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Ragupathi KP
PhD Scholar, Dept. of Seed Science & Technology, TNAU, Coimbatore, Tamil Nadu, India

PR Renganayaki
Professor and Head, Dept. of Seed Science & Technology, TNAU, Coimbatore, Tamil Nadu, India

S Sundareswaran
Director, Directorate of Seed Center, TNAU, Coimbatore, Tamil Nadu, India

S Mohan Kumar
Director, CPMB & B, TNAU, Coimbatore, Tamil Nadu, India

A Kamalakannan
Professor, Dept. of Plant Pathology, TNAU, Coimbatore, Tamil Nadu, India

Corresponding Author:
Ragupathi KP
PhD Scholar, Dept. of Seed Science & Technology, TNAU, Coimbatore, Tamil Nadu, India

Seed mycobiome of tomato cultivar PKM 1

Ragupathi KP, PR Renganayaki, S Sundareswaran, S Mohan Kumar and A Kamalakannan

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Abstract
Seed borne diseases can easily spread from one place to another and serve as an initial source of inoculum. Mycoflora are causative agents of devastating tomato diseases like early blight, fusarium wilt, root and fruit rots. A total of 100 tomato seed samples of cultivar PKM 1 were collected from seed producers and farmers across different agro climatic regions of Tamilnadu and screened for their seed borne mycoflora. A total of 14 species of fungi *viz.*, *Alternaria solani*, *Alternaria alternata*, *Stemphylium solani*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Colletotrichum capsici*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus nigricans*, *Helminthosporium solani*, *Pythium debaryanum*, *Rhizoctonia solani* and *Phytophthora infestans* were recovered from the collected seed samples using various seed health testing methods. Isolated plant pathogens, were tested for their pathogenicity and transmission on tomato cultivar PKM 1 seedlings.

Keywords: Tomato, mycoflora, seed borne diseases, pathogenicity, transmission

Introduction
Tomato also called as "golden apple" belongs to nightshade family *Solanaceae* is the second extensively grown vegetable crop after potato due to its tangy fruit with high nutritive value. Tomato is cultivated worldwide due to its adaptability to wide range of soils and climate is prone to insect pests, abiotic and biotic stresses due to their tenderness as compared to other crops. Over 200 diseases caused by various pathogens have been reported on tomato worldwide (Jones *et al.*, 1991) [11]

Major insect pests of tomato includes Aphids, Tomato Fruit worms & Horn worms, Leaf-footed Bugs & Stink Bugs, Flea Beetles, Whiteflies, Thrips, Spider Mites and Cutworms. (Fouche *et al.*, 2000) [6]. Often tomato is also affected by several nematodes including *Meloidogyne* spp., *Nacobbus aberrans, Ditylenchus dipsaci, Globodera rostochiensis, G. pallida*,*Pratylenchus* spp., *Paratrichodorus* spp., *Tylenchorhynchus* spp., *Xiphinema facolum*, *Rotylenchulus reniformis* and *Dolychodorus heterocephalus* (Greco and Vito, 2011) [8]. Seed-borne diseases can easily spread from one place to another and serve as an initial source of inoculum (Nishikawa *et al.*, 2006) [17]. Seed borne diseases are caused by microorganisms including fungi, bacteria, virus and nematodes. Among the parasitic organisms fungi are most frequently encountered on seeds. The infected seeds served as a source of primary infection and establishment of plant diseases have adverse effect on seed health, reducing germination ability, poor seedling vigour and transmit fungus to the seedlings, accelerating the deterioration in storage, introducing pathogens into new areas and increasing the inoculum source in the field (Alves *et al.*, 2012) [1]. On tomato, numerous seed-borne fungi *viz.*, *Alternaria solani*, *Aspergillus flavus*, *Rhyzopus stolonifer* and *Curvularia* spp. etc. occur that cause abnormalities to the seeds such as seed toxification, seed rotting, necrosis and seed abortion (Neergard, 1997) [16]. Considering the economic importance of seed-borne fungi and their effect on seed vigor various seed health detection assays are being adopted by seed pathologists to screen and eliminate infested seed lots before planting (ISTA, 1976) [9]. On the basis of the above information the present study was designed with aim to study the mycobiome associated with the tomato seeds for their effective management.

Materials and Methods
Seed samples Collection
A total of 100 tomato seed samples of cultivar PKM 1 were collected from seed producers and farmers across different agro climatic regions of Tamil nadu. Seeds were extracted from

mature fruits and brought down to the safe moisture content and were subjected to various experiments.

Detection of Tomato cultivar PKM 1 Seed Mycobiome by various Seed health testing methods

Standard Blotter Method

Detection of seed borne fungi in seed samples was done by following ISTA procedures. In this method, three layers of blotter paper was soaked in sterilized water and placed in the petri plates. 100 seeds were sterilized in 0.2% Sodium hypochlorite solution for 2 to 3 minutes and seeds taken randomly from each sample and were placed in petri plates and incubated for seven days in the laboratory under alternating cycles of 12 hrs light and 12 hrs darkness. The incubated seeds were examined under stereo binocular microscope to ascertain the presence of fungi. (ISTA, 1993) [10]

Potato Dextrose Agar Method

In this method 100 seeds were sterilized with 0.2% Sodium hypochlorite solution for 2 to 3 minutes. Then, the seeds were placed on sterile glass petri plates containing PDA medium and incubated at 40ºC with alternating cycles of 12 hrs light and 12 hrs darkness for seven days and examined under stereo binocular microscope. (ISTA, 1993) [10]

Water Agar Method

In this method 100 seeds were sterilized with 0.2% Sodium hypochlorite solution for 2 to 3 minutes. Seeds were placed on sterile glass petri plates containing 2.5% water agar medium and incubated at 25±2ºC for seven days then examined under stereo binocular microscope (Neergaard, 1977) [15]

2, 4-D Method

In this method, 100 seeds were sterilized with 0.2% Sodium hypochlorite solution for 2 to 3 minutes. The three layers of blotter paper discs were dipped in 0.2% of 2, 4-Dichloro phenoxy acetic acid solution. Seeds were placed equidistantly on moist blotter discs using sterilized forceps under aseptic conditions in laminar air flow chamber and the plates were incubated at room temperature for seven days. Seeds were examined under stereo binocular microscope on the seventh day (Limonaard, 1968) [11]

Deep Freezing Blotter Method

In this method, three layers of blotter paper were soaked in sterilized water and placed in the petri plates. 100 seeds were sterilized in 0.2% Sodium hypochlorite solution for 2 to 3 minutes and seeds taken randomly from each sample and were placed in petri plates and incubated at 25±2ºC for first 24 hrs under alternate cycles of 12 h NUV light and darkness, for next 24 hrs the plates were incubated at -20ºC and then kept back under original conditions for next six days and examined under stereo binocular microscope to ascertain the presence of fungi. (ISTA, 1993) [10]

Screening of Tomato cultivar PKM 1 Seed Mycobiome

The incubated seeds were screened on eighth day using stereo binocular and compound microscope. The associated fungi were recorded and identified with the help of standard guides and manuals. Barnett (1960) [3], Sigourd and Funder (1961) [20] Subramanian (1983) [21], Van Arx (1981) [23]

Pathogenicity testing of Tomato cultivar PKM 1 Seed Mycobiome

Isolated fungi were selected for disease transmission studies in the experimental plot. The seed samples were sterilized by 2% Sodium hypochlorite solution for 2-3 minutes and washed in the distilled water before sowing. Sterilized seeds were sown in the fields and the proper agronomical practices were followed for raising the plants. Severity of the disease was assessed by using 0-9 scale in the randomly selected plants and percentage of diseases index was calculated (Mayee and Datar, 1986) [12] Seed to seed transmission of isolated fungi was also studied. Confirmation of pathogens were done by pathogenicity tests. The specific pathogenicity test was performed by the following method as reported by Giri *et al.*, 2001 [7].

Statistical analysis

The data obtained from various experiments were analysed statistically by adopting the procedure described by Panse and Sukhatme (1985) [18] The laboratory experiments were laid out in completely randomized design (CRD) and field trials were designed in randomized block design (RBD). The data recorded on per cent values were arc-sine transformed before analysis and the critical differences (CD) were calculated at 5 per cent probability level.

Results and Discussion

Detection of Tomato seed mycobiome by various Seed health testing methods

Seeds play a vital role in the production of healthy crops. Seed-borne fungi are microorganisms that may harbour seeds internally causing infection or externally as contaminant, causing pre and post germination death, making them toxic and reduced its quality for consumption as well as for food industry. In the present investigation the collected seeds samples were used for the isolation of mycoflora. The present study with tomato cultivar PKM 1 seeds reveals the incidence of 14 different species of fungi *viz., Alternaria solani*, *Alternaria alternata*, *Stemphylium solani*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Colletotrichum capsici*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus nigricans*, *Helminthosporium solani*, *Pythium debaryanum*, *Rhizoctonia solani* and *Phytophthora infestans* were recovered from the collected seed samples using various seed health testing methods. (Table 1)

Table 1: Percent incidence of tomato cultivar PKM 1 seed mycobiome under different seed health testing methods

Fungal species	Seed health testing methods (% incidence)									
	Non-surface sterilized					Surface sterilized				
	SBM	PDA	WA	2,4-D	DFB	SBM	PDA	WA	2,4-D	DFB
Alternaria solani	9.3	9.6	8.1	8.4	7.4	6.1	6.5	5.4	4.5	4.2
Alternaria alternate	8.7	5.6	4.5	5.0	4.2	4.6	3.7	3.4	2.1	3.5
Stemphylium solani	4.3	3.8	3.9	3.2	2.5	1.5	1.4	1.6	1.5	1.2
Fusarium moniliforme	3.1	4.5	3.3	4.6	4.7	2.1	1.4	2.3	2.9	1.4

Fusarium oxysporum	4.4	*4.1*	4.5	2.6	2.1	3.2	2.6	2.1	1.2	1.7
Colletotrichum capsici	6.9	5.2	4.4	3.2	3.4	3.7	2.6	1.8	1.3	1.5
Curvularia lunata	5.3	3.9	2.7	3.3	2.7	2.7	1.4	2.6	1.0	1.3
Aspergillus niger	5.2	4.2	2.8	1.2	1.4	1.5	1.4	1.5	2.0	1.7
Aspergillus flavus	6.1	6.8	4.5	3.1	3.6	2.7	2.1	1.1	1.3	1.6
Rhizopus nigricans	3.8	3.5	2.3	3.8	2.0	1.8	2.5	1.2	1.4	1.4
Helminthosporium solani	2.2	2.4	3.4	3.5	3.2	1.0	1.2	1.4	1.5	1.2
Pythium debaryanum	1.7	1.0	1.9	1.3	1.1	2.1	1.0	2.8	2.8	1.5
Rhizoctonia solani	4.1	3.7	3.0	2.9	3.5	1.4	2.9	1.4	1.4	1.9
Phytophthora infestans	2.9	2.1	2.2	2.4	2.6	1.4	1.5	1.2	1.9	1.4
SEd	0.12	0.09	0.10	0.10	0.08	0.07	0.06	0.07	0.05	0.04
C D (P=0.05)	0.27	0.19	0.20	0.21	0.15	0.14	0.12	0.14	0.10	0.08

*SBM – Standard Blotter Method *PDA – Potato Dextrose Agar *WA – Water Agar
*2,4-D - 2,4-D Blotter Method *DFB – Deep Freeze Blotter Method

Perveen (1996) [19] reported *Fusarium solani*, *F. moniliformae*, *Alternaria alternata* and *Drechslera australiensis* as predominant seed-borne mycoflora on tomato. *Alternaria solani*, *Fusarium oxysporum*, *Aspergillus flavus* and *A. fumigatus* were also detected on tomato, causing severe damage to seeds (Fakir, 2001) [5]. Likewise, Bhatti *et al;* (2010) [4] recorded *Bipolaris* spp, *Curvularia lunata*, *F. moniliformae* and *F. semitectum* from tomato seeds. Nation *et al*., (2011) [13] reported *Alternaria alternata*, *Aspergillus flavus* and *A. niger* whilst *Aspergillus amstelodami* and *Cunnnin ghameliaechinulata* were new records in tomato. The importance of seed health testing cannot be underestimated. To boost food production and crop yield sowing healthy seed is major concern. The present study with tomato cultivar PKM 1 seeds reveals the highest incidence of different fungi under standard blotter method compared with other seed health testing methods. (Table 1) Natsugah *et al*., (2004) [14] evaluated seed health status of tomato seeds through blotter incubation method. Sultana and Ghaffar (2009) [22] revealed blotter and deep-freezing methods more efficient compared to agar plate method. Nation *et al;* (2011) [13] found agar plate method superior over blotter method. Asha *et al.* (2011) [2] with blotter test isolated *Fusarium oxysporum* from infected seeds of PKM 1 tomato cultivar.

Pathogenicity testing of isolated tomato seed mycobiome

All the fungi which were isolated from collected tomato seed samples were tested for their pathogenicity on tomato seeds and seedlings. Pathogenicity test results reveals that the isolated fungi were virulent and their aggressiveness was recorded. Among the fungi isolates *Alternaria solani* and *Alternaria alternata* had higher seed transmission. (Table 2).

Table 2: Pathogenicity of isolated tomato cultivar PKM 1 seed mycobiome

Fungal species	SBM% infection	Pre-emergence mortality (%)	Post-emergence mortality (%)	Diseased plants (%)
Alternaria solani	98	12	6	80
Alternaria alternata	96	15	6	76
Stemphylium solani	79	14	8	57
Fusarium moniliforme	81	11	10	60
Fusarium oxysporum	84	16	9	59
Colletotrichum capsici	93	21	7	65
Curvularia lunata	88	12	8	68
Aspergillus niger	98	19	13	66
Aspergillus flavus	99	23	11	65
Rhizopus nigricans	86	12	7	67
Helminthosporium solani	94	18	7	69
Pythium debaryanum	77	16	9	52
Rhizoctonia solani	86	13	5	68
Phytophthora infestans	73	17	12	44
SEd	2.48	0.33	0.16	1.62
C D (P=0.05)	5.12	0.69	0.33	3.24

*SBM – Standard Blotter Method

Conclusion

Tomato being a highly nutritive vegetable crop is cultivated throughout the world due to its large consumption. Seed borne pathogens of tomato are of serious concern in seed production industry as they adversely influence the germination and vigour of the seedlings, and consequently yield and quality. Use of healthy seed accompanied with sound seed certification program is a key factor to increase its quality and production. Seed treatments are essential in controlling seed borne diseases, therefore, integrated approach is recommended to minimize the incidence of diseases carried through seeds.

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