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Seed mycoflora associated with different varieties of chilli

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ABSTRACT: Isolation and identification of seed mycoflora associated with seeds of four varieties of chilli *viz.*, GVC-101, GVC-111, GVC-121 and AVNP-C-131 were conducted by standard blotter method and agar plate method. The fungi isolated were *Aspergillus niger*, *A. flavus*, *Colletotrichum* sp., *Fusarium* sp., *Alternaria* sp., *Penicillium* sp., *Curvularia* sp., *M. phaseolina*, unidentified sterile fungi with septate mycelium and unidentified sterile fungi with aseptate mycelium. The fungi isolated by standard blotter method were *A. niger* (1.50-2.00%), *A. flavus* (1.50-3.00%), *Colletotrichum* sp. (1.25-2.75%), *Fusarium* sp. (0.75-2.50%) and *Alternaria* sp. (0.75%), whereas fungi isolated by agar plate method were *A. niger* (7.50-14.00%), *A. flavus* (2.25-4.75%), *Colletotrichum* sp. (10.50-11.50%), *Fusarium* sp. (8.50-12.00%), *Alternaria* sp. (2.00-2.50%), *Penicillium* sp. (0.75-2.25%), *Curvularia* sp. (1.00 - 1.75%), *M. phaseolina* (1.00-1.50%), unidentified sterile fungi with septate mycelium (0.75%) and unidentified sterile fungi with aseptate mycelium (0.75-1.00%). The common and dominant fungi recorded were *A. niger* (0.00-14.00%), *Colletotrichum* sp. (1.25-11.50%) and *Fusarium* sp. (0.00-12.00%). Genotype GVC 101 and GVC 111 were found as most susceptible as maximum number of mycoflora were detected from these varieties. Agar plate method was found more effective as total ten fungal species were isolated by this method as compared to only five fungal species in standard blotter method.

Keywords: *Aspergillus niger*, chilli, *Colletotrichum* sp., *Fusarium* sp., seed mycoflora

INTRODUCTION

Chilli (*Capsicum annuum* L.), of family Solanaceae is an important vegetable cum spice crop having diverse commercial and therapeutic value. It is grown in almost all parts of tropical and subtropical regions of world and is propagated by seed. Seed is the basic and the primary material for commercial propagation hence, seed health plays an important role for successful cultivation and higher yield of crop species. High yielding variety seed has high genetic and physical purity and high germination percentage (Chamling, 2011). Seeds also can be a source of transmission of plant pathogens over long distances. The pathogen may be externally or internally seed borne or associated with the seed as contaminant. Development and spread of plant diseases at domestic and international level as a result of usage of seeds infected with pathogens were known from long back.

The seed mycoflora reduce the quality, quantity and longevity of seeds. The seed borne fungi not only affect the nutritive value of the produce but also adversely affect the market value. Several seed borne fungi are known to be associated with chilli seed and are responsible for seed abortion, seed rot, seed necrosis, reduction in seed germination, seedling mortality (post and pre emergence) and development of disease at later stages of plant growth like discolouration of fruit and fruit rot. Seed borne mycoflora reported in chilli includes, *Aspergillus niger* Tiegh., *A. flavus* Link*, Fusarium oxysporum, P. aphanidermatum* (*Edson)* Fitz*., Colletotrichum* sp., *F. moniliforme* J. Sheld., *Alternaria alternata* (Fr.) Keissl., *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Drecheslera* sp., *M. phaseolina* (Tassi) Goid*., Mucor* sp. and *Rhizopus* sp. (Kumari, 2011 and Anonymous, 2017). Sporulating structures emerging out from the dead chilli seedlings serves as the potential source of inoculum for further spread of pathogen in field. Hence, it is of vital importance that seeds must be treated before they are sown in the field. The main aim of the present study is to enumerate and identify the mycoflora associated with chilli seeds of varieties GVC-101, GVC-111, GVC-121 and AVNP-C-131.

MATERIALS AND METHODS

Chilli varieties *viz.,* GVC-101, GVC-111, GVC-121 and AVNP-C-131 were selected for experimental purpose and were collected from Regional Horticultural Research Station (RHRS), Navsari Agricultural University, Navsari during 2018. Isolation of seed mycoflora was done by two different incubation methods i.e. standard blotter method

Table 1: Frequency of seed mycoflora associated with chilli varieties by standard blotter method

Fungus	Frequency in different varieties (%)			
	GVC 101	GVC 111	GVC 121	AVNP-C-131
Aspergillus niger	1.50	2.00	1.50	0.00
A. flavus	3.00	1.50	2.75	1.75
Colletotrichum sp.	2.75	2.25	1.75	1.25
Fusarium sp.	2.50	1.75	0.00	0.75
Alternaria sp.	0.75	0.00	0.00	0.00

and agar plate method prescribed by International Seed Testing Association (ISTA, 1999).

Standard blotter method

Random samples of 400 seeds were taken from each of four different varieties of chilli for isolation of seed mycoflora. A set of three sterilized blotter discs was dipped in a beaker containing sterilized distilled water with the help of a forceps. The excess water was allowed to drip off from the blotters. Then the blotters were placed at the bottom of each Petri plate. 25 seeds from each of the variety were taken and then transferred aseptically in Petri plates containing 3 layers of (9cm diameter) moistened blotter paper. The seeds were placed at equal distance in Petri plates by keeping 15 seeds in the outer ring first, 9 seeds in middle ring and 1 in the centre. The labelled plates were incubated for 7 days at 25 ± 2^0C under 12h alternating cycles of light and darkness. Care was taken while handling the dishes in the tray and transferring them to the incubation room so that the plated seeds were not displaced from their original position. After seven days of incubation period, fungal growth on each of seed developed was observed.

Agar plate method

Twenty five seeds from sample of each of the variety were randomly taken and transferred aseptically in Petri plates containing 20 ml solidified sterilized Potato Dextrose Agar (PDA) medium under laminar air flow cabinet. The seeds were placed at equal distance in Petri plate by keeping 15 seeds in the outer ring first, 9 seeds in middle ring and 1 in the centre. The plates were incubated usually for 7 days at 25 ± 2^0C under 12h alternating cycles of light and darkness. After 7 days of incubation period, fungal growth on each of seed developed was observed.

The developing colonies of a particular seed borne fungi exhibiting slightest variation in their characters were critically examined after incubation. The fungal growth of different fungi obtained on seeds in both methods were purified and maintained on PDA slants for further studies. The pure culture of seed borne fungi were preliminary identified on the basis of cultural and morphological characters through naked eye and microscopic examination. Cultural characteristics were usually observed by colony characteristics of each fungus, sporulation characters like asexual or sexual spore or fruiting structure. Detailed morphological examination of fungal isolates was done under compound microscope and their identification was confirmed by comparing with relevant literature (Campbell *et. al*, 1996).

RESULTS AND DISCUSSION

Cultural and morphological characteristics of the isolated mycoflora

After purification, each of the isolate was identified on the basis of its cultural and morphological characteristics.

***Colletotrichum* sp.:** The colonies in PDA appeared as flat to raised fluffy with white colour growth. Conidiophores are 3-45 × 2-6µm, hyaline, cylindrical, unicellular or septate. Conidia are 7-14 × 2.5-3.5µm and one-celled.

***Fusarium* sp.:** *Fusarium* sp. grew rapidly on PDA and produced white woolly to cottony, flat, spreading colonies. Macroconidia (4-9 x 13-65µm) was produced from phialides on unbranched or branched conidiophores. They are two or more celled and sickle shaped with pointed distal ends. Microconidia (2-4 x 4-8µm) are formed on long or short simple conidiophores. They were one celled, hyaline, ovoid to cylindrical.

***Aspergillus niger* :** The fungus initially produced thick white wooly growth which turned black due to accumulation of spore. The culture appeared white to light yellow from the reverse. Conidiophores were unbranched with foot cell basally. The conidia appeared as long chain of spherical beads. Chain contained a large number of black coloured spherical conidia that are easily detachable from one another.

***Aspergillus flavus* :**Colony on PDA was yellow-green to olive green with creamy edge. Conidiophores were apically swollen with numerous conidia bearing cells in

long chains. Conidiophores were less than 1 mm length and 12- 22µm diameter. Conidia typically spherical measured about 2.5-3.5µm.

***Alternaria* sp.:** The fungus produced profuse mycelial growth on PDA with initial hyaline mycelium that turned to greyish brown colour in its later growth. The conidiophores were septate, light brown to olivaceous brown and slightly swollen at apex. They bear muriform shaped conida having tapered apex with 1 to 4 transverse and 1 to 2 longitudinal septa. The conidial size was found to be 11.30 to 52.65µm x 4.38 to 14.40µm.

***M. phaseolina* :** Initially the fungus produced dirty white mycelial growth which later changed to brown black in centre due to the formation of numerous small sclerotia, which are black in colour. The mycelium was hyaline to brown, branched somewhat at right angles, septate and 1.63 to 6.62µm in width. The sclerotia formed in culture were black, hard and 62.60 to 117.22µm in diameter.

***Penicillium* sp.:** The fungus growth in PDA was rapid with velvety blue green colour and creamy white to white border. Reverse side appeared pale yellowish brown. The conidiophores were brush like spore bearing structures that were simple or branched and terminated by clusters of flask shaped phialides. Conidia were pale green, unicellular, round to ovoid in shape and found in chains.

***Curvularia* sp.:** C*urvularia* produced rapidly growing, woolly colonies on potato dextrose agar. From the front, the colour of the colony was white to pinkish gray initially and turned to olive brown or black as the colony matures. From the reverse, it was dark brown to black. It produced septate brown hyphae and conidiophores were simple or branched. The conidia (8-14 x 21-35µm) were brown and multiseptate. The septa were transverse and the central cell was typically darker and enlarged compared to the end cells in the conidium. The swelling of the central cell usually gave the conidium a curved appearance.

Aseptate sterile fungus: The isolated fungus on PDA produced fast growing white colonies. The mycelium was aseptate, hyaline and there was no production of conidia or spores.

Septate sterile fungus: The isolated fungus on PDA produced fast growing light brownish colour colonies. The mycelium was septate, brown in colour and there was no production of conidia or spores.

The isolated fungi were identified as *Colletotrichum* sp., *Fusarium* sp., *A. niger*, *A. flavus*, *Alternaria* sp., *M. phaseolina*, *Penicillium* sp., *Curvularia* sp., aseptate sterile fungi and septate sterile fungi. Kumari (2011) isolated and identified *A. niger, A. flavus, A. alternata, F. moniliforme* and *Penicillium* sp. as most dominant fungi from chilli seeds based on cultural and morphological characteristics.

The results on nature of mycoflora of chilli seeds using standard blotter method showed that in all the four varieties *viz.*, GVC 101, GVC 111, GVC 121 and AVNP-C-131, fungi like *A. flavus* (1.50-3.00%) and *Colletotrichum* sp. (1.25-2.75%) were dominant and more frequently detected. *A. niger* (1.50-2.00%) was isolated from GVC 101, GVC 111 and GVC 121. *Fusarium* sp.

Table 2: Frequency of seed mycoflora associated with chilli varieties by agar plate method

Fungus	Frequency (%)			
	GVC 101	GVC 111	GVC 121	AVNP-C-131
Aspergillus niger	7.50	12.75	14.00	10.00
A. flavus	4.75	2.25	0.00	0.00
Colletotrichum sp.	11.50	10.50	11.00	11.00
Fusarium sp.	8.50	9.25	12.00	10.00
Alternaria sp.	2.00	2.50	0.00	0.00
Penicillium sp.	1.25	2.25	0.00	0.75
Curvularia sp.	1.00	1.75	0.00	0.00
M. phaseolina	1.00	1.50	0.00	0.00
Unidentified fungi with septate mycelium	0.75	0.75	0.00	0.00
Unidentified fungi with aseptate mycelium	0.75	1.00	0.00	0.00

(0.75-2.50%) was found in GVC 101, GVC 111 and AVNP-C-131. While, *Alternaria* sp. (0.75%) was found only in GVC 101.

The results on nature of mycoflora of chilli seeds using agar plate method showed that in all the four varieties *viz.*, GVC 101, GVC 111, GVC 121 and AVNP-C-131 fungi like *A. niger* (7.50-14.00%), *Colletotrichum* sp. (10.50-11.50%) and *Fusarium* sp. (8.50-12.00%) were dominant and frequently detected. Whereas, *A. flavus* (2.25-4.75%), *Alternaria* sp. (2.00-2.50%), *Curvularia* sp. (1.00-1.75%) and *M. phaseolina* (1.00-1.50%) were only found with seeds of GVC 101 and GVC 111. *Penicillium* sp. (0.75-2.25%) was found to be associated with GVC 101, GVC 111 and AVNP-C-131 but was absent in GVC 121. Non sporulating aseptate and septate sterile fungi was less frequently detected, only from GVC 101 and GVC 111.

The fungi isolated by standard blotter method were *A. niger* (1.50-2.00%), *A. flavus* (1.50-3.00%), *Colletotrichum* sp. (1.25-2.75%), *Fusarium* sp. (0.75-2.50%) and *Alternaria* sp. (0.75%), whereas fungi isolated by agar plate method were *A. niger* (7.50-14.00%), *A. flavus* (2.25-4.75%), *Colletotrichum* sp. (10.50-11.50%), *Fusarium* sp. (8.50-12.00%), *Alternaria* sp. (2.00-2.50%), *Penicillium* sp. (0.75-2.25%), *Curvularia* sp. (1.00 - 1.75%), *M. phaseolina* (1.00-1.50%), unidentified sterile fungi with septate mycelium (0.75%) and unidentified sterile fungi with aseptate mycelium (0.75-1.00%).

From overall results it can be stated that, the common and dominant fungi recorded were *A. niger* (0.00-14.00%), *Colletotrichum* sp. (1.25-11.50%) and *Fusarium* sp. (0.00-12.00%) as they were recorded from almost all the seed samples of four chilli seed varieties used for investigation. Kumari (2011) detected *A. niger* (34.42%), *A. alternata* (14.62%) and *Penicillium* sp. (14.27%) in chilli seeds of variety GVC 101 and GVC 111 by standard blotter method. Machenahalli *et al.* (2014) detected maximum frequency of *Colletotrichum* sp. (72.85%) followed by *A. alternata* (5.20%) and *F. oxysporum* (4.30%) in chilli seeds by standard blotter method. From GVC 101 and GVC 111 Kumari (2011) detected maximum frequency of *F. moniliforme* (78.61%), *A. alternata* (34.75%) and *A. niger* (23.99%) by agar plate method. Jat (2015) isolated *Colletotrichum* sp. (0.50-5.50), *A. niger* (2.50-9.00), *A. flavus* (2.00-8.50), *A. alternata* (3.50-9.25), *Rhizopus* sp. (3.75-7.25) and *Fusarium* sp. (2.00-6.50) from chilli seeds by agar plate method.

Variety GVC 101 and GVC 111 were found as most susceptible as maximum number of mycoflora (10) were detected from these varieties. Kumari (2011) reported the susceptibility of GVC 101 and GVC 111 because of isolation of around twenty five fungi from chilli seeds of these two variety.

Out of the two isolation methods used for detection and isolation of fungi from seeds of different varieties of chilli, agar plate method was found more effective as total ten fungal species were isolated by this method as compared to only five fungal species in standard blotter method. Telang (2010) reported agar plate method as best method in isolation of seed mycoflora due to the isolation of fifteen fungi from agar plate method, whereas only twelve from standard blotter method. Kumari (2011) reported isolation of about twenty one fungi from agar plate method and eight fungi from standard blotter method.

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