Biological Forum – An International Journal **15(9): 805-812(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Different Culture Media on Growth of *Alternaria* Isolates

Manisha Solanki[1], J.J. Kadam[1] and Jeetu Narware[2]*
[1]Department of Plant Pathology,
Dr. Balasaheb Sawant Konkan Krishi Vidhyapeeth, Dapoli (Maharashtra), India.
[2]Department of Mycology and Plant Pathology,
Institute of Agricultural sciences, BHU (Uttar Pradesh), India.

(Corresponding author: Jeetu Narware)*
(Received: 06 July 2023; Revised: 07 August 2023; Accepted: 05 September 2023; Published: 15 September 2023)
(Published by Research Trend)

ABSTRACT: *Alternaria* blight is a devastating disease among the different cultivated and wild host. Diseases caused by *Alternaria* are prevalent in all agro climatic zones of Maharashtra. A number of disease cause by *Alternaria* spp. to cereals, vegetables, fruit, and ornamental crops with huge crop loss. A large variation observed in genus *Alternaria* with respect of symptom development, morphological characteristics and radial growth. In this experimental study we collected the 58 samples, out of which 26 isolates were taken for the variability study of *Alternaria* spp. The findings of this study highlight the variation observed in mean radial mycelial growth and dry mycelial weight of *Alternaria* spp on six different media (PDA, OMA, RAM, AHM, SAM and V8 juice agar). It was recorded that the maximum mean radial mycelial growth of *Alternaria* isolates was observed on PDA medium followed by OMA medium and minimum growth was recorded on V8 medium. Whereas highest dry mycelial weight was recorded on PDA the and lowest mean dry mycelial weight was recorded on V8.

Keywords: *Alternaria* spp., radial mycelial growth, dry mycelial weight and six different media.

INTRODUCTION

Alternaria is frequently one of the most prevalent fungal genera, according to several recent assessments of different plant microbiomes (Dettman *et al*., 2023). India has diverse agroclimatic conditions. A humid climate is a zone of climate characterized by hot, usually humid summers and mild to cool winters. Diseases caused by *Alternaria* are prevalent in agroclimatic conditions of Maharashtra.

The members of the genus *Alternaria* are anamorphs of the fungus belonging to genus *Lewia* which is a member belongs to Phylum Ascomycota, sub-division Pezizomycotina, class Dothiomycetes and order Pleosporales of the family Pleosporaceae (Kirk *et al*., 2008). *Alternaria* species are potential global fungus that may be found in soil, plants, food, feed, and indoor air (Nayyar *et al.*, 2014). Most of the *Alternaria* species are destructive pathogens of cultivated crops and wild hosts. It is an opportunistic pathogen that affects a variety of hosts and is responsible for at least 20% of agricultural spoilage and, in the most severe cases, up to 80% of the yield losses (Nowicki *et al*., 2012). Several prominent plant pathogenic species fall within the sub-generic *Alternaria* section *Alternaria* (Lawrence *et al*., 2013), including one of the most widely distributed and commonly found species, *A. alternata*. A large variation observed in genus *Alternaria*, there are 299 species in this genus (Kirk *et al.*, 2008). At present 746 species of *Alternaria* recorded in the index fungorum. Some *Alternaria* species are initiators of decomposition of the plant residues. Researchers have reported yield loss due to *Alternaria* ranging from 36.88 % to 70.00 % (Talukdar *et al*., 2017). Purple blotch of onion caused by *Alternaria porri* is one among the serious fungal diseases that affect onion, causing yield loss ranging from 2.5 to 87.8 per cent during kharif season (Behera *et al.*, 2017). *A. japonica* causes yield loss up to 58% on rapeseed in Australia (Al-lami *et al*., 2019b).

The spores are airborne and found in the terrestrial as well as aquatic environments. A number of disease cause by *Alternaria* spp to cereals, vegetables, fruit, and ornamental crops with huge crop loss. Some *Alternaria* spp. act as post-harvest pathogen and cause extensive spoilage of agricultural output. The pathogen produces distinctive leaf spots and can also cause stem lesions, ring spots and fruit rot. Initial infection on the leaves is in the form of small, circular dark spots, which gradually enlarge to form larger patches with concentric rings.

MATERIALS AND METHODS

The diseased leaves of different plants species showing typical symptoms of *Alternaria* leaf blight were collected from different cultivated and wild hosts from different locations. Out of the 58 samples collected, cultures of 26 isolates of *Alternaria* were obtained (Table 1) and pathogenicity was proved.

For the observation of variation in radial mycelial growth and dry mycelial weight of the *Alternaria* spp used three Synthetic and three non-synthetic solid and liquid media. The different media i.e., Potato Dextrose Agar, Oat Meal Agar, Richard's medium, Asthana& Hawker's agar medium, Sabouraud's agar medium and

V8 Juice Agar media were prepared with given composition. The pH of all the media were maintained at 6.5. Before autoclaving of the media dispended in different conical flasks. The flasks were plugged with non-absorbent cotton plugs and sterilized in an autoclave at 15 lbs pressure for 20 minutes. Petri plates were sterilized in hot air oven at 160ºC for 1 hour. Sterilized Petri plates were poured with 20 ml of molten medium and allowed to solidify. Five-millimetre diameter disc of the test fungus was cut with the help of incinerated cork borer and inoculated at the centre of Petri plates. The inoculated plates were then incubated at room temperature (27±2ºC) for 7 days.

For the growth of *Alternaria* isolates on liquid media the composition and preparation of media used were the same as that of solid media except that the agar was not added. The pH of media was adjusted to 6.5. Twenty millilitre of different liquid media was dispended into each of 100 ml conical flasks. These flasks were then sterilized at 15 lbs psi for 20 minutes. The flasks were inoculated with 5 mm culture discs obtained from 7 days old culture and incubated at 27 ± 2ºC for 7 days. After 7 days the growth of the fungus was estimated by harvesting mycelial mat onto Whatman No. 1 filter paper from each medium and measured the dry mycelial weight. Three replications were maintained for each treatment.

Table 1: Twenty-six *Alternaria* spp.

Designation	*Alternaria* spp	Designation	*Alternaria* spp
A1	*Alternaria alternata*	A14	*Alternaria brassicicola*
A2	*Alternaria alternata*	A15	*Alternaria brassicicola*
A3	*Alternaria alternata*	A16	*Alternaria brassicicola*
A4	*Alternaria alternata*	A17	*Alternaria brassicicola*
A5	*Alternaria alternata*	A18	*Alternaria brassicae*
A6	*Alternaria alternata*	A19	*Alternaria brassicae*
A7	*Alternaria alternata*	A20	*Alternaria brassicae*
A8	*Alternaria alternata*	A21	*Alternaria raphani*
A9	*Alternaria alternata*	A22	*Alternaria macrospora*
A10	*Alternaria alternata*	A23	*Alternaria sesami*
A11	*Alternaria alternata*	A24	*Alternaria cucumerina*
A12	*Alternaria alternata*	A25	*Alternaria porri*
A13	*Alternaria brassicicola*	A26	*Alternaria solani*

RESULT AND DISCUSSION

The different media influenced the colony diameter of *Alternaria* isolates (Table 2). The maximum mean radial mycelial growth (7.49 cm) was recorded in A1 (Isolated from marigold), it was at par with A24 (7.33cm). The least mean colony diameter was recorded in the isolate A7 (3.77 cm). Among the six solid media evaluated to record colony diameter, maximum mean radial mycelial growth was observed on PDA medium (6.85 cm). This suggests that the microorganism grew very well on this medium. The mean radial mycelial growth on OMA medium was 6.55 cm, which is slightly less than mean radial mycelial growth on PDA, as it is at par with PDA. Whereas the least mean mycelial growth was recorded on V8 medium (3.49 cm). This medium appears to be less favourable for the growth of the microorganism compared to PDA and OMA. The results of the present experiment are lined with previous study done by Arunakumara (2006); Ramjegathesh and Ebenezar (2012); Agale *et al.* (2014); Sangeetha and Ashtaputre (2015); Tiwari *et al*. (2016); Valvi *et al*. (2019); Archana *et al*. (2022).

Arunakumara and Kulkarni (2006) reported that the radial growth of *A. solani* was the maximum on potato dextrose agar with colony diameter of 89 mm followed by oat meal agar (85.6 mm), among the non-synthetic media and two synthetic media tried, Richard's agar promoted maximum colony diameter of 87.4 mm. According to Ramjegathesh and Ebenezar (2012) higher radial growth (8.14cm) of *A. alternate* was reported on potato dextrose agar. Agale *et al*. (2014) reported that the maximum mycelial growth of *A. porri* was recorded on oat meal agar (90.00 mm) followed by Richard's agar (70.00 mm) and Asthana & Hawkar's agar (70.00 mm). Tiwari *et al.* (2016) reported that the maximum mycelial growth of *Alternaria porri* was recorded on PDA and minimum on Asthana & Hawker medium, the maximum mycelial growth of *A. brassicae* was recorded on PDA medium and maximum mycelial growth of *Alternaria sesami* was occurred on PDA media while, minimum on Asthana & Hawker's media. Valvi *et al.* (2019) used eight culture media and were tested among that; the potato dextrose agar medium was found most suitable and encouraged maximum radial mycelial growth (90.00 mm) of *A. brassicae*. Archana *et al*. (2022) used common semi-synthetic, synthetic, and natural media, in both solid and liquid form, culture the fungus and reported that the *A. alternata* showed maximum mycelial growth (86.67) on Potato dextrose agar (PDA) medium.

The results of Akbari (2005); Naik *et al*. (2010) are contradictory to present findings. Akbari (2005) reported that Richard's medium (81.66 mm) performed the best for radial growth of *A. alternata*. PDA was also adjudged better for radial growth of *A. alternata*. Naik *et al*. (2010) reported that the Maximum radial growth of *Alternaria solani* was recorded in Sabouraud's agar (89.67 mm) followed by PDA supplemented with $CaCO_3$ (87.67mm).

The effect of media on colony diameter of the isolates under study was statistically significant. The isolate A1 (8.57 cm) recorded maximum radial growth on OMA followed by A1 (8.40 cm) on PDA, A24 (7.97 cm) on

SB, A1 (7.93 cm) on RA, A24 on AH (7.23 cm) and V8 (6.17 cm). Among the twenty-six Isolates, the least radial mycelial growth was recorded in isolate A5 (2.13 cm) on V8 medium.

From Table 3, it was confirmed that different media affected the dry mycelial weight of test isolates. The maximum mean dry mycelial weight was recorded by A24 (309.23 mg) isolated from watermelon, it was at par with isolate A1 (270.83 mg), A22 (240.60 mg), A6 (240.29). The lowest dry mycelial weight was recorded on isolate A25 (96.78 mg), it was significantly less as compared to the rest of isolates.

Among the liquid media maximum mean dry mycelial weight was recorded on PDA (316.61 mg) followed by OMA (253.94 mg), Richard's medium (194.87mg), Asthana & Hawker's agar medium (156.45 mg), Sabouraud's agar medium (168.54 mg) and lowest mean dry mycelial weight was recorded on V8 (93.24 mg). The results of the present experiment are in accordance with Archana *et al.* (2022). But contradictory with Akbari (2005); Agale *et al*. (2014); Koley and Mahapatra (2015).

Archana *et al.* (2022) reported that the potato dextrose broth was observed most supportive for its growth of *Alternaria alternata*. Akbari (2005) reported that the higher dry mycelial weight (559.0 mg) of *A. alternata* was recorded on Richard's solution. OMA (225.0 mg) and PDA (212.0 mg) supported medium to poor growth and the least dry mycelial weight was recorded on Asthana and Hawker's medium (68.0 mg). Agale *et al.* (2014) reported that the OMA (181.67 mg) was the best for dry mycelial weight of *A. porri*. Richard's medium recorded dry mycelial weight of 167.50 mg, on Asthana & Hawkarsmedium, it was 150.67 mg and on PDA it was 80.67 mg. Koley and Mahapatra (2015) reported that the highest dry mycelial weight (713.33 mg) of *A. solani* was recorded on Richard's broth medium followed by Sabouraud's broth (533.33 mg). Potato dextrose broth (289.33 mg) and oat meal broth (281.33 mg) also supported good dry weight mycelial growth of *A. solani*. Minimum dry mycelial weight was recorded on Asthana and Hawker's broth (76.67 mg).

The interaction of isolates × media was statistically significant. The maximum mean dry mycelial weight was recorded on PDA by the isolate A24 (600.00 mg) followed by A1 (510.19 mg) on OMA, A23 (319.39 mg) on SB, A5 (317.35 mg) on RA, A24 (266.94 mg) on AH and A1 (143.33 mg) on V8.

Table 2: Effect of different culture media (solid) on growth of *Alternaria* isolates.

Media / Isolate	Potato dextrose agar medium (cm)*	Oat meal agar medium (cm)*	Richard's medium (cm)*	Asthana & Hawker's agar medium (cm)*	Sabouraud's agar medium (cm)*	V8 juice agar medium (cm)*	Mean
A1	8.40	8.57	7.93	6.53	7.40	6.13	7.49
A2	7.77	6.70	6.53	5.97	5.50	4.00	6.08
A3	8.07	8.30	7.23	6.27	5.20	3.83	6.48
A4	7.77	7.87	7.43	6.93	7.77	2.90	6.78
A5	5.50	5.13	6.13	4.40	4.23	2.30	4.65
A6	7.70	7.47	6.97	3.70	6.27	3.60	5.95
A7	5.33	4.57	3.70	3.10	3.07	2.87	3.77
A8	7.33	7.30	6.50	5.13	5.07	3.43	5.79
A9	4.67	4.53	4.47	3.63	3.47	2.47	3.87
A10	6.73	6.50	6.47	6.20	5.83	3.43	5.86
A11	6.70	6.33	6.07	5.83	4.80	3.17	5.48
A12	6.23	5.27	4.93	4.73	4.13	2.33	4.61
A13	6.93	6.23	6.03	5.30	5.27	3.53	5.55
A14	6.73	6.23	5.90	5.27	4.97	3.40	5.42
A15	6.63	5.77	5.73	5.00	4.67	3.00	5.13
A16	6.57	5.70	5.60	4.57	4.53	3.20	5.03
A17	6.53	5.53	5.47	3.50	4.10	3.07	4.70
A18	6.83	6.80	6.27	6.10	5.53	4.13	5.94
A19	6.60	6.37	6.33	3.43	5.03	2.97	5.12
A20	6.57	6.33	6.27	5.97	5.00	3.33	5.58
A21	6.90	6.53	6.50	5.63	5.23	4.33	5.86
A22	7.67	7.73	6.30	5.87	5.40	3.87	6.14
A23	6.90	7.43	5.80	4.70	7.47	3.67	5.99
A24	8.27	8.13	6.20	7.23	7.97	6.17	7.33
A25	5.20	4.97	4.83	3.27	3.10	2.43	3.97
A26	7.53	7.90	6.20	5.67	5.17	3.27	5.96
Mean	**6.85**	**6.55**	**6.08**	**5.15**	**5.24**	**3.49**	

	Culture	Media	Culture x Media
S. Em. ±	0.18	0.08	0.44
C.D. at 1 %	0.67	0.32	1.63

*Mean of three replications.

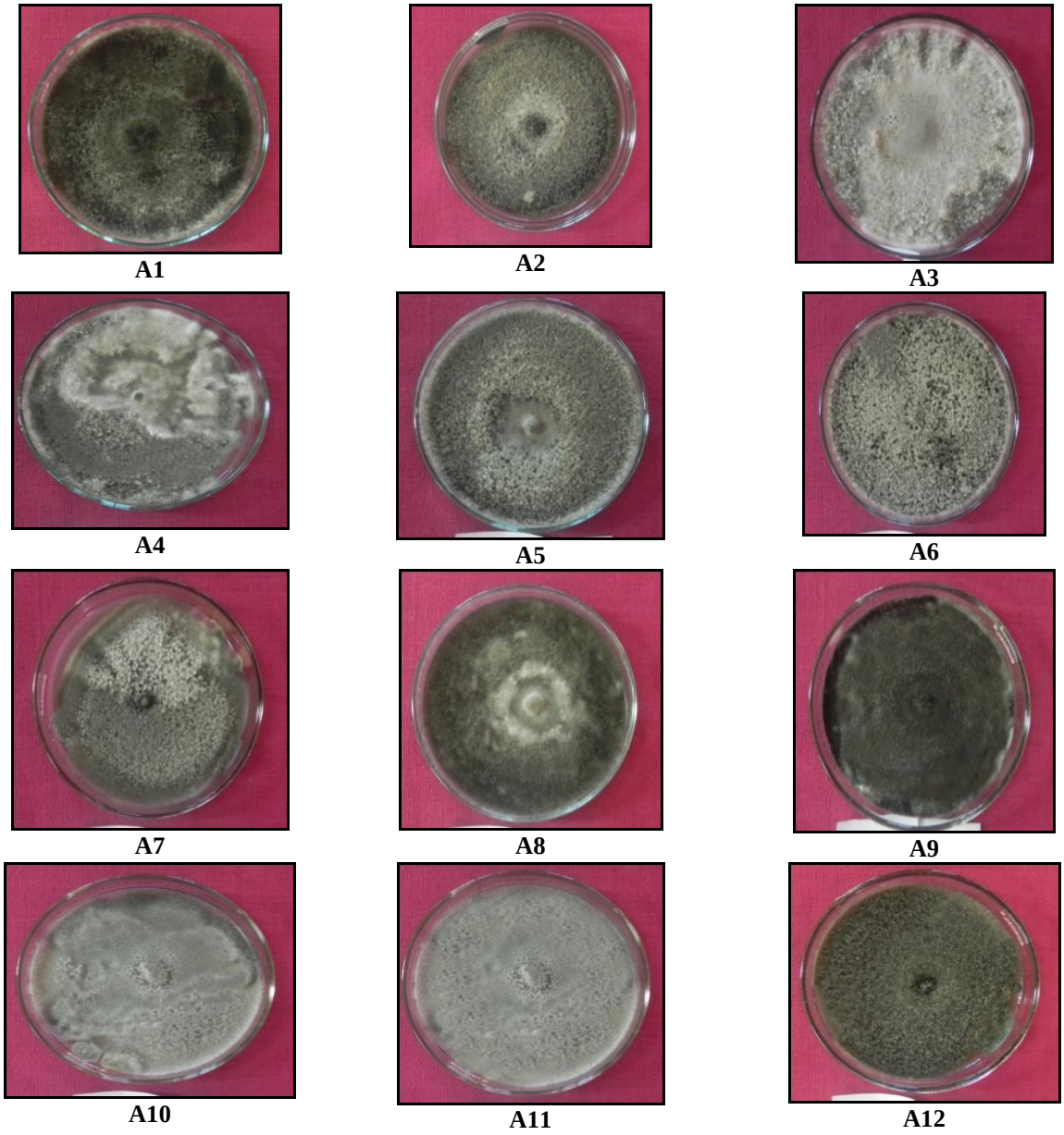

Fig. 1a. Variability in the growth of *A. alternata* isolates on PDA medium.

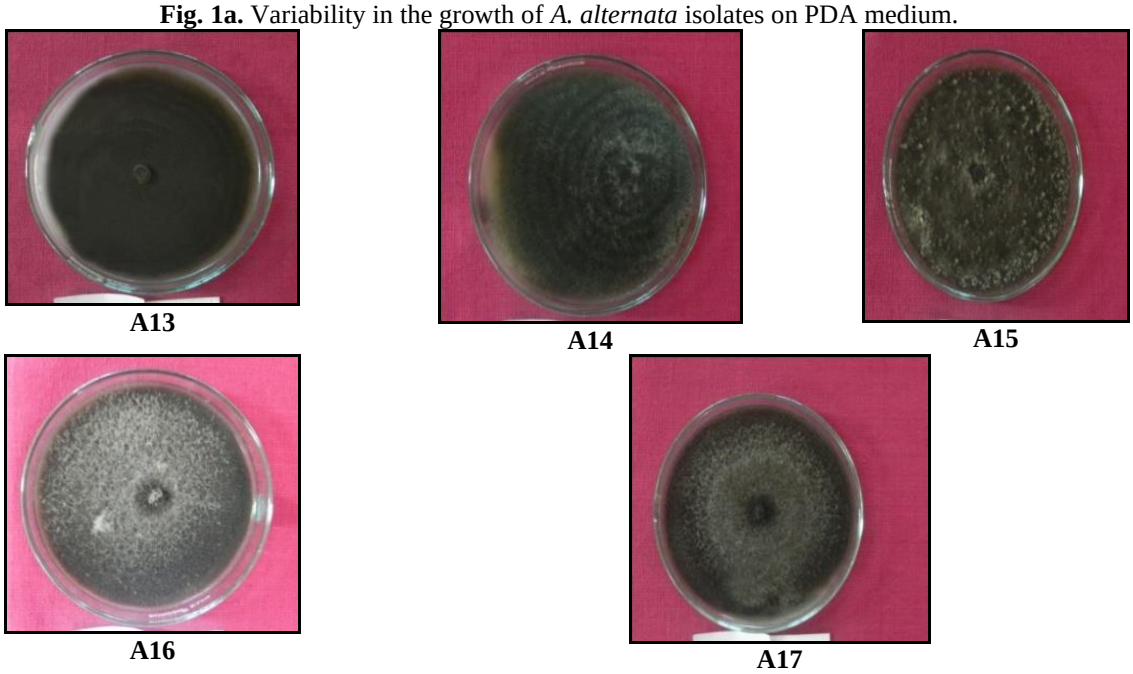

Fig. 1b. Variability in the growth of *A. brassicicola* isolates on PDA medium.

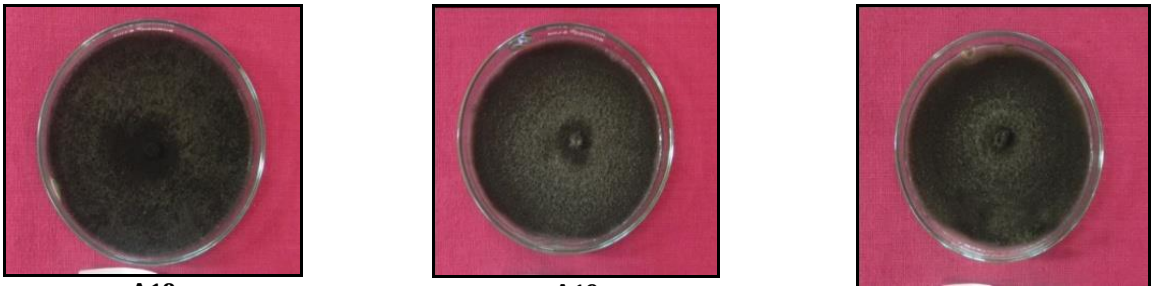

Fig. 1c. Variability in the growth of *A. brassicae* isolates on PDA medium.

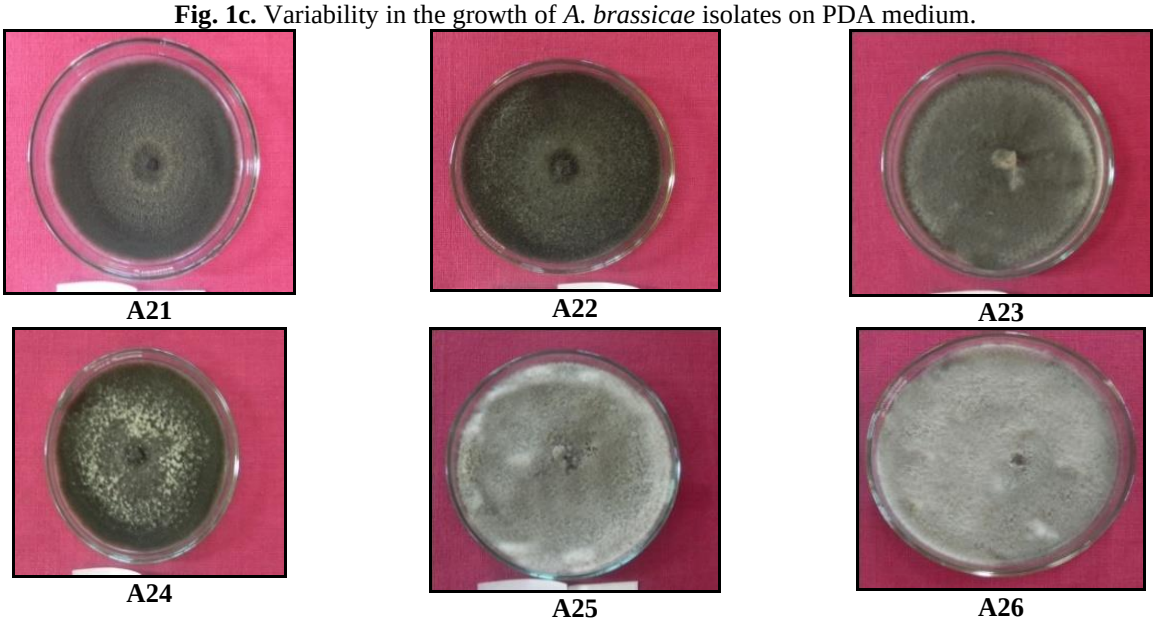

Fig. 1d. Variability in the growth of *A. raphani* (A21), *A. macrospora* (A22), *A. sesame*(A23), *A. cucumerina* (A24), *A. porri* (A25), *A. solani* (A26) on PDA medium.

Table 3: Effect of different culture media (liquid) on dry mycelial weight of *Alternaria.*

Media / Isolate	*Dry mycelial weight (mg)						
	Potato dextrose agar medium	Oat meal agar medium	Richard's agar medium	Asthana & Hawker's agar medium	Sabouraud's agar medium	V8 juice agar medium	Mean
A1	333.74	510.19	248.60	164.55	224.55	143.33	**270.83**
A2	403.15	186.00	173.06	104.33	96.67	86.04	**174.88**
A3	324.80	436.25	224.33	171.25	146.11	87.69	**231.74**
A4	300.28	271.45	221.93	176.07	266.03	84.04	**219.97**
A5	260.84	200.00	317.35	167.33	162.89	83.04	**198.57**
A6	500.00	252.33	203.53	174.75	203.00	108.14	**240.29**
A7	254.00	324.12	252.41	232.67	196.67	83.33	**223.87**
A8	312.33	248.33	190.00	155.00	123.33	91.33	**186.72**
A9	190.00	180.67	171.00	102.00	98.33	82.00	**137.33**
A10	300.07	244.00	217.00	209.97	110.67	97.67	**196.56**
A11	403.33	256.70	213.00	168.67	119.00	89.00	**208.28**
A12	400.00	175.00	123.33	88.95	118.00	80.29	**164.26**
A13	303.41	213.33	202.02	177.69	175.36	96.48	**194.71**
A14	302.33	193.17	154.67	144.67	98.67	88.00	**163.58**
A15	300.67	173.33	157.67	135.67	128.00	85.67	**163.50**
A16	217.00	213.93	209.67	193.13	190.13	106.55	**188.40**
A17	202.33	151.67	141.00	109.00	130.00	94.33	**138.06**
A18	312.21	240.03	216.47	204.78	183.00	104.29	**210.13**
A19	221.29	211.85	201.18	149.77	207.11	81.35	**178.76**
A20	231.74	180.13	177.44	126.17	123.48	86.00	**154.16**
A21	450.00	259.56	230.12	168.43	168.15	97.00	**228.88**
A22	274.95	500.00	216.81	196.53	157.00	98.30	**240.60**
A23	207.00	330.18	188.81	104.00	319.39	87.33	**206.12**
A24	600.00	335.00	216.67	266.94	300.42	136.33	**309.23**
A25	193.00	101.67	87.33	77.00	71.00	50.67	**96.78**
A26	400.00	175.00	123.33	88.95	118.00	80.29	**164.26**
Mean	**316.61**	**253.94**	**194.87**	**156.45**	**168.54**	**93.24**	-
		Isolates		**Media**		**Isolates × Media**	
SE (m)±		20.58		9.88		50.42	
CD @ 1%		75.45		36.25		184.82	
* Mean of three replications							

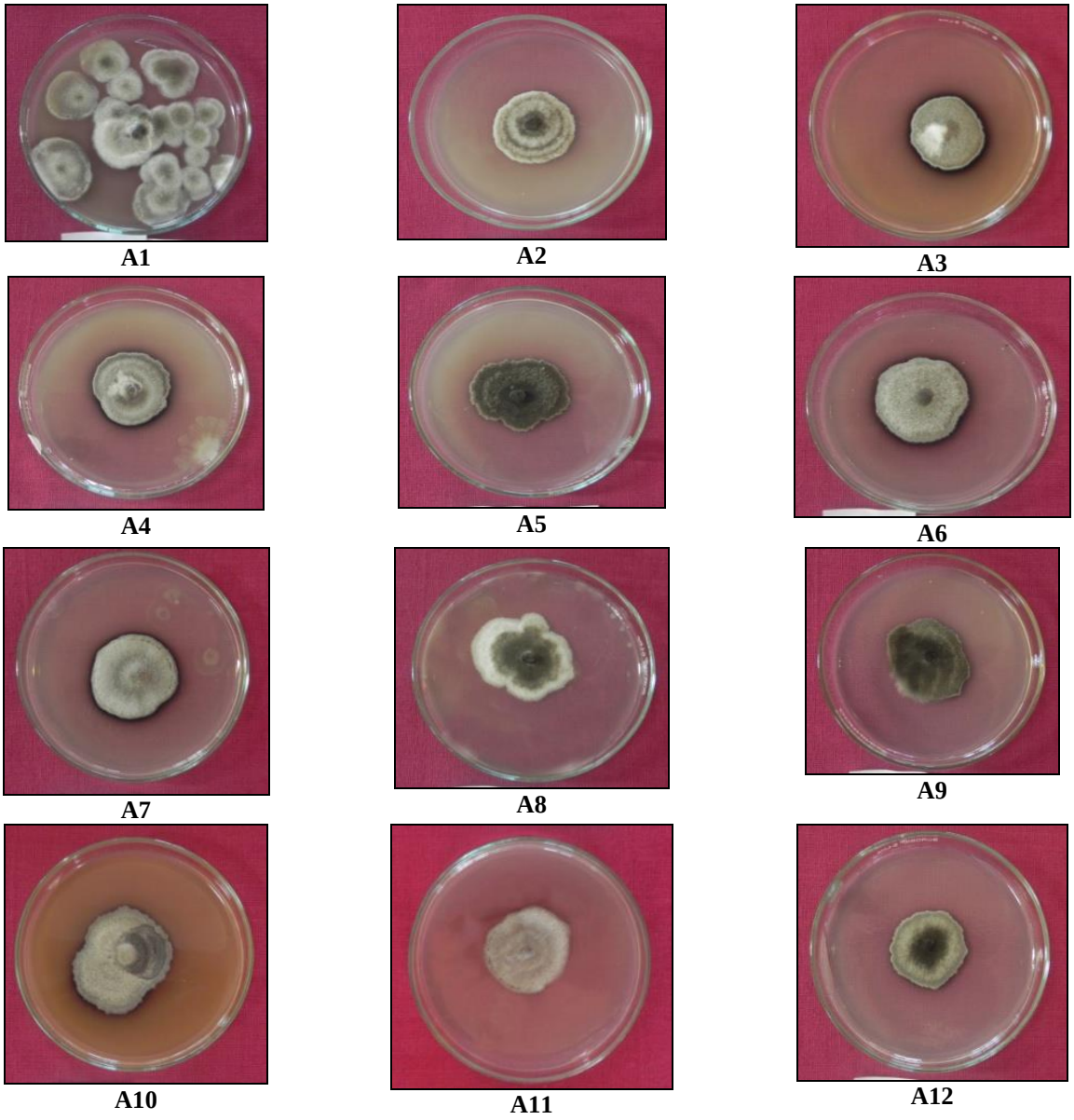

Fig. 2a. Variability in the growth of *A. alternata* isolates on V8 medium.

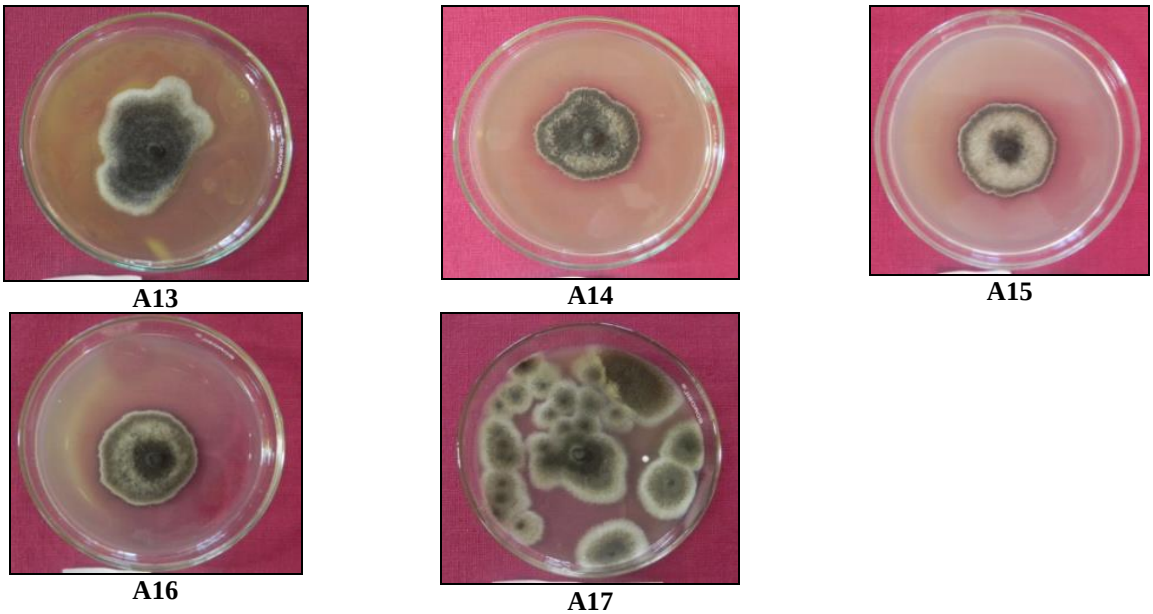

Fig. 2b. Variability in the growth of *A. brassicicola* isolates on V8 medium.

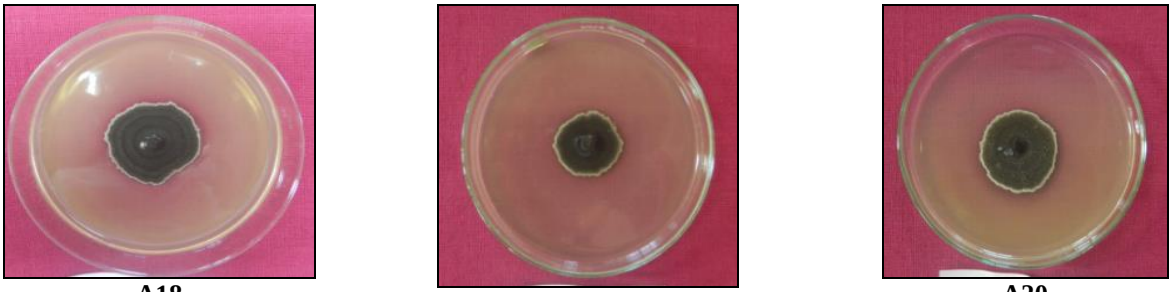

Fig. 2c. Variability in the growth of *A. brassicae* isolates on V8 medium.

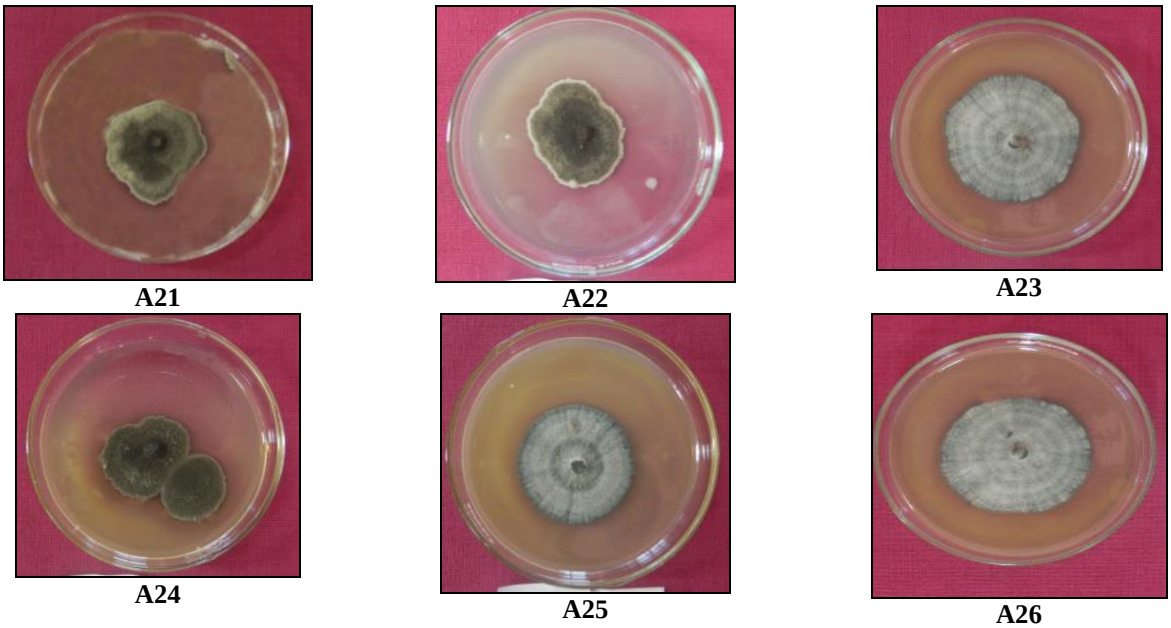

Fig. 2d. Variability in the growth *Alternaria* isolates of *A. raphani* (A21), *A. macrospora* (A22), *A. sesami* (A23), *A. cucumerina* (A24), *A. porri* (A25), *A. solani* (A26) on V8 medium.

CONCLUSIONS

In this study twenty-six isolates of *Alternaria* were studied for their cultural variation on different media which are isolated from different host plants. Among the six solid and liquid media evaluated to record colony diameter and dry mycelial weight, the maximum mean radial mycelial growth and dry mycelial weight was observed on PDA medium (Fig. 1a, 1b, 1c, 1d). The least mean mycelial growth and dry mycelial weight was observed on vegetable juice agar (V8) medium (Fig. 2a, 2b, 2c, 2d). The present study will be helpful in the research being carried out by different workers studying pathogen *Alternaria* to understand its variation in cultural characteristics and its related studies.

FUTURE SCOPE

The study will help the researchers in selecting the best media which influence growth of *Alternaria*, their sporulation and morphological characteristics. It will be helpful to understand pathogenicity, genetic study and host specificity of *Alternaria*. Climate change and environmental factors can influence the distribution and behaviour of *Alternaria* species. Future studies can explore how the climatic change effect on the variability and virulence of these fungi.

Acknowledgments. Author acknowledged the facilities provided by Department of Plant Pathology at Dr. Balasaheb Sawant Konkan Krishi Vidhyapeeth, Dapoli to conducting the research work.

Conflicts of Interest. None.

REFERENCES

Agale, R. C., Kadam, J. J., Rite, S. C., Kadam, J. S. and Pawaskar, J. R. (2014). Physiological studies on *Alternaria porri* causing purple blotch disease in white onion. *J. Pl. Dis. Sci.*, *9*(2), 202–208.

Akbari, L. F. (2005). Epidemiology and management of blight (*Alternaria alternata* (Fr.) *Keissler*) of sesame (*Sesamum indicum* L.). Ph.D. Thesis, Junagadh Agricultural University, Junagadh, India.

Al-lami, H. F. D., You, M. P. and Barbetti, M. J. (2019b). Role of foliage component and host age on severity of *Alternaria* leaf spot (caused by *Alternaria japonica* and *A. brassicae*) in canola (*Brassica napus*) and mustard (*B. juncea*) and yield loss in canola. *Crop & Pasture Science*, *70*, 969–980.

Archana, A. M., Lokesha, M. S., Mahesh, Y. S., Shirol, A. M. and Ashoka, N. (2022). Cultural and Physiological studies on *Alternaria alternata* causing Blight of Marigold. *Biological Forum – An International Journal*, *14*(1), 1269-1273.

Arunakumar, K. T. (2006). Studies on *Alternaria solani* (Ellis and Martin) Jones and Grout. Causing early blight of tomato. M.Sc. (Agri.) Thesis. Unvi. Agril. Sci., Dharwad, Karnataka, India.

Behera, S., Rai, A. K. and Rout, R. (2017). Efficacy of fungicides against *Alternaria porri* causing purple blotch of onion. *Int. J. Curr. Microbiol. App. Sci., 6*(12), 1520-1524.

Dettman, J. R., Eggertson, Q. A., and Kim, N. E. (2023). Species diversity and molecular characterization of *Alternaria* section *Alternaria* isolates collected mainly from cereal crops In Canada. *Frontiers in Microbiology*, *14*, 1194911.

Kirk, P. M., Cannon, P. F., Minter, D. W. and Stalpers, J. A. (2008). Dictionary of the Fungi. 10th ed. Wallingford: CABI. p. 22.

Lawrence, D. P., Gannibal, P. B., Peever, T. L. and Pryor, B. M. (2013). The sections of alternaria: Formalizing species-group concepts. *Mycologia*, *105*(3), 530–546.

Nayyar, B. G., Akhund, S. and Akram, A. (2014). Management of *Alternaria* and its mycotoxins in crops. *J. Agrl. Sci., 4*(7), 432-437.

Nowicki, M., Nowakowska, M. and Niezgoda, A. (2012). *Alternaria* black spot of crucifers: symptoms, Importance of disease and perspectives of resistance breeding. *Veg. Crp. Res. Bulletin*, *76*, 5-19.

Ramjegathesh, R. and Ebenezar, E. G. (2012). Morphological and physiological characters of *Alternaria alternate* causing leaf blight disease of onion. *International Journal of Plant Pathology*, *3*, 34-44.

Sangeetha, K. D. and Ashtaputre, S. A. (2015). Morphological and cultural variability in isolates of *Alternaria* spp. causing leaf blight of cotton. Karnataka. *J. Agric. Sci*., *28*(2), 214-219.

Talukdar, D., Baruah,A. R.,Debnath, P. and Dey Utpal (2017). Variation in *Alternaria brassicae* population causing *Alternaria* blight of rapeseed and mustard in Assam. *Int. J. Curr. Microbiol. App. Sci., 6*(8), 3203-3215.

Tiwari, G. P., Tiwari, S. P. and Sushma, N. (2016). Variability of Cultural Media and pH of *Alternaria* spp Pathogenic to Different Crops. *International Journal of Agriculture Sciences* (8), 1835-1837.

Valvi H. T., Kadam J. J. and Banger V. R. (2019). Isolation, Pathogenicity and Effect of Different Culture Media on Growth and Sporulation of *Alternaria brassicae* (berk.) Sacc. causing *Alternaria* Leaf Spot Disease in Cauliflower. *Int. J. Curr. Microbiol. App. Sci., 8*(4), 1900-1910.

How to cite this article: Manisha Solanki, J.J. Kadam and Jeetu Narware (2023). Effect of Different Culture Media on Growth of *Alternaria* Isolates. *Biological Forum – An International Journal, 15*(9): 805-812.