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"Tomato seed mycoflora of Vidarbha region"

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Abstract: The tomato has many medicinal uses. The pulp and juice of the fruit is degestiable and mild aperients, a promoter of gastric secretion and a blood purifier. It is also considered to be an intestinal antiseptic as it has a cleaning effect in the enteric portion of the alimentary canal. It is also said to be useful in canker of the mouth "nurses sore mouth" etc. it stimulate torpid liver and is good in chronic dyspepsia. Fresh edible portion of tomato contain 94.1% water, 1 % protein, 0.3 % fat, 4.0 % carbohydrate, 0.6% fibre, 0.3% Nicotonic acid, 0.13% Pantothenic acid, 23 mgm/100 gm Vitamin C, 0.27% Vitamin E, 0.004% Biotin, 150 mgm/100 mg Malic acid, 390 mgm/100 mg Citric acid, 7.5 mgm/100 gm Oxalic acid respectively. Tomato is an economically important vegetable crop, suffering from many fungal diseases. Seed fungal mycoflora are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. Infected seed plays a key role in the dissemination of plant pathogens and disease establishment, they are carriers of some important seed-borne diseases caused by microorganisms which results in considerable losses in yields. Some of the seed-borne fungi were found to be very destructive, caused seed rot, and decreased seeds germination. Also, cause pre and post germination death. Seed-borne fungi are, however, easily controlled compared to air-borne or soil-borne fungi. There is no much work on seed-borne fungi from Vidarbha region, hence present crop is taken for detailed study.
Key words: Tomato seed, mycoflora, Vidarbha region.

Tomato (*Lycopersicon esculentum* L.**)** is a well known and very popular vegetable grown successfully throughout Vidarbha region of Maharashtra State, belonging to the family Solanaceae. The fruit is available in cities almost all the year around. It is cooked as a vegetable alone or mixed with potato and brinjal. When it is ripe, it is also taken as raw or it's made into salads, soups, preserves, pickles, sauce, ketchups and many other products. The popularity of tomato and its products increasing day to day due to its high nutritive value. The districts viz., Nagpur, Chandrapur and Akola are well known for their extensive cultivation.

The tomato has many medicinal uses. The pulp and juice of the fruit is degestiable and mild aperients, a promoter of gastric secretion and a blood purifier. It is also considered to be an intestinal antiseptic as it has a cleaning effect in the enteric portion of the alimentary canal. It is also said to be useful in canker of the mouth "nurses sore mouth" etc. it stimulate torpid liver and is good in chronic dyspepsia. Fresh edible portion of tomato contain 94.1% water, 1 % protein, 0.3 % fat, 4.0 % carbohydrate, 0.6% fibre, 0.3% Nicotonic acid, 0.13% Pantothenic acid, 23 mgm/100 gm Vitamin C, 0.27% Vitamin E, 0.004% Biotin, 150 mgm/100 mg Malic acid, 390 mgm/100 mg Citric acid, 7.5 mgm/100 gm Oxalic acid respectively. Tomato is an economically important vegetable crop, suffering from many fungal diseases[1]. Seed fungal mycoflora are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. Infected

seed plays a key role in the dissemination of plant pathogens and disease establishment, they are carriers of some important seed-borne diseases caused by microorganisms which results in considerable losses in yields. Some of the seed-borne fungi were found to be very destructive, caused seed rot, and decreased seeds germination. Also, cause pre and post germination death[2,3]. Seed-borne fungi are, however, easily controlled compared to air-borne or soil-borne fungi[4]. There is no much work on seed-borne fungi from Vidarbha region, hence present crop is taken for detailed study.

Report on the seed mycoflora of Tomato

Jamison reported the *Phoma destructiva* on tomato seed which cause fruit rot of tomato [5]. Crossier reported that *Phytophthora infestans* harboued in seed coat from which it may transmit cause heavy losses in tomato[6].

Kendrik reported seed-borne nature of *Fusarium oxysporum*, which cause wilt of tomato [7]. Younkin noted the pathological condition of fruits of tomato wilt where they found *Colletotrichum phomoides* consistently associated [8]. Rudolph reported the fungus *Verticillium sp*. on seeds of tomato, the infected seeds associated with fungus did not germinate [9]. Gattani and Kaul reported *Pythium aphanidermatum* causing rotting of seeds of tomato and often severe pre- and post emergence damping-off . Wavde studied the seed mycoflora of tomato and isolated species of *Alternaria, Aspergillus, Curvularia, Rhizoctonia* and *Rhizopus* as internally seed-borne fungi[10]. Harne and Nema isolated *Alternaria tenuis, Aspergillus niger, Curvularia lunata, Oospora lactis parasitica, Rhizoctonia bataticola* and *Rhizopus nigricans* from surface sterilized tomato seeds [11]. Fruit rot in tomato crop causes heavy damage and resulted in losses of tomato yield. Many seed-borne fungi were responsible for causing tomato fruit rot. *Trichothecium* fruit rot caused by *Trichothecium roseum* [12], *Gibberelella* fruit rot caused by *Gibberelella persicaria* [13], *Rhizoctonia* fruit rot caused by *Rhizoctonia solani* [14]. Sharma and Sohi noticed the *Phytophthora parasitica* causing buckeye rot and blossom blight of tomato [15]. They critically studied the symptoms of the disease and morphology of the seed-borne organisms viz., *Geotrichum candidum, Colletotrichum phomoides, Phoma destructiva, Phytophthora parasitica* and bacteria were responsible for causing damage to tomato fruit in storage whereas *Fusarium sp. and Rhizoctonia solani* were the chance contaminants causing negligible loss. Surynarayana enlisted seed-borne fungal diseases of tomato viz., damping-off (*Pythium* sp. and *Phytophthora* sp.), early blight (*Alternaria solani*), fruit rot (*Phytophthora* sp.) and *Phoma* rot (*Phoma destructiva*) [4]. Besei reported *Fusarium oxysporum f.* sp. *lycopersici* which remain viable in dried pulp fragment on the surface of the tomato seeds for many years [16]. Raicu and Stan isolated *Phytophthora parasitica*, *Pythium sp.* and *Rhizoctonia sp.* from tomato seeds and described as the pathogens of seed rot and damping-off of tomato seedlings [17].

Verma isolated *Pythium inflatum* from fields of tomato, causing pre-and post-emergence, damping-off and had serious effects on the growing crop. He had confirmed the pathogenecity of fungus [18]. Khulbe and Sati isolated first time *Alternaria raphani* from tomato seeds [19]. Tomato seed infected with *Fusarium semitectum* showed loss in germination and seedling vigour [20]. *F. solani* produces post harvest soft rot [21]. *Phytophthora infestans*, the late blight fungus, produced a loss of upto 40% in Baden, Germany [22].

There are reports where seed-borne organisms cause post harvest rot of tomato fruits eg. *Fusarium oxysporum* produces decayed water soaked area with white or pinkish mould growth on the lesions [23]. *Cladosporium* sp., produce sunken and jet black lesions on tomato fruits [24], *Alternaria alternata* produces black or brown lesion which reduces the market value of the fruits [25, 26], *Rhizopus* sp., also produce water soaked discolored lesion on tomato [27].

Materials and Methods

Collection of seed samples

Field survey on Tomato (*Lycopersicon esculentum* L.) was made in the year 2009-10 and 10 seed samples of each crop were collected from different localities and different farmer's fields of Vidarbha region. The collected seed samples were dried in sunlight to bring down the safe storage seed moisture and stored in cloth bags at room temperature for further use.

Prior to study of the seed-borne fungal flora, it was necessary to study the germination percentage of the collected seeds samples. For this, roll-towel technique was used [28]. The germination papers were washed with 1 % hydrochloric acid to remove the impurities and any contamination. The papers were thoroughly washed with distilled water. Four hundred seeds from each sample were placed with the help of sterilized forceps, on a sheet of damp paper toweling, covered by another layer, the lower 5 cm turned over and whole sheet rolled up secured by elastic bands. The rolls were incubated at 27°C in incubator. Seeds were watered regularly when required. The seed germination counts were taken on 10^{th} day. The seedlings were classified into normal and abnormal whereas ungerminated seeds were classified into dead seeds. Only normal seedlings were considered for standard germination.

Isolation and Identification of seed borne fungi:

The surface mycoflora of selected seeds were isolated by blotter paper test as well as agar plate method as recommended by International Seed Testing Association ISTA [29, 30].

Standard blotter paper method

This is the very simple, most convenient and efficient of all the incubation methods. Doyer was first and then De Temp adopts blotter paper method in seed health management. A pair of sterile white blotter papers of 8.5 cm diameter was soaked in sterile distilled water and was placed in pre-sterilized petriplates of 90 mm diameter. Ten seeds of test sample per petriplate were then placed at equal distance on moist blotter. 400 seeds were used in each experiment. The plates were incubated at 27°± 2°C under diurnal conditions. On seventh day of incubation, seeds were first examined under stereoscopic microscope for determining the various fungal growths. The fungi were isolated, purified and subculture maintained on Czapek-Dox medium for further studies.The identification and further confirmation of seed borne fungi was made by preparing slides of the fungi.

Agar plate method

In Northern Ireland, Musket first used this method for seed health management [28]. In this method, pre sterilized petriplates were poured with 15 mL of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described for blotter test method.

The fungi occurring on seeds plated on moist blotter paper and agar plate were preliminary identified on the basis of sporulation characters. Detail examination of fungal characters was done by using compound microscope and identification was confirmed [33,34.]

Total percentage of fungal incidence of fungi was calculated by using the following formula,

$$\text{Percentage Frequency} = \frac{\text{Total no. of seeds in which a particular fungus appeared}}{\text{Total no. of seeds studied}} \times 100$$

Observations and Results

Germination capacity or viability of seeds:

The data on seed germination of freshly collected seed samples of Tomato from different localities of Vidarbha region is presented in (Table-1).

In Seeds of Tomato, the maximum 73% germination recorded in T7 and minimum germination 56% in T5. Remaining seeds showed germination ranging from 59% to 71.5%.

Tomato (*Lycopersicon esculentum* L.) seeds:

Table-1: Percentage germination of freshly collected seed samples of Tomato from various locations of Vidarbha region.

Place of collection	Sample No.	% Germination
Khapri	T1	71.5
Katol road	T2	64.5
Akola	**T3**	**60.5**
Pardi	**T4**	**59.0**
Kalmeshwar	**T5***	**56.0**
Chandrapur	T6	64.0
Kamptee	T7	73.0
Amravati	T8	62.5
Wardhamna	T9	64.0
Wardha	T10	65.0
S.E.±		1.55
C.D at p=0.01		5.75
C.D at p=0.05		3.79

***Data based on mean of 100 seeds (each sample in four replicates).**

Fungi like *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium fulvum, Colletotrichum capsici, Curvularia lunata, Fusarium moniliforme, F. lycopersici, Penicillium oxalicum, Rhizopus stolonifer* and *Syncephalastrum* sp. were recorded by both standard blotter as well as agar plate method.

However, *Aspergillus ochraceus, A. sulphureus, Curvularia* sp. *Fusarium solani* and *Penicillium* sp. were isolated by agar test only where as *Cercospora capsici, Chaetomium globosum, Drechslera* sp. *Phytophthora infestans* and *Trichothecium roseum* were restricted to blotter test only.

Among these fungi, *Alternaria porri, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium fulvum, Peniciliium oxalicum, Rhizoctonia solani, Rhizopus stolonifer* and *Rhizopus sp.* were found to be most dominant fungi on tomato seeds ranged their frequency from 9 to 18%.

Some of the fungi were recorded in frequency ranged from 5 to 8%, on tomato seeds. These fungi were *Alternaria solani, Aspergillus sulphureus, Colletotrichum capsici, Curvularia lunata, Fusarium moniliforme, Helminthosporium* sp. *Phytophthora infestans* and *Syncephalastrum* sp. However, fungi like *Aspergillus ochrcious, Botrytis cinera, Cercospora capsici, Chaetomium globosum, Curvularia* sp., *Drechslera* sp., *Fusarium solani, F. lycopersici, Paecilomyces* sp., *Peniciliium sp., Pythium* sp. and *Trichothecium roseum* were occurred in low frequency ranged from 0.5 to 4.5% only.

Table-2: Isolation of seed borne fungi from the seeds of Tomato, Chilli and Brinjal using Standard blotter and Agar plate method.

Sr N.	Fungal isolates	Frequency of fungal incidence					
		Tomato					
		T5		T4		T3	
		B	A	B	A	B	A
1	*Acremonium* sp.	-	-	-	-	-	-
2	*Alternaria porri* (Ell.)	9.5	5.0	8.0	4.0	5.0	4.0
3	*A. solani* (Ellis & Mart.)	7.5	6.0	5.5	2.5	7.5	4.5
4	*Alternaria* sp.	-	-	-	-	-	-
5	*Aspergillus amstelodami* M.	-	-	-	-	-	-
6	*A. flavus* Link	9.0	5.5	7.5	5.5	5.5	5.0
7	*A. fumigatus* Fres	15.0	7.5	13.5	5.0	8.5	8.0
8	*A. nidulans* (Eidam) winter	-	-	-	-	-	-

9	*A. niger* Van Tieghan	18.5	7.5	17.5	8.0	15.0	7.5
10	*A. terreus* Thom	-	-	-	-	-	-
11	*A. ochraceus* Wihelm	-	2.0	-	-	-	-
12	*A. sulphureus* (Fresenius)	-	5.0	-	1.0	-	-
13	*Beltrania* sp.	-	-	-	-	-	-
14	*Botrytis cinera* Pers.	2.5	1.5	2.5	0.5	1.5	1.5
15	*Cercospora capsici* Heald.	2.5	-	1.0	-	-	-
16	*Chaetomium globosum* Knz	4.5	2.5	3.5	-	3.0	-
17	*Cladosporium fulvum* Link	14.0	3.5	9.0	2.5	2.5	2.0
18	*Colletotrichum capsici* Syd.	5.5	3.0	6.0	2.5	5.0	2.0
19	*Curvularia lunata* Bat.	6.0	2.0	4.0	3.0	4.0	3.0
20	*Curvularia* sp.	-	2.5	-	2.0	-	0.5
21	*Drechslera* sp.	2	-	-	-	2	-
22	*Fusarium moniliforme* Seld	4.5	6.5	4.0	2.0	2.5	2.5
23	*F. oxysporum* Schlecht	-	-	-	-	-	-
24	*F. semitectum* Berk & Rav.	-	-	-	-	-	-
25	*F. solani* (Mart.)	-	4.0	-	2.0	-	2.0
26	*F. lycopersici* Brushi	4.0	1.0	3.5	3.0	2.5	1.0
27	*Helminthosporium* sp.	5.5	-	-	4.5	-	-
28	*Mucor* sp.	-	-	-	-	-	-
29	*Neurospora* sp.	-	-	-	-	-	-
30	*Paecilomyces* sp.	3.5	0.5	-	1.0	-	-
31	*Penicillium oxalicum* Curr.	15.5	2.5	2.5	3.5	4.0	2.0
32	*Penicillium* sp.	3.0	-	-	2.5	-	2.0
33	*Phoma* sp.	-	-	-	-	-	-
34	*Phytophthora infestans* De.	-	-	5.5	-	-	-
35	*Pythium* sp.	2.5	0.5	3.5	-	-	-
36	*Rhizoctonia solani* Kuehn.	18.0	-	14.0	-	14.0	-
37	*Rhizopus stolonifer* Ehrarb.	18.0	4.0	11.0	2.5	10.0	2.5
38	*Rhizopus* sp.	14.0	3.5	12.0	4.0	6.0	-
39	*Sporotrichum* sp.	-	-	-	-	-	-
40	*Syncephalastrum* sp.	7.5	2.0	4.5	3.0	4.5	4.0
41	*Trichothecium roseum* Link	3.0	-	4.5	-	4.5	-
S.E.±		1.16	0.45	0.97	0.37	0.87	0.51
C.D at p=0.01		3.25	1.27	2.76	1.05	2.50	1.48
C.D at p=0.05		2.40	0.93	2.02	0.77	1.82	1.08

Discussion and Conclusions

Vegetable seed is an important constituent in day to day life and seed lots used from stored condition are susceptible to fungal attack. Many specific chemicals substances needed by our body for growth and maintenance of health are attained through important constituents like protein, carbohydrates, fats, vitamins and minerals. The fungi bring about an appreciable deterioration in nutritive potency of the seeds. The secretion of certain toxic metabolites by fungi in the seed commodities creates an alarming situation with regard to their consumption by the human beings. To overcome this situation various control measures have been developed. With this end view in mind, the experiments conducted to study the presence of seed-borne fungi on Tomato, Chilli and Brinjal seeds from Vidarbha region of Maharashtra State.

In the present investigation, the seed samples of Tomato had been collected from different localities of Vidarbha region and tested for their germination capacity following roll-towel technique [30] and percent seed germination for each sample was recorded (Table-1). The minimum germination recorded for Tomato was 56% in T5, 59% in T4 and 60.5% in T3. This indicated that the seed germination percentage for all seed samples was below 75%. Early workers as Hicks, advocated standard germination 85-90% for Tomato seeds [35]. Mc Carthy, recommended as standard of germination 85% for Tomato seeds respectively[36]. Musson prescribed a

germination standard of 90% for Tomato[37]. Bosewell studied tomato and nine other vegetable seeds for viability, they recorded an initial germination of 93% in tomato and prescribed 80% as standard viability[38]. The data published by ISTA suggested, the standard germination 70% for tomato seeds[39].

From the seed germination study, it has been concluded that the seed germination of the collected seed samples of Tomato was below the standard.

Seed mycoflora

Seeds play a vital role in the production of healthy crops. They are known to carry pathogens which cause heavy yield losses. In the present investigation the collected seeds samples were used for the isolation of fungal flora. Internal seed-borne flora isolated by agar plate method whereas externally seed-borne flora was isolated by blotter method (ISTA)[29].

Tomato (*Lycopersicon esculentum* L.) seeds:

Tomato seeds exhibited the association for twenty nine fungi (Table-1). Fungi like *Alternaria porri, A. solani, Aspergillus flavus, A. fumigatus, A. niger, Botrytis cinera, Cladosporium fulvum, Colletotrichum capsici, Curvularia lunata, Fusarium moniliforme, F. lycopersici, Penicillium oxalicum, Rhizopus stolonifer* and *Syncephalastrum* sp. were found to be most dominant fungi on tomato seeds ranged their frequency from 9 to 18%. Orlava studied the mycoflora of tomato seeds grown in the Moscow area[40]. Of 23 sp. of fungi, 15 were isolated from the seed surface and 8 from the internal parts. *Penicillium* and *Aspergillus* sp. predominated on surface. *Alternaria, Chaetomium, Ulocladium* and *Fusarium sp.* (*F. culmorum, F. gibbosum, F. equiseta* and *F. solani*) were most frequent causing internal infections. They studied the pathogenecity of 3 sp. of *Fusarium* isolated from tomato seeds and observed that pathogenecity was higher when tomato seeds were dipped in spore suspension for 1 hour before sowing. Naseema obtained *Aspergillus niger* and *Rhizopus stolonifer* from the tomato seeds as an internally seed-borne fungi[41]. Saifutdinova observed that root rot of tomato glasshouse crop in Tashkent district are mainly caused by seed-borne fungi, *Fusarium sp*. and *Rhizoctonia sp*[42]. Vartanian reported *Phytophthora infestans* in discoloured seeds of tomato[43]. In these seeds, hyphae were observed on and in the seed coat, in the remnants of funiculus and between the endosperm and the seed coat. *Phytophthora infestans* was most common fungus causing 95% loss of tomato. Ram Nath reported *Fusarium solani* from tomato seeds [44].

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