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Identification and isolation of different *Colletotrichum* spp. along with other fungi associated with chilli seeds collected from infected fruits

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Abstract
The study was undertaken to evaluate Identify the different *colletotrichum* spp. along with other fungi associated with chilli seeds collected from infected fruits. Each sample was categorized in five groups deformities (Shriveled) and discoloration (black and brown) were noticed in all the samples. Maximum deformity, in the form of shriveling (12.87%) was observed in sample 'B' followed by sample 'C' (12.32%) and it was minimum in sample 'F' (1.30%). Discoloration in the form of black / brown was maximum in sample 'B' (6.80% / 4.70%), while it was minimum in the sample 'F' (0.90% / 0.50%). Maximum mechanically damaged (3.32%) seeds occurred in sample 'B' and they were minimum in sample 'F' (0.60%). Impurities in the form of plant debris' and inert material were observed in all the samples. Maximum apparently healthy seeds were observed in sample 'F' (96.62%) and it was minimum (65.79%) in sample 'B'.

Keywords: *Colletotrichum* spp., *Fusarium* spp., *Rhizopus* spp., discoloration

Introduction
Chilli (*Capsicum annuum* L.) commonly known as "Mirchi" or "Mirch" is an important spice and cash crop of India belongs to the family Solanaceae and is a native of tropical America and west India. Chilli is a perennial sub-shrub produces fruit in groups. Chilli is a self as well as cross pollinated crop and mature fruits are red in colour. Chilli fruits has importance in our daily diet and is especially liked for its pungency and spicy taste. The pungency of fruit due to the presence of a crystalline volatile alkaloid called capsaicin. It is also used to flavour, soups, stews, sauces, chutney, salad for preparing pickle curry, samber, rasam and other savoury dishes. Extracts of chillies are used in the production of ginger beers and other beverages. It has several medicinal properties and also used as counter irritants in lumbago, neuralgia, rheumatic disorders and also useful in atomic dyspepsia (Pruthi, 1993) [26]. Its paste is externally used as a rubefacient and as a local stimulant for the tonsil and in tonsillitis (Nadkarni, 1972) [23].

Chilli is an important and widely grown cash crop in India and it cultivated in largest area of the world with the production over one million tonnes (anonymous, 1999) [2]. In Rajasthan, chilli cultivated in its all regions with an average yield 10.8 q / ha. Amongst different chilli growing region, Jaipur region (Ajmer, Jaipur, Dausa, Sikar and Jhunjhunu) standes 4th in its area and production with an average yield 8.05 q / ha and which is less in comparison to state average yield (10.83 q / ha) as well as average yield of Jodhpur (10.18 q / ha), kota (13.62 q / ha) and Bharatpur (22.25 q / ha) region (anonymous, 2003-04) [3].

One of the important factors which limits the production in Jaipur region and specially in its, Ajmer, Sikar, Dausa, Jhunjhunu districts in comparison to other region is the poor health of seeds where most of the farmers generally used the seed collected from stored ripe red fruits infected with rots for raising the seedlings in nurseries and that take heavy toll of the crop at all the stages right from sowing to harvest and also in transit, marketing and storage. Chilli suffers from several seed borne fungal fruit rot disease *viz.*, Alternaria rot, Alternaria fruit rot, Anthracnose, Aspergillus rot, black rot, Black fruit rot, Die-back, Ripe fruit rot, *Cladosporium* fruit rot, Charcoal fruit rot, grey mold rot, Phytophthora and Rhizopus rot caused by *Alternaria alternata, A. solani, Alternaria tenuis, Aureobasidium pullulans, A. capsici annuum, Aspergillus niger, Botrytis cinerea, Brachysporium senegalensis, Cladosporium herbarum, Colletotrichum* and *P. parasitica*, respectively (Sultana and Khan, 1988; Mirdha and Siddiqui,

1989; Sultan *et al.*, 1992; Basak, 1994; Basak *et al.*, 1991; Datar, 1995; Dhyani *et al.*, 1990; Prabhavathy and Reddy, 1995; Kumar and Mahmood, 1986; Raut *et al.*, 1989, Rout and Rath, 1972, Kulshrestha *et al*., 1976 and Suryawanshi and Deokar, 2000, respectively) [34, 35, 5, 7, 11, 13,24, 18, 8, 29, 17, 36].

Among different rots reported on chilli fruits, ripe fruit rot disease (*Colletotrichum* spp.) was alone reported to be a serious and destructive disease of chilli and it causes 12 to 25% and 50% loss in the crop by infecting them right from its each cropping stage *i.e.* seedling, flowering, fruiting, maturity and storage to transit, marketing, storage of the fruit under suitable environmental condition (Singh, 1999 and McColloch *et al.* 1968, respectively) [32, 21]. Literature search revealed that no systematic seed pathological study has been conducted on seed borne inoculum of *Colletotrichum* in chilli seeds. Therefore, it was considered imperative to investigate, the following aspects.

Materials and Methods

Collection of seed samples

Six seed samples were collected from Jaipur region. Out of six, first five samples were collected from farmers houses belongs to Ajmer, Dausa, Jaipur, Sikar and Jhunjhunu districts which comes under Jaipur region (Table 1). From each district 10-12 seed samples were collected from chilli fruits stored by farmers belong to 5-10 km away from district head quarter in different geographical directions where chilli is generally cultivated and stored by using old traditional practices under variable environment conditions. At each district headquarter all collected seed samples were mixed to represented a composite sample of particular district. Sixth sample was collected from Department of Horticulture, Agricultural Research Station (ARS), Durgapura where chilli crop cultivated using improved cultural practices. Seed samples was collected 2-3 weeks before sowing in nurseries. These collected samples were kept in cloth bags, brought to the laboratory and stored at 10 °C temperature for further studies. Sampling was done by the method suggested in ISTA, 1976 [15].

Table 1: Place of samples collection in Jaipur region and their code number

S. No.	District	Code No.
1.	Ajmer	A
2.	Dausa	B
3.	Jaipur	C
4.	Jhunjhunu	D
5.	Sikar	E
6.	Department of Horticulture,	F
	ARS, Durgapura	

Examination of dry seeds

The method suggested by Agarwal and Sinclair (1987) with 20 g seed from each sample were taken at random and divided into 4 fractions (5 g per fraction). Each fraction was spread on bottom of a Petri-dish and examined with the help of a hand-lens or if required under stereo-binocular microscopes. The inspected materials were categorized as follows:

Deformed seed: Shrivelled

Discoloured seeds

a) Black
b) Brown

Damaged seeds: Mechanically

Impurities

a) Plant debris (pieces of petiole and fruit)
b) Inert material

Apparently healthy

Seeds impurities of each category were polled separately and weight on modern electric balance and per cent contain by weight was calculated.

Incidence of *Colletotrichum* spp. in particular category will also be recorded by using standard incubation methods.

Seed Washing Test

The Seed Washing Test suggested by Agarwal and Sinclair (1987) [1] was followed with slight modification. From each sample 200 seeds taken at random were divided into two groups of 100 seeds and were immersed in 20 ml distilled water in 100 ml. conical flask and shaken for 15 minutes on a mechanical shaker. The seeds were discarded and the liquid was centrifuged at 2500 rpm for 15 minutes. The supernatant was also discarded and sediment was re suspended in 2 ml of lacto phenol. A drop of suspension was placed at the center of the haemocytometer and spores of different fungi were counted in 10 squares, chosen at random under stereo binocular microscope. Spore load per seed was calculated by using following formula:

$$\text{Spore load per seed} = \frac{N \times V}{X \times n}$$

Where,

N = Total number of spores counted / number of squares

X = Volume of mounting solution between the cover glass and above the square covered (area of squares × depth of chamber)

V = Volume of the mounting fluid added to the sediment and

n = Number of seeds washed.

Isolation of mycoflora associated with chilli seeds

For isolation of external and internal mycoflora of chilli seeds, two inoculation methods *viz*., Blotter Method and Agar Plate Method (ISTA, 1976) [15] were used, respectively.

Blotter Method

From each sample four hundred seeds selected at random were analysed. Blotting papers were cut into circles of 10 cm diameter and sterilized at 1.045 kg/cm^2 for 15 minutes. Three circles of blotting papers were placed at the bottom of sterilized Petri-dishes aseptically and moistened by sterilized distilled water. Twenty seeds were placed at an equal distance in each Petri-dish. These dishes were incubated at 22°±1 °C with 12 hours of light alternating with 12 hours of dark period. The seeds were examined on 7th day of incubation.

Agar Plate Method

Two hundred seeds from each of the sample selected at random were taken for isolation of internally seed-borne mycoflora. Seeds were surface sterilized with 0.1 percent mercuric chloride solution for 1-2 minutes followed by 3 washing with sterilized distilled water. Sterilized Petri-dishes, each containing 20 ml. Potato Dextrose Agar medium were used for incubation of seeds. Ten seeds per Petri-dish were

equi spaced aseptically and incubated at 22±1 °C with 12 hours of light alternating with 12 hours of dark period. The fungal colonies emanating from seeds were examined from 3rd to 7th day of incubation.
Isolation of mycoflora from chilli seeds was carried out and maintained on 2 per cent Potato Dextrose Agar medium. Observations on per cent incidence of seed mycoflora were recorded in both Blotter and Agar Plate Method.

Seedling Symptom Test
In order to observe symptoms of disease incited by seed-borne *Colletotrichum* sp. and other fungi associated with seeds in the seedling stage, two methods *viz*; (a) Soil Test and (b) Agar Test in Test Tubes were employed for this purpose with slight modification.

Soil Test
One hundred seeds of each sample taken at random were tested. The seedlings were raised in 30 cm plastic pots. The pots were sterilized with 0.1 per cent mercuric chloride for 2-3 minutes followed by 3 washing with sterilized water and filled with sterilized soil (Soil: FYM = 3.1, autoclaved at 1.045 kg.cm^2 for 1 hour for 3 consecutive days). Five seeds were sown at equal distance in each pot (5 seeds/pot × 20). After sowing, pots were kept in a cage house where the temperature ranged from 20 °C to 25 °C. The pots were watered as and when required. After 20-30 days of sowing, seedlings were examined for appearance of disease symptoms.

Agar Test in Test Tubes
One hundred seeds of each sample selected at random were tested. The seedlings were raised in 160 × 16 mm test tubes, each containing 10 ml of 1 per cent Water Agar (10 g agar in 1000 ml distilled water) and plugged with a loose cotton plug and sterilized at 1.045 kg / cm^2. One seed was placed in each test tube and incubated at 22±1 °C under alternating cycle of 12 hours light and 12 hours darkness. After 10 to15 days of incubation, seedlings were examined for symptoms.
The infected plant tissues (in both the methods i.e., Soil Test and Agar Test in Test Tube) were collected, surface sterilized and placed on Potato Dextrose Agar medium for isolation of the fungi associated.

Result and discussion
Examination of dry seeds
Each sample was categorized in five groups (Table-2). Deformities (Shriveled) and discoloration (black and brown) were noticed in all the samples. Maximum deformity, in the form of shriveling (12.87%) was observed in sample 'B' followed by sample 'C' (12.32%) and it was minimum in sample 'F' (1.30%). Discoloration in the form of black / brown was maximum in sample 'B' (6.80% / 4.70%), while it was minimum in the sample 'F' (0.90% / 0.50%). Maximum mechanically damaged (3.32%) seeds occurred in sample 'B' and they were minimum in sample 'F' (0.60%). Impurities in the form of plant debris' and inert material were observed in all the samples. Maximum apparently healthy seeds were observed in sample F (96.62%) and it was minimum (65.79%) in sample 'B'

Table 2: Seed abnormalities and impurities in six chilli seed samples

S. No.	Categories of seed / impurities	Per cent content (by weight basis) / sample*					
		A	B	C	D	E	F
1.	**Deformed seed**						
a.	Shriveled	10.45	12.87	12.32	9.71	10.7	1.30
2.	**Discoloured seeds**						
a.	Black	3.85	6.80	5.49	3.67	4.09	0.90
b.	brown	3.20	4.70	3.92	2.94	3.46	0.50
3.	**Damaged seeds**						
a.	Mechanically	1.50	3.32	2.87	1.24	2.37	0.60
4.	**Impurities**						
a.	Plant debris (pieces of petiole and fruit)	0.95	1.28	1.26	0.87	1.17	0.05
b.	Inert material (stone and sand)	3.70	5.24	4.62	3.11	4.42	0.03
5.	Apparently healthy	76.35	65.79	69.52	78.46	73.79	96.62

*Average of 4 replications (5g seeds / replication)

Fungal Spore Load
Seeds of different samples tested in Seed Washing Test revealed the presence of spores of *Alternaria* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp. and *Rhizopus* sp. on their tests (Table - 3). All the above-mentioned fungi, were observed in all the six samples.

The total spore load / seed in various samples ranged from 3.25 x 10^3 to 12.0 x 10^3. The range of spore load/seed was observed maximum in sample `B' (12.0 x 10^3) followed by 'C' (9.0 x 10^3) 'A' (8.75 x 10^3) and 'E' / 'D' (7.00 x 10^3) and it was minimum in sample 'F' (3.25 x 10^3).

Table 3: Spore load of different chilli samples detected by Seed Washing Test

Sample no.	Number of spores / seed (× 10^3)*					Total spore load (×10^3)
	Alternaria sp.	*Aspergillus* sp.	*Colletotrichum* sp.	*Fusarium* sp.	*Rhizopus* sp.	
A	2.50	3.00	1.00	0.25	2.00	8.75
B	3.00	4.00	1.50	0.50	3.00	12.00
C	2.00	3.00	1.00	0.50	2.50	9.00
D	2.00	2.00	0.50	1.00	1.50	7.00
E	2.00	2.00	0.50	1.00	1.50	7.00
F	1.00	1.00	0.25	0.25	0.75	3.25
Average	2.08	2.50	0.79	0.58	1.87	

*Average of (100 × 2) 200 seeds.

Mycoflora of chilli seeds

Blotter method

Six fungal species belonging to five genera were isolated from chilli seeds (Table- 4). Fungi and their respective per cent incidence were *Alternaria alternata* (3.00-11.50), *Aspergillus flavus* (5.25-8.90), *Aspergillus Niger* (4.25-12.00), *Colletotrichum capsici* (1.20-10.00), *Fusarium* sp. (2.25-4.35) and *Rhizopus* sp. (4.00-11.35).

Total per cent mycoflora was maximum in sample 'B' (50.75) followed by 'C' (46.65), 'D' (27.10), 'E' (26.0) and 'A' (24.35) while it was minimum in sample 'F' (20.95). Per cent incidence of *Colletotrichum capsici*, out of total per cent mycoflora was highest in case of sample 'B' (19.71) followed by 'C' (15.05), 'D' (11.07), 'A' (10.18) and 'E' (8.65), while it was minimum in sample 'F' (5.72).

Table 4: Per cent incidence of mycoflora on chilli seed isolated by Blotter Method with special reference to *Colletotrichum capsici*

Mycoflora	Per cent incidence / sample*						Average
	A	B	C	D	E	F	
Alternaria alternate	3.00	11.50	8.50	4.25	3.50	4.00	5.79
Aspergillus flavus	6.25	8.50	8.90	5.35	7.25	5.25	6.91
Aspergillus niger	5.30	10.00	12.00	6.50	5.50	4.25	7.25
Colletotrichum capsici	2.50	10.00	7.00	3.00	2.25	1.20	4.32
Fusarium sp.	4.75	4.25	3.75	2.50	3.00	2.25	3.08
Rhizopus sp.	11.75	6.50	6.50	5.50	4.50	4.00	4.20
Total per cent mycoflora	24.55	50.75	46.65	27.10	26.0	20.95	
Colletotrichum capsici	10.18	19.70	15.05	11.07	8.65	5.72	

* Sample size 400 seeds

Agar Plate Method

Six fungi were detected by Agar Plate Method from all the six seed samples (Table- 5). Fungi and their per cent occurrence recorded were *Alternaria alternata* (3.00-10.00), *Aspergillus flavus* (2.0-9.50), *Aspergillus Niger* (2.50-10.50), *Colletotrichum capsici* (0.50-12.50), *Fusarium* sp. (1.50-3.00), *Rhizopus* sp. (3.00-8.50).

Total per cent mycoflora was maximum in sample 'B' (53.50) followed by 'E' (35.00), 'C' (34.00), 'D' (33.50) and 'A' (29.0), while it was minimum in sample 'F' (14.00). Per cent incidence of *Colletotrichum capsici*, out of total per cent mycoflora was highest in sample 'B' (23.36) followed by 'C' (16.00), 'A' (12.06) and 'D' (7.46) while it was minimum sample 'F' (3.57).

Table 5: Per cent incidence on mycoflora of chilli seed isolated by Agar Plate Method with special reference to *Colletotrichum capsici*

Mycoflora	Per cent incidence / sample*						Average
	A	B	C	D	E	F	
Alternaria alternate	6.50	10.00	8.00	6.00	3.50	3.00	6.91
Aspergillus flavus	5.50	9.50	6.50	7.00	8.50	2.00	6.50
Aspergillus niger	6.50	10.50	5.50	9.00	9.50	2.50	7.25
Colletotrichum capsici	3.50	12.50	5.50	2.50	4.50	0.50	4.83
Fusarium sp.	1.50	2.50	2.00	3.00	2.50	3.00	2.41
Rhizopus sp.	5.50	8.50	6.50	6.00	6.50	3.00	6.00
Total percent mycoflora	29.0	53.50	34.0	33.50	35.00	14.00	
Colletotrichum capsici	12.06	23.36	16.00	7.46	4.28	3.57	

* Sample size 200 seeds

Seedling symptom test

Soil test

Symptoms like seed rot, yellowing, seed leaf blight / decay, stem blacking, leaf spot, leaf blight and root rot were observed on seedlings. Range of such seedlings were 1.00 to 10.00 per cent and it was maximum in sample 'B (10.00%) and minimum in 'F' (1.00%).

Agar Test in Test Tubes

Symptoms as observed in above test were observed on seedlings in all the samples except rotting of roots. Seedlings showing symptoms ranged from 2.00 to 15.00 per cent. Maximum percentage of such seedlings was in sample 'B' (15.00%) while it was minimum in sample 'F' (2.00%).

Fungi were isolated from infected tissues of seedlings in both the seedling symptom tests. The isolation revealed the following association:

Seed rot	-	*Alternaria* sp., *Aspergillus* sp., *Colletotrichum capsici*, *Fusarium* sp.
Seedling blight / decay, stem blackening leaf spot and leaf blight	-	*Alternaria* sp and *Colletotrichum capsici*
Yellowing	-	None
Root rot	-	*Fusarium* sp.

Pathogenicity Test

Among various fungi isolated from seeds and seedlings showing symptoms, *Colletotrichum capsici* were most predominantly associated with them. Hence for further studies on pathogenicity was tested for *Colletotrichum capsici* only.

Soil Inoculation

In soil inoculation, *C. capsici* caused higher pre- and post-emergence mortality (21.00% and 17.76%, respectively) and reduction in vigour index (355.00) in comparison to control (496.00). Amongst the seedling raised in the test, 35.75 per cent seedlings showed symptoms like seed rot, seeding blight / decay, leaf blight and yellowing of leaves / seedling (Table-6).

Table 6: Pathogenicity of seed-borne *Colletotrichum capsici* of chilli by Soil Inoculation Technique

***Colletotrichum* sp.**	**Germination (%)**	**Per cent seedling mortality**		**Elongation (cm)**		**Per cent seedlings showing symptoms***	**Vigour index**	***Type of symptoms***
		Pre-emergence	**Post-emergence**	**Root**	**Shoot**			
Colletotrichum capsici	72.0	21.00	17.76	2.6	2.4	35.75	355.00	Seed rot, seedling blight, leaf spot and blight.
Control (un inoculated soil)	83.0	0.00	0.00	3.1	2.9	0.00	498.00	-

* Based on emerged seedlings.

Seed Inoculation

Inoculation of healthy seeds with *C. capsici* caused both pre- and post-emergence mortality (18.00% and 12.50%, respectively), reduction in vigour index (352.00) in comparison to control (535.5). Amongst the seedling raised in the test, 30.00% seedling showed symptoms and they were seed rot, seedling blight and yellowing of leaves / seedling (table 7).

Table 7: Pathogenicity of seed-borne *Colletotrichum capsici* of chilli by Seed Inoculation Technique

Colletotrichum sp.	Germination (%)	Per cent seedling mortality		Elongation (cm)		Per cent seedlings showing symptoms*	Vigour index
		Pre-emergence	Post-emergence	Root	Shoot		
Colletotrichum capsici	75.0	18.00	12.50	2.6	2.1	30.00	352.50
Control (apparently surface sterilized seed)	85.0	00.00	00.00	3.0	3.3	0.00	535.5

* Based on emerged seedlings.

Foliar inoculation

Out of 100 seedlings sprayed with conidial suspension of *Colletotrichum capsici*, 45 per cent of seedlings showed symptoms. Symptoms observed at seedling stage were chlorotic / necrotic leaf spots and seedling blight. Re-isolation from affected tissue revealed the presence of the pathogen.

Fruit Inoculation

Table 8 revealed the susceptibility of fruits of both green and red (ripe) category. Ripe fruit observed to be more susceptible as they have highly per cent disease incidence and intensity *i.e.* 70.0% / 30.0% in comparison green fruits *i.e.* 25.0% / 10.0%, respectively.

Table 8: Effect of *Colletotrichum capsici* on chilli fruits Tested by Fruit Inoculation Technique

Colletotrichum sp.	Disease incidence (%)*			Disease intensity (%)*
	Green fruit, red (ripe) fruit	Red (ripe) fruit, green fruit	Green fruit, red (ripe) fruit	Red (ripe) fruit, green fruit
Colletotrichum capsici	70.0	25.0	30.0	10.0
Control (i) with fruit injury	0.00	0.00	0.00	0.00
Control (ii) without fruit injury	0.00	0.00	0.00	0.00

* Average of 4 replications (25 fruits / replication).

Seeds play a vital role in the production of healthy crops. They are carrier of many important seed borne pathogens inciting various diseases, which results in considerable losses in the yield. Chilli seeds carry a number of fungi. Although majority of them are saprophytes, a few are potential pathogens capable of ruining the crop. Weather during the harvesting period plays a decisive role in the infection of seed by certain fungi. Storage of seeds results in dominance of storage fungi (Christensen, 1973) [9]

Examination of dry seed samples revealed the presence of deformed (shriveled), discoloured (brown and black) and damaged seeds, in addition to inert materials (impurities) and apparently healthy seeds. It is likely that different types of seed mycoflora during storage might have caused such deformation / discoloration in seeds. Presence of such seeds and other impurities as seed concomitant, contaminate and contaminate in chilli and other crops like cumin, sesame and pearl millet have also been reported by Singh, 1993 [31], Kumhar, 1997 [20], Shekhawat, 1989 [30], Singh and Singh, 1983 [33] and Randhawa and Aulakh, 1984 [27], respectively.

Six fungi including *Colletotrichum capsici* detected as micro and macro conidia by the Seed Washing Test in all the samples. A possible reason for the occurrence of fungi on seed coat might have been due to favourable weather conditions during maturity of the crop in the field and in storage for survival and multiplication of fungi associated with mature chilli fruit / seeds on their surface. Non-adoption of improved cultural practices by the farmers might be another reason. Presence of different fungal spore on seed coat of cumin and fenugreek have been also reported.

During present investigation, Blotter Method, Agar Plate Method and Seedling Symptoms Test were employed for the detection of *Colletotrichum* spp. from seeds.

The studies revealed that in all, six fungi belong to five genera were detected from six seed samples including *Colletotrichum capsici*. Other fungi detected were *Alternaria alternata*, *Aspergillus Niger*, *A. flavus*, *Fusarium* sp. and *Rhizopus* sp.

The studies revealed that in all six fungi belongs to five genera were detected from six samples including species of *Colletotrichum capsici*. Other detected were *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium* sp. and *Rhizopus* sp. Ten species of *Colletotrichum capsici* including *C. capsici* (Grover and Bansal, 1970, Rout and Rath, 1972, Asalmol *et al.*, 2001) [14, 29, 4], *C. acutatum* (Kulshrestha *et al.*, 1976) [17], *C. coccodes* (Yu *et al.*, 1987 and Govern and Polstion, 1985) [37,], *C. dematium* (Kulshrestha *et al.*, 1976 and Raut *et al.*, 1989) [17, 28], *C. gloeosporioides* (Kulshrestha *et al.*, 1976 and Khodke and Gahukar, 1995) [17, 16], *C. piperatum* (Rout and Rath, 1972) [29], *C. multisetorum* (Rout and Rath, 1972) [29], *C. graminicola* (Kulshrestha *et al.*, 1976) [17], *C. lindemuthianum* (Kulshrestha *et al.*, 1976) [17] and *C. lini* (Kulshrestha *et al.*, 1976) [17] have already been reported along with other fungal species such as *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium* sp. and *Rhizopus* sp. on seeds of chilli (Asalmol *et al.*, 2001 and Rout and Rath, 1972 and Basak *et al.*, 1996) [4, 29, 38].

In general, little variation was observed in Blotter Method and Agar Plate Method in the presence of *Colletotrichum capsici* and other fungi recorded on seeds. Per cent of *Colletotrichum capsici* was higher in Agar Method as comparison to Blotter Plate Method. This variation might be due to the reasons that some of the weak and slow growing fungi could not grow in Agar culture in comparison to fast growing saprophytic fungi. Pre-surface sterilization of seeds and different substratum used in the method employed may be another reason (de Tempe, 1961; Neergaard and Saad, 1962) [12, 25]. To have a complete spectrum of the mycoflora, it seems essential to deploy both the methods. Neergaard and Saad (1962) [25] also observed that Blotter Method and Agar Plate Method are equally valuable and supplementary to each other.

Isolation from infected tissues showing symptoms like seed rot, yellowing, seedling blight / decay, stem blackening, leaf spot / leaf blight and root rot) as observed on seedlings raised in both the Seedling Symptoms Tests, yielded *Colletotrichum capsici*. Association of *Colletotrichum capsici* and its other species with symptoms like seed rot, leaf spot, decay and anthracnose have also been observed by (Chaurasia, 1976, Yu *et al.*, 1987 and Grover and Bansal, 1970) [8, 37, 14].

Seed germination and seedling, growth is generally influenced by seed-borne fungi (Christensen and Kaufmann, 1965) [10]. In the present investigation, seed, soil, fruit and foliar inoculation were used to prove the pathogenicity of *Colletotrichum capsici* from chilli seeds.

Conclusion

Six seed samples were collected from Jaipur region showing deformed (Shriveled) and discoloured (black, brown) damaged (mechanically) seeds. Impurities of one kind or other were also found in all the six samples.

A total of six seed-borne fungi were obtained in Blotter and Agar Plate Method. These were *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Colletotrichum capsici*, *Fusarium* sp. and *Rhizopus* sp. Maximum mycoflora were observed in sample `B' and minimum in sample `F'. Maximum incidence of total *Colletotrichum* sp was observed in sample `B' while it was minimum in sample `F'. Higher number of fungi were isolated by Agar Plate Method in comparison to Blotter Method.

Maximum number of seedlings of sample `B' showed symptoms specific to seed rot, seedling blight / decay, stem blackening, leaf spot, leaf blight, root rot and yellowing disease while number of seedlings showed symptoms were minimum in sample `F'. Most of the symptoms were incited by *Colletotrichum capsici*. Hence, the pathogenicity of *Colletotrichum capsici* were tested and taken for further studies.

Discoloured (black and brown) and deformed (shriveled) seeds showed association of different *Alternaria alternata*, *Aspergillus flavus*, *A. Niger*, *Colletotrichum capsici*, *Fusarium* sp. and *Rhizopus* sp.

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