ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Biological Sciences 5 (1): 30-38, 2012
ISSN 1996-3351 / DOI: 10.3923/ajbs.2012.30.38

Regional Variation in Genetic Structure and Pathogenecity of *Fusarium oxysporum* f. sp. *cumini* Isolated from *Cuminum cyminum* L.

[1,2]R.K. Deshwal and [1]N. Kumari
[1]School of Biosciences, Suresh Gyan Vihar University, Jaipur-302 025, India
[2]Apex Institute of Management and Sciences, Jaipur-302 020, India

Corresponding Author: R.K. Deshwal, School of Biosciences, Suresh Gyan Vihar University, Jaipur-302 025, India

ABSTRACT

Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cumini*, can lead to significant yield losses in cumin (*Cuminum cyminum* L.) locally known as Zeera. Ten *F. oxysporum* isolates from cumin plant were obtained from diseased plants of different cultivated areas of Rajasthan and characterized for pathogenicity and Vegetative Compatibility Grouping (VCG). The isolates were placed into six VCG groups based on pairing of complementary mutants. To find out correlation between genetic diversity and pathogenecity RAPD analysis was carried out using 40 UBC (University of British Columbia, Vancouver, Canada) primers, generated 41 polymorphic markers out of 65 amplicons with 63.07% average polymorphism. Based on Ntsys analysis of RAPD data was clustered all the genotypes in two major groups (group A and B) at 66.25% between group similarities. The results suggest that phylogenetic relationships among fungal isolates, collected from different regions of Rajasthan correlates with variation exist at pathogenicity.

Key words: *Cuminum cyminum* L., *Fusarium oxysporum* f. sp. *cumini*, VCG, RAPD

INTRODUCTION

Cumin (*Cuminum cyminum* L.) locally known as Zeera in Hindi, belongs to order umbellales and family Umbelliferae and believed to have originated from Egypt (Edison *et al*., 1991). It is grown extensively in South-Eastern Europe and North Africa bordering the Mediterranean Sea and in India and China (Chattopadhyay and Maiti, 1990). In India, cumin is one of the most important seed spice crop grown in the states of Rajasthan, Gujarat, Uttar Pradesh and Tamil Nadu. The area under cumin cultivation in India is about 430840 ha with annual production of 227829 tones. Rajasthan stands first in acreage (202980 ha) and production (120981 tones). Major cumin producing districts in Rajasthan are Barmer, Jalor, Nagaur and Jodhpur. During 2004-05, 13750 metric tones of cumin seed were exported earning a foreign exchange of Rs. 10190.00 lakhs. Cumin seeds preferred for their typical pleasant aroma due to an aromatic alcohol (animol) and spicy taste. It is largely used in mixed spices and curry powders.

The average yield of this crop is however low due to lack of superior varieties, scientific crop production technology and vulnerability to diseases like Wilt, blight and Powdery mildew incited by *Fusarium oxysporum* f. sp. *cumini*, *Alternaria burnsii* and *Erysiphe polygoni*, respectively. In these diseases wilt is most common, results in yield losses up to 35% in cumin in some districts of Rajasthan (Vyas and Mathur, 2002). Many other plants are also adversely affected by these pathogens (Gupta *et al*., 2010; Srivastava *et al*., 2011; Kelaniyangoda *et al*., 2011).

Certain difficulties have been encountered for the management of this disease in plant because of scanty availability of known and recommended fungicides in the market. In addition, these chemicals are costly and not fully efficacious in some cases. In fact, during last two decades, problems of resistance against bactericides and fungicides (Silva and Singh, 1974; Nene and Thapliyal, 2000) have increased as well as some new important pathogenic strain have been identified and documented (Champawat and Pathak, 1991; Chakraborty, 2005; Mamatha and Rai, 2004). Management of wilt by resistance breeding is an economically viable and ecologically desirable strategy. Many scientists have also tried to manage it by use of biocontrol agents (Reid *et al*., 2002; Mujeebur *et al*., 2004; Jegathambigai *et al*., 2009). Knowledge of diversity and relatedness among the pathogen populations is, however, a prerequisite for exploitation of resistance breeding. *F. oxysporum* isolates from different agroclimatic areas exhibit considerable diversity (Singh and Kumar, 2011) with respect to cultural characteristics (Elwakil and Ghoneem, 1999; Haglund, 1984) also seen most often in areas of fields that are low and compacted, especially during periods of high temperatures (Hanson and Jacobsen, 2009).

There are a number of techniques used to observe genetic variation within fungal pathogens. One of the techniques is VCG which is based on the ability of the mycelium to anastomose to form heterokaryon to determine genetic relatedness (Mohammadi and Mofrad, 2009). Now a day's attention has been focused on studying polymorphism at the DNA level for genetic characterization because different molecular markers, apart from elucidation of genetic variability, can also be used to study evolution and monitoring movement/shift of pathogen population over time and space Previously study on genetic relationship and phylogeny among *Fusarium* species have been conducted at DNA levels by many scientists (Bogale *et al*., 2006, 2007; Suga *et al*., 2000; Benthy *et al*., 1998; Attitalla *et al*., 2011; Hill *et al*., 2011).

The aim of this study was to better understand phylogenetic relationships among *F. oxysporum* isolates collected from different regions of Rajasthan and determine if this diversity correlates with pathogenicity or geographic origin.

MATERIALS AND METHODS

Collection and identification: Collection of fungal isolates from cumin plants exhibiting symptoms of *F. oxysporum* were collected from different cumin cultivated areas of Rajasthan. Tap root and side root samples were trimmed, washed in running tap water to remove soil, blotted dry and cut into 10 mm segments. Root segments were sterilized with 0.5% NaCl for 1 min Each longitudinal section was transferred to Petri dishes containing potato dextrose agar (PDA; Hi media). Plates were incubated on the laboratory bench for 5 days at 25±2°C. Fungi were transferred to freshly prepared PDA to eliminate contamination. Single spore isolates were either prepared from cultures of *Fusarium* species (Nelson *et al*., 1983; Leslie *et al*., 2006) or isolates were subcultured onto 2% (w/v) water agar (Hi media) for hyphal tip transfer (Nelson *et al*., 1983) to obtain pure cultures. Single spore isolation made from each colony. Isolates were identified morphologically to species based on characteristics of macroconidia, phialides, microconidia, chlamydospores and colony growth traits (Leslie *et al*., 2006).

Pathogenicity screening: Ten seeds of cumin were planted in each of 304 cm diameter plastic pots containing a soil mixture. After seed germination, a plug of *F. oxysporum* culture was put on the base of a seedling. Negative controls were treated same way but lacked fungi. The pots were placed in a greenhouse at 25±2°C. Symptoms were evaluated after 3-6 days of incubation (Kavak and Boydak, 2006; Keinath, 1994; Valizadeh *et al*., 2007).

Vegetative compatibility grouping (VCG): Nitrate non-utilizing (nit) mutants were generated and the phenotypes were characterized as nit1, nit3 or nitM as described by Correll *et al.* (1987). Pairings of all possible combinations or complementation of the nit mutants was made on Minimal Media (MM) (Puhalla, 1985) and amended with kalium chlorate concentration ranging from 1.5% (w/v) to 5.0% (w/v). The MM plates were incubated at 25°C. Vegetatively compatible isolates produced abundant aerial mycelium at the interface of two colonies and were placed in the same group.

DNA extraction/PCR amplification and electrophoresis: Total genomic DNA was extracted using the method of Doyle and Doyle (1990) from five days old fungal mycelium grown on PDA. The quality of extracted DNA after RNase treatment was assessed on 0.8% agarose gel and finally the DNA was quantified using spectrophotometer (Optigen 2020 plus). The DNA samples were diluted to 25 ng μL^{-1} for PCR amplification.

For RAPD analysis 40 primers from set No. 1 and 10 primers from set No. 2 obtained from the University of British Columbia, Vancouver, Canada were screened. Out of 50 primers only 9 primers were amplified and eight of these primers were removed because of their monomorphic nature and poor reproducibility. The PCR reactions were performed in a 25 μL reaction mixture containing 1x assay buffer, 0.5 units of Taq DNA polymerase, 200 μM of each dNTPs (Bangalore Genei), 0.2 μM primers and 50 ng of template DNA. The PCR reactions were carried out in DNA thermal cycler (Model-CGI-96, Corbett Research, Australia) using a single primer in each reaction. The PCR reactions were repeated thrice for each primer to ensure the reproducibility of RAPD results. Only highly reproducible and polymorphic primers were included in the study.

The PCR amplification conditions for RAPD consisted of initial extended step of denaturation at 94°C for 4 min followed by 44 cycles of denaturation at 94°C for 1 min, primer annealing at 37°C for 1 min and elongation at 72°C for 2 min followed by a final step of extension at 72°C for 4 min. The PCR reaction products were mixed with 4 μL of 6x DNA loading buffer and fractionated on 1.2% agarose for RAPD containing 0.5 μg μL^{-1} ethidium bromide. Gels were electrophoresed until the indicator dye reached 10 cm from the well at 55 mA for 4 h. After separation gels were documented using Avigene Gel Doc syastem (Koria).

Scoring and data analysis: Only clear and reproducible bands were scored for the data analysis, but a major band corresponding to a faint band in repetition was also included in the study. RAPD data were scored for the presence (1) or absence (0) and each band was regarded as a locus. λ DNA EcoRI/HindIII double digest marker was used as a standard for the estimation of molecular weight of the RAPD products. Bands with same molecular weight and mobility were considered as a single locus. The total number of alleles, polymorphic alleles, average number of alleles per primer and polymorphism percentage were calculated.

Similarity matrix and cluster analysis: The statistical calculations were done using Ntsys pc 2.02e. Similarity matrix for RAPD primers was constructed using the Jaccard's similarity coefficient values to find out genotypic relationship. The average distance of a single variety from rest of the genotypes was also calculated. The 0/1 matrix data obtained from RAPD were arranged to get separate similarity matrices which were subjected to UPGMA (unweighted pair-group method with arithmetic averages) analysis to generate dendrogram and compared using the Mantel matrix correspondence test (MxComp module of NTSYSpc).

RESULTS AND DISCUSSION

Total ten isolates of *F. oxysporum* were recovered from root rotted parts of cumin plants collected from the major producing areas of Rajasthan (Table 1). Colonies of isolates were woolly to cottony with cream to white aerial mycelium and purple pigment. Conidiophores had simple or branched monophialides, microconidia are generally one celled, very seldom two celled, hyaline measuring 2-3.5×5-12 μm. Macroconidia are mostly 3- septate but some have 4-5 septa. They are boat shaped to oblong and 3-4×25-45 μm in size.

Pathogenecity and VCG grouping: Isolates of *F. oxysporum* were examined for their pathogenecity on seedlings of cumin. Results of pathogenecity tests revealed that all isolates were pathogenic to cumin and produce interveinal yellowing of leaves followed by wilting (Bowden and Leslie, 1992).

F. oxysporum isolates produced chlorate-resistant sectors on media complemented with chlorate. One hundred and twelve nit mutants were generated from 10 isolates using PDA amended with 3 and 5% potassium chlorate. The number of sectors was observed at 3.0% (w/v) of chlorate concentration. The nit mutants were divided into three classes; nit1 (a mutation of nitrate reductase structural locus), nit3 (a mutation of nitrate-assimilation pathway specific locus) and nitM (mutations that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity). The most frequent phenotype was nit1 (50%), followed by nit3 (20%) and nitM (30%) among total 112 mutants. According to the literature data the frequency of mutant nit1 is higher than the frequency of other types of nit mutants. Similarly, Klittich and Leslie (1988) examined twelve strains of *F. moniliforme* for their ability to sector spontaneously on toxic chlorate medium and concluded that genetic control of nitrate reduction in *F. mo*niliforme is similar to that in *Aspergillus* and *Neurospora*, but that the overall regulation of nitrogen metabolism may be different.

Based on pairing complementary nit mutants of all isolates, mainly with nit1 and nitM mutants, the 10 isolates were grouped into six VCGs that B, and C VCGs have two members. VCGs A had 3 members and D, F and E had only single member (Table 2).

VCG diversity can be calculated by dividing the number of total VCG by the total number of isolates (Smith-White *et al*., 2001). In the present study, the overall VCG diversity for *F. oxysporum* was 50% which suggest that VCG analysis showed considerable variations among the isolates. In VCG analysis, the variation could be cause by a single base changes with compatible loci which may divide two almost identical isolates into separate groups. Isolates in a same VCG often share pathological and physiological traits as well as geographical origins (Swift *et al*., 2002; Sharifi *et al*., 2008). In this study, this attributes can be seen in *F. oxysporum* isolates in which the VCGs were grouped according to the symptom and locations. This study was also revealed by various previously carried research works (Hawthorne and Rees-George, 1996; Kistler *et al*., 1998; Latiffah *et al*., 2009).

Table 1: Localities and their attributes selected for collection and study of cumin

Location	Latitude (N)	Longitude (E)	Average temperature (°C)
Ahore	25° 21'	72° 51'	30-36
Jalore	25° 22'	73° 00'	30-35
Jodhpur	26° 18'	73° 04'	27-35
Jobner	26° 58'	75° 23'	28-32

Table 2: Vegetative compatibility groups of *F. oxysporum* used in the study

Isolates	Location	VCG group
A-1	Ahore	A
A-2	Ahore	A
J-1	Jalore	A
J-2	Jalore	B
J-3	Jalore	B
Jod-1	Jodhpur	C
Jod-2	Jodhpur	C
Jod-3	Jodhpur	D
Job-1	Jobner	E
Job-2	Jobner	F

Table 3: List of single arbitrary primers showing total and polymorphic amplicons generated along with PIC of each pattern for 10 fungal genotypes

Primers	Sequences (5'¬3')	Total No. of bands (a)	Total No. of polymorphic bands (b)	Polymorphism (b/ax 100)	PIC
GCC 106	CGTCTGCCCG	3	1	33.33	0.106
GCC 116	TACGATGACG	5	3	60.00	0.184
GCC 126	CTTTCGTGCT	5	5	100.00	0.312
GCC 132	AGGGATCTCC	14	4	28.57	0.084
GCC 137	GGTCTCTCCC	7	6	85.71	0.322
GCC 184	CAAACGGCAC	6	1	16.66	0.070
GCC 189	TGCTAGCCTC	8	5	62.50	0.197
GCC 193	TGCTGGCTTT	4	4	100.00	0.425
GCC 196	CTCCTCCCCC	13	12	92.30	0.392
Total		65	41	63.07 (Aver.)	

RAPD analysis: VCG analysis has its limitations especially some isolates were unable to form mutants on chlorate medium and to form heterokaryon in complementation test that why molecular diversity analysis using RAPD analysis was also include in this study for better characterization of isolates from more diverse locations and different cumin species.

RAPD analysis was performed using 40 primers from set No. 1 and 10 primers from set No. 2 obtained from the UBC. Out of 40 primers only 9 primers were amplified and eight of these primers were removed because of their monomorphic nature and poor reproducibility. The total number of bands generated by eleven amplifying primers was 65 with an average amplification of 7.20 bands per primer. The average polymorphism generated by these bands was 63.07%. The size of the amplicons generated varied from 272 to 3615 bp (Hyun and Clark, 1998; White *et al*., 1990; Cooley, 1992). Polymorphic Information Content (PIC) ranges from 0.070 (GCC 184) to 0.425 (GCC 193) (Table 3, Fig. 2a, b).

Genetic relationship among the accessions and cluster analysis: Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard's coefficient analysis (Table 4). The similarity coefficient matrix generated was subjected to algorithm Unweighted Pair Group Method for Arithmetic Average (UPGMA) to generate clusters using NTSYS 2.02 pc program (Rohlf, 1998, 2000). The dendrogram showing relationship among various genotypes was constructed using these clusters (Fig. 1).

Table 4: Jaccard's similarity coefficient

	A1	Jod-1	Jod-2	A-2	J-2	Job-1	J-1	Jod-3	Job-2	J-3
A1	1.00									
Jod-1	0.65	1.00								
Jod-2	0.62	0.67	1.00							
A-2	0.76	0.58	0.65	1.00						
J-2	0.76	0.58	0.65	0.79	1.00					
Job-1	0.77	0.75	0.79	0.76	0.76	1.00				
J-1	0.82	0.60	0.67	0.93	0.81	0.78	1.00			
Jod-3	0.64	0.76	0.73	0.67	0.67	0.75	0.69	1.00		
Job-2	0.65	0.70	0.74	0.68	0.71	0.69	0.70	0.73	1.00	
J-3	0.72	0.61	0.65	0.75	0.79	0.76	0.77	0.67	0.62	1.00

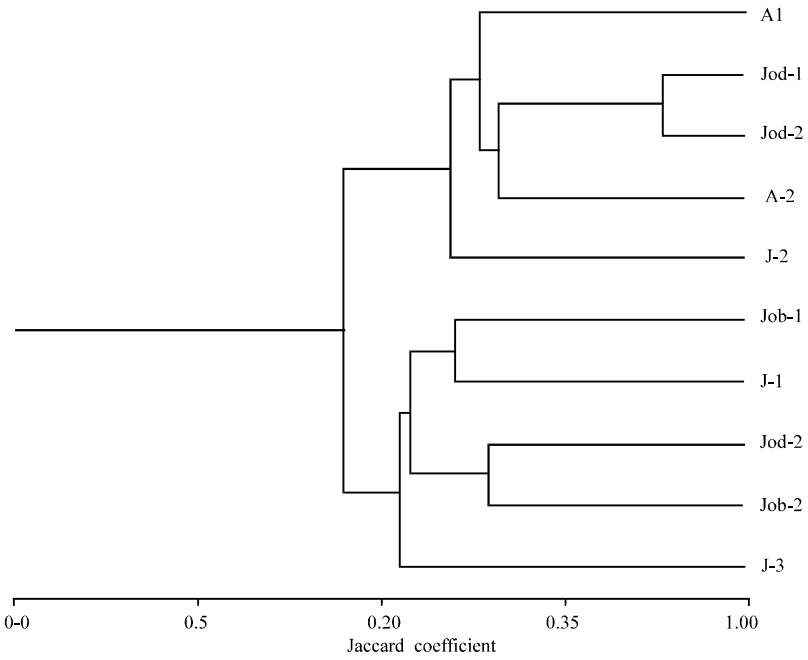

Fig. 1: Dendrogram showing relationship among ten fungal genotypes generated by UPGMA analysis based on single primers

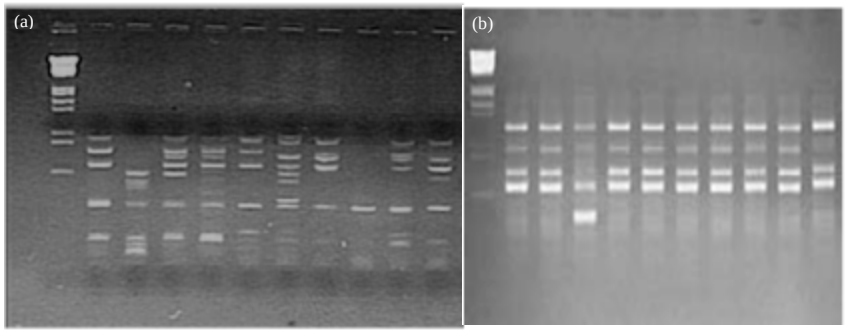

Fig. 2(a-b): RAPD analysis using primer No. (a) 137 and (b) 116

The Jaccard's pairwise similarity coefficient values ranged from 0.58 (A2 and Jod-1, J2 and Jod-1) to 0.93 (J-1 and A-2) with an average of 0.711, for single primer based RAPD patterns (Table 4). The clusters constructed through NTSYS (2.02 pc) presented in the form of dendrogram has been shown in Fig. 1. The dendrogram has put all the genotypes in two major groups (group A and B) at 66.25% between group similarity. Group A consists A-1, A-2, J-2, J-1 and J-3 total 5 genotype having 79.0% within group similarity and group B consists Jod-1, Jod-2, Jod-3, Job-1 and Job-2, total 5 genotype with 73.5%. Zakaria *et al.* (2009) and Kubelik and Szabo (1995) also used RAPD PCR for characterization and diversification of fungal isolates and found that this technique efficiently divide isolates into clear groups according to their genetic differences.

CONCLUSION

The results suggested phylogenetic relationships among fungal isolates, collected from different regions of Rajasthan correlates with variation exist at pathogenicity.

REFERENCES

Attitalla, I.H., Z. Latiffah, B. Salleh and S. Brishammar, 2011. Biology and partial sequencing of an endophytic Fusarium oxysporum and plant defense complex. Am. J. Biochem. Mol. Biol., 1: 121-144.

Benthy, S., K.G. Pegg, N.Y. Moore, R.D. Davis and I.W. Buddenhagen, 1998. Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cubense* analyzed by DNA fingerprinting. Phytopathology, 88: 1283-1293.

Bogale, M., B.D. Wingfield, M.J. Wingfield and E.T. Steenkamp, 2006. Characterization of Fusarium oxysporum isolates from Ethiopia using AFLP, SSR and DNA sequence analyses. Fungal Diversity, 23: 51-66.

Bogale, M., B.D. Wingfield, M.J. Wingfield and E.T. Steenkamp, 2007. Species-specific primers for *Fusarium redolens* and a PCR-RFLP diagnostic technique for distinguishing among three clades of Fusarium oxysporum. FEMS Microbiol. Lett., 271: 27-32.

Bowden, R.L. and J.F. Leslie, 1992. Nitrate non-utilizing mutants of *Gibberella zeae* (*F. graminearum*) and their use in determining vegetative compatibility. Exp. Mycol., 16: 308-315.

Chakraborty, S., 2005. Potential impact of climate change on plant-pathogen interactions. Aust. Plant Pathol., 34: 443-448.

Champawat, R.S. and V.N. Pathak, 1991. Effect of fungicidal seed treatment on wilt disease of cumin. J. Turk. Phytopathol., 20: 23-26.

Chattopadhyay, S.B. and S. Maiti, 1990. Diseases of Betelvine and Spices. 2nd Edn., Indian Council of Agricultural Research, New Delhi, pages: 160.

Cooley, R.N., 1992. The use of RFLP Analysis, Electrophoretic Karyotyping and PCR in Studies of Plant Pathogenic Fungi. In: Molecular Biology of Filamentous Fungi, Sthl, U. and P. Tudzynski (Eds.). VCH., Weinheim, Germany, pp: 13-26.

Correll, J., C.J.R. Klittich and J.F. Leslie, 1987. Nitrate non-utilizing mutants of Fusarium oxysporum and their use in vegetative compatibility tests. Phytopathology, 77: 1640-1646.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Edison, S., A.K. Johny, K. Nirmal Babu and A. Ramadasan, 1991. Spices varieties: A compendium of morphological and agronomic characters of improved varieties of spices in India. National Research Centre for Spices (ICAR), Kerala, pp: 63.

Elwakil, M.A. and K.M. Ghoneem, 1999. Detection and location of seed-borne fungi of black cumin and their transmission in seedlings. Pak. J. Biol. Sci., 2: 559-564.

Gupta, V.K., A.K. Misra, R.K. Gaur, P.K. Jain, D. Gaur and S. Sharma, 2010. Current Status of Fusarium Wilt Disease of Guava (*Psidium guajava* L.) in India. Biotechnology, 9: 176-195.

Haglund, W.A., 1984. Fusarium Wilts. In: Compandium of Pea Diseases, Hagedorn, D.J. (Eds.). The American Phytopathological Society, St. Paul, Minnesota, USA.

Hanson, L.E. and B.J. Jacobsen, 2009. Fusarium Yellows. In: Compendium of Beet Diseases and Pests, Harveson, R.M., L.E. Hanson and G.L. Hein (Eds.). APS Press, USA., pp: 28-30.

Hawthorne, B.T. and J. Rees-George, 1996. Use of nitrate non-utilizing mutants to study vegetative incompatibility in *Fusarium solani* (*Nectria haematococca*) especially members of mating populations I, V and VI. Mycol. Res., 100: 1075-1081.

Hill, A.L., P.A. Reeves, R.L. Larson, A.L. Fenwick, L.E. Hanson and L. Panella, 2011. Genetic variability among isolates of Fusarium oxysporum from sugar beet. Plant Pathol., 60: 496-505.

Hyun, J.W. and C.A. Clark, 1998. Analysis of *Fusarium lateritium* using RAPD and rDNA RFLP techniques. Mycol. Res., 102: 1259-1264.

Jegathambigai, V., R.S.W. Wijeratnam and R.L.C. Wijesundera, 2009. Control of Fusarium oxysporum wilts disease of *Crossandra infundibuliformis* var. *Danica* by *Trichoderma viride* and *Trichoderma harzianum*. Asian J. Plant Pathol., 3: 50-60.

Kavak, H. and E. Boydak, 2006. Screening of the resistance levels of 26 sesame breeding lines to Fusarium Wilt disease. Plant Pathol. J., 5: 157-160.

Keinath, A.P., 1994. Pathogenicity and host range of *Fusarium oxysporum* from sweet basil and evaluation of disease control methods. Plant Disease, 78: 1211-1215.

Kelaniyangoda, D.B., A.S.A. Salgadoe, S.J.B.A. Jayasekera and R.M. Gunarathna Banda, 2011. Wilting of bell pepper (*Capsicum annuum* L.) causal organism isolation and a successful control approach. Asian J. Plant Pathol., 5: 155-162.

Kistler, H.C., C. Alabouvette, R.P. Baayan, S.B. Bentley and A. Coddington *et al*., 1998. Systemic numbering of vegetative compatibility groups in the plant pathogenic fungus Fusarium oxysporum. Phytopathology, 88: 30-32.

Klittich, C.J.R. and J.F. Leslie, 1988. Nitrate reduction mutants of Fusarium moniliforme (*Gibberella fujikuroi*). Genetics, 118: 417-423.

Kubelik, A.R. and L.J. Szabo, 1995. High GC primers are useful in RAPD analysis of fungi. Curr. Genet., 28: 384-389.

Latiffah, Z., M.Z.N. Hayati, S. Baharuddin and Z. Maziah, 2009. Identification and pathogenicity of Fusarium species associated with root rot and stem rot of *Dendrobium*. Asian J. Plant Pathol., 3: 14-21.

Leslie, J.F., B.A. Summaerel and S. Bullock, 2006. The Fusarium Laboratory Manual. Blackwell Publication, New York.

Mamatha, T. and V.R. Rai, 2004. Evaluation of fungicides and plant extracts against Fusarium solani leaf blight in *Terminalia catappa*. J. Mycol. Plant Pathol., 34: 306-307.

Mohammadi, A. and N.N. Mofrad, 2009. Genetic diversity in population of Fusarium solani from cumin in Iran. J. Plant Prot. Res., 49: 283-286.

Mujeebur, K., R. Shahana, M. Khan and A.M. Fayaz, 2004. Biological control of Fusarium wilt of chickpea through seed treatment with the commercial formulation of *Trichoderma harzianum* and/or *Pseudomonas fluorescens*. Phytopathol. Mediterr., 43: 20-25.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. Fusarium Species an Illustrated Manual for Identification. Pennsylvania State University, UK.

Nene, Y.L. and P.N. Thapliyal, 2000. Poisoned Food Technique. Fungicides in Plant Disease Control. 3rd Edn., Oxford and IBH Publishing Company, New Delhi, pp: 531-533.

Puhalla, J.E., 1985. Classification of strains of Fusarium oxysporum on the basis of vegetative compatibility. Can. J. Bot., 63: 179-183.

Reid, T.C., M.K. Hausbeck and K. Kizilkaya, 2002. Use of fungicides and biological controls in the suppression of Fusarium crown and root rot of asparagus under greenhouse and growth chamber conditions. Plant Dis., 86: 493-498.

Rohlf, F.J., 1998. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, Version 2.02. Exeter Software, New York.

Rohlf, F.J., 2000. NTSYSpc: Numerical Taxonomy and Multivariate Analysis System. Version 2.02, Exeter Software, Setauket, New York, USA.

Sharifi, K., R. Zare and J. Rees-George, 2008. Vegetative compatibility groups among Fusarium solani isolates causing potato dry rot. J. Boil. Sci., 8: 374-379.

Silva, A.P. and K. Singh, 1974. Soil Fungicides. Vol. 1, CRC Press, USA.

Singh, P.K. and V. Kumar, 2011. Variability among isolates of *Fusarium oxysporum* f.sp. *chrysanthemi* pathogenicto chrysanthemum. Int. J. Plant Pathol., 2: 136-143.

Smith-White, J.L., L.V. Gunn and B.A. Summerell, 2001. Analysis of diversity within Fusarium oxysporum populations using molecular and vegetative compatibility grouping. Australas. Plant Pathol., 30: 153-157.

Srivastava, S., V.P. Singh, R. Kumar, M. Srivastava, A. Sinha and S. Simon, 2011. In vitro evaluation of carbendazim 50% WP, antagonists and botanicals against *Fusarium oxysporum* f. sp. *psidii* associated with rhizosphere soil of Guava. Asian J. Plant Pathol., 5: 46-53.

Suga, H., T. Hasegawa, H. Mitsui, K. Kageyama and M. Hyakumachi, 2000. Phylogenetic analysis of the phytopathogenic fungus Fusarium solani based on the rDNA-ITS region. Mycol. Res., 104: 1175-1183.

Swift, C.E., E.R. Wickliffe and H.F. Schwartz, 2002. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cepae* from onion in Colorado. Plant Dis., 86: 606-610.

Valizadeh, M., S.K.K. Tabar and G.A. Nematzadeh, 2007. Effect of plant growth regulators on callus induction and regeneration of cumin (Cuminum cyminum). Asian J. Agric. Res., 1: 17-22.

Vyas, R.K. and K. Mathur, 2002. Distribution of Trichoderma spp. in cumin rhizosphere and their potential in suppression of wilt. Indian Phytopathol., 55: 451-457.

White, T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White (Eds.). Academic Press, New York, pp: 315-322.

Zakaria, L., N.S. Ali, B. Salleh and M. Zakaria, 2009. Molecular analysis of *Ganoderma* species from different hosts in peninsula Malaysia. J. Biol. Sci., 9: 12-20.