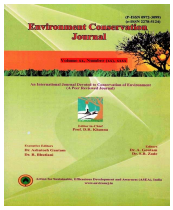
Environment Conservation Journal 24 (1):217-224, 2023

Journal homepage:https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Occurrence of *Phytophthora nicotianae* causing collar and root rot disease of Chrysanthemum in India

Priti Sonavane ✉
Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bangalore, India
Venkataravanappa V
Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bangalore, India
Krishna Reddy M
Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bangalore, India
Pavithra R S
Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bangalore, India

ARTICLE INFO

Received : 07 April 2022
Revised : 31 July 2022
Accepted : 28 August 2022

Available online: 15 January 2023

Key Words:
Chrysanthemum
Collar rot and Root rot
Multi-locus phylogeny
Phytophthora nicotianae

ABSTRACT

Chrysanthemum is an important flower crop grown in different parts of the world. Apparently there are new reports of chrysanthemum collar rot and root rot caused by *Phytophthora* sp. around the world. In recent years there has been increase in incidence of *Phytophthora* root and collar rot in chrysanthemum and no information is available about this disease in India. Therefore the aim of the present study is to isolate and characterize the pathogen causing collar rot and root rot disease of chrysanthemum. During year 2021, the plants exhibiting collar rot and root rot disease symptoms were received from the local farmers. Based on initial micro-morphological observation it was identified as *Phytophthora* sp. Further the pure culture of pathogen was isolated and confirmed its identity through cultural, morphological and amplification of the three regions/genes (ITS, *tef-1α* and *β-tubulin*) of *Phytophthora* sp. Phylogenetic analysis of concatenated sequence derived from ITS, *tef-1* and *β-tubulin* sequences of five *Phytophthora* isolates (PhN1, PhN2, PhN3, PhN4 and PhN5) showed close clustering of these isolates with *Phytophthora nicotianae* isolates infecting different crops. This is first detailed characterization of *Phytophthora nicotianae* causing collar rot and root rot in chrysanthemum in India.

Introduction

Ornamental flower plants are primarily cultivated for beautification and also to increase aesthetic value of public places, gardens and landscaping. Growing of flower crops is one of the prime businesses throughout the worldwide due to increase in demands for flowers at national and international market. In India, the area under flower crops production was 324 thousand hectares and the production of 1962.03 MT during 2017-18 (www.agricoop.nic.in). Among the flower crops, chrysanthemum (*Chrysanthemum indicum* L.) is one of the oldest flowering plant commercially grown in different parts of the world including India. Chrysanthemum belongs to the family Asteraceae and is cultivated globally for its cut as well as loose flowers and also used for herbal tea and preparation of pesticides (Bhattacharya and Silva, 2006). In India the crop is being cultivated commercially in Karnataka, Maharashtra, Rajasthan, Gujarat, Haryana, West Bengal, Delhi, Uttar Pradesh and Tamil Nadu. The crop is more popular due to wide range of colours, size and shape. So far, around 200 species of chrysanthemum comprising 20,000 varieties are reported around the world (Joshi *et al.,* 2010) of which nearly 200 varieties are grown in India (Singh and Kumar, 2014). The intensive cultivation of chrysanthemum is hampered by different disease caused by numerous pathogens such as bacterial, fungal and viral diseases (Bhattacharya and Silva,

Corresponding author E-mail: priti9883@gmail.com
Doi:https://doi.org/10.36953/ECJ.11922310

2006; Cook, 2001; Luong *et al.,* 2010; Nishi *et al.,* 2009). Among them collar and root rot is one of the major limiting factor for commercial cultivation of chrysanthemum in other countries. So far, different *Phytophthora* species has been well documented around the world causing disease on chrysanthemum that includes *Phytophthora chrysanthemi* from Japan (Naher *et al.,* 2011), Croatia (Tomic and Ivic, 2015), Germany (Götz *et al.,* 2017), United States (Lin *et al.,* 2017); Randall-Schadel, 2016), *Phytophthora drechsleri* and *Phytophthora nicotianae* from United States (Krasnow *et al.,* 2021; Mullen *et al.,* 2001). In India *Phytophthora* species causing flower blight of chrysanthemum has been identified in 1997, but no report of collar and root rot disease caused by *Phytophthora* in chrysanthemum. Since there is no much information available about *Phytophthora* species causing collar and root rot disease in chrysanthemum in India, Present study was attempted to characterize the collar and root rot disease of chrysanthemum based on morphological and molecular method for accurate identification of incite of the disease.

Material and Methods

Collection of Chrysanthemum collar rot and root rot disease samples

In September 2021, chrysanthemum plants (variety: Scent white and Scent yellow) exhibiting collar rot and root rot were submitted by the local chrysanthemum growing farmers from the two different locations (Shivakote and Bylakere) to the Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru.

Since the disease appeared to be new and based on initial observation the pathogen was tentatively confirmed as *Phytophthora* sp. based on the microscopic observation. So, far there were no reports of *Phytophthora* causing collar and root rot disease in chrysanthemum, therefore field visit was undertaken in chrysanthemum growing areas to collect more number of samples in different farmers field in Bengaluru rural Area *i.e.* Shivakote (13°08'00.3"N,77°30'33.1"E), Madappanahalli (13°08'50.2"N,77°31'38.9"E), Linganahalli (13°09'19.2"N, 77°31'14.5"E) and Bylakere (13°06'16.9"N,77°30'43.5"E), Karnataka (India). From each location one infected chrysanthemum plants showing the collar and root rot disease samples were collected and transported to Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru. For isolation of causal agent standard pathological procedure was followed as mentioned above.

Isolation of causal agents

The infected leaf, flower, stem segments of chrysanthemum plants collected during field visit were observed under the microscope to check for any sporulation. Further the infected root and collar region of the stem of chrysanthemum samples were washed in running water to remove soil and air-dried on blotting paper. The infected stem and root region were cut into 5 mm segments and surface sterilized using 1% sodium hypochloride followed by rinsing in distilled water for three times. The procedure for isolation of *Phytophthora* fungi was followed as mentioned by Sonavane and Venkataravanappa (2017). Then stem and root segments were dried on blotting paper and then inoculated aseptically on plates containing Potato Dextrose Agar with 100mg/L streptomycin. After 2 days of incubation at room temperature, a mycelia disc of 5 mm were cut using cork borer and transferred to petriplates containing sterile distil water for sporangia formation and incubated for 2 days. The isolated pure cultures were further maintained on PDA slants for further use. Infected samples were also surface sterilized and incubation at 25°C in sterile distill water for sporulation.

Morphological identification

The pure culture of *Phytophthora* isolates were used for morphological identification (colony growth, sporangia, oospore formation) using microscope at 100X magnification based on morphological keys (Waterhouse, 1963; Santos *et al.,* 2005). To study sporangia and oospore formation, mycelia disc from pure culture of each isolate was cut into 5mm disc using cork borer and dispersed in petriplates containing sterile distill water. After 48 hours of incubation at room temperature, abundant sporangia were observed at terminal end of mycelia. Sporangia from each isolate were collected and observed under 100X magnification. Colony characteristic (colour and texture) was observed after 5 days and colony diameter was measured at 2, 4, 8 and 10 days after inoculation.

Pathogenicity test:

For pathogenicity test two cultivars of Chrysanthemum (Scent white and Scent yellow) grown locally were used. For sporangia production procedure mentioned earlier in morphological identification was carried out. Produced sporangia were incubated at 8°C for 10 minutes for zoospore release which was confirmed under microscope. Chrysanthemum plants were maintained in polyhouse and zoospore suspension (1 x 10^6 zoospores/ml) of the pathogen was inoculated to the root zone. In control distil water was inoculated. The plants were kept in growth chamber at 28°C. Collar rot symptoms were visible 3 days after inoculation. The pathogen was re-isolated from infected roots.

Genomic DNA extraction, PCR amplification and sequencing

The pure culture of the fungus was grown on Potato Dextrose Broth and incubated for 7 days at 28°C. The mycelia mat was harvested by filtration through whatman filter paper no. 1 and washed with sterile distilled water and dried. Two grams of mycelia mat was used for total DNA isolation using CTAB method (Doyle and Doyle, 1990). The quality and concentration of the DNA was assessed using NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific). For molecular identification, the total genomic DNA extracted from the *Phytophthora* fungi was subjected to the PCR amplification using universal primer pairs of ITS rDNA, Translational elongation factor one alpha gene and *β-tubulin* gene regions of rDNA (Table 1).

Table 1: Primers used in this study for DNA amplification and sequencing

Locus	Primer name	Primer sequence (5'- 3')	Annealing temperature (°C)	References
ITS	ITS-1	TCCGTAGGTGAACCTGCGG	55°C	White et al., 1990
	ITS-4	TCCTCCGCTTATTGATATGC		
Beta-tubulin	T1	AACATGCGTGAGATTGTAAGT	60°C	O'Donnell & Cigelnik, 1997
	T2	TAGTGACCCTTGGCCCAGTTG		
Elongation Factor1 alpha	EF1	ATGGGTAAGGAAGACAAGAC	60°C	O'Donnell et al., 1998
	EF2	GGA(G/A)GTACCAGT(G/C)ATCATGT		

The PCR reactions were carried out in a GeneAmp PCR system 9700 (PE Applied Biosystems, Foster City, CA) thermocycler. All amplifications were performed in volumes of 25 µL PCR mix containing 2 µL DNA templates, 1.5 U Taq DNA polymerase, 25mM MgCl2, 2 mM dNTPs and 20 pmol of each primer. The PCR was programmed with the initial denaturation at 94ºC for 2 min, followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing temperature for ITS, tef-1α, and β-tubulin is mentioned in table 1 and the final extension of 72ºC for 90 seconds. PCR products were electrophoresised (1 h at 80 V) in 0.8% agarose gels in Tris-Borate-EDTA buffer, pH 8. Gels were stained with ethidium bromide (10 mg/mL) and viewed in a gel documentation system (Alpha Innotech, USA). The amplified PCR products of Internal transcribed spacer, Translational elongation factor one alpha gene and β-tubulin gene of *Phytophthora* isolates was purified from agarose gels using the QIA quick gel extraction kit (Qiagen, Hilder, Germany) and sequenced using automated DNA sequencing facility at Eurofins Genomics India Pvt. Ltd., Bangalore, India.

Sequence analysis

The sequences of ITS rDNA, Translational elongation factor one alpha gene and *β-tubulin* gene of the *Phytophthora* isolates were subjected to NCBI BLASTn to search for similar sequences in the database. The related sequences retrieved from the database belong to different *Phytophthora* species infecting diverse hosts were used for phylogenetic analysis. The ITS, Translational elongation factor one alpha gene and *β-tubulin* gene nucleotide sequences were concatenated with Mesquite version 3.61 (Maddison and Maddison, 2019). Sequences were aligned using clustalW method implemented in SEAVIEW program. A phylogenetic tree of the ITS, Translational elongation factor one alpha gene and *β-tubulin* gene

was constructed by maximum likelihood method using MEGA X version software (Kumar *et al.,* 2018) with 1,000 bootstrapped replications to estimate evolutionary distances between all pairs of sequences simultaneously.

Results and Discussion

Survey and collection of diseased chrysanthemum samples

During survey five fields were surveyed in diverse location of Bengaluru rural area for the incidence of collar rot and root rot disease of chrysanthemum. Typical symptoms of collar rot and root rot, yellowing, defoliation, leaf blight, blossom blight and complete dead of the plants was observed on two predominately grown local chrysanthemum varieties scent white and scent yellow in different farmer's field (Figure1). The infection appeared to be isolated in different areas of the field rather than complete loss at one place. The disease incidence in different location of Bengaluru rural Area ranged from 19.5 - 32.2 percent. Based on initial observations the pathogen was identified as *Phytophthora* sp. During field visit infected flowers, leaves, stem, roots and soil was collected (Figure 1).

There was no mycelia growth or sporulation on infected samples. Infected leaf, flower, stem and roots were used for isolation after surface sterilization. Among these only from roots the pathogen could be isolated. There was no sporulation on any of the infected material even after incubation in moist chamber.

Morphological characterization

The isolated pathogen was characterized as *Phytophthora nicotianae* on the basis of morphological and cultural characteristics. Mycelia growth of *Phytophthora nicotianae* on potato dextrose agar can be described as dense cottony mycelium with slightly petaloid pattern and growth was found to be abundant at 30°C (Figure 1). Intercalary hyphal swelling was abundant in solid culture.

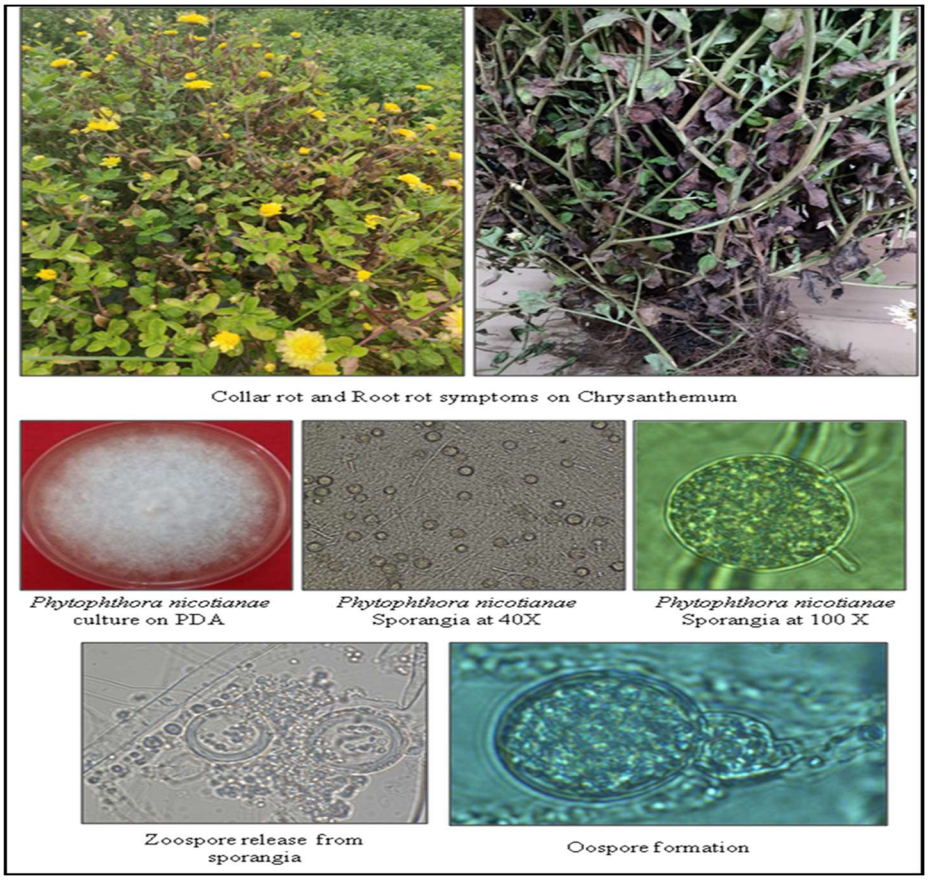


Figure 1: Symptoms, cultural and morphological characterisation of *Phytophthora nicotianae*

There was no sporangia formation on PDA agar but abundant sporangia was observed when disc were immersed in sterile distill water after 48h of incubation. The sporangia were mainly terminal, papillate, spherical, ovoid, 50-60µm in length, non-caducous. Zoospores are discharged through an exit pore 10-15µm wide (Figure 1). Isolates are homothallic, amphigyous based on oospores formation in 4 days after incubation. Oogonia are abundantly produced after 4 days. Oogonium is terminal, spherical. Anthridium is terminal and amphiygnous (Waterhouse, 1963 ; Santos *et al.,* 2005) (Figure 1).Pathogenicity test was conducted to determine the collected isolates of *P. nicotianae* were capable of causing similar observed symptoms under field conditions. The test was conducted on two popular chrysanthemum varieties i.e. Scent white and Scent yellow mentioned earlier. Plants were inoculated with zoospore suspension (10^6 spores per ml) of sterile water and control plants were left un-inoculated. In inoculated plants collar rot symptoms were visible 3 days after inoculation in both the varieties of chrysanthemum.

Molecular characterization

PCR amplification of different genomic regions of *Phytophthora* isolates

Total genomic DNA of the five *Phytophthora* isolates (PhN1, PhN2, PhN3, PhN4 and PhN5) causing collar and root rot disease of chrysanthemum were subjected to PCR for the amplification using universal primers pairs specific to ITS, *tef-1* and *β-tubulin* gene (Table 1). It resulted in the amplicons size of 550bp, 871bp and 799 bp respectively. The amplified PCR products of ITS, *tef-1* and *β-tubulin* was cloned and subjected to sequencing. The sequence analysis of three regions of five *Phytophthora* isolates (PhN1, PhN2, PhN3, PhN4 and PhN5) showed they belongs to *Phytophthora nicotianae* having nucleotide identity of 98-100 % with the respective from NCB. The consensus nucleotide sequence data of ITS, *tef-1* and *β-tubulin* was deposited in the GenBank under the following accession numbers listed in Table 2.

Table 2: List of the isolates sequenced for molecular phylogenetic analysis in this study

Species	Isolate no.	DNA database accession		
		ITS rDNA	β-tubulin	Elongation factor 1α
Phytophthora nicotianae	1	MZ396857	MZ502251	MZ447850
Phytophthora nicotianae	2	MZ396871	MZ502252	MZ447851
Phytophthora nicotianae	3	MZ411440	MZ502253	MZ447852
Phytophthora nicotianae	4	MZ411441	MZ502254	MZ447853
Phytophthora nicotianae	5	MZ411443	MZ502255	MZ447854

The nucleotide sequences of ITS, *tef-1* and *β-tubulin* regions of five *Phytophtho*ra *nicotianae* isolates infecting chrysanthemum in the present study were compared with the different *Phytophthora* species. The concatenated sequence derived from ITS, *tef-1* and *β-tubulin* sequences analysis indicated that, five *Phytophthora* isolates (PhN1, PhN2, PhN3, PhN4 and PhN5) in the current study shared nucleotide identity between 99.8-100% similarity per cent, among themselves. There were no sequences of *Phytophthora nicotianae* infecting chrysanthemum in the database, therefore other *Phytophthora* species identified in different parts of the world infecting chrysanthemum were used for phylogenetic analysis. Further, ITS sequence of these isolates shared maximum nucleotide (nt) identity of 99.74% per cent with the *Phytophthora nicotianae* infecting pomegranate from Turkey. While translation elongation factor shared highest nt identity with *Phytophthora nicotianae* infecting Nagpur orange from India. In case of *β-tubulin* showed more identical to *P. nicotianae* infecting ornamental crops in Italy. The phylogenetic analysis also showed close clustering of these isolates with *Phytophthora nicotianae* isolates infecting different crops (Figure 2). Phylogeny studies based on single locus DNA sequence was the common practice in fungi. This may not always elucidate the taxonomic status of the organisms (Berbee, 2001) and also give wrong conclusion about the relationship of a fungus within the same members of the same species or even the same genus (Lang *et al.,* 1999). The nuclear rDNA is the most popular and highly conserved genes, 18S (SSU), 5.8S (ITS), and 28S (LSU) for molecular phylogenetic studies (Kroon *et*

al., 2004). Therefore, analysis of concatenated sequences derived from multi-gene loci is becoming more acceptable in taxonomic positioning of fungi in the recent days (Kroon *et al.,* 2004). In the present study the analysis of concatenated sequence derived from ITS, *tef-1* and *β-tubulin* regions of five *Phytophthor*a isolates infecting chrysanthemum with reference sequences

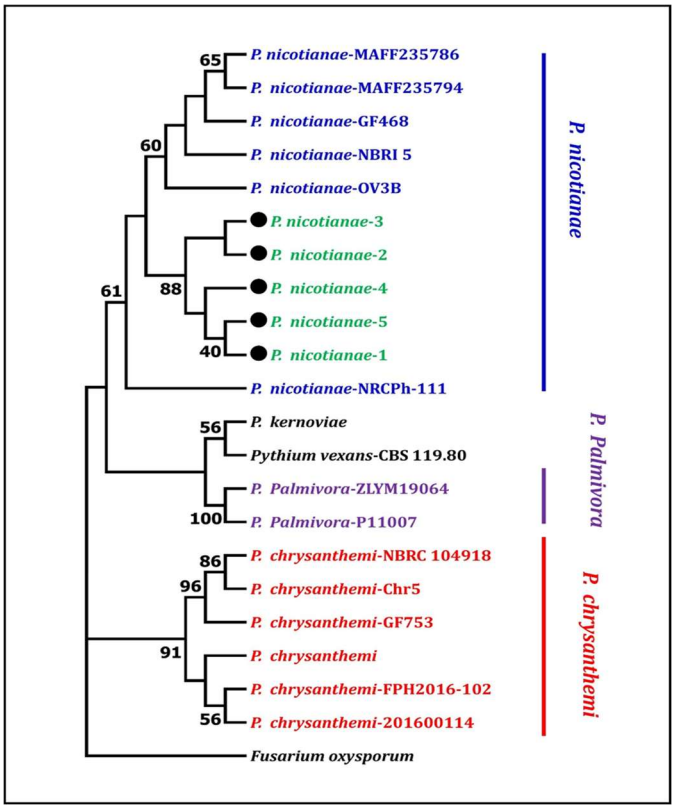


Figure 2: Phylogenetic tree showing the relationship of *Phytophthora nicotianae* within the genus *Phytophthora* based on gene sequences of (ITS, translation elongation factor 1α and β-tubulin) inferred by Bayesian analysis.

of *Phytophthora chrysanthemi, Phytophthora nicotianae* and *Phytophthora drechsleri* and different *Phytophthora* sequences available in the NCBI database revealed that all the five *Phytophthora* isolates infecting chrysanthemum along with several isolates of *Phytophthora nicotianae* formed monophyletic clade in the phylogentic analysis. Literature surveyed also showed similar conacatenated sequences derived from multi-gene loci analysis was carried out in fungi infecting different crops (Blair *et al.,* 2008; Camacho, 2009; Cook *et al.,* 2000; Donahoo *et al.,* 2006; Frezzi, 1950; Ivors et al., 2004; Lang *et al.,* 1999; Martin and Tooley, 2003ab; Molnar *et al.,* 2020). So, far three different species of *Phytophthora* has been identified around the world in chrysanthemum that includes *Phytophthora chrysanthemi* from Japan (Naher *et al.,* 2011) and United States (Lin *et al.,* 2017) and *Phytophthora drechsleri* from United States causing stem and blight of chrysanthemum (Krasnow *et al.,* 2021). However, this is first attempt of using concatenated sequence for identification of *Phytophthor*a *nicotianae* infecting chrysanthemum as per our knowledge. In conclusion, the morphological and molecular analysis of five *Phytophthora* isolates infecting chrysanthemum from Karnataka State, India are identified as *Phytophthor*a *nicotianae.* More sampling may provide insights into its population structure pathogen and better information for resistance breeding programmes and designing management strategies to contain the disease.The collar rot and root rot disease of chrysanthemum caused by *Phytophthor*a *nicotianae*

is becoming major threat for the production of chrysanthemum in recent years in different parts of the world. Collar and root rot disease of chrysanthemum initially starts with yellowing, dwarfing, no flower bud initiation, partial or complete wilting of plants and finally death plants. Under humid environmental conditions, the disease develops around the stem and root region of chrysanthemum plant later spreading over the soil. *Phytophthora nicotianae* infects several horticultural crops causing huge loss to the growers. In the present study, field visit was undertaken in different chrysanthemum growing areas of Bengaluru rural of Karnataka State to collect more number of infected chrysanthemum samples. During surveys, it was observed that the Collar and root rot disease of chrysanthemum was prevelling in almost all surveyed areas. The infected plants are exhibiting collar and root rot and complete dead of the plants. The incidence collar and root rot disease of chrysanthemum is ranged from 19.5 - 32.2 percent per cent different surveyed areas of Bengaluru rural. The higher disease incidence might be due to growing of local chrysanthemum scent white and scent yellow variety continuously every year in the same land and location which is considered as susceptible to collar rot and root rot disease of chrysanthemum (RF).

Conclusion

Chrysanthemum is an important flower crop cultivated in different parts of the country attacked by different biotic stress leads to huge loss to the growers. In past few years there have been several reports of *Phytophthora* species infecting chrysanthemum from all over the world, but very limited information is available in India. Therefore In the present study of *Phytophthora nicotianae* was identified for cause of collar rot and root rot disease of chrysanthemum in India Further, survey and research work needs to be carry out to estimate disease severity, pathogen diversity and management practices to tackle the losses in chrysanthemum flower production.

Acknowledgement

The authors are grateful to the Director, Indian Institute of Horticultural Research, Bangalore for providing research facilities and his keen interest in this study.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Berbee, M. L. (2001). The phylogeny of plant and animal pathogens in the Ascomycota, *Physiological and Molecular Plant Pathology*, *59*(4), 165-187.

Bhattacharya, A. & Teixerra da Silva, J. A. (2006). Molecular systematic in *Chrysanthemum* x *grandiflorum* (Ramat.) Kitamura, *Scientia Horticulturae, 109***,** 379–384.

Blair, J. E., Coffey, M. D., Park, S.Y., Geiser, D. M. & Kang, S. (2008). A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences, *Fungal Genetic and Biology, 45*(3), 266-277. doi: 10.1016/j.fgb.2007.10.010.

Camacho, E. R. (2009). M. S. Thesis, Clemson University, Clemson, SC,Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. & Brasier, C. M. (2000). A molecular phylogeny of *Phytophthora* and related Oomycetes, *Fungal Genetic and Biology, 30,* 17–32.

Cook, R. T. A. (2001). First report in England of changes in the susceptibility of *Puccinia horiana*, the cause of chrysanthemum white rust, to triazole and strobilurin fungicides, *New Disease Reporter, 50,* 792.

Donahoo, R., Blomquist, C. L., Thomas, S. L., Moulton, J. K., Cooke, D. E. L. & Lamour, K. H. (2006). *Phytophthora foliorum* sp. nov., a new species causing leaf blight of *azalea*, *Mycological Research, 110,* 1309–1322.

Doyle, J. J. & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue, *Focus, 12***,** 13-15.

Frezzi, M. J. (1950). *Review Invest Agriculture, 4***,** 47.

Götz, M., Ulrich, R. & Werres, S. (2017). First detection of *Phytophthora chrysanthemi* on *Chrysanthemum indicum* in Germany. *New Disease Reporter, 35,* 6. http://www.ndrs.org.uk /article.php?id=035006.

Ivors, K. L., Hayden, K. J., Bonants, P. J. M., Rizzo, D. M. & Garbelotto, M. (2004). AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum, Mycological Research, 108*, 378–392.

Joshi, M., Verma, L. R. & Masu, M. M. (2010). Performance of different varieties of chrysanthemum in respect of growth, flowering and flower yield under north Gujarat condition. *The Asian Journal of Horticulture, 4*(2), 292-294.

Krasnow, C., Rechcigl, N., Olson, J., Schmitz, L. & Jeffers, S. N. (2021). First Report of Stem and Foliage Blight of Chrysanthemum Caused by *Phytophthora drechsleri* in the United States, *Plant Disease,* https://doi.org/10.1094/PDIS-03-21-0631-PDN

Krimitzas, A., Pyrri, I., Kouvelis, V. N., Gotsi, E. K. & Typas, M. A. (2013). Phylogenetic analysis of greek isolates of *Aspergillus* species based on morphology and nuclear and mitochondrial gene sequences, *BioMed Research International, 18* http://dx.doi.org/10.1155/2013 /260395

Kroon, L. P. N. M., Bakker, F. T., van den Bosch, G. B. M., Bonants, P. J. M. & Flier, W.G. (2004). Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences, *Fungal Genetic Biology, 41,* 766–782.

Kumar, S., Glen, S., Michael, L., Christina, K., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution, 35*(6): 1547–1549.

Lang, B. F., Gray, M. W. & Burger, G. (1999). Mitochondrial genome evolution and the origin of eukaryotes. *Annual Review of Genetics, 33:* 351-397.

Lin, S., Martin, D. E., Taylor, N. J., Gabriel, C. K. & Peduto, C. F. (2017). Occurrence of *Phytophthora chrysanthemi* causing root and stem rot on garden mums in the United States, *Plant disease, 101*(6).

Luong, T. M., Huynh, L. M. T., Hoang, H. M. T., Tesoriero, L. A., Burgers, L. W., Phan, H. T. & Davies, P. (2010). First report of *Pythium* root rot of Chrysanthemum in Vietnam and control with Metalaxyl drench. *Plant Disease Notes, 5,* 51- 54.

Maddison, W. & Maddison, D. R. (2019). Mesquite: a modular system for evolutionary analysis. Version 3.61. http://www.mesquiteproject.org

Martin, F. N. & Tooley, P. W. (2003a). Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of the mitochondrially-encoded cytochrome oxidase I and II genes, *Mycologia, 95,* 269–284.

Martin, F. N. & Tooley, P. W. (2003b). Phylogenetic relationships of *Phytophthora ramorum*, *P. nemorosa* and *P. pseudosyringae*, three species recovered from areas in California with sudden oak death, *Mycological Research, 107,* 1379–1391.

Molnar, C., Nikolaeva, E., Kim, S., Olson, T., Bily, D., Kim, J. & Kang, S. (2020). *Microorganisms, 60*(8), 1056. https://doi:10.3390/microorganisms8071056

Mullen, J. M., Hagan, A. K. & Carey, D. K. (2001). First Report of *Phytophthora* blossom blight of chrysanthemum caused by *Phytophthora nicotianae. Plant disease, 85*(8), 923. doi: 10.1094/PDIS.2001.85.8.923B. PMID: 30823076.

Naher, M., Motohash, K. & Watanabe, H. (2011). *Phytophthora chrysanthemi* sp. nov., a new species causing root rot of chrysanthemum in Japan, *Mycological Progress, 10,* 21–31. https://doi.org/10.1007/s11557-010-0670-9.

Nishi, N., Muta, T., Ito, Y., Nakamura, M. & Tsukiboshi, T. (2009). Ray speck of Chrysanthemum caused by *Stemphylium lycopersici* in Japan. *Journal of General Plant Pathology, 75:* 80 - 82.

Randall-Schadel, B. (2016). NPAG Report - *Phytophthora chrysanthemi* :Crown and root rot of chrysanthemum. *New Pest Advisory Group,* United States Department of Agriculture.

Santos, D. A. F., Edmn, L. & De Souza, J. T. (2005). *Phytophthora nicotianae :* agente etiológico da gomose da acácia negra no Brasil, *Fitopatologia Brasileira, 30,* 81–84.

Singh, P. K. & Kumar, V., (2014). Fusarium Wilt of Chrysanthemum – Problems and Prospects. *Plant Pathology & Quarantine, 4*(1), 34–44, Doi 10.5943/ppq/4/1

Sonavane, P. and Venkataravanappa, V. (2017). Status of *Phytophthora* and Huanglongbing Diseases of Citrus Causing Decline in Coorg Mandarin in India. *Journal of Agricultural Science and Technology B. 7*(4). https://doi.org/10.17265/2161-6264/2017.04.002

Tomic, Z. & Ivic, D. (2015). *Phytophthora chrysanthemi* Naher, Motohash, Watanabe, Chikuo, Senda, Suga, Brasier & Kageyama - new cause of chrysanthemum disease in Croatia. *Glasilo biljne zaštite, 15*(4), 291-300.

Waterhouse, G. M. (1963). Key to the species of *Phytophthora* de Bary. *Mycological Papers, 92.* Commonwealth Mycological Institute, Kew, UK.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., White T. J., eds. PCR Protocols: a guide to methods and applications. San Diego: Academic Press. 315–321.