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Original Research Article

Transmission and Histo-Pathological Studies of Seed-borne Fungal Infections of Tomato (*Solanumlycopersicum* Mill.)

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A B S T R A C T

Tomato (*Solanum lycopersicum* Mill.) is the most popular vegetable crop grown in the world. Crop is affecting many of seed-borne fungal infection. These seed-borne fungi are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. Thus, the seed transmission studies helps to confirm the seed to plant transmission to prove the pathogenicity and also to design the suitable management strategy. Hence an attempt was made to know the mode of transmission and type of damage caused by *viz*., *Alternaria. solani*, *A. alternata and Fusarium* sp., the results revealed that the highest disease incidence was observed in treatment T_1 and T_2 (10^6 and 10^5 conidia/1 ml) in case of all three fungi, whereas lower disease incidence was observed in T_3, T_4 and T_5 (10^3 , 10^4 conidia per 1ml and un-inoculated apparently healthy seeds). This showed that seed inoculum levels of T_1 and T_2 (10^6 and 10^5 conidia/1 ml) can cause maximum disease incidence. The histopathological results revealed that the location of seed borne fungi in the infected tomato seeds, *Alternaria alternata*, *Phoma* sp and *Aspergillus* spp., were confined to pericarp only, where as *Fusarium* sp. and *Alternaria solani* were noticed on other than pericarp also.

Keywords

Seed-borne, Tomato, Histo-pathology

Introduction

Seed-borne infections or infected seed is very important discouraging factor, which posses a serious problem in seed certification. Although infected seeds which may otherwise be viable with prescribed germinability as per certification standards, may not be acceptable as seed because of poor physical appearance, high incidence of seed-borne fungi and mycotoxin such as aflatoxin. Tomato (*Solanum lycopersicum* Mill.) is the most popular vegetable crop grown in the world, crop offers significant nutritional advantages, as it contains significant source of dietary lycopene, β-carotene, vitamin C and antioxidant properties in a low energy dense food (Britt and Kristin, 2011). Several human studies indicated a relationship between a high intake of tomato products and a decreased risk of several types of cancer, atherosclerosis and cardiovascular diseases (Cecilia *et al*., 2010). Recently, this crop is recognized as a model for plant-pathogen interactions (Arie*et al*., 2007). The infection of tomato seeds caused by economically important diseases like early blight, late blight, fusarium wilt, septoria leaf spot, damping off and fruit rot. Fungal pathogens may be externally or internally seed-borne, extra- or intra-embryal, or associated with the seeds as contaminants (Singh and Mathur, 2004). Other fungi, including saprophytes and very weak

pathogens, may lower seed's quality causing discoloration, which reduces the commercial value of the seeds (Al-Askar*et al*., 2012).

The pathogenicity test of seed-borne fungi, *Alternaria alternata, Fusarium solani* and *Fusarium oxysporum* on ten tomato cultivars. All the germinated seeds were infected by these fungi with varying degree of variability or aggressiveness and each cultivar significantly reduced germination and produced more abnormal seedlings compared to control. They also reported the transmission of *Alternaria alternata, F. solani*, and *F. oxysporum* from seeds to seedlings. The highest transmission was observed during germination stage followed by seedling stage on leaves and on stem (Hayat *et al.,* 2014).Shovan*et al.* (2008) pathogenicity test with 33 isolates of *Fusarium solani* under pot culture, all the tested isolates were found to be pathogenic on tomato seeds. Seed transmission studies helps to confirm the seed to plant transmission to prove the pathogenicity and also to design the suitable management strategy. Hence an attempt was made to know the mode of transmission and type of damage caused by viz., *A. solani, A. alternata and Fusarium* sp. under existing microclimatic conditions. Keeping these factors in view and considering their importance in present scenario of seed production, seed industry and quarantine, present investigation was undertaken.

Materials and Methods

Effect of different seed inoculum levels on disease incidence

This study was under taken by using apparently healthy seeds to know the effect of different seed inoculum densities and extent of disease incidence. *Alternaria solani, A. alternate* and *Fusarium* sp. isolated from naturally infected tomato seeds, was cultured on PDA, at 20 ± 2ºC for one week. The fungal propagules (mycelial bits) were isolated by taking a 0.5 cm disc using a cork borer. The discs were gently mixed with 5ml of sterile water by using a magnetic stirrer and propagules were adjusted to required concentration by using haemocytometer.

No of propagules in the diluted suspension/milliliter = Average no of propagules above one large square × 1ml / 0.004 mm3 (or)

No of propagules in the diluted suspension/milliliter = No of propagules counted × 250,000

This number has to be multiplied by the original dilution of the suspension to ascertain the density of original suspension in number of cells /ml (Karuna and Kolte, 2005).

Apparently healthy seeds of tomato cultivars were surface sterilized for 3 min with 0.5 % sodium hypochlorite solution, and washed in sterile distilled water. The sterilized seeds were soaked in different inoculum level under vacuum for 30 minutes, and dried at room temperature overnight. The seeds of control treatments were similarly treated except that they were soaked in sterile distilled water. Treatments were laid out in comlete randomized block design (CRD) with 4 replications. Ten seeds were sown in each pot and replicated 4 times in each treatment. The pots were incubated under glasshouse condition at 26 ± 2ºC. Observations were recorded on the rapidity of disease incidence in each treatment, every alternate days and the per cent disease incidence and per cent seed germination were calculated by using the formula,

$$\% \text{ disease incidence} = \frac{\text{No. of diseased plants /pot}}{\text{Total no. of plants /pot}} \times 100$$

$$\% \text{ seed germination} = \frac{\text{No. of seed germinated /pot}}{\text{Total no. of seed sown /pot}} \times 100$$

Histopathological studies

The location of fungi in seed was studied by employing component plating technique (Madene*t al*, 1975). Naturally infected tomato seed samples, of variety PKM-1 was used for the study. Twenty five seeds were washed four times with tap water then surface sterilized in one per cent sodium hypochlorite solution for one minutes. These seeds were again washed with sterile water and soaked in water for 1 hr and then the seeds were dissected aseptically using sterile needle and forceps. The pericarp was separated and other seed parts were difficult to separate they were fused together (*i.e.*, embryo endosperm and hilum). The separated parts were plated immediately before drying on potato dextrose agar plates. The plates were incubated at 20 ± 2ºC for seven days, the seed component were examined under stereoscopic binocular microscope for the presence of fungal structures in different seed parts.

Results and Discussion

Effect of inoculum levels, transmission studies and proving pathogenicity

Pot culture studies

The results of pot culture studies conducted to study the seed to plant transmission of *Alternaria solani, A. alternate* and *Fusarium* sp. revealed that apparently healthy and artificially inoculated tomato seeds exhibited severe reduction in per cent germination and disease incidence was noticed after 20DAS. The results of the experiment are in Table 1 to 3. In case of *A. solani*, seeds soaked in 2.4 × 10^6 and 2.4 × 10^5 conidia/ 1ml recorded maximum per cent seedling infection. The characteristic symptom *i.e.* brown to dark brown concentric rings (target board effect) was observed on older leaves at 30 DAS onwards. The maximum infection was observed in treatments T_1 and T_2 whereas lower infection was observed in T_3 and T_4 (seeds soaked in 2.4 × 10^4 and 2.4 × 10^3 conidia/ 1 ml respectively). In case of *A. alternate* seeds soaked in 2.12 × 10^6 and 2.12 × 10^5 conidia/ 1ml showed maximum per cent seedling infection. The characteristic symptom *i.e.* minute water soaked specks with yellow halo on leaves was recorded after 45 days of sowing. The maximum infection was in treatments T_1 and T_2 (seeds soaked in 2.12 × 10^6 and 2.12 × 10^5 conidia/ 1 ml concentration), whereas lower infection was recorded in T_3 and T_4 seeds soaked in 2.12 x 10^4 and 2.12 x 10^3conidia /1ml treatments, might be due to the increased level of toxin produced by the fungi.

This study reveals the significance of seed-borne fungal infection. Seeds with high dose of inoculum yielded more of disease incidence and the incidence was noticed upto10^{-3} and at 10^{-4}there was no significant visible symptoms, indicating that 10^{-3}*i.e.*, conidial concentration of ($2.4x10^4$) is the minimum threshold inoculum required for causing the disease under Dharwad conditions. Similar result was obtained by Hayat *et al.* (2014).

In case of *Fusarium* seeds soaked in 3.25 x 10^6 conidia/1ml and 3.25 x 10^5 conidia/1ml

concentration showed least germination percentage but germination per cent increased with corresponding decrease in concentration of fungal propagules. Wilting of seedlings was noticed after 20 DAS. The maximum wilt incidence was recorded in treatments T_1, T_2 and T_3 with $3.25x10^6$, $3.25x10^5$ and 3.25×10^4 conidia/1ml. Wilt incidence was also recorded upto T_4 and also in T_5 (Apparently healthy seeds) this might be because of traces level infection of the pathogen in apparently healthy seeds and also there was no inoculum available for infection through sterilized soil. Similar observation was obtained by Abdulaziz *et al*., (2014).

Table.1 Effect of seed inoculum levels of *Alternaria solani* on per cent germination and seedling infection under pot culture

Treatments	Per cent	
	Seedling infection	**Seed germination**
T_1- Seeds soaked in 2.4×10^6 conidia/1 ml	82.75 (65.47)*	37.50 (37.73)
T_2-Seeds soaked in 2.4×10^5 conidia /1 ml	78.25 (62.21)	47.50 (43.56)
T_3-Seeds soaked in 2.4×10^4 conidia /1 ml	62.50 (52.27)	57.50 (49.33)
T_4- Seeds soaked in 2.4×10^3 conidia /1 ml	42.50 (40.67)	82.00 (65.01)
T_5-Un-inoculated (Apparently healthy seeds)	22.50 (28.23)	97.00 (80.67)
S.Em±	**1.22**	**1.62**
CD at 1%	**5.08**	**6.77**

*Figures in parentheses indicates arcsine transformed values

Table.2 Effect of seed inoculum levels of *A. alternata* on per cent germination and seedling infection under pot culture

Treatments	Per cent	
	Seedling infection	**Seed germination**
T_1-Seeds soaked in 2.12×10^6 conidia /1 ml	66.67 (54.37) *	37.50 (37.72)
T_2-Seeds soaked in 2.12×10^5 conidia /1 ml	62.49 (52.33)	48.75 (44.30)
T_3-Seeds soaked in 2.12×10^4 conidia /1 ml	45.83 (42.59)	56.66 (53.77)
T_4 -Seeds soaked in 2.12×10^3 conidia /1 ml	41.58 (40.10)	83.08 (65.75)
T_5- Un-inoculated (Apparently healthy seeds)	15.25 (22.99)	80.25 (63.65)
S.E.m±	**2.36**	**2.16**
CD at 1%	**9.84**	**9.00**

*Figures in parentheses indicates arcsine transformed values

Table.3 Effect of seed inoculum levels of *Fusarium* sp. on per cent germination and wilt incidence under pot culture

Treatments	Per cent	
	Wilt incidence	**Seed germination**
T_1- Seeds soaked in 3.25×10^6 conidia /1 ml	99.25 (87.51)*	2.50 (9.05)
T_2- Seeds soaked in 3.25×10^5 conidia /1 ml	97.00 (80.17)	32.50 (34.72)
T_3- Seeds soaked in 3.25×10^4 conidia /1 ml	87.50 (69.53)	67.50 (55.28)
T_4- Seeds soaked in 3.25×10^3 conidia /1 ml	62.50 (52.27)	85.00 (67.50)
T_5- Un-inoculated (Apparently healthy seeds)	18.50 (25.42)	100.00 (90.00)
S.Em±	**1.73**	**1.44**
CD at 1%	**7.19**	**5.99**

*Figures in parentheses indicates arcsine transformed values

Location of fungi in seed

Results of the component plating technique to know the location of seed borne fungi in infected samples, revealed the presence of *A. alternata*, *Phoma* sp. and *Aspergillus* sp. in the pericarp only. Similar result was obtained by Saad *et al.* (1988).Whereas *Fusarium* sp. and *A. solani* were noticed on other than pericarp also *i.e.*, endosperm embryo and hilum. These results indicate the requirement of systemic seed dressing molecules (like combi-products) for the effective control of the pathogen as the molecules can penetrate up to the embryo. Histopathological studies conducted by Thippeswamy*et al*. (2006) who studied the location and transmission of *Fusarium oxysporum* and *Alternaria solani* in naturally infected tomato seeds. The results revealed that both pathogens were located in seed coat, cotyledons and in embryonic axis of tomato seedlings at various concentrations.

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