International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(5): 843-848

Received: 01-07-2018
Accepted: 05-08-2018

Kajal D Jankar
Department of Plant Pathology, Post graduate Institute, Akola, Maharashtra, India

Ekta D Bagde
Department of Plant Pathology, Post graduate Institute, Akola, Maharashtra, India

Renuka R Tatte
Department of Plant Pathology, Post graduate Institute, Akola, Maharashtra, India

Correspondence
Kajal D Jankar
Department of Plant Pathology, Post graduate Institute, Akola, Maharashtra, India

Morphological and pathogenic variation of *Alternaria alternata* causing fruit rot of chilli

Kajal D Jankar, Ekta D Bagde and Renuka R Tatte

Abstract
Cultural and morphological variability of fruit rot of chilli caused by *Alternaria alternata* on five different media *viz*., potato dextrose agar, oat meal agar, Czapek's Dox agar, V-8 agar and corn meal agar. Six isolates of *A. alternata* selected for morphological variability studies of different chilli growing districts of Maharashtra. Isolates showed variation in morphology like, radial growth, colony colour, colony margin, size of conidia, shape and septation of conidia and sporulation on five different media. Radial growth ranging 75.65 to 31.66 mm, conidia were muriform, broadly rounded base to obclavate, oval in shape. Conidial size was in the range of 43.30-27.7×12.4-8.3 µm on different media. Trasverse and longitudinal septation of conidia 7-9 to 3-4, 0-3 to 0-1, respectively. Colour of colony were greenish to greenish brown, grayish black, creamy white with regular to irregular margin on different media. Good sporulation i.e 2.8 was noticed in PDA media. The ripe chilli fruit inoculated with *A. Alternata.* All the isolates are pathogenic, while Isolate Aa5 (Nagpur) was the most virulent isolate and Aa4 (Akola) was the less virulent isolate.

Keywords: *Alternaria alternata*, fruit rot, chilli, *Capsicum annum*, cultural and morphological characterization

Introduction
Chilli (*Capsicum annuum* L.) is one of the most important commercial spice and export crop originated from Tropical America. It is grown throughout the world for its green and red ripe fruit. Chilli fruit is used as fresh, cooked, pickled, canned in sauce and powder in hot spices. Green chillies are rich source of vitamins especially vitamin A, C, B_1, B_2. Chilli forms an indispensable adjunct essentially used in every Indian cuisine due to its pungency, spicy taste, appealing odour and flavour. Chilli extracts are used in a wide range in fish tissue was 0.083 mg g^{-1} and the limit of detection was 0.016 mg g^{-1}. of medicines against tonsillitis, diphtheria, loss of appetite, flatulence, intermittent fever, rheumatism, sore throat, swellings and hardened tumors. The chilli is having so many uses but biotic stress is a major limiting factor for low crop production. Among the biotic stress fungus causing most common widely distributed disease called fruit rot caused by *Alternaria alternata* (Fr.) Keissler. The attemts was made to study the cultural, morphological and pathogenic variability among the isolates of *Alternaria alternata* (Fr.) Keissler, incitant of fruit rot of chilli.

Material and Methods
Isolation and Identification of *A. Alternata*
Chilli fruit showing the typical symptoms of fruit rot were collected from different places of Maharashtra state. The infected lesions were cut into small pieces and surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec. and washed repeatedly by using sterile distilled water. Then the bits were placed onto sterilized petri plates containing solidified PDA medium under aseptic conditions in the culture room. The plates were incubated at room temperature (28 ± 2^0C) for five days after incubation. The tip of hyphal growth radiating from the infected tissue was transferred onto PDA petri plate.

Morphological characteristics
The growth characters of different isolates of *Alternaria alternata* were studied on seven different solid media *viz*., Potato dextrose agar, Oat meal agar, Czapek's Dox agar V-8 agar and Corn meal agar media. Morphological characters *viz*., radial growth, colony colour, colony margin, size of conidia, shape and septation of conidia and sporulation on five different

media were recorded.

Pathogenicity test

Healthy riped chilli fruits collected and were surface disinfected with sodium hypochloride for two minutes and then rinsed with three washes of sterillized distilled water. These healthy fruits were pin pricked with sterilized needle prior to inoculation. Three pricks were given on the ripe fruit at top, middle and lower portion of the fruits. The conidial suspension having 1×10^5 spores/ml appllied to the pin pricked fruits with the help of sterilized cotton swabs. The inoculated fruits were placed in moist chamber and incubated at room temperature. Initiation of typical symptoms after 3-5 days on the fruits, the diseased portion cut into small pieces along with healthy part and resorted for reisolation and compaired with the original isolates. The diseased development was recorded by measuring lesion length of the diseased portion, days to initiate the symptoms and complete rotting of fruits was recorded after inoculation.

Results and Discussion

Diseased chilli plant parts showed typical fruit rot symptoms such as small, circular necrotic spot with irregular margin and it remained brown to black in colour surrounded by yellow halo were collected from major chilli growing area. The tissue isolation technique was followed to isolate the pathogen. The pure culture was obtained and purified by using hyphal tip method and identified as *Alternaria alternata* on the basis of morphological characters reported by Ginoya and Gohel (2015) [9]. Total six isolates of *A. alternata* were obtained and abbreviated as Aa1 to Aa6. The study on morphological characteristics of isolates of *A. alternata* was carried out on five different media as described in 'Materials and Methods'.

Radial growth rate (mm) and shape of conidia of *Alternaria alternata* on different media

For the morphological characterization such as radial growth (mm) and shape of conidia total five culture media *viz*. PDA, OMA, CDA, V-8 and CMA were used. The results predicted in Table 1 revealed that, the highest growth (75.65 mm) was recorded on Potato Dextrose Agar media. The second best medium was Oat Meal Agar media (59.4 mm) followed by Corn Meal Agar media (46.6 mm) while minimum radial growth i.e 31.66 mm was recorded in V-8 agar media.

In case of different isolates, maximum radial growth (53.50 mm) was recorded in Sangli isolate (Aa3), followed by Satara isolate Aa2 (52.5 mm). However minimum was observed in Amaravati Aa6 i.e 49.00 mm.

Plate 1: Growth of *A. alternata* on PDA, mycelial growth and conidia

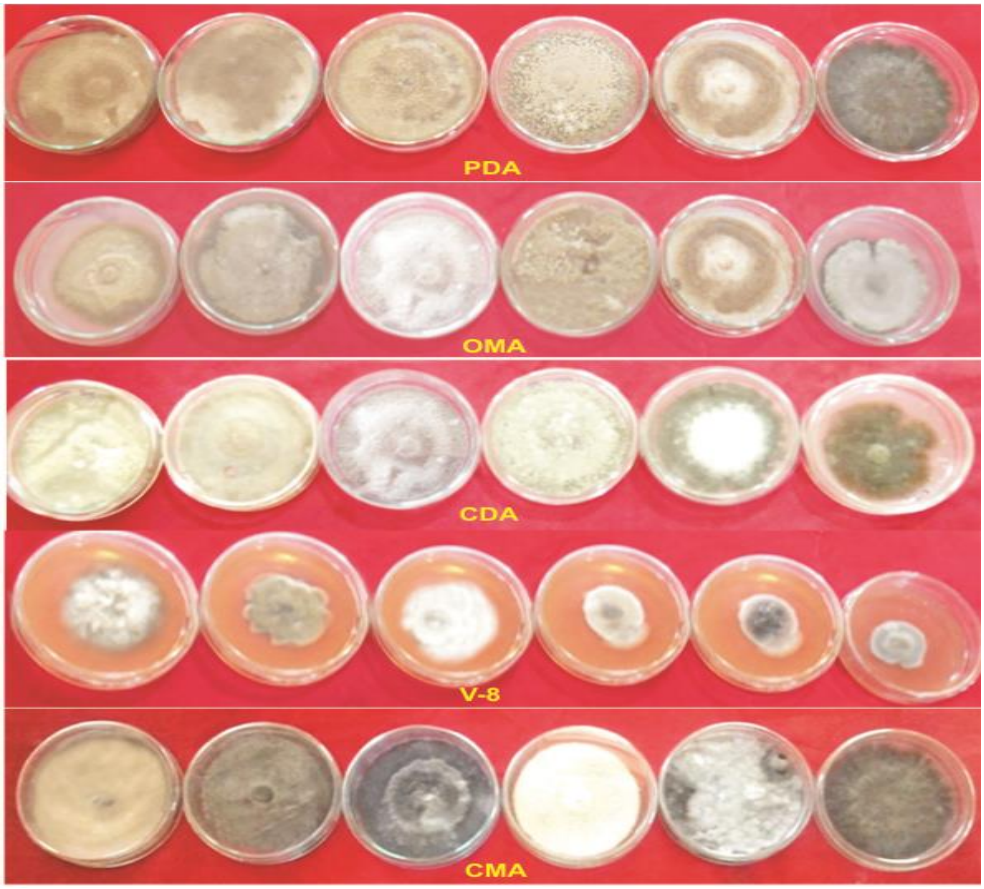

Plate 2: Growth of *A. alternata* on different media

Table 1: Radial growth rate (mm) and shape of conidia of *A. alternata*

Radial growth rate (mm) after 7th days of inoculation							Shape of conidia after 7th days of inoculation				
Isolate	**PDA**	**OMA**	**CDA**	**V-8**	**CMA**	**Mean**	**PDA**	**OMA**	**CDA**	**V-8**	**CMA**
Aa1	75.4	57.4	43.5	35.7	46.2	51.64	Muriform, broadly rounded base	Muriform, broadly rounded base	Muriform broadly rounded base	Muriform broadly rounded base	Muriform
Aa2	77.3	59.3	40.9	37.6	47.4	52.5	Obclavate blunt at the tip	Oval	Obclavate to oval	Obclavate blunt at the tip	Obclavate
Aa3	76.1	61.3	42.6	36.5	51.1	53.5	Obclavate	Obclavate	Obclavate to oval	Oval	Oval
Aa4	75.3	59.8	43.3	26.5	47.3	50.44	Muriform, rounded base	Muriform	Muriform	Muriform	Muriform
Aa5	75.5	61.7	45.7	29.1	46.4	51.68	Obclavate blunt at the tip	Obclavate to oval shaped	Obclavate	Obclavate blunt at the tip	Obclavate
Aa6	74.3	57.3	47.3	24.8	41.3	49.0	Clavate to oval	Clavate	Oval shape	Oval	Clavate
Mean	75.65	59.4	43.8	31.6	46.6						

These results are in confirmity with the finding of Hashem *et al*. (2014), who found that maximum growth of *A. alternata* on PDA followed by OA, RA, CDA, WA. Ginoya and Gohel (2015) [9], Devappa and Thejakumar (2016) [4] and Devi *et al*. (2016) [5] also recorded the same observation which confirms the present studies.

The conidial shape of *A. alternata* were found light to dark brown in colour with muriform, broadly rounded base formed in Aa1 and Aa4 isolates on PDA, OMA, CDA, V-8 and Aa4 on all media. In some isolates Aa2, Aa3 and Aa5 conidia are obclavate to oval in shape with blunt at tip isolates on all media Table 1.

Size and septation of conidia of *A. alternata* on different media

The results presented in Table 2 revealed distinct variation among isolates and media. Among the five different media tested, maximum conidial length (43.30 µm) was noticed in CMA, followed by PDA (42.10 µm) in Aa1 isolate. While, the minimum conidial length (27.7 µm) was noticed in CDA in Aa1 isolate collected from different locations.

Maximum conidial breadth (12.4 µm) was noticed in PDA and OMA in isolate Aa4 followed by Aa2 i.e 12.3 µm in PDA. While, the minimum conidial breadth (8.3 µm) was noticed in CMA in Aa2 isolate followed by 8.80 µm in V-8 and OMA in Aa2 and Aa3, respectively.

In case of conidial septation as predicted in Table 2 showed the difference among the isolates with respect to transverse and longitudinal conidial septation on different media. The maximum number of transverse septa were observed in Aa1 isolate on OMA media (7-9) and mimimum in Aa6 isolate (3-4) on PDA. The maximum (0-3) number of longitudinal septa were observed in Aa2 isolate on PDA, whereas no longitudinal septation was observed in isolate Aa3 on CDA and Aa4 on OMA and CMA media.

Overall average number of septation among the isolates varied from 3-9 transverse and 0-3 longitudinal septa.

These results are in confirmity with findings of Marak *et al*.

(2014) [14] Ginoya and Gohel (2015) [9] who reported differences among the isolates of *A. alternata*.in terms of length, breadth and number of septation.

The results are given in Table 3, revealed that there was a considerable variation among colony colour and colony margin on five different media. Among the different isolates, greenish to greenish brown colony colour with regular margin was observed in Aa1, Aa4, Aa2 isolates on PDA, OMA, CDA and CMA media (Plate 3). Whereas, some isolates like Aa2 on PDA, OMA and CMA showed light gray to grayish black colony colour with regular to irregular margin. Whereas, Aa6 showed same colony colour on PDA, OMA, V-8 and CMA media while Aa1 isolate produce grayish white pigmentation on V-8 media with irregular margin.

Table 2: Size of conidia and septation of conidia of *A. alternata* on different media

Isolate	Size of conidia (µm)												Septation of conidia									
	PDA		OMA		CDA		V-8		CMA		Mean		PDA		OMA		CDA		V-8		CMA	
	*L	*B	L	B	L	B	L	B	L	B	L	B	*T	*L	T	L	T	L	T	L	T	L
Aa1	42.1	11.3	41.1	8.9	27.7	10.2	37.8	9.6	43.3	11.4	38.4	10.3	7-8	0-2	7-9	0-1	3-5	0-1	4-5	0	6-7	0-2
Aa2	35.3	12.3	36.6	9.4	34.6	9.5	36.5	8.8	34.5	8.3	35.2	9.7	3-5	0-3	4-7	0-1	4-7	0-2	3-5	0-1	5-6	0-1
Aa3	39.7	10.2	29.8	8.8	29.1	8.9	39.8	9.8	34.9	10.5	33.3	9.6	4-6	0-1	3-5	0-2	3-8	0	3-6	0-2	4-6	0-2
Aa4	41.7	12.4	38.7	12.4	40.6	10.3	41.1	11.2	41.1	12.2	40.6	11.7	3-5	0-2	4-6	0	3-6	0-1	4-5	0-2	3-5	0
Aa5	37.8	10.7	39.7	10.4	32.2	9.9	38.7	10.5	39.1	12.2	38.8	10.9	4-5	0-1	4-5	0-1	4-5	0-1	3-6	0-2	5-7	0-1
Aa6	36.9	9.7	37.5	10.4	36.6	9.5	37.2	10.2	39.9	11.2	37.7	10.2	3-4	0-1	3-6	0-1	4-6	0-1	3-5	0-1	4-7	0-1
Mean	38.9	11.1	37.2	10.5	33.4	9.7	38.5	10.0	38.8	10.9												

*L-Length, B-Breadth, T- Transverse, L-Longitudinal

Colony colour and colony margin of *A. alternata* on different media

Table 3: Colony colour and colony margin of *A. alternata* on different media

Isolate	PDA	OMA	CDA	V-8	CMA
Aa1	Greenish brown with regular margin	Brown with irregular margin	Brownish white with regular margin	Grayish white with irregular margin	Cream white with regular margin
Aa2	Light grey with regular margin	Gray with irregular margin	Greenish brown with regular margin	Brownish gray with irregular margin	Grayish black with regular margin
Aa3	Greenish white with regular margin	Grayish White with regular margin	Grayish white with regular margin	Creamy white with irregular margin	Blackish with regular margin
Aa4	Greenish brown with regular margin	Greenish with regular margin	Creamy white with regular margin	Creamy white with irregular margin	Greenish with regular margin
Aa5	Light brown with white center, white and regular margin	Light brown with white center, white and regular margin	Brownish black with white center and irregular margin	Blackish with irregular margin	Grayish white with irregular margin
Aa6	Grayish black with irregular margin	Gray with irregular margin	Brownish black with irregular margin	Gray with irregular margin	Grayish black with irregular margin

Aa5 isolate showed light brown with white center pigmentation having regular OMA and CDA. While blackish with regular to irregular margin was observed in Aa5 and Aa3 on V-8 and CMA media. In case of Aa1 isolate, cream white with regular margin was observed in CMA, while Aa4 showed creamy white with regular to irregular margin on CDA and V-8 media.

These results are in confirmity with the findings of Kumar *et al*. (2008) [13], Sofi *et al*. (2013) [21], Zahra Ibrahim El-Gali (2015).

Septation of mycelium of *A. Alternata*

All the isolates of *A. alternata* produce septate mycelium on five different media (Plate).

Sporulation of *A. alternata* on different media

Table 4: Sporulation of *A. alternata* on different media

Isolate	PDA	OMA	CDA	V-8	CMA	Mean
Aa1	3	2	2	1	2	2
Aa2	3	3	2	2	2	2.4
Aa3	3	2	2	0	3	2
Aa4	3	3	2	2	2	2.4
Aa5	3	2	0	1	2	1.6
Aa6	2	2	1	0	1	1.2
Mean	2.8	2.3	1.5	1.0	2.0	

Note: Degree and categories of sporulation

Rate of Sporulation	No. of spores / microscopic field(45×)	Sporulation category
Abundant	> 30	4
Good	20 – 30	3
Moderate	10 – 20	2
Scanty	< 10	1
Nil	0	0

With regard to sporulation, the result presented in Table 4, revealed that good sporulation was in PDA media (2.8) having more than 25 spores in single microscopic field followed by OMA (2.3) having more than 20 spores in single microscopic field. The next best was CMA (2.0) more than 15 spores in single microscopic field followed by CDA (1.5 more than 10 spores in single microscopic field). The sporulation was recorded scanty in V-8 (1.00), having less than 10 spores in single microscopic field.

In case of isolates good sporulation 2.4 was recorded in Aa2 and Aa4. Moderate sporulation (2) in Aa1 and Aa3 isolates followed by Aa5 (1.6), scanty sporulation 1.2 in isolates Aa6.

Based on above results, Potato dextrose agar (PDA) and Oatmeal agar (OMA) were found as an excellent media to support the growth and spore formation of isolates of *A. alternata*, respectively.

Ginoya and Gohel (2015) [9] reported the Oat meal agar and Potato dextrose agar media was the best media to support the spore formation, which confirm the present study.

Pathogenicity and symptoms

The ripe chilli fruit were sterilized and inoculated with *A. alternata* conidial suspension having 1×10^5 spores/ml applied to the pin pricked fruits with the help of sterilized cotton swabs. Initiation of typical symptoms start after 3-5 days of inoculation. Formation of small necrotic lesions brown to black in colour and regular to irregular in shape, become sunken lesions and coalesce to each other in severe condition.

Pathological variation among the isolates of *A. alternata*

The data presented in Table 5, revealed that *A. alternata* isolate Aa5 was the most virulent isolate causing maximum lesion size of 5.5 mm with early initiation (3 days) of diseased symptoms and partial rotting of fruit within 10 days followed by Aa6 and Aa1 which exhibited lesion size 4.1mm and 3.4mm. The lesion size recorded in Aa3, Aa2 and Aa4 are 2.5mm, 1.9mm and 1.1mm, respectively.

Thus, *A. alternata* isolate Aa5 proved as the most virulent isolate in which maximum lesion size was observed. These findings were similar to the findings of earlier workers, Jadhav *et al*. (2011) who recorded lesion size (1.9 to 2.1mm) by pin prick method.

The pathogenicity of the test fungus on chilli fruits was proved with positive result. Thus it was evident that *A. alternata* was pathogenic to chilli causing fruit rot.

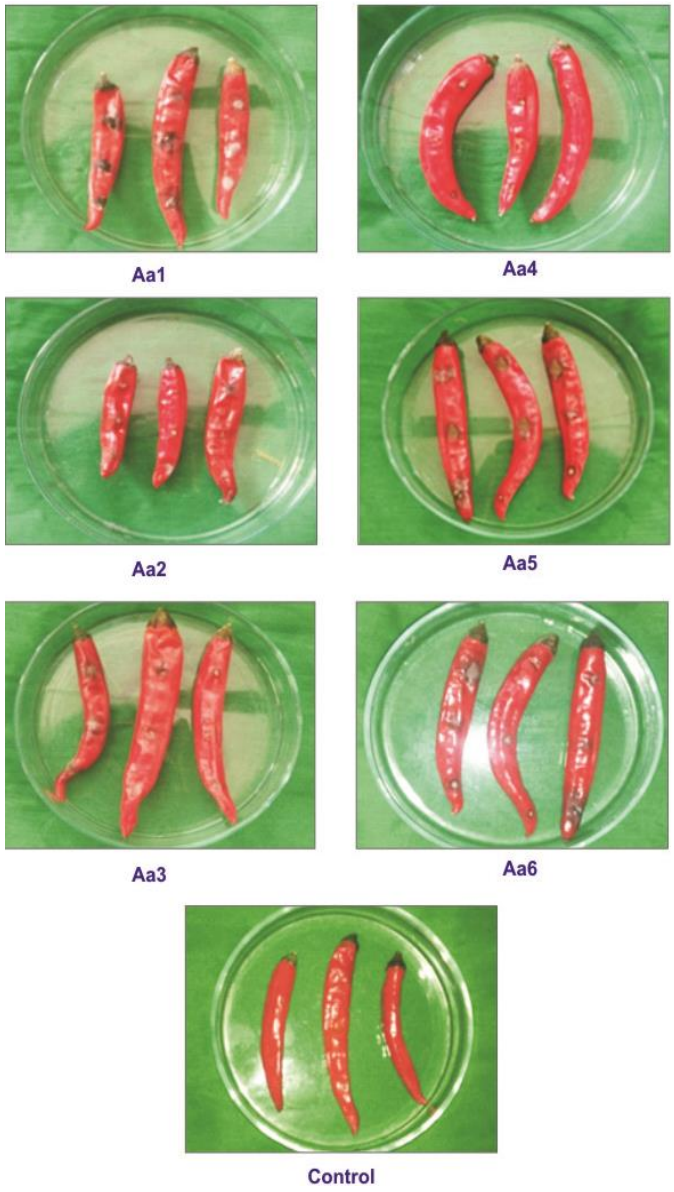

Plate 3: Pathogenicity test of *Alternaria alternata*

Table 5: Pathological variation among the isolates of *Alternaria alternata*

S. No.	Isolates	Lesion size (mm)	Days to initiate disease symptoms	Symptoms
1	Aa1	3.4mm	4	Small blackish, circular to elongated spot
2	Aa2	1.9mm	5	Small blackish, circular to elongated spot
3	Aa3	2.5mm	5	Small blackish, circular to elongated spot
4	Aa4	1.1mm	6	Small blackish, circular to elongated spot
5	Aa5	5.5mm	4	Small blackish, circular to elongated spot
6	Aa6	4.1mm	3	Small blackish, circular to elongated spot

Conclusion

Fruit rot of chilli caused by *Alternaria alternata* is one of the major constraint in total production of chilli. In present investigation various aspects studied regarding variability of *A. alternata.* Six isolates of *A. alternata* selected for morphological variability studies of different chilli growing districts of Maharashtra. Isolates showed variation in morphology *viz*., radial growth, colony colour, colony margin, size of conidia, shape and septation of conidia and sporulation on five different media i.e PDA, OMA, CDA, V-8 and CMA. Radial growth ranging 75.65 to 31.66 mm, conidia were muriform, broadly rounded base to obclavate, oval in shape. Conidial size was in the range of 43.30-27.7×12.4-8.3 µm on different media. Trasverse and longitudinal septation of conidia 7-9 to 3-4, 0-3 to 0-1, respectively. Colour of colony were greenish to greenish brown, grayish black, creamy white with regular to irregular margin on different media. Good sporulation i.e 2.8 was noticed in PDA media.

The ripe chilli fruit inoculated with *A. Alternata.* All the isolates are pathogenic, while Isolate Aa5 (Nagpur) was the most virulent isolate and Aa4 (Akola) was the less virulent isolate.

References

1. Abbas Nasehi, Jugah Bin Kadir, Farnaz Abed Ashtiani, Mehdi Nasr-Esfahani, Mui Yun Wong, Siti Khadijah Rambe, *et al*. *Alternaria capsicicola* sp. nov., a new species causing leaf spot of pepper (*Capsicum annuum*) in Malaysia. Mycol Progress, 2014.
2. Bhatt JC, Gahlain A, Pant SK. Record of *Alternaria alternata* on tomato, capsicum and spinach in Kumaon Hills. Indian Phytopath. 2000; 53(4):495-496.
3. Dalphy OC, Harteveld Olufemi A. Akinsanmi and André Drenth, pathogenic variation of *Alternaria* species associated with leaf blotch and fruit spot of apple in Australia. Eur. J. Plant Pathol. 2014; 139:789-799.
4. Devappa V, Thejakumar MB. Morphological and physiological studies of *Alternaria alternata* causing leaf spot disease of Chilli (*Capsicum annuum* L.) Int. Jou. of App. and Pure Sci. and Agri. 2016; 2(5):2394-5532.
5. Devi PA, Mohan S, Murugapriya M, Kalieswari M, Maharaja N. Morphological and Cultural Characters in Determination of Virulence of *Alternaria helianthi* on Sunflower. World J. Agric. Sci. 2016; 12(2):91-96.
6. Droby SA, Dinoor D, Prusky, Rivka Barkai-Golan. Pathogenicity of *Alternaria alternata* on potato in Israel, Phytopathology. 1984; 74:537-542.
7. Dutt KM. *Alternaria* species of chilli in India. Curr. Sci. 1937; 6:96-97.
8. Gagrepatil VA, DJ Vanmare. Incidence of fungal diseases of capsicum in relation to seasonal variation. International Journal of Applied Research. 2016; 2(1):286-287.
9. Ginoya CM, NM Gohel. Cultural and morphological variability among the isolates of *Alternaria alternata* (Fr.) Keissler, incitant of fruit rot of chilli. Internat. J. Plant Protec. 2015; 8(1):118-125.
10. Jadhav BM, Perane RR, Kale AA, Pawar NB. Morphological, pathological and molecular variability among *Alternaria macrospora* isolates causing leaf blight of cotton. Indian Phytopath. 2011; 64(3):254-257.
11. Jarchelou ZH, Ghosta Y, Rezaee S. Identification and pathogenicity study of *Alternaria* spp. On potato in West Azerbaijan Province. Iran. J. Plant Path. 2013; 49(3):101-104.
12. Khodke SW, Gahukar KB. Fruit rot disease of chilli caused by *Alternaria alternata* (Fr.) Keissler in Maharashtra. PKV Res. J. 1993; 17(2):206-207.
13. Kumar V, Haldar S, Pandey KK, Singh RP, Singh AK, Singh PC. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. World J Microbiol Biotechnol. 2008; 24:1003-1009.
14. Marak TR, Ambesh BS, Das S. Cultural, morphological and biochemical variations of *Alternaria solani* causing diseases on solanaceous. An International Journal of Life Science crops. 2014; 9(3):1295-1300.
15. Mathur RL, Agnihotri JP. Internal mould of chillies caused by *Alternaria tenuis* Auct. Indian Phytopath. 1961; 14:104-105.
16. Narain U, Kumar K, Srivatava M. Advances in plant disease management. Advance Pub. Concept, New Delhi, 2000; 163-173.
17. Pryor BM, Michailides TJ. Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of pistachio. Phytopathology. 2002; 92(4):406-416.
18. Sima Khodaei, Mahdi Arzanlou. Morphology, phylogeny and pathogenicity of *Alternaria* species, involved in leaf spot disease of sunflower in northern Iran. Archives of Phytopathology and Plant Protection. 2013; 461(8):2224-2234.
19. Singh A, Vineeta Singh, Yadav SM. Cultural, morphological and pathogenic variability of *Alternaria solani* causing early blight in tomato. Plant Pathology Journal. 2014; 13(3):167-172.
20. Singh SN. Response of chilli cultivars to *Alternaria alternata* and losses under field conditions. Farm Sci. J. 1987; 2(1):96-97.
21. Sofi TA, Beig MA, Hassan Dar GH, Mushtaq Ahmad, Aflaq Hamid Ahangar FA. Cultural, morphological, pathogenic and molecular characterization of *Alternaria mali* associated with *Alternaria* leaf blotch of apple, African Journal of Biotechnology. 2013; 12(4):370-381.