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Diversity of fungi associated with Onion (*Allium cepa*) and Ginger (*Zingiber officinale*) produced from Karnataka, India

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ABSTRACT

A mycological survey was conducted in *Allium cepa* (Onion) and *Zingiber officinale* (Ginger) samples collected from different regions of Karnataka state. The analysis revealed the occurrence of diverse group of fungi including *Fusarium verticililoides, F. oxysporum, F. solani F. equiseti, A. flavus, A. niger, P. citrinum, Trichoderma, Rhizopus,* and *curvularia* in Ginger samples. Onion samples associated with *Aspergillus niger, A. flavus, P.citrinum, Rhizopus, Trichoderma, and Cladosporium.* Highest frequency of *Fusarium* (78.9%) in Ginger and *Aspergillus niger* (89.5%) in Onion was recorded. Further toxigenic fungi like *Fusarium* species and *Aspergillus flavus* were subjected to molecular characterization. *Fusarium* isolates were confirmed by PCR amplification using ITS1 and ITS4 primers. As the data represented toxigenic fungal species, DNA sequencing and phylogenetic tree was constructed to know authenticity of toxigenic fungi. The present data represents the occurrence of toxigenic fungi in Ginger and Onion which may cause serious health hazards upon consumption of the contaminated vegetables.

Key words: *Ginger, Onion, Mycotoxins, Fusarium, Aspergillus Niger, Polymerase Chain Reaction, Phylogentic tree.*

Introduction

Ginger (*Zingiber officinale* Rosc.) (Family: *Zingiber aceae*) is a herbaceous perennial, grown for its aromatic rhizomes, used both as a spice and a medicine (Ramteke and Kamble, 2011). India is the leading producer of ginger in the world and during 2016-17 the country produced 1087560 tonnes of the ginger from an area of 161670 hectares. Karnataka, Assam, Arunachal Pradesh, Meghalaya, Gujarath and Orissa together contribute 65 % to the total production in the country. (ICAR - Indian Institute of Spices Research). Ginger has been extensively used in Ayurvedic, Chinese, and Tibb-Unani herbal formulations, since ancient times for the treatment of diseases such as fever, sore throat, indigestion, vomiting, constipation, hypertension, arthritis, rheumatism, cramps, pains, sprains, helminthiasis, and infectious diseases (Ali *et al.,* 2008).

Onion *(Allium cepa*) is a widely consumed vegetable across the country throughout the year. India is the second largest producer of onion after China (FAOSTAT. 2018) and contributes for 24.8% global production. Karnataka, Maharashtra, Madhya Pradesh, Gujarat and Bihar states are the major producers of onion, during 2017-18 the country produced 23262000 Metric tonnes of the onion from an area of 1285000 hectares. Onions are an important dietary resource and have also been of interest for medical purposes. Onion is associated with many

pharmacological effects, onions and plants belonging to the genus *Allium* are being used as herbal remedies for a wide range of diseases (Orpin *et al*., 2017).

Zingiber ofiicinale and *Allium cepa* are exposed to a wide range of microbial contamination during pre and post-harvest. Such contamination can occur during processing, storage, distribution, sale and/or use. Spoilage causing microorganisms may be seed borne, the contamination may also take place during crop emergence in the field, during harvesting, postharvest handling, or even during the storage.

Fungi such as *Fusarium, Aspergillus* and *Penicillium* species are the filamentous fungi that can cause decrease in germination capability, weight loss, discoloration, mustiness, chemical, nutritional changes and mycotoxin contamination (Adebayo-Tayo *et al.,* 2012). Mycotoxins pose serious risks to public health. Mycotoxins can cause immune function suppression in humans and reduce immunity power against infections. In chronic circumstances, mycotoxins may cause the development of tumors in vital organs, or high morbidity and premature death among humans and animals (Calado *et al.,* 2014). Therefore early detection of such fungi is of paramount importance. PCR is the best tool for confirmation of mycotoxigenic fungi. Ribosomal DNA sequence based PCR protocols are widely used for the accurate identification of fungal species. Such sequences are considered as stable and occur in multiple copies, possessing characteristics appropriate for the identification of pathogens up to the species level (Maheshwar *et al.,* 2009). Hence the purpose of this study was to investigate the diversity of fungal species associated with naturally infected bulbs and rhizome of *Allium cepa* and *Zingiber ofiicinale* respectively.

Materials and Methods

Sample collection- A total of 56 samples (1kg/sample) of Onion and 57 samples (250 g/sample) of Ginger were collected from markets, grocery stores and agricultural fields from different places of Karnataka state in sterile polythene bags. Samples were brought to the laboratory, stored at room temperature and subjected to mycological evaluation within 5 days.

Sample preparation : Samples were selected by hand halving method based on International Seed Testing, Association (ISTA).

Ginger : 50g of Ginger rhizome was selected from the primary sample, washed with sterile water, air dried and cut into small bits of 1cm with sterile knife and surface sterilized by dipping in 1% Sodium hypochlorite solution for 2 min followed by rinsing with sterile water twice.

Onion : 03 Onion bulbs were picked from 1kg of onion sample, outer two layers were discarded and inner part of the bulb was cut into small bits of 1cm with sterile knife and surface sterilized by dipping in 2% sodium hypochlorite solution for 1 min followed by rinsing with sterile water.

Mycological analysis : Mycological analysis using 0.1 g of surface sterilized sample was carried out to determine percent infection, frequency and relative density of fungi associated with Ginger and Onion samples by agar plate and blotter method (Z. I. El-Gali, 2014).

Agar plate method- Onion and Ginger samples were placed on to potato dextrose agar medium supplemented with Chlorampenicol (0.050g/l) at the rate of 8 bits per plate and incubated at 25±2°C for 5-7 days.

Blotter method : Onion and Ginger Samples were plated onto blotter paper moistened with sterilized water at the rate of 15-20 bits per petriplate and incubated at 25±2°C for 7 days.

Identification of fungi: Fungal colonies developed on the samples were observed under stereo binocular microscope for colony morphology. Colonies were pure cultured onto PDA medium and incubated 25±2°C for 7 days. Isolated pure cultures were identified based on micro morphological characteristics using identification keys and manuals (Barnett and Hunter 1972; Booth 1977; Leslie and Summerell, 2006). The following formula was used to record Percent infection, Relative density and Frequency of isolated fungi described by Ghiasian *et al.,* (2004).

$$\text{Incidence}(\%) = \frac{\text{Number of samples infected by genus/ species}}{\text{Total number of samples plated}}$$

$$\text{Fr}\ (\%) = \frac{\text{Number of samples in which a genus/species occurred}}{\text{Total number of samples analyzed}}$$

$$\text{RD}\ (\%) = \frac{\text{Number of isolates of a genus/species}}{\text{Total number of fungi/genus isolates}}$$

a. Molecular characterization

DNA isolation- All the fungal colonies were pure cultured and were subjected to molecular characterization. Fungal isolates were inoculated to 1.5 ml

sterile Potato dextrose broth in 2 ml eppendorf tubes and incubated at 25±2°C for 4 days. The resulting mycelial mat was centrifuged at 1000rpm for 8 minutes (REMI C24 Cooling Centrifuge). The supernatant was discarded and the pellet was treated with 500 µL of lysis buffer (2M Tris Hcl, 0.1M EDTA, 20%SDS, 5% PVP, 10M Licl, pH 8.0). The mixture was crushed with blunt ends of micropipette tips and incubated in water bath at 65°C for 20 min, with brief vortexing at every 5 min interval. After incubation, 500 µL of freshly prepared Phenol : Chloroform(1:1) was added to each mixture and vortexed for 1min. The resulting solution was centrifuged at 3000rpm for 5min. Supernatant was transferred to fresh microfuge tube, equal volume of ice cold propanol was added and incubated at -20°C for overnight to allow DNA precipitation. After incubation, samples were centrifuged at 12000rpm for 12 min. The resultant pellet was rinsed with 70% alcohol twice, air dried, dissolved in 50 µL of nucleic acid free water and can be used for PCR directly or can be stored at -20°C until further analysis (Maheshwar *et al.*, 2009).

b. Primers for Polymerase chain reaction-Genus specific primers: ITS 1 and 4: The isolated fungal DNA was amplified using ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers as described by I.M. Singha *et al.*, (2016), the expected amplicon size was 600 bp.

c. Polymerase chain reaction- The DNA amplification was carried out in the Thermocycler (Sure cycler 8000 Agilent technologies) using PCR tubes with 25 µL of reaction mixture consisting of 1µL DNA, 0.5 µL of each primers (20picomoles),1 µL of dNTP's, 0.125 µL of Taq DNA polymerase, 1.25 µL of Taq buffer, 1.25 µL of $MgCl_2$ and 19.375 µL nucleic acid free water.

The amplification cycle involved initial denaturation at 94 ºC for 4 min followed by 30 cycles of denaturation at 94 ºC for 1 min, primer annealing at 57.8ºC for 1 min, primer extension at 72 ºC for 1.5 min. and final extension at 72 ºC for 5 min. The PCR products (8 µL+ 3µL loading dye) were visualized by 1.5% agarose gel in 1X TAE buffer, stained with ethidium bromide. The size of the amplicon was determined with 100bp DNA ladder and the gel was documented using gel documentation system.

d. Sequencing and phylogenetic analysis of isolated species-The PCR amplicons of all isolates were sequenced and the resultant sequences were compared with previously published NCBI BLAST database for the identification of the isolates. The sequence reads were deposited at NCBI GenBank and obtained accession numbers. Phylogenetic tree was built in Mega X (10.1.6) online software using Neighbor Joining method. The evolutionary distances were determined by Maximum Composite Likelihood method.

Results

Mycological examination of Ginger and Onion samples resulted in the occurrence of diverse mycoflora including *Fusarium verticillioides, F. oxysporum, F. solani, F. equiseti, A. niger, Aspergillus flavus, Penicillium citrinum, Trichoderma, Rhizopus, Curvularia* and *Cladosporium species.*

Percent incidence of fungal isolates : Highest incidence of *Aspergillus niger* (78.6%) was observed in Onion samples followed by *Penicillium citrinum* (5.2%) and *Aspergillus flavus* (4.6%). Least incidence was recorded for *Cladosporium* species (4.3%), *Rhizopus* (3.0%) and, *Trichoderma* (1.9%). (Table 1 and fig 1). *Fusarium* species were recorded for highest incidence (44.9%) followed by *Aspergillus niger* (9.1%). Other fungal species including *Penicillium citrinum* (5.1%), *Aspergillus flavus* (4.3%), *Trichoderma* (2.1%), *Rhizopus* (1.9%) *and Curvularia* (1.1%) were recorded for lowest incidence in Ginger samples (Table 2 and Fig. 1).

Table 1. Fungal species isolated from Onion

Sl. No.	Fungal isolate	Percent incidence (%)	Frequency (%)
1	*Aspergillus niger*	78.6	89.5
2	*Aspergillus flavus*	4.6	28.1
3	*Penicillium citrinum*	5.2	56.1
4	*Trichoderma* spp.	1.9	38.6
5	*Rhizopus* spp.	3.0	35.1
8	*Cladosporium* spp.	4.3	33.3

Fungal percent frequency - The data shows that *Aspergillus niger* recorded the highest frequency (89.5%) in Onion samples followed by *P. citrinum* (56.1%), *Trichoderma species* (38.6%), *Rhiozopus species* (35.1%) and *Cladosporium species* (33.3%)(table:1and fig:2). Highest frequency of *Fusarium* species (78.9%) were occurred in Ginger samples followed by *A.niger* (46.2%), *Trichoderma* (23.1%), *P.citrinum* (21.1%) and *A.flavus* (15.3%). *Rhizopus* and

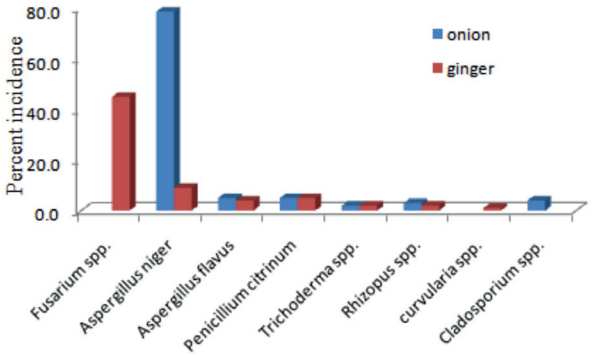

Fig. 1. Percent incidence of fungal species isolated from Ginger and Onion

Curvularia species showed the lowest frequency with 13.5% and 8.2% respectively (Table 2 and Fig. 2).

In the current study, it was observed that in each and every onion sample *Aspergillus niger* was more predominant and was not allowing other genera of fungi to grow in PDA plates, even after increasing the sodium hypochlorite concentration to 3% for surface sterilization. Hence blotter method was found useful for the isolation of diverse fungi from Onion samples.

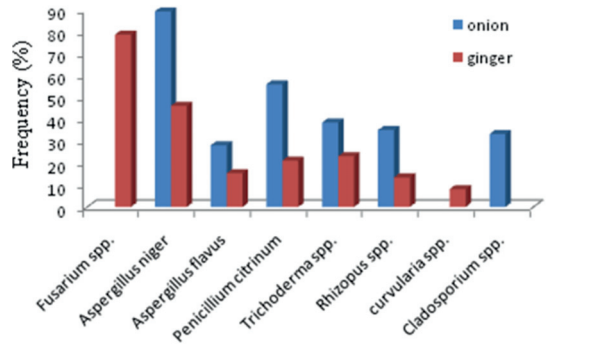

Fig. 2. Frequency percentage of fungal species isolated from Ginger and Onion

Relative density percentage among Fusarium isolates - Among the Fusarium isolates relative density was calculated and represented in Table 3. *Fusarium oxysporum* recorded the highest relative density (56.4%) followed by *F. verticillioides* (26.3%), *F.solani* (9.9%), *F. equiseti* (9.4%).

PCR analysis- Quantification of all the representatives of different *Fusarium* isolates and *Aspergillus flavus* was done by PCR analysis using ITS1 and ITS 4 primers. All the fungi subjected to PCR were positive for the ITS regions. All the *Fusarium and A.flavus* species recorded the expected 550-600bp amplicon size (Fig. 3).

Phylogenetic tree-Gene sequence data of selected fungal isolates show that all of the isolates having>99 to 100%similarity of previously deposited within the GenBank. The sequence reads were deposited at NCBI GenBank and obtained accession numbers (Table 4). The phylogenetic tree analysis of identified fungal species was constructed to determine their affiliations (Fig. 4-6). The interpretation of the phylogenetic tree of *Fusarium* species, *Aspergillus flavus* and *Penicillium citrinum* exhibited well resolved two major clades with boot strap values significantly >80 % for *Fusarium*, *A.flavus* and >60 % for *P.citrinum* respectively which are considered as positive values for determining the genetic resemblance within the species. The minor clades

Table 2. Fungal species isolated from Ginger

Sl No.	Fungal isolate name	Percent incidence (%)	Frequency(%)
1	*Fusarium* spp.	44.9	78.9
2	*Aspergillus niger*	9.1	46.2
3	*Aspergillus flavus*	4.3	15.3
4	*Penicillium citrinum*	5.1	21.1
5	*Trichoderma* spp.	2.1	23.1
6	*Rhizopus* spp.	1.9	13.5
7	*Curvularia* spp.	1.1	8.2

Table 3. Relative density among *Fusarium* isolates

Sl No.	Fungal isolate name	Total number of isolates	Relative density (%)
1	*F.oxysporum*	234	56.4
2	*F.verticilloides*	109	26.3
3	*F.solani*	41	9.9
4	*F.equiseti*	39	9.4

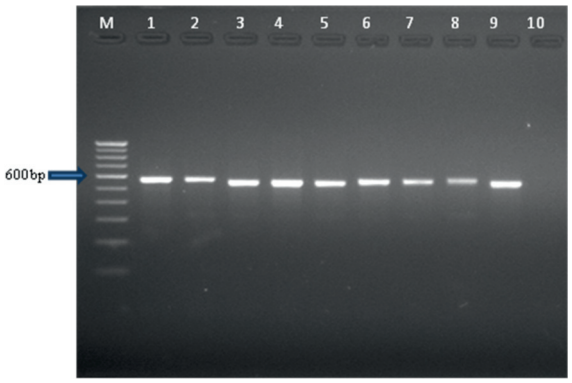

Fig. 3. PCR products amplified from *Fusarium* species using ITS 1 and 4 primers. M-DNA Marker 100Kb to 100bp, 1 to 9 - *Fusarium* isolates, 10- *Aspergillus niger* (negative control).

Table 4. Gen bank Accession numbers of the fungal isolates

Sl. No.	Fungal species	Isolate number	NCBI Accession number
1	*F.verticilloides*	3A	MN818601
2	*F.verticilloides*	21A	MN796069
3	*F.oxysporum*	11A	MN784505
4	*F.oxysporum*	13A	MN796045
5	*F.solani*	10A	MN784486
6	*F.solani*	29A	MN796074
7	*F.equiseti*	25A	MN796071
8	*F.equiseti*	18A	MN796063
9	*A.flavus*	8S	MN658670
10	*P.citrinum*	17S	MN658672

show heterogeneity. The optimal phylogenetic tree with the sum of length of branches was 11.395, 16.71 and 26.07 for *A.flavus* , *Penicillium* and *Fusarium* species respectively.

Discussion

In this study we collected Ginger and Onion samples from various districts of Karnataka state, India and subjected to mycological examination. Percent infection, frequency and relative percentage of the associated fungal isolates were studied to know their epidemiological significance on Ginger rhizomes and Onion bulbs. The isolates were confirmed using PCR, which revealed contamination of Ginger rhizomes and Onion bulbs with toxigenic fungi. Identification of the individual species of *Fusarium* was done on the basis of differences in the sequence of ITS region. Identification of fungal species by using phenotypical characteristics is often

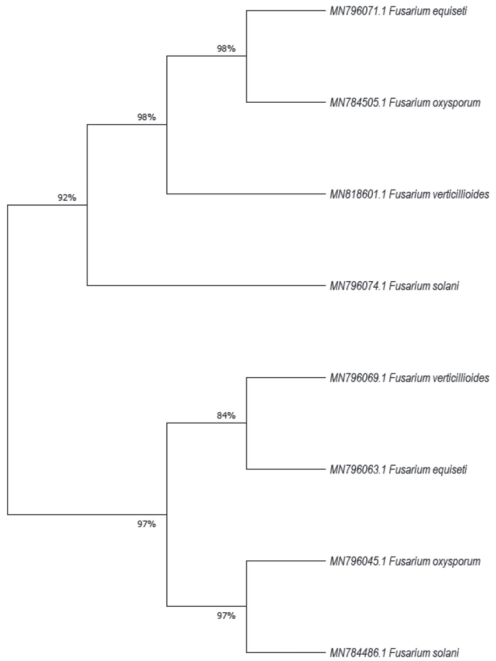

Fig. 4. Phylogenetic tree constructed for *Fusarium* species

confusing, this is particularly complicated in case of genus *Fusarium* since various and often contradictory taxonomic approaches exist. Hence in the present investigation PCR analysis with DNA sequencing was used to identify *Fusarium* species associated with test samples.

Most dominate fungal genera isolated from Ginger was *Fusarium* species and this complies with the earlier findings wherein *F. oxysporum* has been iso-

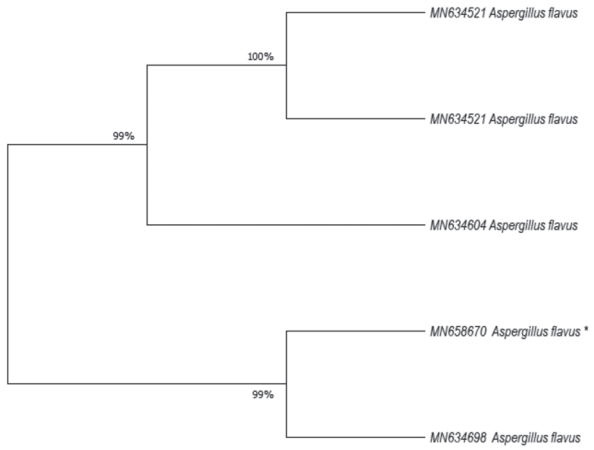

Fig. 5. Phylogenetic tree constructed for *Aspergillus flavus* (MN658670) isolated in the present study is indicated by * symbol.

lated from rhizome rot of *Zingiber ofiicinale* and characterized by ITS 1and ITS4 primers (Li *et al.*, 2014). *Fusarium, penicillium*, *Aspergillus, Rhizopus, Eurotium, Mucor, Curvularia and Nigrospora* were reported to cause soft rot disease in harvested, drying and stored Ginger with percentage incidence of *Fusarium* (34.7%) (Berza *et al*., 2012). Thirty endophytic fungi including *Fusarium solani, F. oxysporum*, *Curvularia affinis* and *Acremonium macroclavatum* were identified and characterized by ITS rDNA sequence in rhizome of medicinal plant red ginger from Indonesia. Highest occurrence of *F.oxysporum* (74%) and *Fusarium oxysporum* (51%) was reported by Moreira (2013); Sharma (2017) and their colleagues respectively in rhizome rot of Ginger. Park *et al.*, (2018) isolated and identified ten fungal isolates by ITS sequencing as *Hypocrea virescentiflava, Fusarium oxysporum, Acremonium strictum, Fusarium solani,* and *Cadophora fastigiata* from 120 days stored non-spoiled and spoiled Ginger samples. Pathogenicity test revealed the casual agent for storage rot was *F. Oxysporum*. The causative agent for Ginger soft rot in Mizoram state was isolated and identified as *F. oxysporum*, *F. solani* and *Plectosphaerella cucumerina*. Identification up to species level was achieved by ITS rDNA sequence analysis with amplicon size ranged from 478bp - 513bp (Rosangkima *et al.*, 2018). *P. brevicompactum* was identified in 85% of the infected ginger rhizome samples which produced a potent immunosuppressant mycophenolic acid in tissue extracts (Overy and Frisvad, 2005).

The result from mycological analysis of Onion indicates the high intensity of fungal infection associated with Onion. This is in agreement with Varga and colleagues (2008) who pointed out that *A.niger, A.flavus* and *Penicillium* species are the frequently encountered fungal pathogens from Onion and the major causal organism of black mould disease of Onion in storage was *Aspergillus niger*. *Aspergillus niger, Fusarium oxysporum, Fusarium moniliforme, Aspergillus flavus and Penicillium spp.,* was isolated from infected Onion samples (Ara *et al.*, 2008). *Aspergillus niger* was reported as the most predominant fungi among twelve fungal species isolated from Onion seeds (Dumbrel *et al.*, 2011). *Aspergillus niger*, *A. fumigatus, A. flavus, Fusarium oxysporum*, *Rhizopus stolonifer, Alternaria porri,* and *Penicillium citrinum were isolated from spoiled onion bulbs in which highest frequency (30%) of A. niger was observed followed by A. flavus (*18%) and least frequency was found to be *P. citrinum* (5%) (Shehu and Muhammad, 2011). Wani and Taskeen-Un-Nisa (2011) reported that hexaconozole and mancozeb fungicides can control *Aspergillus niger* associated with *Allium cepa* causing black mold rot. Kumar and colleagues (2015) reported that *Aspergillus niger* is the most virulent fungal pathogen in the field and

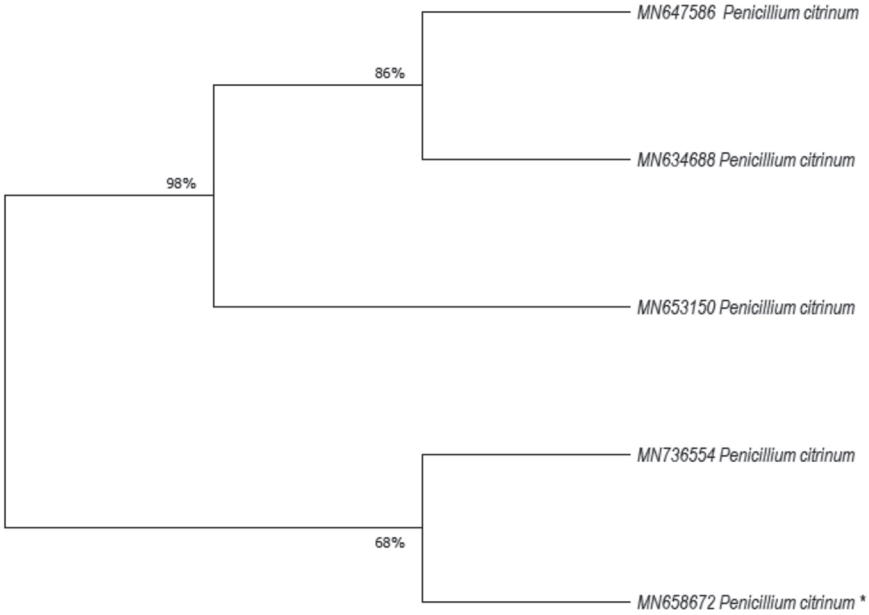

Fig. 6. Phylogenetic tree constructed for *Penicillium citrinum* (MN658672) isolated in the present study is indicated by * symbol.

during the post-harvest storage.

Conclusions: Zingiber ofiicinale and *Allium cepa* have been neglected in terms of mycological investigation reports on diversity of fungi associated with Onion and Ginger are very limited. The data on frequency, percent incidence and relative density represents the extent of fungal contamination and damage with respect to nutritional and biochemical quality. Occurrence of toxigenic fungi in significant numbers as reported in the present study can result in loss of weight, reduction of germination capability, discoloration and mustiness. It also reflects the possible risk to human health, as these fungi are the sources of highly potent mycotoxins which are carcinogenic. The problem is augmented by the fact that *Zingiber ofiicinale* and *Allium cepa* are not only consumed raw but also used in ayurvedic and traditional medicines, which affects consumers adversely. Hence appropriate control measures in countering spoilage and good storage practices must be followed to prevent health issues among consumers.

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1. MN818601*Fusarium verticillioides* 3A
CAATTGTTGCCTCGACGGATCA GCCCG CTCC
CGGTAAAACGGAA CGGC CCGCCA GAGGAC
CCCTAAACT CTGTTTCTATATGTAACTTCT
GAGTAAAACCATAAATAAATCAAAAC
TTTCAACAACGGATC TCTTG GTTCTGGCAT
CGATGAAGAGCGCAGCAAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGTGAA
TCATCGAATCTTTGAACGCACATTGCGCC
CGCCAGTATTCTGGCGGGCATGCCTGTTC
GAGCGTCATTTCAACCCTCAAGCCCCCGGG
TTTGGTGTTGGGGATCGGCGAGCCTCA
CGGCAAGCCGGCCCCGAAATACAGTGG
CGGTCTCGCTGCAGCTTCCATTGCGTAGT
AGTAAAACCCTCGCAACTGGTACGCGG CGCG
GCCAA GCCGTT AA AC CCCCAAC TTCTGAA

2. MN796074 *Fusarium solani* 29A
TACCTATAACGT TGCCTCGGCGG GAACAGA
CGGCCCCGTAACACGGGCCGCCCCCGCCA
GAGGACCCCCTAACTCTGTTTCTATAATG
TTTCTTCTGAGTAAACAAGCAAATAAA
TTAAAACTTTCAACAACGGATCTCTTGGC
TCTGGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCGCCAGTATTCTGGCGGGCA
TGCCTGTTCGAGCGTCATTACAACCCT
CAGGCCCCCG GGCCTGGCGTTGGGGATC
GGCGGAAGCCC CCTGCGG GCACAACGC
CGTCCCCCAAATACAGTGGCGGTCCCGCC
GCAGCTTCCATTGCGTAGTAGCTAACACC
TCGCAACTGGAGAGCGGCGCGGCCACG
CCGCAAAACACCCAACTTCTGAATG

3. MN796071 *Fusariumequiseti*25A
ACCCCTGTGACATACCTATAC GTTGCCTCGG
CGGATCAGCC CGCGCCCT GTAAAACGG
GACGGCCCGCCCGAGGACCCTA AACTC
TGTTTTTAGTGGAA CTTCTGAGTAAAACAA
ACAAATAAATCAAAACTTTCAACAA
CGGATCTCTTGGT TCTGG
CATCGATGAAGAACGC AGCAAAATGCGAT
AAGTAATGTG AATTGCAGAATTCAGTGA
ATCATCGA ATCTTTGAACGCACATT
GCGCCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTC
ATTTCAACCCTCAAGCTCAGCTTG
GTGTTGGGACTCGCGGTAACCCGCGTTCCC
CAAATCGATTGGCGGTCACGTCGAG
CTTCCATA GCGTAGTAATCATACACCTCGTTA
CTGGTAATCGTCGCGGCCACGC
CGTTAAACCCCAACTTCTGAATGTTGACCTCG
GATCAGGTAGGAATACCC
GCTGAACTTAAGCATATCAATAAGCG

4. MN796069 *Fusarium verticillioides*21A
ATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCG
GTAA AACGGG ACGGCCCGCC AGAGGAC
CCCTAAACTCTGTTTCTATATGTAACTTC
TGAGTAAAACCATAAATAAATCAAAAC
TTTCAACA ACGGATCTCTTGGTTCTGGCAT
CGATGAAGAACGCAGCAAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGT GAATC
ATCGA ATCTTTGAA CGCACATTGC GCCCG
CCAGTATTCTGGCGGGCATGCCTGTTCGA
GCGTCATTTCAAC CCTCAAGCCCTCG GGTTT
GGTGTTGGGGATCGGCGAGCCCTTGCGGCA
AG CCGGCCCCGAAATCTAGTGGCGGACTCG
CTGCAGCCTCCATTGCGTAGTAGGAAGACC
CTCTTTTTTTTTTTGCGGCGCGGCC

5. MN796063 *Fusarium equiseti* 18A
CATACCTATACGTTGCCTCGGCGGATCA
GCCCGCGCCCTGTAAAACGGGACGGCCC
GCCCGAGGACCCTAAACTCTGTTTTTAGTGG
AACTTCTGAGTAAAACAAACAAATA
AATCAAAACTTTCAACAACGGATCTCTT
GGTT CTGGCATCGATGAAGAA CGCAG CAAA
ATGCGATAAGTAATGTGAATTGCAGA ATTCA
GTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGTATTCTGGCGGG
CATGCCTGTTCGAGCGTCA TTTC AACC CTCA
AGCTCAGCTTGGTGTTGGGACTCGCGGTAA
CCCGCGTTCCCCAAATCGATTGGCGGT
CACGTCGAGCTTCCATAGCGTAGTAATCA
TACACCTCGTTACTGGTAATCGTCGCGGCCA
CGCCGTAAAACCCCAACTTCTGAATG
TTGACCTCGGATCAGGTAGGAATACCCGC
TGAACTTAAGCATATCAATAAGCG

6. MN796045 *Fusariumo xysporum* 13A

ACCACTTGTTGCCTCGGCGGATCAGCCCGCT
CCCGGTAAAACGGGACGGCCCGCCAGA
GGACCCCTAAACTCTGTTTCTATATGTAACTT
CTGAGTAAAACCATAAATAAATCAA AACTT
TCAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAG AACGCAGCAAAA TGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCAT

CGAATCTTTGAACGCACATTGCGCCC
GCCAGTATTCTGGCGGGCATGCCTGTTCG
AGCGTCATTTCAACCCTCAAGCACAGC
TTGGTGTTGGGACTCGC GTTAAT TCGCGTT
CCTCAAATTGATTGGCGGTCACGTCGAG
CTTCCATAGCGTAGTAGTAAAACCCTCGTT
ACTGGTAATCGTCGCGGCCACGCCGTTAAAC
CCCAACTTCTGAATGTTGACCTCGGATCA
GGTAGGAATACCCGCTGAACTTAAGCATA
TCAATAA

7. MN784505 *Fusariumo xysporum* 11A
TACCACTTGTTGCCTCGGCGGATCAGCCCGCT
CCCGGTAAAACGGGACGGCCCGCCAGAG
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CAACAACGGATCTCTTGGTTCTGGCATCGA
TGAAGAACGCAGCAAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCGCCAG TATT
CTGGCGGGCATGCCTGTTCGAGCGTCATT
TCAACCCTCAAGCACAGCTTGGTGTTG GG
ACTCGCGTTAATTCGCGTTCCTCAAATTGATT
GGCGGTCACGTCGAGCTTCCATAGCG
TAGTAGTAAAACCCTCGTTACTGGTAATCG
TCGCGGCCACGCCGTTAAACCCCAACT
TCTGAATGTTGACCTCGGATCAGGTAGGAAT
ACCCGCTGAACTTAAGCATATCAATAAGCG
GAGGA

8. MN784486 *Fusarium solani* 10A
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CTTGGCTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACG
CACAT TG CGCCCGCC AGTATTCTGGCGGGCA
TGCCTGTTCG AGCGTCATT ACAACC CTCAGG
CCCCCGGGCCTGGCGTTGGGGATCGGCGG
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AAATACAGTGGCGGTCCCGCCGCAGCTTCC
ATTGCGTAGTAGCTAACACCTC GCAACTG
GAGAGCGGCGCGGCCACGCCGTAAAACA
CCCAACTTCTGAATGTTGACCTCGAATCAG
GTAG GAATACCCGCTGAACTTA AGCATATCA
9. MN658672 *Penicillium citrinum* SLGIN17S
CCCGTGTTGCCCGAACCTATGTTGCCTCGGCGG
GC CCCGCGCCCGCCGACGGCCCCCCTGAA
CGCTGTCTGAAGTTGCAGTCTGAGACC
TATAACGAAATTAGTTAAAACTTTCAACAAC
GGATCTCTTGGTTCCGGCATCGATGAAGAA
CGCAGCGAAATGCGATAACTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAGTC
TTTGAAC GCACATTGCGCCCTCTGGTATTC
CGGAGGGCATGCCTGTCCGAGCGTCATTG
CTGCCCTCAAGCCCGGCTTGTGTGTTGG
GCCCCGTCCCCCCCGCCGGGGGGACGGGC
CCGAAAGGCAGCGGCGGCACCGCGTCCGG

10. MN658670 *Aspergillus flavus* SLGIN8S
CCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCG
GCGGGCCCGCCATTCATGGCCGCCGGGG
GCTCTCAGCCCCGGGCCCGCGCCCGCCGG
AGACACCACGAACTCTGTCTGATCTAGTGA
AGTCTGAGTTGATTGTATCGCAATCAGTTA
AAACTTTCAACAATGGATCTCTTGGTTCC
GGCATCG ATGAAGAACGCAGCGAA ATGCGA
TAACTAGTGTGAATTGCAGAATTCCGTGAAT
CATCGAGTCTTTGAACGCACATTGCGCC
CCCTGGTATTCCGGGGGG CATGCCTG TCCGA
GCGTCATTGCTGCCCATCAAGCACGGCTTGT
GTGTTGGGTCGTCGTCCCCTCTCCGGGGG
GGACGGGCCCCAAAGGCAGCGGCGGCAC
CGCGTCCGATCCTCGAGCGTATGGGGCTTT
GTCACCCGCTCTGTAGGCCCGGCCGGCGC
TTGCCGAACGCAAATCAATCTTTTTCCAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTG AA
CTTAAGCATATCAATAA