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CULTURAL, MORPHOLOGICAL AND BIOCHEMICAL VARIATIONS OF *ALTERNARIA SOLANI* CAUSING DISEASES ON SOLANACEOUS CROPS

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ABSTRACT

Alternaria solani is an economically important pathogen causing diseases on Solanaceae crops. Significant morphological variations in length and breadth of conidia, numbers of horizontal, vertical and oblique septations were observed in the test isolates. The conidia varied from 20.68-43.10 x 10.53-17.99μm in brinjal, 19.86-43.73 x 7.52-13.05μm in chilli, 21.5-33.21 x 8.03-17.85μm in potato and 30.31-75.47 x 7.26-27.42μm in tomato isolates. Variability with respect to cultural characters showed that brinjal, tomato and potato isolates produce cottony mycelia growth in all the tested media. Maximum growth was recorded on PDA for chilli and potato (93.33mm and 95.00mm respectively), CDA for brinjal (85.33mm) and PCA for tomato (87.00mm). Isozyme tested in PAGE showed that tomato produces three bands and potato, brinjal and chilli produces four bands each for α- esterase. For β- esterase tomato and potato produces two bands each and chilli produces three bands. The cluster analysis of morphological characters showed that brinjal, tomato and potato were in same cluster and in isozyme studies potato, chilli and brinjal were in same and tomato in different cluster. Existence of variation among different *Alternaria* isolates is evident from the results obtained.

INTRODUCTION

Leaf spot or Early blight incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is an economically important and widely distributed disease throughout the world on crops belonging to Solanaceae family. The characteristic symptoms of the disease are dark brown to black concentric rings, which produce target board or bullseye effect. Variability is a well known phenomenon in *A. solani* and variability in morphological, cultural and biochemical criteria indicate the existence of different patho-types. The existence of the high level of variability was reported by many workers (Castro *et al.*, 2000; Pryor and Gilbertson, 2002; Pryor and Michailides, 2002; Quayyum *et al.*, 2005; Kumar *et al.*, 2008).The variants within population may affect the rate of disease development and induce infection in more host lines, which might have implication for stability of cultivar resistance (Naik *et al.*, 2010). Molecular techniques such as isozyme analysis, Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR) have been used to study variability of *A. solani* isolates in different parts of the world (Morris *et al.*, 2000; van der Waals *et al.*, 2004). However, little is known about the variability of *A. solani* isolates in India as well as in West Bengal. Analysis of pathogenic and genetic variation within pathogen populations is helpful in understanding host-pathogen co-evolution, epidemiology and developing strategies for resistance management (Leung *et al.*, 1993). Understanding the pathogen variability will help in developing the effective management strategies. So, the present investigation was carried out to find a comprehensive understanding of this causal organism with reference to morphological, cultural and molecular variability within the four different host cultivars from West Bengal region. It will contribute a preliminary idea to develop an effective programme of breeding for disease resistance.

MATERIALS AND METHODS

The pathogen *Alternaria solani* was isolated from infected leaves of tomato, potato, brinjal and chilli. Isolation was made by cutting a small section of infected portion along with healthy areas, which was surface sterilized with 0.1% $HgCl_2$ solution and rinsed repeatedly in sterilized distilled water. It was then placed into the sterilized petri plates containing solidified potato dextrose agar (PDA) medium, and incubated at 27 ± 1ºC. The pure cultures were maintained for each isolate by single spore isolation technique (Johnston and Booth, 1983).

Morphological studies of the fungus

Five different media i.e., Czapek dox agar (CDA), potato carrot agar (PCA), carrot agar (CA), potato dextrose agar (PDA) and oatmeal agar (OA) were prepared as suggested by Ainsworth (1961) and used for the cultural studies of the fungus. 20ml of each medium was poured into 90mm diameter petri plates. After solidification 5mm discs from 5-7 days old cultures of *A. solani* were cut by using a cork borer and were placed at the centre of the plate. Each set of the experiment was replicated

thrice and the plates were incubated at 27 ±1ºC for 9 days. The colony diameter, colour of the colony, nature of colony margin and the zonation of the colony were recorded from 24 h after inoculation to 9 days after inoculation. The photographs of the conidia were taken and by use of micrometry (Meena *et al.*, 2005) measurements of the conidia were done.

Studies on isoenzyme

A. solani isolates were grown in 250ml conical flask containing 50ml potato dextrose broth. Three flasks were used for each isolate. Each flask was inoculated with 2 discs each of 5mm diameter cut from the periphery of actively growing 5 day old culture grown on PDA. The inoculated flasks were incubated at 27 ± 1ºC for 10 days. Electrophoresis of esterase and peroxidase isozyme was done in 7.5% gel according to the method proposed by Kahler and Allard (1970). The Rm (Relative mobility) value of band(s) in gel was estimated.

$$\text{Rm value} = \frac{\text{Distance of the band from origin}}{\text{Distance of buffer front}}$$

Statistical analysis

Radial growth and other morphological characteristics, isozyme patterns of different isolates were statistically analyzed in a cluster analysis to find out the variations among these four *A. solani*.

RESULTS AND DISCUSSION

Morphological variability

The different *A. solani* isolate on different host species produces different morphological characters. The morphology of the fungus on carrot agar media was different in different isolate. In case of brinjal, length of conidia with beak varied between 20.68-43.10µm, length of conidia without beak 18.66-31.54µm, the beak length 3.4-14.3µm, width of conidia is 10.53-17.99µm the number of horizontal septation varied between 3-4, vertical septation 0-1 and oblique septation 0-1. In chilli isolate, the length of conidia with beak varied between 19.86-43.73µm. Beak length and width of conidia varied between 1.2-9.8µm and 7.52-13.05μm respectively. Similarly, length of conidia without beak varied between 18.05-33.27µm. The number of horizontal septation varied between 4-6, oblique septation 0-1 and vertical septation 1-2 and some produce no vertical septation. Isolates from potato showed varied length of conidia with beak *i.e.*, 21.5-33.21μm, beak length 2.18-7.4μm and width of conidia 8.03-17.85μm. Length of conidia without beak also varied between 15.90-28.16μm. The conidia of the fungal isolate from potato are also having 3-4 horizontal septations, 1-2 vertical septations and 0-1 oblique septations whereas in tomato (Table 1) the number of horizontal septation varied between 3-8 and the vertical septation 0-2 numbers and no oblique septation was noticed. It was observed that the length of conidia was more, among the other three *Alternaria* affected on chilli, potato and brinjal as the length of conidia varies between 30.31-75.47μm, beak length and width of conidia was 3.43-10.98ìm and 7.26-27.42μm respectively. Length of conidia without beak was also maximum in tomato isolate *i.e.*, 15.75-54.55μm. It was observed that *A. solani* affecting different host produces morphological variability (Table 1). So it was concluded that the conidial dimensions of the strains of *A. solani* are different on different host species. This conidial morphology of *A. solani* isolates in different hosts was in accordance with those described by Ellis and Ellis (1985) and Naik *et al*. (2010). However, Kaul and Saxena (1989) concluded that spore dimensions were not useful in distinguishing *A. solani* strains. The DMRT (Dunkan Multiple Range Test) analysis were done among the morphological characters particularly number of horizontal septation, beak length, total length of conidia, length of conidia without beak and width of conidia to find the group among the similarity of four isolates (Table 2). It was observed that brinjal and potato were similar in case of number of horizontal septation whereas chilli and tomato were different. In case of beak length, chilli and potato produced similar character and brinjal and tomato also showed the similar pattern. Chilli and potato also produced similar type of total length of conidia whereas, brinjal and tomato were different. Length of conidia without beak showed that all the four isolates were different though chilli was to some extent similar to brinjal and potato. Similarly, width of conidia was different among themselves though tomato was in between brinjal and potato. The cluster analysis of morphological characters showed that tomato, potato and brinjal are in the same cluster whereas chilli was in different cluster (Fig.1).

Cultural variability

Alternaria solani isolates showed variability in colony growth of the fungus on different solid media *viz.*, Potato Dextrose

Table 1: Morphological characters of *Alternaria solani*

Sl. No.	*Alternaria* isolate on host	No. of horizontal septation	No. of vertical septation	No. of oblique septation	Length of conidia with beak (μm)	Length of conidia without beak (μm)	Beak length (μm)	Width of conidia (μm)
1	Brinjal	3-4	0-1	0-1	20.68-43.10	18.66-31.54	3.4-14.3	10.53-17.99
2	Chilli	4-6	1-2	0-1	19.86-43.73	18.05-33.27	1.20-9.80	7.52-13.05
3	Potato	3-4	1-2	0-1	21.5-33.21	15.90-28.16	2.18-7.40	8.03-17.85
4	Tomato	3-8	0-2	0	30.31-75.47	15.75-54.55	3.43-10.98	7.26-27.42

Table 2: DMRT test of morphological characters of *Alternaria solani*

No. of horizontal septation	Total length of conidia	Length of conidia without beak	Width of conidia	Beak length
Brinjal^{c}	Brinjal^{b}	Brinjal^{b}	Brinjal^{a}	Brinjal^{a}
Chilli^{b}	Chilli^{c}	Chilli^{bc}	Chilli^{c}	Chilli^{b}
Potato^{c}	Potato^{c}	Potato^{c}	Potato^{b}	Potato^{b}
Tomato^{a}	Tomato^{a}	Tomato^{a}	Tomato^{ab}	Tomato^{a}

Table 3: Growth and cultural characteristics of the isolates of *Alternaria solani* from brinjal on different media

Sl. No.	Media	Colony size (Diameter)(mm)	Colony colour	Mycelial growth	Margin of colony	Zonation
1	Czapek's dox	11.33-85.33	Whitish- brownish with grey colouration	Cottony	Irregular with white margin	Concentric zonation
2	Potato Dextrose Agar	11.00-80.66	Whitish- brown with grey colouration	Cottony	Irregular with white margin	Concentric zonation
3	Carrot Agar	13.00-78.00	Whitish-Brownish or olive green, grey	Cottony	Irregular with white margin	Concentric zonation
4	Potato carrot agar	11.67-83.00	Whitish- brownish green, grey	Cottony	Irregular with white margin	Concentric zonation
5	Oatmeal agar	9.66-85.00	Whitish-Greenish with white colouration	Cottony	Irregular with white margin	Concentric zonation

Table 4: Growth and cultural characteristics of the isolates of *Alternaria solani* from chilli on different media

Sl. No.	Media	Colony size (Diameter) (mm)	Colony colour	Mycelial growth	Margin of colony	Zonation
1	Czapek's dox	11-79.33	Whitish- brownish with black centre	Not cottony	Irregular with white margin	Concentric zonation
2	Potato Dextrose Agar	13.33-93.33	Whitish- blackish green or dark green	Not cottony	Round with white margin	Concentric zonation
3	Carrot Agar	11.00-91.00	Whitish- blackish green	Not cottony	Irregular with white margin	Concentric zonation
4	Potato carrot agar	12.33-93.00	Whitish-Greenish or dark green	Not cottony	Round with white margin	Concentric zonation
5	Oatmeal agar	11.33-90.66	Whitish-Greenish or dark green	Not cottony	Irregular with white margin	Concentric zonation

Table 5: Growth and cultural characteristics of the isolates of *Alternaria solani* from potato on different media

Sl. No.	Media	Colony size (Diameter) (mm)	Colony colour	Mycelial growth	Margin of colony	Zonation
1	Czapek's dox	12.30-90.66	Whitish- greenish	Cottony, sparse	Irregular with white margin	Concentric zonation
2	Potato Dextrose Agar	13.66-95.00	Whitish- brownish green	Cottony	Irregular with white margin	Concentric zonation
3	Carrot Agar	11.66-90.66	Whitish-greenish	Cottony	Irregular with white margin	Concentric zonation
4	Potato carrot agar	12.33-90.33	Whitish green-Blackish green or dark green	Cottony	Irregular with white margin	Concentric zonation
5	Oatmeal agar	11.66-93.33	Whitish green-Blackish green or dark green	Cottony, sparse	Irregular with white margin	Concentric zonation

Table 6: Growth and cultural characteristics of the isolates of *Alternaria solani* from tomato on different media

Sl. No.	Media	Colony size (Diameter)(mm)	Colony colour	Mycelial growth	Margin of colony	Zonation
1	Czapek's dox	10.66-81.33	Whitish- whitish brown or olive green	Cottony	Irregular with white margin	Concentric zonation
2	Potato Dextrose Agar	11.66-85.66	Whitish- blackish brown with grey centre	Cottony	Irregular with white margin	Concentric zonation
3	Carrot Agar	12.00-84.66	Whitish- blackish brown with grey centre	Cottony	Round with white margin	Concentric zonation
4	Potato carrot agar	11.33-87.00	Whitish- blackish brown with grey centre	Cottony	Round with white margin	Concentric zonation
5	Oatmeal agar	9.66-85.66	Whitish- greenish with grey colouration	Cottony	Irregular with white margin	Concentric zonation

Agar, Czapek's Dox Agar, Carrot Agar, Oatmeal Agar and Potato Carrot Agar. The diversity in growth was studied on different days after inoculation up to 9 days. Four different isolates produced different colony size on different media. The size of the colony was increased with increase in incubation period. Brinjal isolate showed maximum colony diameter on Czapek's Dox media followed by Oatmeal Agar. This is in accordance with the results obtained by Somappa *et al*. (2013). Chilli and

Table 7: Relative mobility (Rm) values Esterase and Peroxidase isozyme in different isolates of *Alternaria solani*

Sl. No.	Isolates	α- Esterase Rm value	No. of bands	β-Esterase Rm value	No. of bands	Peroxidase Rm value	No. of bands
1	Potato	0.23 0.28 0.32 0.38	4	0.29 0.39	2	0.05 1.14	2
2	Brinjal	0.22 0.23 0.28 0.32	4	0.29	1	0.05 1.14	2
3	Tomato	0.22 0.23 0.28	3	0.15 0.29	2	0.05 1.14 1.19	3
4	Chilli	0.23 0.28 0.32 0.38	4	0.29 0.37 0.39	3	0.05 1.14 1.19	3

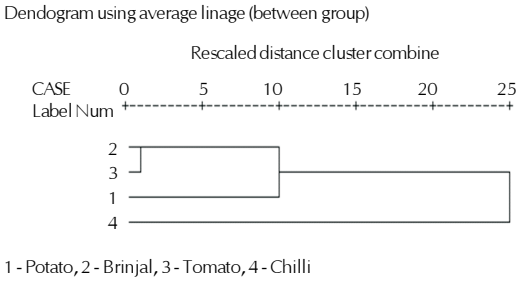

Figure 1: Morphological dendrogram of *Alternariasolani* isolates

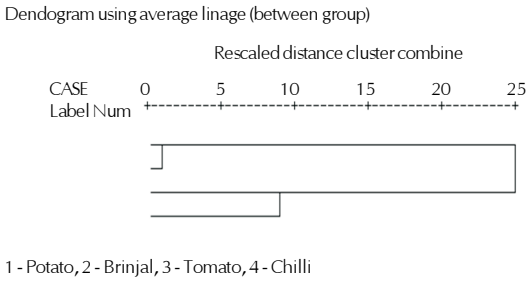

Figure 1: Dendrogramon isozyme pattern of different isolates of Alternariasolani

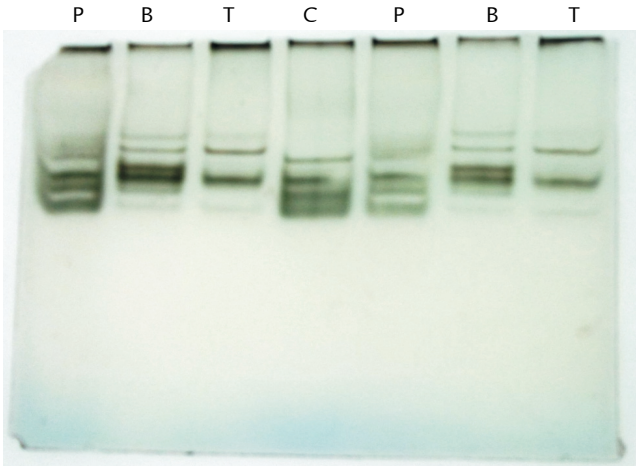

Plate 1: Isomers of α- Esterase from different fungal isolates of *A. solani* (P-Potato, B- Brinjal, T- Tomato, C- Chilli)

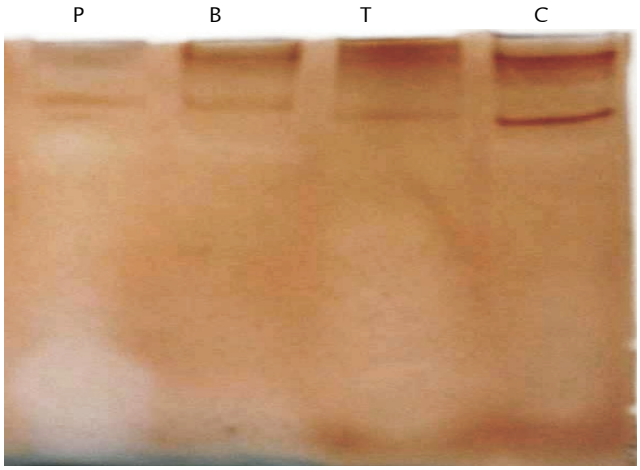

Plate 2: Isomers of peroxidase from different fungal isolates of *A. solani* (P-Potato, B- Brinjal, T- Tomato, C- Chilli)

potato showed maximum growth on PDA. In case of tomato, maximum growth was observed on Potato Carrot agar and minimum in Czapek's Dox agar. The colour of the colony changes from whitish, greenish and brownish to dark green to dark brown with increase in age of the fungal culture. Growth behavior of four isolates showed different type of characters. Diversity in cultural characters such as colony colour, its margins and topography were noticed among the isolates of *A. solani* (Naik *et al.*, 2010). Brinjal produced cottony growth in all the media tested whereas chilli produces non cottony growth in every media from days after inoculation to 9 days of incubation. In case of potato, all the media produce cottony growth from initiation of the growth to 9 days after inoculation, though cottony growth added with sparse growth were observed in Czapek's Dox and Oatmeal agar in every days after inoculation; whereas in tomato, all the media produce

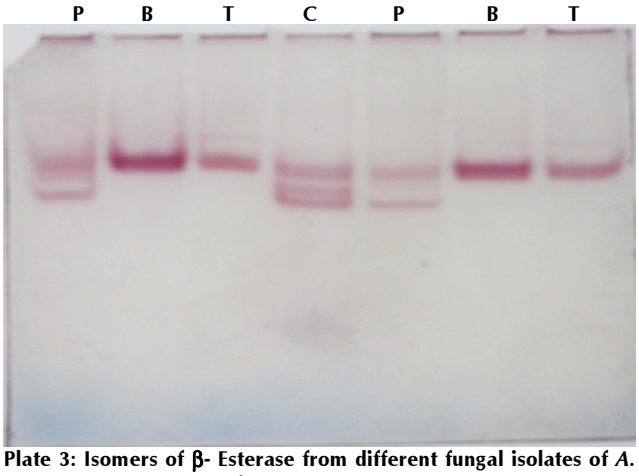

Plate 3: Isomers of β- Esterase from different fungal isolates of *A. solani*. (P-Potato, B- Brinjal, T- Tomato, C- Chilli)

cottony growth, though Czapek's dox produced thicker growth than others. It was observed in all the four isolates that the margin of the colony was different as on chilli, PDA and Potato Carrot agar produces round white margin and others produces irregular white margins. The results are in agreement with Sofi *et al*. (2013) who reported that the colonies had velvety or cottony mycelia growth with slight variations and regular to irregular margins. In potato, first day of incubation produced irregular margin of colony whereas during second day incubation to ninth days incubation, all the media produced irregular with white margin colony. In case of tomato, all the media produced irregular white margin colony growth except Potato Carrot agar and Carrot agar where round and white margin of the colony was observed. Generally, *Alternaria* produces some toxin on the culture media which was noted by a zonation around the mycelia growth of the fungus and it was observed that all the four isolates produces concentric zonation in all the media or substrate except in first day of incubation (Tables 3, 4, 5 and 6). Many workers reported the presence of zonation in the mycelia growth of *A. solani* (Pramila *et al*., 2014; Singh *et al*., 2014 and Ambesh *et al*., 2014).

Isozyme variability

Isozyme variability of four isolates of *A. solani* differing in their effect on four different host *i.e.*, tomato, potato, brinjal and chilli and their UPGMA (unweighted paired group method of arithmetic average) cluster analysis were done. Three different enzymes *i.e.*, α- and β- esterase and peroxidase was examined and they all showed positive activity. α- esterase enzyme showed the highest enzyme activity in terms of maximum numbers of banding loci among all the three isozyme tested (Plates 1, 2 and 3). Isolates potato and chilli exhibited isozyme pattern with four banding loci sharing only one locus of Rm 0.38 in all the isolates. In case of tomato isolate, only three bands have been observed which are already present in other three isolates. Among the four bands of brinjal, one locus showing maximum Rm value is 0.32. Potato and chilli isolate share the similar banding pattern with the maximum Rm value of 0.38, whereas brinjal and tomato produces three banding patterns with similar Rm value with an exception in brinjal, which produces another band of Rm value 0.32 (Table 7)

In case of β-esterase, potato and tomato produces two bands, brinjal one band and chilli three bands. Potato and tomato isolate though produces two bands but their Rm value is different. Although, one moving band of Rm 0.29 was common to all the four isolates (Table 7). Another two moving bands 0.29 and 0.39 was also common in potato and chilli, whereas another band of Rm value 0.37 was observed in chilli isolate.

In peroxidase, potato and brinjal produces two bands whereas tomato and chilli produces three bands each. Though all the isolates produce two similar moving bands with an exception of tomato and chilli where another one band of Rm value 1.19 was added (Table 7). Clustering analysis to group the isolates based on their overall similarities showed that four isolates are to be grouped into two clusters (Fig. 2). In the first cluster, potato isolate and chilli isolate were grouped together and second cluster consisted of brinjal and tomato isolate. Although brinjal and tomato isolate produces similar type bands they were to some extent different; and potato and chilli produces similar type of banding pattern. The similar result was reported by Ambesh *et al*. (2014).

So, it can be concluded from this study that *A. solani* exhibits high morphological and isozyme variability among the isolates themselves. The present investigation opens up the scope for further studies in details regarding physiological, ecological, genetic and pathogenic variation of strains within the hosts and their DNA analysis for molecular variation of the pathogen which will help in developing an effective breeding programme for disease resistance of the crops.

REFERENCES

Ainsworth, G .C. 1961. Dictionary of Fungi. Common Wealth Mycological Institute, Kew, Swerry. England, pages. p. 547

Ambesh, B. S., Ngomle, S., Marak, T. and Das, S. 2014. Morphological and biochemical variation of some *Alternaria* species infected on different floricultural plants. *The Ecoscan*. Special issue. **IV:** 361-368.

Castro, M. E. A., Zambolim, L., Chavos, G. M., Cruz, C. D. and Matsuoka, K. 2000. Pathogenic variability of *Alternaria solani*, the causal agent of tomato early blight. *Summa Phytopathologica*. **26:** 24-28.

Ellis, M. B. and Ellis, J. P. 1985. Micro fungi on land plants. *Croom Helm, Sydney, Australia,* p. 425.

Johnston, A. and Booth, C. 1983. Plant Pathologist's Pocket Book. Common-wealth Mycological Institute Kew, Surrey England.

Kahler, A. L. and Allard, R. W. 1970. Genetics of isozyme variants in barley. I. Esterases. *Crop Sci*. **10:** 444-448.

Kaul, A. K. and Saksena, H. K. 1989. Conidial morphology of isolates of *Alternaria solani* showing cultural and pathogenic variability. *Plant Disease Research*. **4(2):** 184-186.

Kumar, V., Haldar, S., Pandey, K. K., Singh, R. P., Singh, A. K. and Singh, P. C. 2008. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. *World J. Microbiol. Biotechnol*. **24:** 1003-1009.

Leung, H., Nelson, R. J. and Leach, J. E. 1993. Population structure of plant pathogenic fungi and bacteria. *Adv. Plant Pathol*. **10:** 157-205.

Meena, P. D., Chattopadhyay, C., Kumar, V. R., Meena, R. L. and Rana U.S. 2005. Spore behaviour in atmosphere and trends in variability of *Alternaria brassicae* population in India. *J. Mycol. Plant Pathol*. **35:** 511.

Morris, P. F., Connolly, M. S. and Clair, D. A. 2000. Genetic diversity of *Alternaria alternata* isolated from tomato in California using RAPD. *Mycol. Res*. **104:** 286-292.

Naik, M. K., Prasad, Y., Bhat, K. V. and Devika Rani, G. S. 2010. Morphological, physiological, pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. *Indian Phytopath*. **63(2):** 168-173.

Pramila, G. P., Tasleem, M., Taj, G., Mal, R. and Kumar, A. 2014. Morphological, cultural, pathogenic and molecular variability amongst Indian mustard isolates of *Alternaria brassicae* in Uttarakhand. *African J. Biotechnol*. **13(3):** 441-448.

Pryor, B. M. and Gilbertson, R. L. 2002. Molecular phylogenetic relationship amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. *Mycol. Res*. **104:** 151-160.

Pryor, B. M. and Michailides, T. J. 2002. Morphological, pathogenic and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of Pistachio. *Phytopathol*. **92:** 406-416.

Quayyum, H. A., Dobinson, K. F. and Traquair, J. A. 2005. Conidial morphology, virulence, molecular characterization, and host parasite interactions of selected Alternaria panax isolates on American ginseng. *Can. J. Bot*. **83:** 1133-1143.

Singh, A., Singh, V. and Yadav, S. M. 2014. Cultural, morphological and pathogenic variability of *Alternaria solani* causing Early Blight in tomato. *Plant Pathol J.* **13(3):** 167-172.

Somappa, J., Srivastava, K., Sarma, B. K., Pal, C. and Kumar, R. 2013. Studies on growth conditions of the tomato *Alternaria* leaf spot causing *Alternaria solani* L. *The Bioscan*. **8(1):** 101-104.

Sofi, T. A., Beig, M. A., Dar, Gh. H., Ahmad, M., Hamid, A., Ahangar, F. A., Padder, B. A. and Shah, M. D. 2013. Cultural, morphological, pathogenic and molecular characterization of *Alternaria mali* associated with Alternaria leaf blotch of apple. *African J. Biotechnol*. **12(4):** 370-381.

van der Waals, J. E., Korsten, L. and Slippers, B. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Dis*. **88:** 959-964.