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Validation of detection techniques and management of seed borne diseases of Chilli (*Capsicum annum*)

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Abstract

Diagnosis of seed borne pathogens is an important step for seed health and management of crop diseases. This study was carried out to assess seeds of two chilli varieties, namely, Garima-12 and HPH-12 for inoculum of seed borne pathogens. Agar plate method, Blotter paper method and Rolled paper towel method were used for germination percentage and presence of seed borne pathogens. Five seed borne pathogens viz., *Aspergillus flavus*, *A. niger*, *Colletotrichum capsici*, *Penicillium citrinum* and *Fusarium annuum* were predominant. Efficacy of seed treatment with fungicides and bioagents in reducing the seed borne fungi was also studied using three methods. Carboxin, Metalaxyl and Carbendazim in combination with *Trichoderma* were effective in reducing the seed borne fungi.

Keywords: Seed health, seed borne pathogens, blotter paper method, agar plate method, rolled paper towel method

Introduction

Chilli (*Capsicum annum* L.) is an important vegetable cum spice crop grown in almost all tropical and subtropical countries of the world. The production of chilli was 1.30 mt. with an area of 0.806 mha. during 2013-14 in India. In Madhya Pradesh it occupied an area of 54410 ha. with a production of 93570 tons of chilli. The seeds of chilli hybrids and varieties are most priced input to the farmers and delayed and erratic germination of chilli seeds is one of the reasons for low yield and increasing the cost of cultivation of this commercial crop. The success of chilli crop dependent on raising of healthy seedling to transplanting in desirable land by following good agronomical practices and proper soil moisture maintenance. There are many biotic and abiotic constraints responsible for the delayed and erratic germination, among these, seed borne pathogens are predominant. Chilli suffers many of the fungi among them *Fusarium oxysporum*, *Colletotrichum capsici*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Alternaria alternata* and *Penicillium citrinum* as leading seed borne fungal pathogens associated on seed either internally or externally, which reducing seed germination and seedling vigour, and result in poor yield. *Colletotrichum capsici*, *Aspergillus niger*, *A. flavus*, *Fusarium* and *Penicillium* were the most frequency isolated fungi with 54.75%, 44.00%, 29.75%, 1.25%, and 0.25% (Ekhuemelo and Ebenezar, 2013) [3]. To validate the promising fungicides for raising of seedling are immensely required for successful cultivation and producing more vigour seedling, complete elimination of pre-existing inoculum of fungi is the key criteria for successfully harvesting of crop, because management of that type of pathogen at the time of growing phase of the crop is comparatively more difficult and some time due to delayed detection and diagnosis, it may incite tremendous irreparable losses to the crop. The present investigation was undertaken to study the validation of detection techniques from two varieties of chilli and assessment of different fungicides and their impact on seed germination and associated fungi with two types of seed.

Materials and Methods

Untreated seed of two varieties of chilli viz., variety Garima-12 and hybrid HPH-12 were procured from Kargone, and Khandwa district of Madhya Pradesh, respectively. Identification of pathogens found to be associated with different types of seeds and varieties were made on the basis of their cultural and morphological character appears on petri plates and microscopic observation.

Detection of seed borne pathogens

Following three methods were evaluated *in vitro* condition to identify the pathogens on chilli varieties. The possible reduction in the associated seed borne pathogens assessed by dressing

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the seeds with fungicides and/or bio-agents were assessed by using three methods viz., Blotter paper, Agar plate and Rolled paper towel method. Efficacy of different fungicides in combination and alone along with biocontrol agent viz., Carbendazim, Chlorothalonil, Streptocycline, Thiram+Metalaxyl, Ametoctradin 300g/l + Dimethomorph 225g/l SC, Dimethomorph 50%WP, Cymoxanil 8% + Mancozeb 64% Wp, Thiophanate methyl 450g/l + Pyraclostrobin 50g/l FS, Pyraclostrobin 12.8 + Boscalid 25.2%WG, *Trichodermaspp.*, *Pseudomonas spp.*, *Trichoderma*+Carboxin, *Trichoderma*+metalaxyl and *Trichoderma*+Carbendazim, were evaluated on these varieties as the effect on seed germination and percent association of types of seed borne pathogens. The seed dressing with different treatments of fungicides were made @ 0.1% with described combination of treatment, separately. Total 100 seeds of chilli varieties were maintained in each treatment with three replications. Same number of seeds were maintained in control. The determination of germination and colonization of associated seed borne pathogens were determined.

Blotter method

The collected seed samples of chilli were analyzed for the presence of major seed borne fungal pathogens by blotter method following the International rules for Seed Testing. One hundred seeds were tested for each variety maintaining three replications. Ten seeds were placed on moist blotting paper (Whatman No.1) in each glass petridish. The petridishes were incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed was observed under stereomicroscope in order to record the presence of fungal colony at 7 days after incubation based on growth habit. In

doubtful cases temporary slides were prepared from the fungal colony and observed under compound microscope. The results were presented as percent incidence for individual pathogen. Germination and associated inoculum of seed associated pathogen were recorded in each treatment separately.

Agar plate method

In the agar plate method, One hundred seeds were tested for each treatment with three replications. Surface disinfected seeds (0.1% sodium hypochloride) were plated on the PDA medium and the plated seeds were usually incubated for 7-10 days at 25±1°C under 12h altering cycles of light and darkness. At the end of the incubation period, fungi growing out from the seeds on the agar medium were examined and identified. Identification was done based on colony characters and morphology of sporulation structures under a compound microscope. In the agar plate method more than one type of fungal colonies were produced. The identification of the different colonies were done visually and then under a stereomicroscope and followed by an examination of the spores and fruiting structures under a compound microscope.

Rolled paper towel method

The method for detection of seed borne pathogen evolved by Warham (1990) [12] was followed. Towel paper were wetted with distilled sterilized water poured in tray and then One hundred seeds were kept in such a way that ten seeds in each of the ten rows following by wetting another towel paper with sterilized water for covering the seeds and rolled down along with butter paper the trays were the incubated at 26±1°C. The germination counts were taken after 14 days and associated fungi were detected.

Table 1: Effect of seed treatment with fungicide/or Antagonist on germination percentage of different chilli varieties by standard blotter method.

Methods	Standard blotter method				Agar plate method				Rolled paper towel method			
	Garima-12		HPH-12		Garima-12		HPH-12		Garima-12		HPH-12	
Varieties	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
Treatments	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
Carbendazim	85.33	79.67	79.33	74.67	87.67	78.33	82.67	74.67	82.33	77.33	81.67	64.67
Chlorothalonil	79.67	72.67	80.00	70.00	80.33	74.00	80.67	70.67	79.33	70.33	78.33	68.67
Streptocycline	72.33	68.33	69.00	67.33	74.67	66.33	73.33	64.33	72.33	62.33	69.33	60.67
Thiram+Metalaxyl	81.00	78.33	80.33	73.33	84.33	76.33	81.67	75.33	80.33	73.67	80.67	72.33
Zampro 525 SC	78.67	72.00	78.33	70.00	78.67	71.33	75.33	68.00	75.67	72.33	75.67	68.67
Ametoctradin 300g/l + Dimethomorph 225g/l SC	82.33	75.67	77.33	71.33	79.67	73.33	78.67	71.67	78.33	66.33	77.67	66.67
Cymoxanil 8% + Mancozeb 64% Wp	80.67	75.33	79.00	72.00	85.33	75.67	81.33	73.33	82.67	71.33	79.67	67.33
Thiophanate methyl 450g/l + Pyraclostrobin 50g/l FS	79.67	71.00	79.33	65.67	82.33	73.67	81.67	70.67	77.67	71.67	77.33	68.33
Pyraclostrobin 12.8 + Boscalid 25.2%WG	82.67	72.33	80.33	67.33	83.33	75.33	80.67	72.67	80.33	69.33	77.67	66.67
<i>Trichoderma spp.</i>	77.33	69.00	76.33	69.67	82.67	69.00	81.67	69.67	72.67	69.67	71.67	66.33
<i>Pseudomonas spp.</i>	71.33	70.67	72.00	65.33	75.33	68.33	74.33	65.33	68.67	66.33	68.33	62.33
<i>Trichoderma</i> +Carboxin	90.67	82.33	88.33	74.67	90.33	84.67	89.33	82.33	88.33	81.33	85.67	81.33
<i>Trichoderma</i> +metalaxyl	85.67	79.00	82.00	75.00	88.67	81.67	86.67	79.33	83.33	78.67	72.67	68.67
<i>Trichoderma</i> +Carbendazim	87.33	78.33	84.33	77.00	87.67	82.33	86.33	79.00	84.67	79.33	75.67	80.33
Control	68.67	56.00	63.33	51.67	71.33	55.33	66.67	51.67	68.67	53.33	65.33	49.33
Mean	79.85	73.37	77.95	69.66	82.15	73.71	80.06	71.24	78.35	70.88	75.82	67.48
SEm±	1.39	1.40	1.21	0.99	1.80	0.71	0.91	1.11	0.84	1.49	1.21	1.07
CD at5%	3.85	3.89	3.36	2.75	4.99	1.97	2.52	3.08	2.32	4.13	3.35	2.98

C1-Normal seed, C2- Abnormal seed, Mean of three replication

Table 2: Effect of seed treatment with fungicide on micro flora associated (%) with chilli varieties as determined by Standard blotter paper

Fungi/Bacterium→	<i>A.flavus</i>		<i>A.niger</i>		<i>C.capsici</i>		<i>P.citrinum</i>		<i>F.annum</i>		MEAn			
	Garima-12	HPH-12	Garima-12	HPH-12	Garima-12	HPH-12	Garima-12	HPH-12	Garima-12	HPH-12				
Varieties→	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2		
Treatments↓	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2		
Carbendazim	2.3	4.3	2.3	4	2	3.3	2.3	3.3	2.3	4	2	3	2.9	
Chlorothalonil	4	6	3.7	4.7	2.3	4	3.7	3.7	3	4.7	3.3	5.7	3.7	
Streptocycline	7.7	9.7	8.7	5.7	6	8	6.7	9	4.7	7.3	10.7	13.3	6.7	
Thiram+Metalaxyl	2.3	4.7	2.3	4	2	3.7	2.3	3.3	3.3	3	3	1.3	3	3.0

Result and Discussion

Per cent germination of varieties influenced by different treatment combinations

Assessment of different combinations of fungicides and bio-agents were carried out to get the best possible combination of treatments for percentage of normal and abnormal seeds of varieties Garima -12 and HPH-12 by using three detection techniques namely standard blotter, agar plate and rolled paper towel method (Table 1). Among the detection techniques agar plate technique was found superior and maximum germination of seeds recorded in both the varieties (Garima-12 and HPH-12) and seed types (Normal and abnormal seed). Garima-12 variety of chilli was found to be more germination than the HPH-12. Seed treatment with native strain of *Trichoderma* along with fungicides, carboxin, metalaxyl and carbendazim were found to be best combination for seed germination of chilli tested in different detection technique. However, seed treatment with carbendazim also found effective in reducing the seed borne diseases and increasing the seed germination. In abnormal seeds, which supposed to be more contaminated by the seed borne fungi, maximum germination (84.67%) recorded in variety Garima-12 treated with *Trichoderma* + carbon under agar plate method detection technique, followed by 82.33% germination reported in seed treatment with *Trichoderma* + carbendazim and *Trichoderma* + carboxin in standard blotter method.

Detection of seed associated fungi in combinations of fungicides as determined by different detection technique. To quantify the individualised associated fungi with normal and abnormal chilli varieties of Garima-12 and HPH-12 as influenced by the different combination of fungicides and bio-agents were determined by standard blotter paper, agar plate and rolled paper towel method.

In standard blotter paper technique of seed borne borne detection showed more number (15%) of *C. capsici* was recorded in abnormal seeds of variety HPH-12, followed by association of *A. flavus* (11.7%). Seed treatment with *Trichoderma* + carboxin found to be most effective in reducing the soil borne fungi (1.4%) subsequently by 2.1% fungi associated in *Trichoderma* + carbendazim and 2.3% fungi associated in *Trichoderma* + metalaxyl. Association of *C. capsici* with seeds were also found least association in treatment of fungicides and *Trichoderma* in both the variety of chilli. Normal seeds of both the variety was comparatively least association of fungi than the normal seeds of variety. The fungi *A. flavus*, *A. Niger*, *C. Capsize*, *P. Citrinum* and *F. annum* were mostly found associated with normal and abnormal type seeds of Garima-12 and HPH-12 (Table 2). The seed treatment with carbendazim was also showed there effectiveness in reducing the seed borne fungi and equally effective against all the fungi associated with seeds of both varieties.

Agar plate method of seed borne pathogen detection technique was found to be most efficient technique and detected more number of pathogen (10.1%) associated with seeds as compared to other two techniques. (Table 3) Seed treatment with *Trichoderma* along with carboxin, carbendazim and metalaxyl were equally effective against seed borne pathogens (*A. flavus*, *A. niger*, *C. capsici*, *P. citrinum* and *F. annum*).

The seed associated pathogens were determined in normal and abnormal seeds of two varieties of chilli (Garima-12 and HPH-12) under the rolled paper towel method of seed borne detection treated with different combinations of fungicides

(0.1%) and bio agents. The seed treatment with *Trichoderma* + Carboxin (0.1%) was comparatively found to be best combination of treatment and reducing the associated seed borne pathogens in abnormal and abnormal type of seeds of both the varieties.

Solanke *et al.* (2001) ^[10] reported presence of *F. moniliformae*, *C. capsici*, *A. niger*, *A. flavus*, *A. alternata* and *Curvularialunata* for the seed samples of different chilli varieties. Asalmol *et al.* (2001) ^[2] reported that *A. flavus*, *Rhizopusstolonifer*, *F. moniliformae*, *C. capsici* and *A. niger* were the predominant seed borne fungi in the chilli seeds. Solanke *et al.* (2001) ^[10] studied the seed borne mycoflora by different methods in different chilli cultivars and reported that higher percentage of seed mycoflora was recorded on agar plate method as compared to standard blotter paper method, rolled paper method and the moist sand method.

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