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MANAGEMENT OF SEED BORNE FUNGAL DISEASES OF TOMATO: A REVIEW

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A B S T R A C T

Plant diseases caused by different kinds of microorganisms either carried through air, water or present in soil, seeds or propagative planting materials have adverse impact on agriculture production and economy worldwide. Apart from other crops vegetables are also subjected to several seed borne fungal, bacterial and viral pathogens, which cause substantial yield loss upto 10 percent in Pakistan. This article gives vast information regarding significance and prevalence of various kinds of seed borne mycoflora (*Alternaria solani*, *Fusarium oxysporum*, *F. solani*, *Botrytis cineria*, *A. alternata*, *Chaetomium globosum*, *Curvularia lunata*, *Aspergillus niger*, *Drechslera specifer* and *Rhizoctonia solani*) particularly associated with seeds of tomato. These mycoflora are causative agents of devastating tomato diseases like early blight, fusarium wilt and foot rots, grey mold, root and fruit rots. A range of conventional and modern techniques employed for seed borne fungal detection and different control strategies including chemical and biological methods opted by researchers have been reviewed in present paper. A variety of factors like availability of susceptible plants, favorable environmental conditions and overhead irrigation are serious constraints for plant disease development. Under these conditions, monitoring of plant health and detection of diseases particularly using seed detection assays to screen infested seed lots before planting provide effective disease management strategy.

Keywords: Detection methods, seed borne fungi, seed treatments, tomato.

INTRODUCTION

Tomato (*Lycopersicun esculentum* Mill.) also called as "golden apple" belongs to nightshade family *Solanaceae* is the second extensively grown vegetable crop after potato due to its tangy fruit, taste and high nutritive value throughout the world (Saltueit, 2003). Tomato is high in vitamins particularly vitamin A, C, β-carotene and essential minerals. A powerful natural antioxidant-Lycopene is present in red ripe and cooked tomatoes good for both preventing heart diseases and cancer (Madhavi and Salunke, 1998; Olson, 2004).Tomato being short-duration, low price and high economic returns has fetched attraction of growers to produce the crop throughout the year even in places with warmer climates (Naika *et al*., 2005). Since 1993, tomato production and consumption have almost doubled in the world (Van de vooren *et al*., 1986). Due to its assorted fruits, it is considered as an important cash and industrial crop in many countries including United States, China, Turkey, India and Egypt. According to world's statistics, China is the leading tomato producing country in the world with annual production of about 34 million tones. World production of tomato is estimated at 145 million tones annually over an area of 4.36 million ha, yielding on average basis up to 336 thousand kg/ha. In Pakistan tomato covers an area of 58.1 thousand hectares with an annual production of about 574 thousand tones (GOP, 2013).

Though, tomato is cultivated worldwide due to its adaptability to wide range of soils and climate (Ahmad, 1976), however, tomatoes are prone to insect pests, abiotic and biotic stresses due to their tenderness as compared to other crops (Ketelaar and Kumar, 2002). Plant diseases are the main limiting factors to total crop losses all over the world and are of growing importance

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as the world population increases. Over 200 diseases caused by various pathogens have been reported on tomato worldwide (Jones *et al.,* 1991). However, seed-borne diseases can easily spread from one place to another and serve as an initial source of inoculum potential (Nishikawa *et al*., 2006). Therefore a high quality seed have significant role in production of sustainable and profitable vegetables. 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 (Agarwal, 1981), thus, have adverse effect on seed health, reducing germination ability, poor seedling vigor 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*., 2006).

Fungi being an imperative group of microorganisms are also responsible for tomato seed-borne diseases and cause a substantial yield loss. On tomato, numerous seed-borne fungi viz *Fusarium 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, 1977; Fakir and Khan, 1992). 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). Farmers are facing financial constraints with considerable crop losses due to seed-borne mycoflora on their crops. Primary step in any agricultural crop production and protection program is control of seed-borne pathogens through various methods. Therefore, substantial control of seed-borne pathogens can be achieved by using single or combination strategies of mechanical, physical, biological and chemical methods.

Prevalence of Seed-borne Mycoflora- A historical Perspective: Seed-borne fungi are microorganisms that may harbor seeds internally causing infection or externally as contaminant (Agarwal and Sincair, 1997), causing pre and post germination death, making them toxic and reduced its quality for consumption as well as for seed industry. Seed-borne mycoflora may be present in the form of hyphae, chlaymydospores, oospores, conidia and sclerotia (Behura *et al.,* 2000; Bhameapravati, 2006). Tomato being used as a model plant in genetics also prone to numerous seed-borne fungal pathogens. In Pakistan tomato production is enormously reduced due to seed-borne fungi along with other pathogens. An estimation of 10 percent crop losses due to seed borne diseases was reported in Pakistan (Bhatti and Bhutta, 1990). Earlier Ahmad *et al;* (1993) recorded15 fungi and Perveen and Ghaffar (1995) reported 22 new species belonging to 15 genera of seed-borne mycoflora on tomato from Pakistan. Most predominant were: *Fusarium solani, F. moniliformae, Alternaria alternata* and *Drechslera australiensis* as predominant fungi. In Bangladesh, similar fungi like *Alternaria solani, Fusarium oxysporum, Aspergillus flavus* and *A. fumigatus* were also detected on tomato, causing severe damage to seeds (Fakir, 2001). Likewise, Bhatti *et al;* (2010) recorded *Bipolaris* spp, *Curvularia lunata, F. moniliformae* and *F. semitectum* with infection percentage of 1.5, 0.5-7.5, 0.5 and 1-3 respectively from tomato seeds.

Storage period also have great impact on occurrence of different mycoflora. Black lesions on tomato fruit when stored at 30°C developed due to seed-borne *Cladosporium* spp (Shersingh *et al.,* 1983). Total 12 fungi were isolated from fruits and seeds of tomato (Dhekle and Bodke, 2013), all of the fungi were associated to tomato fruits as well, except for *Cladosporium* sp that showed incidence only on seeds. Ismael, (2010) detected *Aspergillus clavatus, A. flavus, A.niger, Penicillium digitatum, Pythium sp., Rhizopus arrhizus* and *R. Stolonifer* from tomato seeds of Sulaimania region with frequency percentage rate: 0.42, 0.08, 0.4, 0.04, 0.025, 0.02, and 0.266 whereas with frequency percent of 0.10, 0.10, 0.87 and 0.25% *A. versicolor, P. digitatum*, *R. arrhizus* and *R*. *stolonifer* predominate tomato seeds from Germaine region respectively. As a matter of fact, tomato seeds yielded large number of fungal isolates belonging to *Alternaria, Fusarium, Botrytis, Pythium, Drechslera* and *Rhizoctonia* genus causing seed rot, damping off, root and fruit rot, foliar and wilt diseases (Mathur, 1983; Kassim and Monawar, 2000). Whereas, Mogle and Mane (2010), found twelve species associated with treated and untreated seeds of tomato. Nishikawa *et al;* (2006) while evaluating 109 species of seed-borne fungi from three cultivars of tomato found *Hormonema* spp., one of the most common genera on tomato seeds from old lots of cv. delicious as compared to new lots, additionally; cluster analysis indicated that tomato seed mycoflora is greatly influenced by harvesting year and locality of cultivars.

Depending upon presence of fungi either on seed coat or in the seed, mycoflora are however, isolated both as surface contaminants, internally seed-borne flora and are known to cause serious field diseases. Bankole (1996) isolated 18 fungal species from two tomato varieties, Ibadan and Ife1as externally and internally seed-borne. They found most of the species to be surface contaminants while *Alternaria longissima, Aspergillus flavus, A.niger, Curvularia lunata, Fusarium moniliforme, F. oxysporum* and *Phoma destructive* were present internally. Agarwal and Sinclair (1997) also affirmed that seed may either be infected internally or present as surface contamination. *Fusarium* wilts, one of the most devastating diseases of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* is an internally transmitted fungus (Singh *et al.,* 1980). Sati *et al.,*(1989) isolated five *cladosporium* spp from two tomato cultivars. *Cladosporium oxysporum* and *C. cladosporioides* were most frequently isolated with incidence of 1-25% and 1-10% respectively. Furthermore, *C. cladosporioides* induced 33%, *C. oxysporum* 25% and *C. fulvum* 24% seed and seedling infection in "chaubattia red" variety, whereas, in "shiouse cultivar" caused 39%, 35% and 28% seed and seedling mortality respectively. Similarly earlier workers reported that a total of seventeen fungi were associated with seeds of tomato var. local, among them the predominant fungi were *Aspergillus niger, A. flavus, Fusarium moniliforme, Rhizopus nigricans, Curvularia lunata* and *Alternaria alternate* (Telang, 2010). According to Nation *et al*., (2011), twelve fungi were found coupled with seeds of four tomato cultivars in Gujrat, India. Most predominant were *Alternaria alternata, Aspergillus flavus* and *A. niger* whilst *Aspergillus amstelodami* and *Cunnnin ghameliaechinulata* were new records.

Detection Methods: High quality seed is an important prerequisite for sustainable crop production. Furthermore, asymptomatic infested seeds, low pathogen populations on seeds make testing seed health a difficult task. A range of conventional and modern detection assays exist for different seed fungi, adopted by seed pathologists for detection of seed-borne mycoflora. However, few of them satisfy the minimum requirements for adequate seed tests comprises direct examination, blotter test, agar plate method, seedling growing-on test and serological assays. Though, for preliminary detection of fungi these are the common laboratory seed health testing methods, but blotter test is simplest and most widely used method (ISTA, 1976).

Conventional Methods: The magnitude of seed health testing cannot be underestimated. Hence to boost food production and crop yield sowing healthy seed is of our major concern. Depending upon nature of fungi two methods possibly required for detection of a pathogen (Mathur, 1995). As Perveen and Ghaffar (1995), Kassim and Monawar (2000) and Nation *et al;* (2011) found agar plate method superior over blotter method as it yielded higher number and percentage of seed mycoflora of tomato, eggplant, okra and radish. Whereas, Rashid *et al.,* (2010) evaluated seed health status of tomato seeds through blotter incubation method. *Aspergillus* spp., *Fusarium* spp and *Penicillium* spp were identified from tomato seed samples. Similarly, Natsugah *et al.,* (2004) using blotter plate method, assessed 152 seed samples including sorghum, rice, maize, pearl millet, tomato and watermelon for associated seed-borne fungi. Sultana and Ghaffar (2009) revealed blotter and deep-freezing methods more efficient compared to agar plate method as these methods yielded maximum fungi on bottle gourd seeds both quantitatively and qualitatively. Asha *et al.,*(2011) with blotter test isolated *Fusarium oxysporum* from infected seeds of PKM-I tomato cultivar which showed 7% incidence. Despite the fact that some researchers revealed seed mycoflora through agar plate method, however due to high competition of dominant fungi for nutrition on agar plates, the blotter method isolated higher numbers of fungi than agar method.

Modern Techniques: Besides, pathogenic strains seed mycoflora also include fast growing saprophytes that may overgrow, making isolation of fungal pathogen complicated. Further, some races of pathogenic fungi are morphologically identical. Consequently, use of modern techniques like immunoassays and nucleic acid-based techniques can aid in perceptive detection of seed mycoflora. Serological based seed assays like ELISA are effectively in use for detection of higher percentage of fungi and bacteria compared to agar plate procedure which is time consuming (Afouda *et al.,* 2009). Moreover, Alves and Edson (2012) suggested that adoption of scanning electron microscopy (SEM) methodology in blotter test analysis could be useful to identify fungal structures and finally supportive in seed certification programs as well as to facilitate conduction of more detailed taxonomic classification of seed-borne fungi.

The application of nucleic-acid based detection methods in seed health testing has been facilitated by integrating conventional or real-time PCR with other technologies like BIO-PCR, IMS-PCR etc. Since many PCR-based detection methods have been developed and applied to seed-borne pathogens. Munkvold (2009) reviewed research innovations in seed-borne pathogens detection, use of seed treatments and advancement towards standardization of seed health testing methods. Up till 2005 at least 100 pathogens had been detected with PCR- based seed health tests (Guillemete *et al.,* 2004) hence, acquired more specific detection of *Verticillium* sp. in tomato with real-time PCR assay. However, in the developing world, obstacles like capital costs and technical expertise for establishing PCR capabilities can be challenging in the implementation of PCR-based methods for seed health testing. Subsequently more work is needed to check the role of various detection methods in seed health testing programs.

Effect on Germination: As a result of fungal infection large number of toxins in seeds are produced which may affect seed metabolism at cellular level. These mycotoxins greatly affect seed germination and seedling vigor. Aflatoxins produced by *Aspergillus flavus* disturb the metabolism by inhibiting chlorophyll and various enzymes synthesis consequently reducing seedling growth (Mathur and Jorgensen, 1988). Isolates of *A. alternata, Penicillium waksmanii*, and *Arthrinium sp* detected on tomato seeds significantly inhibited influential root elongation ultimately causing seedling rot three days after germination (Nishikawa *et al*., 2006). Fungal exudates produced by seed mycoflora *Aspergillus niger*, *A. clavatus* and *Sclerotinia sp* significantly decreased the percent germination rate of tomato and pepper seeds (Ismael., 1997; 2000; 2010). Similarly, Telang (2010) also found seed-borne fungi on tomato var. local to be inhibitory for seed germination and caused severe loss in seedling vigor.

Seed Treatments: Seed health is an important step in crop disease management. Due to changing global climate, emergence of diseases in endemic form turns out to be a challenge in safeguarding plant health. Hence, judicious and accurate diagnoses of problem and pathogen surveillance allow time for application of mitigation strategies. Epiphytotic plant diseases have shown to be prevented by treatment of seeds to kill the pathogens carried within or on the seed. Compared to air-borne or soil borne fungi, however, seed-borne fungi are easily controlled. A wide range of chemical, biological physical, and mechanical approaches have been used to reduce pathogens from seeds both internally and externally, and to help protect seeds from soil-borne pathogens (McGee, 1995; Maude, 1996; Neergard, 1977).

Through Chemicals: Seed-borne pathogens results in mycotoxic problems to human health, hence seeds should be treated with suitable fungicides before sowing. Traditionally, seed treatment through fungicides via spraying, soil drenching and dusting has historical importance (Orereke *et al.,* 2007), also protecting seeds and seedlings from the common soil-inhabiting fungi that cause seed rots and damping-off diseases. Kassim and Monawar (2000) treated *(in vitro*) five vegetable seeds including tomato, eggplant, okra etc in Gazan province with 0.2% fungicides viz., Benomyl, Cozib and Mancozeb before incubation. All tested fungicides showed inhibitory effect on most of the isolated fungi. However, benomyl was the most effective against all fungi detected on tomato seeds. Amini *et al.,* (2010) evaluated six fungicides against *Fusarium* wilt causing fungi of tomato *in vitro* and *in vivo*. Prochloraz and bromuconazole followed by benomyl and carbendazim found most effective against *Fusarium oxysporum* f sp. *lycopersici* when applied to seedlings at recommended doses. Additionally, no phytotoxic effects observed on seedlings.

Through Bio-agents: Certainly, chemical fungicides show profound results on seed-borne fungal diseases but on the other hand adversely influence beneficial microorganisms present in the soils and environment. Besides, indiscriminate use of fungicides not only hazardous to animals and human beings but also develops resistance among target pathogen. Since, the fungicides affect the non-target organisms as well (Banerjee *et al*., 2005), researchers are moving towards use of more ecofriendly and cheap alternative methods of treating seeds like acid treatments, antagonistic microbes and plant extracts to control diseases. These are promising alternatives that release plant growth regulators which influence the overall crop growth and improve the morphological characteristics.

In this respect, Kasselaki *et al.,* (2008) tested nitrite solution and resistance inducers in a growth chamber against *Didymella lycopersici*- a seed-borne fungus of tomato. Ten minutes seed soaking with nitrite solution at 300mM concentration and resistance inducer Tillecur (mustard seed extract) at 0.05g/ml reduced seed germination losses and disease incidence in germinating seedlings. Mogle and Mane (2010) evaluated biofertilizers and benomyl fungicide on seed germination and mycoflora of tomato. Seeds treated with *Azotobacter* and *Trichoderma* comparatively showed highest seed germination (96%) followed by *Trichoderma* and *Rhizobium* mixture (95%) while minimum (78%) was recorded with Benomyl. Additionally, seed dressing with biofertilizers greatly reduced the incidence of *Alternaria alternata*, *Rhizoctonia solani* and *Pythium aphanidermatum* whereas; *Fusarium oxysporum* was completely inhibited over control. Effect of various strains of *Trichoderma virens* studied on pathogen growth, seed germination and vigor of tomato seeds. In India, local isolate Tv_1 among tested isolates increased the plant growth and inhibited mycelia growth of *F. oxysporum* f. sp. *lycopersici* under *in vitro* condition. While seed treatment plus soil application of talc based formulation of Tv_1 also significantly reduced disease incidence (54.66%), compared to other isolates of *T. virens* under green house conditions (Christopher *et al.,* 2010).

Likewise, Asha *et al.,* (2011) examined biocontrol potential of *Pseudomonas fluorescens,* isolated from rhizospheres of tomato fields against seed and soil borne *Fusarium oxysporum f sp. lycopersici*. Though, all the ten isolates of *P. fluorescens* inhibited mycelial growth of *F. oxysporum* to various extents, but maximum inhibition zone (2.2cm diameter) was measured for Pf2 isolate and minimum zone (0.2cm diameter) was found for Pf8 isolate. Moreover, *P. fluorescens* increased the seed germination (up to 89%) and also enhanced seedling vigor of tomato.

Through Botanicals: Plants are the richest source of organic chemicals and produce wide variety of eco-friendly secondary metabolites (Okigbo and Nmeka, 2005; Okigbo and Omodemiro 2006; Jamil *et al.,* 2007; Riaz *et al.,* 2010). Instead of chemical fungicides use of botanicals is one of the recent approaches for controlling seed-borne and other plant diseases (Howlader, 2003; Islam *et al.,* 2006). Antifungal activity of various plant extracts viz: garlic clove, neem leaf, allamonda leaf, ginger rhizome, kalijira seed, bel leaf, turmeric rhizome, katamehedi leaf and onion bulb were evaluated against seed-borne damping-off of tomato. Seed treatment with plant extracts showed varied degree of performance towards percent damping-off of tomato. However, the highest seed germination (86.67%) and minimum incidence of damping-off of tomato was recorded with neem leaf extract followed by garlic clove and allamonda leaf extract (Islam and Faruq, 2012). Rashid *et al.,* (2010) treated tomato seeds with garlic tablets at different concentrations against seed-borne fungal diseases. Prevalence of fungal pathogens and increasing seed germination (71.25%) was recorded even at 1:3w/v dose. Moreover, in pot experiment garlic tablets at same concentration (1:3w/v) showed best performance with seedlings having highest germination and reduced blighted, damping off symptoms. Seed-borne fungal diseases were reduced when faba bean, tomato and lettuce grown under soils of *Artemisia afra* shrub. This might be due to presence of biologically active compounds in the soils/roots which may have inhibitory effect on seed germination of faba bean, tomato, peas and lettuce (Materechera and Mbokodi, 1997). Similarly, Ritz (2008) observed the effect of *Calatropis procera* residues alone and in combination with *Fusarium oxysporum* inoculum on tomato cultivars flora-dade, castle rock and strain B. Percent emergence, root and shoot length of tomato cultivars were reduced in the presence of *F. oxysporum* but improved with combination of plant residues and tomato pathogen by suppressing the pathogenic fungus.

CONCLUSIONS

Tomato being a highly nutritive vegetable crop is cultivated throughout the world and placed after potato due to its large consumption. But seed-borne pathogens of tomato are of serious concern in seed production industry as they adversely influence the germination and vigor of the seedlings, and consequently yield and quality of the product deteriorate greatly. Therefore, use of healthy seed and sound seed certification program is a key factor to increase its production. Seed treatments are essential in controlling seed-borne diseases, therefore, integrated approach is recommended to minimize the incidence of diseases carried through seeds of various crops including tomato.

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