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Cultural Characterization of *Alternaria* spp. Associated with Alternaria Blotch of Apple

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A B S T R A C T

Alternaria blotch causes severe foliar defoliation to apple trees in Himachal Pradesh. Sixteen isolates of *Alternaria* spp. were collected from different locations and characterized for cultural variations. *Alternaria alternata* f. sp. *mali* varied in their cultural behavior ranging from velvety to cottony, mostly appressed, with regular to irregular margins. Colour of colonies ranged between light to dark olivaceous. The nutritional studies were undertaken to know the best medium required for the growth and sporulation of the test pathogen. The study revealed that out of six different solid media tested, potato dextrose agar (PDA), malt extract agar and Asthana and Hawker's agar medium proved best for mycelial growth and sporulation of the test pathogen, respectively.

Introduction

Apple (*Malus* × *domestica* Borkh.) belongs to family Rosaceae and it is the most important fruit crop grown extensively in temperate regions of the world. The area under apple cultivation has increased manifold during the last few decades but the production has not increased proportionally and the productivity is quite low in comparison to advanced apple growing countries of the world. Like other horticultural crops apple is also attacked by several pathogens which impair the quality and quantity of the fruit. Huge losses of the crop are incurred mostly by fungal diseases. The major fungal diseases include scab, Alternaria leaf blotch, powdery mildew, collar rot, root rot, sooty blotch, fly speck, etc.

Among these, Alternaria blotch caused by *Alternaria mali*, prevalent in all apple growing areas of the world is an economically important apple disease. *Alternaria mali* was first described in 1924 in the United States by Roberts and become a problem in the southeastern United States. The disease

assumed alarming threat to the crop owing to premature defoliation in North Carolina and has potential of becoming threat especially in those apple and loquat producing regions where susceptible cultivars/strains of Delicious are grown (Filajdic and Sutton, 1991). *A. mali* has attained the status of economically important disease in many Asian countries including Japan and India (Jones and Aldwinckle, 1990). The fungus obtains food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon which the test pathogen can grow and reproduce.

Therefore studies were conducted in different suitable media to identify surface medium for the growth and sporulation of pathogen involved in Alternaria blotch of apple.

Materials and Methods

The experiment was conducted in the Fruit Pathology Laboratory, Dr. YS Parmar University of Horticulture & Forestry, Nauni, Solan (H.P) during the year 2015-16. To find out the suitable medium for the growth and sporulation of the *Alternaria* spp. six solid media viz., potato dextrose Agar, Asthana and Hawker's Agar, Czapek's dox Agar, malt extract agar, glucose asparagine agar and Dimmick's agar were taken for *in vitro* studies.

Inoculated plates were incubated at 25±1^{0}C and observations on mycelial growth (radial) and sporulation were recorded on 2nd, 4th, 6th and 8th day after incubation. Best solid medium was used for further studies. Growth on different solid media was determined by measuring the colony diameter along with the two diagonals passing though the centre of colony by excluding initial diameter (5 mm) of bit and sporulation was recorded by using haemocytometer.

Results and Discussion

Nutrition plays an important role in growth and sporulation of the fungus. In order to determine basal medium for mycelial growth and sporulation of *Alternaria* spp. six solid media were tested *in vitro*. The mycelial growth and sporulation of the test pathogen on nine different solid media was studied as described in the material and methods and results are presented in tables 1, 2 and 3. The data presented in Table 1 indicated that *Alternaria* sp. can grow well on different media. All the isolates tested depicted variation in the growth rate (growth/day) and sporulation (spores/ml) (Table 1 and 2). Isolate 1with mean growth rate of 66.92 mm followed by Isolate 2, 7 and 3 with mean growth rate of 64.13, 61.15 and 60.17 mm, respectively. Least growth rate of 40.08 mm was recorded in Isolate 6 on potato dextrose agar medium. However, maximum growth of the pathogen after 8 days was observed on potato dextrose agar medium followed by malt extract agar and minimum growth was observed on Czapek's dox agar medium. In case of Asthana and Hawker's medium, maximum mean growth rate (51.02 mm) was recorded in Isolate 5 followed by Isolate 6 and 11 with mean growth rate of 48.10 and 47.61mm which were statistically at par with each other. Least growth rate of 35.06 mm was recorded in Isolate 8 in Asthana and Hawker's medium.

Maximum mean growth rate of 51.03mm followed by Isolate 5 and Isolate 3 with mean growth rate of 49.83 and 49.27mm whereas minimum mean growth rate of 35.45mm was recorded on Isolate 8 in case of Czapek's dox agar medium. In malt extract medium, maximum mean growth rate of 51.09mm was observed in Isolate 10 followed by Isolate 7, Isolate 15 and Isolate16 with mean growth rate of 50.91, 50.30 and 49.93 mm, respectively which were statistically at par

with each other. Least growth rate of 34.24 mm was recorded in Isolate 8. In case of glucose asparagine medium, highest mean growth rate (47.33mm) was recorded on Isolate 3 followed by Isolate 12, Isolate 15 and Isolate 2 with mean growth rate of 47.19, 46.40 and 45.21mm, respectively. Whereas in case of Dimmick's agar maximum mean growth rate of 46.71mm was observed in Isolate 9 followed by Isolate 16 and Isolate 15 with mean growth rate of 46.65 and 45.87mm. Least growth rate of 32.90 mm was recorded in Isolate 8.

The sporulation of 20.20×10^3 spores/ml was observed in Isolate 1 followed by Isolate 2, 7 and 3 with sporulation of 20.00×10^3spores/ml, 18.50×10^3 spores/ml and 18.00×10^3spores/ml, respectively. Least sporulation 10.00×10^3 spores/ml was recorded in Isolate 6, 11 and 16 on potato dextrose agar medium. However, maximun sporulation of the pathogen after 8 days was observed on potato dextrose agar medium followed by malt extract agar and minimum sporulation was observed on Czapek's dox agar medium.

In case of Asthana and Hawker's medium, maximum sporulation (3.50×10^3 spores/ml) was recorded in Isolate 5 followed by Isolate 4 and 1 with sporulation of 3.25×10^3 spores/ml and 3.00×10^3 spores/ml, respectively. Least sporulation of 1.50×10^3 spores/ml was recorded in Isolate 11, 14 and 15 in Asthana and Hawker's medium.

Maximum sporulation of 4.80×10^3 spores/ml was observed in Isolate 2 followed by Isolate 1 and Isolate 13 with sporulation of 4.50×10^3 spores/ml and 4.50×10^3 spores/ml whereas minimum sporulation of 2.50×10^3 spores/ml was recorded on Isolate 6, 15 and 16 in case of Czapek's dox agar medium. In malt extract medium, maximum sporulation of 12.00×10^3 spores/ml was observed in Isolate 2 and Isolate 4 followed by Isolate 3 and Isolate1 with sporulation of 11.50×10^3 spores/ml and 11.00×10^3 spores/ml, respectively. Least sporulation of 8.00×10^3 spores/ml was recorded in Isolate 11. In case of glucose asparagine agar medium, highest sporulation (14.00×10^3 spores/ml) was recorded on Isolate 1 and Isolate 7 followed by Isolate 6, Isolate 11 and Isolate 15 with sporulation of 13.50×10^3 spores/ml, 13.45×10^3 spores/ml and 13.40×10^3, respectively. Whereas in case of Dimmick's agar maximum sporulation of 9.50×10^3 was observed in Isolate 1 followed by Isolate 8 and Isolate 10 with sporulation of 8.65×10^3 spores/ml and 8.50×10^3 spores/ml. Least sporulation of 6.90×10^3 spores/ml was recorded in Isolate 4.

Isolates of *Alternaria* spp. differed with respect to their cultural characteristics. The characters *viz*. type and color of colony, growth rate of fungus and pigmentation were recorded.

The *Alternaria* spp. isolates grown on PDA showed variation in their colony characteristics (Table 3). Colony colour varied from light to dark olivaceous with greenish or brownish tinge. Mostly the colonies had velvety or cottony mycelial growth with regular to irregular margin (Plate 1). Colonies developed were either cottony or velvety with slight variations. The velvety growth was observed in ten isolates *viz*. I-2, I-3, I-6, I-7, I-9, I-10, I-11, I-12, I-13 and I-15 and cottony type of growth in six isolates *viz*. I-1, I-4, I-5, I-8, I-14, I-16.

Among the velvety type of colonies variations recorded were as follows: Appressed growth was observed in I-1, I-3, I-4, I-5, I-6, I-7, I-9, I-10, I-11, I-12, I-13, I-15. I-2 though velvety had cottony central growth. Among the cottony type of colonies furrows developed in I-1, appressed centre was recorded in I-5 and sub-aerial mycelial growth in I-16. The colony of I-4 was with slightly appressed margins.

Table.1 Effect of different media on the radial growth of *Alternaria* spp. collected from apple and other hosts

Media →	Potato Dextrose Agar					Asthana and Hawker's Agar					Czapek's Dox Agar					Malt extract Agar					Glucose asparagine agar					Dimmick's agar					Interval Mean				Mean
Days →	Growth (mm/day) at different intervals																																
Isolate ↓	2	4	6	8	Mean	2	4	6	8	Mean	2	4	6	8	Mean	2	4	6	8	Mean	2	4	6	8	Mean	2	4	6	8	Mean	2	4	6	8	
Isolate 1	37.33	60.67	81.00	88.67	**66.92**	20.00	38.17	57.47	68.83	**46.12**	20.83	37.17	53.47	68.53	**45.00**	18.57	32.73	62.53	77.20	**47.76**	17.63	30.73	59.33	73.13	**45.21**	16.47	28.33	57.37	71.57	**43.44**	21.81	37.97	61.86	74.66	49.08
Isolate 2	35.00	57.33	77.33	86.87	**64.13**	21.23	36.83	55.67	68.13	**45.47**	18.93	36.63	52.20	67.53	**43.82**	16.43	31.70	61.97	76.77	**46.72**	17.80	31.27	60.67	73.87	**45.90**	14.37	26.80	55.70	70.43	**41.83**	20.63	36.76	60.59	73.93	47.98
Isolate 3	28.50	54.00	74.77	83.40	**60.17**	22.67	37.33	57.30	69.40	**46.68**	26.57	41.93	58.40	70.17	**49.27**	18.00	33.27	63.43	79.07	**48.44**	17.87	34.63	63.50	73.30	**47.33**	17.13	28.73	57.20	71.03	**43.52**	21.79	38.32	62.43	74.4	49.24
Isolate 4	26.17	55.23	74.00	82.33	**59.43**	21.17	35.73	55.57	67.33	**44.95**	22.77	38.77	53.10	67.17	**45.45**	17.57	33.63	64.13	78.67	**48.50**	16.47	28.00	57.17	69.17	**42.70**	16.50	27.83	55.17	69.77	**42.32**	20.11	36.53	59.86	72.41	47.23
Isolate 5	26.33	54.00	72.00	80.67	**58.25**	29.70	40.93	61.03	72.40	**51.02**	27.77	41.63	58.40	71.50	**49.83**	18.23	34.50	65.23	77.47	**48.86**	16.07	27.30	58.87	70.20	**43.11**	18.00	28.33	56.80	71.67	**43.70**	22.68	37.78	62.06	73.99	49.13
Isolate 6	13.00	24.17	50.47	72.67	**40.08**	28.83	38.47	56.90	68.20	**48.10**	29.90	43.83	59.00	71.40	**51.03**	14.53	24.83	54.57	68.87	**40.70**	13.63	24.73	52.30	62.47	**38.28**	14.17	23.70	52.83	67.43	**39.53**	19.01	29.96	54.35	68.51	42.96
Isolate 7	30.60	55.00	76.00	83.00	**61.15**	27.53	37.50	56.60	67.30	**47.23**	27.17	36.37	54.00	67.50	**46.26**	21.10	36.63	66.73	79.17	**50.91**	12.80	22.77	50.67	60.77	**36.75**	15.63	25.33	53.67	68.50	**40.78**	22.47	35.6	59.61	71.04	47.18
Isolate 8	27.93	54.37	73.53	83.27	**59.78**	12.60	23.53	41.53	62.57	**35.06**	13.43	25.73	41.37	61.27	**35.45**	11.60	19.83	41.87	63.67	**34.24**	11.40	21.10	48.33	59.30	**35.03**	10.50	19.10	39.00	63.00	**32.90**	14.58	27.28	47.61	65.51	38.75
Isolate 9	25.83	53.10	72.00	81.87	**58.20**	22.53	35.83	55.57	69.37	**45.83**	23.30	36.47	55.10	67.63	**45.63**	19.77	34.53	64.67	78.27	**49.31**	17.70	29.47	57.93	70.07	**43.79**	18.67	32.33	62.50	73.33	**46.71**	21.3	36.96	61.3	73.42	48.25
Isolate 10	27.77	54.33	73.60	83.87	**59.89**	20.67	38.83	55.67	68.20	**45.84**	19.23	36.97	54.37	67.77	**44.59**	18.57	38.50	68.17	79.13	**51.09**	18.80	30.93	57.40	69.73	**44.22**	17.00	27.33	60.00	72.33	**44.17**	20.34	37.82	61.54	73.51	48.30
Isolate 11	18.90	30.27	60.00	75.33	**46.13**	28.57	38.30	56.57	67.00	**47.61**	20.10	36.70	56.23	68.00	**45.26**	19.87	33.77	64.83	77.40	**48.97**	16.80	26.57	58.37	69.40	**42.79**	16.67	25.00	53.50	66.00	**40.29**	20.15	31.77	58.25	70.52	45.17
Isolate 12*	20.50	35.00	63.67	80.67	**49.96**	26.50	36.83	57.10	69.67	**47.53**	23.93	37.23	57.43	67.73	**46.58**	18.20	33.33	63.93	78.60	**48.52**	18.87	34.27	62.60	73.00	**47.19**	13.67	23.57	52.93	67.57	**39.44**	20.28	33.37	59.61	72.87	46.53
Isolate 13*	18.87	33.00	62.00	78.67	**48.14**	17.93	22.53	43.50	61.07	**36.26**	17.63	23.53	42.30	62.33	**36.45**	17.87	30.50	60.27	74.77	**45.85**	12.43	20.60	48.10	59.13	**35.07**	15.67	26.67	56.00	70.33	**42.17**	16.73	26.14	52.03	67.72	40.66
Isolate 14*	27.23	54.17	73.73	81.67	**59.20**	17.17	21.97	42.83	58.83	**35.20**	16.77	23.77	41.93	60.00	**35.62**	17.37	34.23	65.87	74.33	**47.95**	15.73	26.53	59.73	70.53	**43.13**	16.53	26.87	53.17	66.00	**40.64**	18.47	31.26	56.21	68.56	43.63
Isolate 15*	20.80	36.77	64.33	81.00	**50.73**	19.70	26.33	43.27	61.90	**37.80**	18.77	22.27	40.47	61.57	**35.77**	18.87	38.03	67.63	76.67	**50.30**	17.73	33.77	62.27	71.83	**46.40**	17.47	32.67	61.00	72.33	**45.87**	18.89	31.64	56.5	70.88	44.48
Isolate 16*	30.53	53.77	74.63	80.43	**59.84**	20.10	26.73	43.17	62.23	**38.06**	19.83	28.30	43.47	62.00	**38.40**	20.50	35.83	66.00	77.37	**49.93**	15.73	26.63	57.93	71.00	**42.82**	19.43	33.83	61.13	72.20	**46.65**	21.02	34.18	57.72	70.87	45.95
Mean	**25.96**	**47.82**	**70.19**	**81.52**	**56.37**	**22.31**	**33.49**	**52.48**	**66.40**	**43.67**	**21.68**	**34.21**	**51.33**	**66.38**	**43.40**	**17.94**	**32.87**	**62.61**	**76.09**	**47.38**	**16.09**	**28.08**	**57.20**	**68.56**	**42.48**	**16.12**	**27.28**	**55.50**	**69.59**	**42.12**	20.02	33.96	58.22	71.42	45.91

*Isolates from hosts other than apple	C. D. (0.05)	Media	0.13	Isolate	0.21	Interval 0.10
		Media× Interval	0.26	Isolate× Media	0.49	Interval× Interval 0.29
		Media ×Isolate× Interval	1.02			

Table.3 Cultural variability of *Alternaria* spp. collected from apple and other hosts

Isolate	Colony			
	Type	**Colour**	**Margin**	**Colour on the underside of Petri-plate**
Isolate 1	Cottony, slightly furrowed with appressed centre	Dark olivaceous, with dark centre	Regular, cottony brownish margin with whitish rim	Smoky grey to dark grey
Isolate 2	Velvety, with cottony central growth	Olivaceous green	Slightly irregular, olivaceous green	Dark grey with light grey margin
Isolate 3	Velvety, appressed	Light olivaceous green	Regular, light brownish margin with white rim	Smoky grey
Isolate 4	Cottony, with slighty appressed margins	Olivaceous green with grayish surface	Regular, brownish with white rim	Grey with light grey margin
Isolate 5	Cottony, appressed centre	Light olivaceous green	Regular, cottony with white rim	Bluish brown with light grey margin
Isolate 6	Velvety, slightly furrowed with appressed centre	Olivaceous with brownish centre and grayish surface	Irregular, appressed, olivaceous green	Dark grey with light grey margin
Isolate 7	Velvety, Appressed	Olivaceous	Irregular, appressed, Olivaceous with white rim	Light grey with brown centre
Isolate 8	Cottony, sub-aerial	Dark green with greyish surface	Regular, dirty white margin	Bluish brown with light grey margin
Isolate 9	Velvety, appressed	Olivaceous green	Slightly irregular, light green with grayish rim	Light grey with brown centre
Isolate 10	Velvety, appressed	Olivaceous	Slightly irregular, brownish with dirty white rim	Brown with light grey margin
Isolate 11	Velvety, appressed	Dark olivaceous green	Irregular, light olivaceous with grayish rim	Smoky grey
Isolate 12*	Velvety, appressed	Dark olivaceous green	Slightly irregular, light olivaceous with grayish rim	Light brown with light grey margin and dark brown centre
Isolate 13*	Velvety, appressed	Olivaceous green	Slightly irregular, light green with grayish rim	Light grey with dark grey centre
Isolate 14*	Cottony, slightly furrowed	Olivaceous	Regular, cottony olivaceous with greyish rim	Greyish with light grey margin and brown centre
Isolate 15*	Velvety, appressed	Olivaceous green	Regular, appressed, olivaceous green	Smoky grey
Isolate 16*	Cottony, sub-aerial	Greenish with grayish surface	Regular, appressed green with white rim	Light grey with dark grey centre

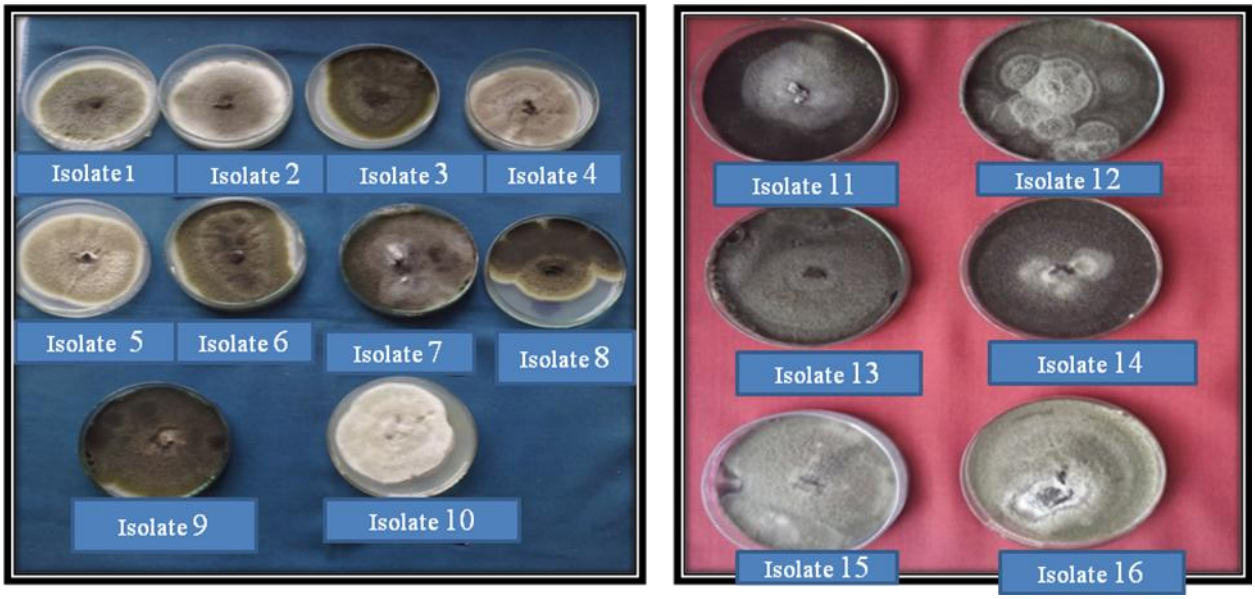
*Isolates from hosts other than apple

Table.2 Effect of different media on the sporulation of *Alternaria* spp. collected from apple and other hosts

Media → / Isolate ↓	Potato Dextrose Agar	Asthana and Hawker's	Czapek's Dox Agar	Malt extract Agar	Glucose asparagine agar	Dimmick's agar	Mean
	Sporulation (Spores/ml)	Sporulation (Spores/ml)	Sporulation (Spores/ml)	Sporulation (Spores/ml)	Sporulation (Spores/ml)	Sporulation (Spores/ml)	
Isolate 1	20.20×10^3	3.00×10^3	4.50×10^3	11.00×10^3	14.00×10^3	9.50×10^3	10.33×10^3
Isolate 2	20.00×10^3	2.00×10^3	4.80×10^3	12.00×10^3	13.00×10^3	8.00×10^3	10.00×10^3
Isolate 3	18.00×10^3	2.50×10^3	3.00×10^3	11.50×10^3	12.00×10^3	8.00×10^3	9.25×10^3
Isolate 4	17.50×10^3	3.25×10^3	3.50×10^3	12.00×10^3	11.00×10^3	6.90×10^3	9.50×10^3
Isolate 5	17.00×10^3	3.50×10^3	4.00×10^3	11.00×10^3	12.00×10^3	7.50×10^3	9.17×10^3
Isolate 6	10.00×10^3	2.20×10^3	2.50×10^3	10.50×10^3	13.50×10^3	7.50×10^3	7.70×10^3
Isolate 7	18.50×10^3	2.00×10^3	3.50×10^3	10.00×10^3	14.00×10^3	8.20×10^3	9.28×10^3
Isolate 8	17.00×10^3	2.20×10^3	3.70×10^3	9.50×10^3	12.00×10^3	8.65×10^3	8.82×10^3
Isolate 9	14.00×10^3	2.50×10^3	4.00×10^3	8.50×10^3	13.00×10^3	8.20×10^3	8.37×10^3
Isolate 10	13.50×10^3	2.00×10^3	3.50×10^3	9.00×10^3	11.50×10^3	8.50×10^3	8.00×10^3
Isolate 11	10.00×10^3	1.50×10^3	4.00×10^3	8.00×10^3	13.45×10^3	7.50×10^3	7.42×10^3
Isolate 12*	15.00×10^3	2.00×10^3	3.20×10^3	9.00×10^3	13.20×10^3	8.00×10^3	8.40×10^3
Isolate 13*	16.20×10^3	2.00×10^3	4.50×10^3	8.50×10^3	11.00×10^3	7.00×10^3	8.20×10^3
Isolate 14*	14.50×10^3	1.50×10^3	4.20×10^3	9.20×10^3	12.20×10^3	8.00×10^3	8.27×10^3
Isolate 15*	12.00×10^3	1.50×10^3	2.50×10^3	8.20×10^3	13.40×10^3	7.50×10^3	7.53×10^3
Isolate 16*	10.00×10^3	2.20×10^3	2.50×10^3	8.50×10^3	13.00×10^3	7.00×10^3	6.64×10^3
Mean	15.21×10^3	2.41×10^3	5.49×10^3	9.78×10^3	12.65×10^3	7.87×10^3	8.55×10^3

*Isolates from hosts other than apple

Plate.1 Cultural characteristics of different isolates of *Alternaria alternata* f. sp. *mali* (1-11) and *Alternaria alternata* (12-16) on PDA medium



Isolates studied also varied in colony colours (Plate 1, Table 3). Dark olivaceous green colonies were observed in two isolates *viz*. I-11, I-12. Four isolates *viz*. I-2,I-4,I-7,I-10,I-14 were olivaceous, one isolate I-1 was dark olivaceous but with dark centre, two isolates *viz*. I-2, I-9, I-13, I-15 were olivaceous green and two isolates *viz*. I-3, I-5 were light olive green. I-6 was olivaceous with grayish surface and brownish centre. The colony margins varied from regular to irregular (Plate 1). Regular margins were observed in eight isolates viz. I-1, I-3, I-4, I-5, I-8, I-14, I-15, I-16. Three isolates (I-6, I-7, I-11) had irregular margins and remaining five (I-2, I-9, I-10, I-12, I-13) were with slightly irregular margins. In some cases margins were followed by white or grey rim.

All the isolates impregnated the media with a colour mostly grey to brown with some variations which were clearly visible from the underside of plates (Table 3). Two isolates (I-2, I-6) imparted dark grey colour with light grey margin, three isolates (I-5,I-8,I-10) were brown with light grey margin and four (I-1,I-3, I-11, I-15) were smoky grey. Two isolates (I-7, I-9) were light grey with brown centre and two (I-13 and I-16) were light grey with dark grey centre. One isolate (I-4) produced grey with light grey margin and I-12 was light brown with light grey margin, while the colour produced by I-14 was greyish with light grey margin and brown centre.

The results of present investigation are in accordance with Sood (1998) who also reported maximum growth of *Alternaria alternata* causing post-harvest rot of tomato on potato dextrose agar medium followed by Richard's medium. Singh *et al.,* (2001) reported that potato dextrose agar medium supported better mycelial growth and sporulation of *Alternaria alternata* followed by Richard's medium, Czapek's dox agar media and Asthana medium. In similar studies little variation in conidial colour of *Alternaria panax* was observed by Quayyum *et al.,* (2005). The results are in agreement with Sofi *et al.,* (2013) who found that the colony color of *A. mali* isolated from apple was light to dark olivaceous with greenish or brownish tinge. In case of *A. alternata* isolated from ribben plants colony colour black to olivaceous black or grayish colour on PDA medium was found (Muthukumar and Venkatesh, 2013). Thirty two isolates of *A brassicicola* for colony color and radial growth were observed by (Deep *et al.*, 2014). Colony colour of *A. brassicicola* varied from olive green to dark olivaceous black on PDA. The isolates colony had cottony and velvety texture on PDA medium. The results are in agreement with (Barry *et al.*, 2002) examined 308 isolates of *Alternaria* spp. colonies generally had a cottony texture on group 4. Alternaria blotch, causal organism *A. mali*, colonies varied in their cultural behavior ranging from velvety to cottony (Sofi *et al.*, 2013). Remarkable variation was observed on spore production and sporulation time on different media and temperature.

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