Seed mycobiome of tomato cultivar PKM 1

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Abstract
Seed borne diseases can easily spread from one place to another and serve as an initial source of inoculum. Mycobiota 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 mycobiota. 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, mycobiota, 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., Tylenchylrhyynchus spp., Xiphinema faculom, Rotylenchulus reniformis and Dolichodorus 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, Rhizopus 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 Tamilnadu. 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 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 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 40°C 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±2°C 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±2°C for first 24 hrs under alternate cycles of 12 h NUV light and darkness, for next 24 hrs the plates were incubated at -20°C 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) [18], 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 alternata           | 8.7 | 5.6 | 4.5 | 4.2 | 4.6 | 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 |

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Perven (1996) \cite{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) \cite{9}. Likewise, Bhatti et al; (2010) \cite{4} recorded *Bipolaris* spp, *Curvularia lunata, F. moniliformae* and *F. semitectum* from tomato seeds. Nation et al., (2011) \cite{13} reported *Alternaria alternata, Aspergillus flavus* and *A. niger* whilst *Aspergillus amstelodami* and *Curvularia flavidaechnulata* 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) \cite{14} evaluated seed health status of tomato seeds through blotter incubation method. Sultana and Ghaffar (2009) \cite{22} revealed blotter and deep-freezing methods more efficient compared to agar plate method. Nation et al; (2011) \cite{13} found agar plate method superior over blotter method. Asha et al. (2011) \cite{23} with blotter test isolated *Fusarium oxysporum* from infected seeds of PKM 1 tomato cultivar.

**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 nutritious 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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