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Occurrence of seed borne pathogens in Chilli (*Capsicum frutescence* L.) cv. GVC 111 *in vitro*

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Abstract
An experiment was to know the occurrence of seed borne pathogens in chilli *in vitro*. Chilli (*Capsicum frutescence* L.) is mainly cultivated for its vegetable green fruits and for dry chilli as the spice of commerce. Chilli is affected by various fungal seed borne pathogens, which affected in chilli yield production. Occurrence of seed borne pathogen was carried out by two different methods *viz.,* Standard blotter paper method and Potato Dextrose Agar (PDA) method. In standard blotter paper method *A. niger* was found dominant fungus in sterilized and unsterilized seeds and in PDA method per cent disease incidence was not recorded in sterilized seeds by *A. niger*, *A. flavus*, *Fusarium* sp., *Rhizopus* sp., *Colletotrichum capsici* and *Penicillium* sp. while maximum per cent disease incidence *A. niger* was found in unsterilized seeds.

Keywords: Seed borne pathogens, *in vitro*

Introduction
Chilli (*Capsicum frutescens* L.) is most widely cultivated vegetable crop in the world. It is a solanaceous fruit vegetable mainly cultivated for its vegetable green fruits and for dry chilli as the spice of commerce. It is a rich source of Vitamin C, A and B. In India, it is an important cash crop, which is grown for the both domestic and export market.
A fungal disease of chilli is very important as it reduces the market value of fruit and seed quality may cause yield losses of up to 50%. This disease was first reported in India on chilli from Coimbatore of Madras Presidency. The disease has been identified in all the chilli growing regions of the world and has become a serious constraint in chilli production. Different species of *Colletotrichum*, namely *C. capsici*, *C. gloeosporioides* and *C. acutatum* also *Alternaria alternata*, *Fusarium oxysporum* are known to cause fruit rot in chilli which also cause seed and seedling rot. Hence, in the present investigation an attempt was made to know the seed borne fungal pathogens associated with seed by standard blotter paper method and Potato Dextrose Agar (PDA) method.

Material and Methods
Popular local cultivar of chilli GVC 111 was obtained from Regional Horticulture Research Station (RHRS) farm, NAU, Navsari. Chilli seed samples were collected and subjected to planting by using blotter paper method as recommended by Mathur and Kongsdal (2003) [6] and Potato Dextrose Agar method. The Petri-dishes with seeds were arranged in seed trays and incubate it for 7-10 days at fixed temperature of 25^{0}C under 12 hours alternate cycles of light and darkness to enhance seed borne pathogen population. Each seed sample at the end of the incubation was examined thoroughly under microscope. Whatsoever pathogens found associated with seeds that were carefully examined and identify based on their habit character. Slides of the respective pathogen were prepared and examine using compound microscope.

Standard blotter paper technique: (ISTA, 1993) [2]
Four hundred seeds from sterilized and non-sterilized seed samples plated on three layer water soaked blotter papers. Fifty seeds (depending upon the size of seeds) were placed in each Petri plate after surface sterilization with 1% sodium hypochlorite (NaOCl) for one min and then washed them thrice in distilled water. These plates were incubated at 25^{0}C under the 12 hours of alternating cycles of day/night under fluorescent light.
After 7 days of incubation, seeds were examined under stereoscopic microscope.

Fungal growth appearing on seeds in Petri plates was directly identified up to the species level on the basis of colony growth, colour and morphological characters of the fungus spores/conidia observed under a compound microscope. Per cent infected seeds of different fungal pathogens were recorded as under:

$$\text{Per cent infected seeds (\%)} = \frac{\text{No. of infected seeds}}{\text{Total number of seed}} \times 100$$

Fungi which were not identified directly from colony growth on seeds was sub cultured on PDA plates and purified by hyphal tip culture technique and preserved in test tubes at 5^0±C for further studies. All these purified cultures were identified on the basis of morphological characteristics of fungi. The experiment was repeated three times in complete randomized design (CRD).

Agar Plate Method: (Muskett and Melone, 1941) [7]

In agar plate, the medium was prepared by mixing 20 g agar, 20 g dextrose and 200 g of potato starch in 1 liter distilled water. Media was sterilized at 15 lbs psi for 20 minutes at 121^0C. About 20ml of sterilized medium was poured in each Petri dish under aseptic conditions. Four hundred seeds from sterilized and non-sterilized seed samples were surface sterilized with 1% NaOCl for one minute and then given thrice washing in sterilized distilled water and was plated as 25 seeds per Petri dish. An incubation condition was kept same as in case of blotter paper technique. The per cent infected seeds were determined by using the following formula:

$$\text{Per cent infected seeds (\%)} = \frac{\text{No. of infected seeds}}{\text{Total number of seed}} \times 100$$

Results and Discussion

Standard blotter paper method

The data on percent occurrence of seed mycoflora on sterilized seeds and unsterilized seeds are presented in table 4.5. The results revealed that percent occurrence of seed mycoflora was ranged from 0.32% to 8.40% in sterilized seeds while, it was ranged from 5.57% to 16.18% in unsterilized seeds. In sterilized seeds highest occurrence of fungal pathogen was recorded by *Aspergillus niger* 8.40% and the next least occurrence of pathogens was recorded in order of *A. flavus* 6.34%, *Penicillium* sp. 2.65% and *Rhizopus* sp. 0.32%. *Fusarium solani* and *Colletotrichum capsici* were not detected in sterilized seeds. While in unsterilized seeds highest occurrence of fungal pathogen was recorded by *A. niger* 16.18% and the next least occurrence of pathogens was recorded in order of *A. flavus* 15.75%, *Fusarium solani* 10.50%, *Rhizopus* sp. 7.43%, *Colletotrichum capsici* 6.81% and *Penicillium* sp. 5.75%. (Table 1) (Fig. 1).

Table 1: Per cent infected chilli seeds with different pathogens in Standard blotter paper method

Sr. No.	Fungi	Per cent infected seeds	
		Sterilized seed	Unsterilized seed
1	*Aspergillus niger*	8.40	16.18
2	*Aspergillus flavus*	6.34	15.75
3	*Fusarium* sp.	0.00	10.50
4	*Rhizopus* sp.	0.32	7.43
5	*Colletotrichum capsici*	0.00	6.81
6	*Penicillium* sp.	2.65	5.75

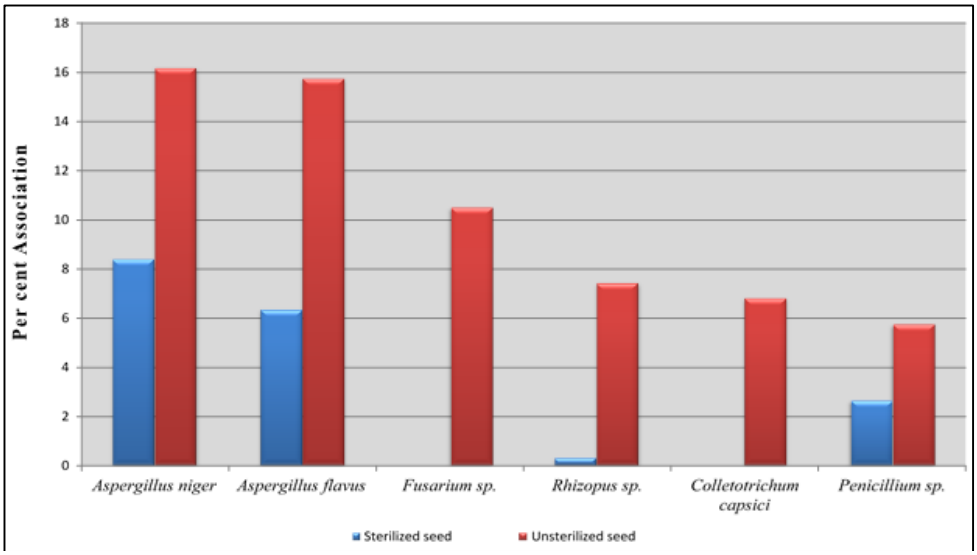

Fig 1: Per cent infected of chilli seeds in Standard blotter paper method

Similar results of occurrence of seed mycoflora was described by Jogi *et al.* (2010) [3] reported seed borne fungi, *C. capsici* and *F. oxysporum* showed disease incidence 6.81% and 10.50%, respectively by using standard blotter paper method, Zakaria (2010) [9] and Machenahalli Santoshreddy *et al.* (2014) [5] found highest incidence of *Colletotrichum capsici* (72.85%) by standard blotter paper method.

Potato Dextrose Agar (PDA) plate method

Generally there was a higher occurrence of fungi in unsterilized seeds. *A. niger* and *A. flavus* were the most

frequently occurred pathogens with 27.54% and 20.62% occurrence, respectively in unsterilized seeds followed by *Fusarium solani* 7.26%, *Penicillium* sp. 3.50% and *Rhizopus* sp. 3.20% recorded. Lowest pathogen occurrence 2.59% was recorded in unsterilized seeds with *Colletotrichum capsici*. In sterilized chilli seeds of cultivar GVC 111, occurrence of pathogens was not recorded. (Table 2) (Fig. 2)

Table 2: Per cent infected chilli seeds with different pathogens in Potato Dextrose Agar (PDA) method

Sr. No.	Fungi	Per cent infected seeds	
		Sterilized seed	Unsterilized seed
1	*Aspergillus niger*	0.00	27.54
2	*Aspergillus flavus*	0.00	20.62
3	*Fusarium* sp.	0.00	7.26
4	*Rhizopus* sp.	0.00	3.20
5	*Colletotrichum capsici*	0.00	2.59
6	*Penicillium* sp.	0.00	3.50

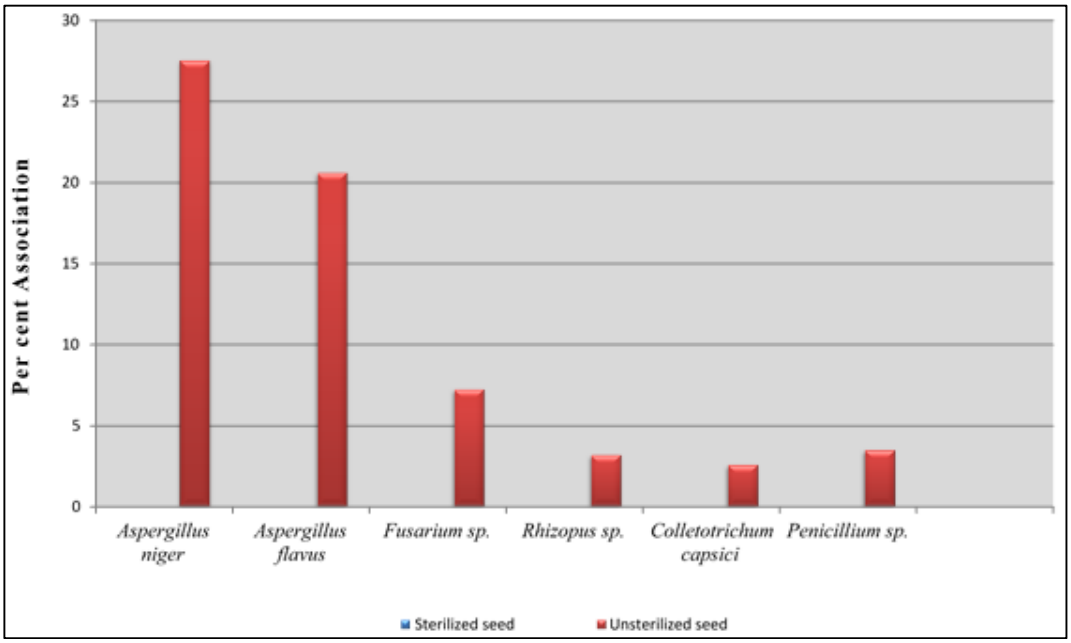

Fig 2: Per cent infected of chilli seeds in Potato Dextrose Agar (PDA) method

The results obtained in this experiment are close with the findings of occurrence of seed borne pathogens was of Chigoziri and Ekefan (2013) [1] they recorded *C. capsici*, *A. niger* and *A. flavus* were the most frequently isolated fungi with 54.75%, 44.00% and 29.75% occurrence, respectively, in agar plate method. Kassam and Monawar (2000) [4] and Sowly and Kodua (2012) [8] also reported similar findings.

Conclusion

Occurrence of seed borne pathogens, in standard blotter paper method *A. niger* was found dominant fungus in sterilized and unsterilized seeds and in PDA method, no any disease incidence was not recorded in sterilized seeds while, maximum per cent disease incidence *A. niger* was found in unsterilized seeds.

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