	C.D.(P<0.05)			0.026	0.017	0.022	0.025	0.024	0.043	0.12	
	CV(%)			0.245	0.663	0.755	0.801	0.713	1.249	1.875	

Data presented was mean of three replications CDA-Czapek Dox Agar, CDA+A-Czapek Dox Agar +Arabitol, CDA+M-Czapek Dox Agar + Mannitol, CDA+Ma- Czpek Dox Agar + Malt extract, PDA-Potato Dextrose Agar, PDA+A- Potato Dextrose Agar +Arabitol, PDA+M- Potato Dextrose Agar + Mannitol, PDA+Ma- Potato Dextrose Agar+ Malt extract, OMA- Oat Meal Agar , OMA+A-Oat Meal Agar +Arabitol, OMA+M-Oat Meal Agar +Mannitol, OMA+Ma- Oat Meal Agar +Malt extract

Figures with similar alphabets do n't differ significantly. '*'-Values in the parenthesis are log transformed values. '**'- Values in the parenthesis are square root transformed

values.

Overall, malt extract enhanced sporulation in both liquid and solid media besides radial growth promotion on solid media. These findings are in accordance with that of Tang *et al*. (2023) who also observed accelerated growth and conidiation of *Ampelomyces quisqualis* using malt extract in various media. Angeli *et al*. (2017) observed high sporulation in PDB. Carbó *et al*. (2020) reported peak sporulation in modified PDB, confirming the benefit of optimized formulations.

A hyperosmotic environment produced in the liquid medium by high concentrations of polyols, such as arabitol and mannitol, may be the cause of the inhibition of sporulation in the liquid media supplemented with these substances. This environment may draw water out of the conidia and possibly upset the delicate balance necessary for germination. It has been observed that mannitol accumulation by a number of fungi improves stress tolerance. According to Son *et al*. (2012), in culture supplemented with high levels of mannitol, the conidia of the cereal head blight fungus, *Gibberella zeae*, transformed into structures resembling chlamydospores. Under low water potentials, mannitol was also found to further impede hyphal development and germination (Estaun, 1990).

CONCLUSION

The present study demonstrates that media composition significantly influences the growth and sporulation of *Ampelomyces quisqualis*. Oat meal-based media were superior in promoting sporulation, while malt extract emerged as the most effective supplement for enhancing both sporulation and biomass yield. Among solid media, CDA+Ma supported the best radial growth, and PDA+Ma was highly effective in promoting all growth parameters. Supplementation of liquid media with high concentrations of arabitol and mannitol were found inhibiting the hyphal growth and sporulation in *A. quisqualis*.

LITERATURE CITED

Angeli D, Saharan K, Segarra G, Sicher C and Pertot I 2017. Production of *Ampelomyces quisqualis* conidia in submerged fermentation and improvements in the formulation for increased shelf-life. *Crop Protection*. 97:135-144.

Estaun M V 1990. Effect of sodium chloride and mannitol on germination and hyphal growth of the vesicular-arbuscular mycorrhizal fungus *Glomus musosseae. Agriculture, Ecosystems and Environment*. 29 (1-4): 123-129.

Carbó A, Torres R, Usall J, Ballesta J and Teixidó N 2020. Biocontrol potential of *Ampelomyces quisqualis* strain CPA-9 against powdery mildew: Conidia production in liquid medium and efficacy on zucchini leaves. *Scientia Horticulturae*. 267: 109337.

Kiss L, Russell J C, Szentiványi O, Xu X and Jeffries P J B S 2004. Biology and biocontrol potential of *Ampelomyces* mycoparasites, natural antagonists of powdery mildew fungi. *Biocontrol Science and Technology*. 14(7): 635-651.

Son H, Lee J and Lee Y W 2012. Mannitol induces the conversion of conidia to chlamydospore-like structures that confer enhanced tolerance to heat, drought, and UV in *Gibberella zeae*. *Microbiological Research*.167 (10): 608-615.

Sztejnberg A, Galper S, Mazar S and Lisker N 1989. *Ampelomyces quisqualis* for biological and integrated control of powdery mildews in Israel. *Journal of Phytopathology*. 124(4): 285.

Tang X, Sun L, Hazila Li W, Dong P, Ren Y and Li G 2023. Investigation and isolation methods of hyperparasites of powdery mildew on landscape plants. *Xinjiang Agricultural Sciences*. 60(7): 1748-1755.

Received on 03.08.2025 and Accepted on 25.08.2025