

MANAGEMENT OF SEED BORNE MYCOFLORA ASSOCIATED WITH CHILLI SEEDS

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ABSTRACT

An investigation entitled “Management of seed borne mycoflora associated with chilli seed” was carried out during the year 2014 - 2015. Seed samples were collected from different location of Nagpur district. *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*, *Curvularia lunata* and *Colletotrichum sp.* were the five different types of seed borne fungi detected on seeds using blotter paper method. Studies revealed that the total association of fungi were highest in variety Deepika followed by Var-334. Fungicides and bioagents were evaluated following poison food and dual culture technique against five fungi. Among fungicides 100% growth inhibition was registered in carbendazim against *Aspergillus niger*, *Fusarium oxysporum* and *Curvularia lunata*. However, benomyl recorded 100% growth inhibition in *Fusarium oxysporum* and *Curvularia lunata*. Mancozeb recorded 100% growth inhibition in *Alternaria alternata*. As regard to bioagents *Trichoderma viride* was found the most effective in arresting the growth of *Aspergillus niger*, *Alternaria alternata*, *Curvularia lunata* and *Colletotrichum capsici*, while *Pseudomonas fluorescens* were found effective against *Fusarium oxysporum* in arresting the growth.

(Key words : Seed microflora, *Trichoderma*, *Pseudomonas*, fungicides)

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important commercial vegetable crop in India belongs to Solanaceae family. It is also called as nature's wonder, hot pepper, cayenne pepper. It is the fourth most important vegetable crops in the world and first in Asia. Chilli is an important spice crop cultivated in tropical and subtropical regions of the world. Seed is an important input for crop production. About 90% of the world food crops including chilli are propagated by seed (Chigoziri and Ekefan, 2013).

Seeds are the passive carriers of some important seed borne diseases caused by microorganisms which usually result in considerable yield losses. Use of fungicides and antagonist as seed treatment has become a necessary and is accepted practice in Agriculture. These fungicides and biological agents were capable of inducing significant effect in germination and seedling vigour index along with disease control (Jogi *et al.*, 2010). The important diseases reported are anthracnose (*Colletotrichum capsici*), Cercospora leaf spot (*Cercospora capsici*), damping-off and root rot (*Rhizoctonia solani*, *Pythium sp.* and *Fusarium sp.*) (Vidhyasekaran and Thiagarajan 1981; Nick, 1980; Pandey *et al.*, 2012). Seed borne pathogens are seed transmissible and cause diseases at various stages of crop

growth from seed germination to crop maturity and causing heavy losses. Therefore, the present investigation was undertaken on “management of seed borne mycoflora associated with chilli seeds”.

MATERIALS AND METHODS

The investigation was carried out during year 2014-15 in Nagpur district, Maharashtra (India) for the study of seed-borne mycoflora of chilli seeds. Chilli seed samples of ten different varieties viz., Chandramukhi, Deepika, Jayanti, Teja-4, Var-334, Var-314, C-5, Loc. Var-1, Wonder hot and Shimla were collected from different locations of Nagpur district. Detection of seed borne mycoflora of chilli seed was carried out by blotter paper method (Anonymous, 1996). The unsterilized 400 seeds were plated at equidistance by sterile forceps in surfaced disinfected transparent plastic petri plates of 90 mm diameter. For blotter paper method 25 seeds were plated in each plastic plate. However, while plating the seed on blotter surface, care was taken that the blotter was sufficiently moist. Plates were incubated at room temperature ($27 \pm 2^\circ\text{C}$) in laboratory for seven days to develop the fungal flora. Observations of fungal colonies on and around the seeds was made by using stereoscopic binocular research microscope and their growth was lifted by sterile inoculating needle and transferred to PDA plates

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for purification. Fungal isolates were purified by periodic transfer and maintained for further investigation. The

morphology and characters of fungi were studied and identified by referring the manual (Chowdhary, 2000).

Treatment details

Treatments	Fungicides / Bioagents	Fungicide dose
T1	Captan 75% WP	2 g kg ⁻¹
T2	Mancozeb 75% WP	2 g kg ⁻¹
T3	Carbendazim 50% WP	1 g kg ⁻¹
T4	Benomyl 50% WP	3 g kg ⁻¹
T5	<i>Trichoderma viride</i> (10 ⁷ CFU)	4 g kg ⁻¹
T6	<i>Pseudomonas fluorescens</i> (10 ⁸ CFU)	10 g kg ⁻¹
T7	Control	—

Poisoned food technique : Efficacy of Four fungicides and two bioagents were evaluated by employing poisoned food technique and dual culture technique respectively.

Four different fungicides were evaluated against seed-borne fungi of chilli by poisoned food technique. The desired concentration was obtained by adding appropriate amount of fungicides in PDA medium and replicated thrice. PDA without fungicide served as control. Each plate was inoculated with a 5 mm mycelial disc of the pathogen taken from the seven day old culture. The inoculated plates were incubated at 27 ± 2°C and radial mycelial growth of pathogen was measured on seventh days after inoculation. The diameter of the colony of the pathogen was measured in both directions and averages were work out and the per cent inhibition on growth of the test pathogen was calculated by using formula as given by Anand *et al.* (2010).

$$\% \text{ inhibition (I)} = (C - T/C) \times 100$$

Where,

C= Growth of mycelial in control (mm)

T= Growth of mycelial in treatment (mm)

Dual culture technique

Autoclaved and warm PDA poured in 90 mm plates and allowed to solidify. Five mm disc of test organisms placed in the centre and disc of antagonist was placed on the four sides of petriplate at equidistance. The antagonists used were *Trichoderma viride* and *Pseudomonas fluorescens*. The plates were incubated at room temperature 27± 2°C along with control. Radial mycelial growth of pathogen was measured at seventh days after inoculation.

Effect of fungicides and bioagents on growth parameters

As per study disinfected seeds were soaked in spore suspension of each culture for two hours and inoculated seeds were dried under shade for one hour. Four hundred pre-inoculated seeds were treated separately with

each concentration of above mentioned fungicides and bioagents. Suitable un-inoculated control was maintained separately. Paper towel method was used to record germination of seed (Anonymous, 1996). In this method 400 seeds were placed on moistened double layered towel paper of 45 cm × 30 cm size lined by blotter paper and covered with another towel paper of same size on it. Then these papers were rolled in many folds and incubated in seed germinator at 27 ± 2°C. Observations on seed germination, shoot length, root length and seedling vigour index were recorded at 14 days after incubation. To record shoot and root length, 10 randomly selected seedlings from each replication were removed and average shoot and root lengths were calculated. Seedling vigour index was work out by using following formula, given by Abdul Baki and Anderson (1973).

$$\text{Seedling vigour index} = \text{Germination per cent} \times [\text{Shoot length (cm)} + \text{Root length (cm)}]$$

RESULTS AND DISCUSSION

The results on per cent association of seed-borne fungi on different varieties of chilli seeds are tabulated in table 1. It was clearly observed from data that there were eight different fungi identified from seeds of chilli in which four belonged to *Aspergillus*, and one each from *Fusarium*, *Alternaria*, *Curvularia* and *Colletotrichum*. Kalyani Kumari *et al.* (2012) reported the dominant presence of fungal pathogen viz., *Aspergillus niger*, *A. flavus*, *Alternaria alternata* on chilli seed. Association of eight fungi belonging to five genera viz., *Aspergillus niger* (11.75 to 53.5 %), *Aspergillus flavus* (11 to 51.25 %), *Aspergillus sp.* (0 to 10.5), *Aspergillus nidulans* (0 to 19.25), *Fusarium oxysporum* (0 to 20.5 %), *Alternaria alternata* (0 to 16.5%), *Curvularia lunata* (0 to 9.25s %) and *Colletotrichum capsici* (9.25 to 28.25) were recorded using blotter technique.

Hemannavar *et al.* (2009) also found blotter paper technique effective in recording seed borne fungi and reported seed borne incidence of anthracnose of chilli in northern Karnataka by using seed health testing method. Among these Standard blotter method was found superior and indicated the dominance of *Colletotrichum capsici* (71.24%) followed by *Cercospora* sp. (14.37%) and *Alternaria* sp. (3.39%) over the other.

Evaluation of fungicides and bioagents were tested against these seed borne fungi by adopting food poison and dual culture technique. The data presented in table 2 clearly reveals that there were significant differences due to various treatments over uninoculated control. Chilli seeds treated with carbendazim (1g kg⁻¹) at VIIth day after inoculation did not show any growth of *Aspergillus niger*, *Fusarium oxysporum* and *Curvularia lunata*. This treatment was found superior over all other treatments recording 100% growth inhibition as compared to all other treatments. Vidhyasekaran *et al.* (1981), Islam (2007) and Wani and Misgar (2007) studied the effect of carbendazim and found that carbendazim treatment was the most effective in controlling these pathogens.

Mancozeb inhibited the fungal growth of *Alternaria alternata*. Similar observations were recorded by Islam (2007), who reported use of rovril and dithane M-45 could significantly reduce the incidence of *Alternaria* sp in radish seeds. Mancozeb inhibited 100 per cent growth over uninoculated control and were found significantly superior over all other treatments. Similarly *Colletotrichum capsici* was tested against fungicides and bioagents and minimum colony diameter was recorded by captan treatment (9.00 mm) followed by carbendazim (13.66 mm) on VIIth DAI over uninoculated control. Similar finding was recorded by Kumud Kumar *et al.* (2004). She tested twenty seed samples of eight chilli (*Capsicum annum*) cultivars to assess the extent of seed infection of *Colletotrichum dematium*. Among the eight fungicides, Companion and Jekstein (carbendazim and bavistin+thiram) were found significantly superior in eliminating the infection from the seed. Among the bioagents *Trichoderma viride* recorded highest growth inhibition over *Aspergillus niger*, *Curvularia lunata*, *Alternaria alternata* and *Colletotrichum capsici* as compared to *Pseudomonas fluorescens*. Gurjar *et al.* (2004), Bharath *et al.* (2010) and Jogi *et al.* (2010) reported the similar results of *Trichoderma viride* showing highest growth inhibition. Minimum growth of *Fusarium oxysporum* (11.66 mm) was observed by *Pseudomonas fluorescens*. *Pseudomonas fluorescens* was found superior over *Trichoderma viride* which was recorded 79.42 per cent growth inhibition over uninoculated control. The findings are in agreement with the reports of Ardebili *et al.* (2011), who

recorded the growth inhibition effect of *Pseudomonas fluorescens* against *Fusarium oxysporum* in increasing seedling vigour index and controlling tomato wilt.

Chilli seeds were treated with fungicides and bioagents which were pre-inoculated with individual fungal culture to record the seed germination, shoot and root length, and seedling vigour index using paper towel and the data are tabulated in table 3, table 4, and table 5. The results indicated that all the fungicides and bioagents found superior over control. The results in respect of *Aspergillus niger* and *Fusarium oxysporum* on germination, shoot and root length and seedling vigour index were found to be significant. Seed treatment of carbendazim and captan recorded highest germination (81.33 per cent), shoot length, root length and seedling vigour index as compared to other treatments.

Trichoderma viride increased seed germination in case of *Aspergillus niger* (73.67 per cent) and *Pseudomonas fluorescens* in *Fusarium oxysporum* (75.33 per cent) treatment. These findings are similar with the findings of Singh *et al.* (2006) and Begum and Lokesh (2008), who reported increase seed germination due to use of fungicides application in chili. Effect of Benomyl was found superior against the seeds treated with *Curvularia lunata* which reflects to high germination (83.33 per cent), shoot length (3.1), root length (6.90) and seedling vigour index (838.56), respectively.

Seeds treated with biological antagonists like *Trichoderma viride* proved its efficacy in reducing the incidence of *Aspergillus niger* and *Curvularia lunata* and increasing the germination, shoot and root length which resulted in the enhancement of seedling vigour index of chilli seeds. These results are in conformity with the reports of Bharath *et al.* (2010) and Jogi *et al.* (2010). They reported maximum seed germination and seedling vigour index in watermelon by using captan and dithane M-45 as seed treatment. The presence of *Pseudomonas fluorescens* in culture medium inhibited the fungal growth of *Fusarium oxysporum*, *Alternaria alternata* and *Colletotrichum capsici*. Application of *Pseudomonas fluorescens* increased the seed germination by 11.28 per cent of *Fusarium oxysporum*, 77.67 per cent of *Colletotrichum capsici* and 75.00 per cent of *Alternaria alternata*. These observations are in agreement with the findings of Rammoorthy and Samiyappan (2001), who tested *Pseudomonas fluorescens* against *Colletotrichum capsici* for inhibition growth *in vitro* and also could effectively controlled fruit rot of chilli. Srinivas *et al.* (2006) also reported seed treatment of *P. fluorescens* could effectively reduced the population of *Colletotrichum capsici* in chilli seeds.

Table 1. Per cent association of seed-borne fungi on different varieties of chilli

Variety	<i>A.n.</i>	<i>A.f.</i>	<i>A.sp.</i>	<i>A.ni.</i>	<i>F.o.</i>	<i>A.a.</i>	<i>C.l.</i>	<i>C.c.</i>	Total Association of Fungi
Chandramukhi	48.75	37.25	0.00	0.75	0.00	17.5	3.75	15.25	123.25
Deepika	49.75	57.25	12.75	19.5	21.0	14.25	10.0	13.25	197.75
Jayanti	5.25	31.25	8.5	4.75	10.75	2.25	1.00	28.5	92.25
Teja-4	6.75	16.5	0.00	3.00	4.00	3.5	3.25	11.75	48.75
Var-334	43.75	43.5	9.75	5.00	15.25	4.5	5.25	21.75	148.75
Var-341	47.25	40.75	6.25	2.75	6.5	12.5	6.75	19.25	142.00
C-5	7.75	9.00	1.75	1.5	3.25	0.00	2.00	4.25	29.5
Loc. Var-1	7.00	16.75	4.25	11.5	8.5	5.5	8.25	23.75	85.5
Wonder hot	2.5	37.25	0.00	20.75	6.75	0.75	0.00	14.5	82.5
Shimla	36.25	41.75	2.25	3.00	10.75	2.00	3.75	3.5	103.25

A.n. - *Aspergillus niger* *A.sp.* - *Aspergillus sp* *F.o.* - *Fusarium oxysporum* *C.l.* - *Curvularia lunata*
A.f. *Aspergillus flavus* *A.ni.* - *Aspergillus nidulans* *A.a.* - *Alternaria alternata* *C.c.* - *Colletotrichum capsici*

Table 2. Evaluation of fungicides and bioagents against different fungi on 7th DAI

Treatments	Con. (%)	<i>Aspergillus niger</i>		<i>Fusarium oxysporum</i>		<i>Alternaria alternata</i>		<i>Curvularia lunata</i>		<i>Colletotrichum capsici</i>	
		Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)	Colony diameter (mm)	Growth inhibition (%)
Captan 75% WP	0.2	21.33	74.40	13.33	76.47	15.33	79.00	15.33	75.00	9.00	80.43
Mancozeb 75% WP	0.2	25.66	69.20	9.66	82.95	0.00	100.00	23.66	61.42	28.33	38.41
Carbendazim 50% WP	0.1	0.00	100.00	0.00	100.00	32.66	55.26	0.00	100.00	13.66	70.30
Benomyl 50% WP	0.3	33.33	60.00	0.00	100.00	27.33	63.01	0.00	100.00	16.33	64.50
<i>Trichoderma viride</i> (10 ⁷ CFU)	0.4	28.66	65.60	14.33	74.71	21	71.23	30	51.08	10	78.26
<i>Pseudomonas fluorescens</i> (10 ⁸ CFU)	1.0	33.00	60.39	11.66	79.42	22.33	69.41	33.66	45.11	13.33	71.02
Control		83.33		56.66		73		61.33		46	
SE(M)±		0.417		0.356		0.454		0.35		0.398	
CD (P=0.01)		1.242		1.060		1.352		1.043		1.186	

Table 3. Effect of seed fungicides and bioagents on germination and SVI of chilli by *Aspergillus niger*, *Fusarium oxysporum*

Treatments	<i>Aspergillus niger</i>					<i>Fusarium oxysporum</i>							
	Conc. (%)	Seed* germination (%)	Increase of seed germination over control (%)	Shoot length (cm)	Root length (cm)	Seedling* vigour index (SVI)	Increase of SVI over control (%)	Seed* germination (%)	Increase of seed germination over control (%)	Shoot length (cm)	Root length (cm)	Seedling* vigour index (SVI)	Increase of SVI over control (%)
Captan 75% WP	0.2	77.67 (61.80)*	18.89	2.47	6.47	711.69	54.2	74.00* (59.34)	9.91	3.73	5.97	718.51	18.73
Mancoze													
b 75% WP	0.2	74.00 (59.35)*	13.27	2.39	5.45	582.87	26.29	77.67* (61.80)	5.36	4.80	5.79	822.50	35.91
Carbenda zim 50% WP	0.1	81.33 (64.41)	24.49	2.62	6.50	743.94	61.19	83.33 (65.90)*	23.76	5.47	6.51	998.83	65.06
Benomyl 50% WP	0.3	71.67 (57.85)*	9.7	2.01	5.42	539.93	6.99	80.67* (63.92)	19.81	4.85	6.50	973.70	60.91
<i>Trichoderma viride</i> (10 ⁷) CFU)	0.4	73.67 (59.12)*	12.77	2.54	5.91	622.83	34.95	72.67* (58.47)	7.93	3.58	6.03	698.34	15.4
<i>Pseudomonas</i>													
<i>fluorescens</i> (10 ⁸) CFU)	1.0	71.33 (57.63)*	9.18	2.81	4.80	543.80	17.83	75.33 (60.22)*	11.88	4.92	6.43	855.30	41.34
Control													
SE(M)± CD (P=0.01)		65.33 (53.93)*	-	2.01	4.85	461.53	54.2	67.33 (55.14)*	-	3.27	5.21	605.13	18.73
		0.760	-	-	-	8.630	-	0.650	-	-	-	6.880	-
		2.264	-	-	-	25.717	-	1.937	-	-	-	20.502	-

Table 4. Effect of seed fungicides and bioagents on germination and SVI of chilli by *Alternaria alternata* and *Curvularia lunata*

Treatments	<i>Alternaria alternata</i>										<i>Curvularia lunata</i>									
	Conc. (%)	Seed* germination (%)	Increase of seed germination over control (%)	Shoot length (cm)	Root length (cm)	Seedling* vigour index (SVI)	Increase of SVI over control (%)	Seed* germination (%)	Increase of seed germination over control (%)	Shoot length (cm)	Root length (cm)	Seedling* vigour index (SVI)	Increase of SVI over control (%)	Conc. (%)	Seed* germination (%)	Increase of seed germination over control (%)	Shoot length (cm)	Root length (cm)	Seedling* vigour index (SVI)	Increase of SVI over control (%)
Captan 75% WP	0.2	83.67 (66.16)*	18.96	2.19	6.03	703.92	52.9	79.00 (62.72)*	15.04	3.07	6.54	61.1	0.2	74.00 (59.34)*	15.04	3.07	6.54	764.1267	61.1	
Mancozeb 75% WP	0.2	85.00 (67.22)	20.86	2.32	6.23	725.26	57.53	77.67 (61.80)*	13.11	2.80	5.19	46.44	0.2	76.33 (60.89)*	13.11	2.80	5.19	690.7833	46.44	
Carbendazim 50% WP	0.1	73.33 (58.90)*	4.27	2.05	5.50	560.06	21.65	82.67 (65.40)*	20.39	3.07	5.32	74.25	0.1	83.33 (65.90)*	20.39	3.07	5.32	821.94	74.25	
Benomyl 50% WP	0.3	76.67 (61.11)*	9.01	2.21	5.84	616.10	33.82	83.33 (65.90)*	21.35	3.1	6.90	77.77	0.3	76.33 (60.89)*	21.35	3.1	6.90	838.56	77.77	
<i>Trichoderma viride</i> (10 ⁷ CFU)	0.4	72.33 (58.26)*	2.84	20.3	5.40	541.99	17.72	76.33 (60.89)*	11.15	2.47	6.89	54.23	0.4	74.00 (59.34)*	11.15	2.47	6.89	727.4867	54.23	
<i>Pseudomonas fluorescens</i> (10 ⁸ CFU)	1.0	75.00 (60.01)*	6.64	2.14	6.11	626.70	36.12	74.00 (59.34)*	7.76	2.70	6.20	40.47	1.0	70.33 (57.00)*	7.76	2.70	6.20	662.6133	40.47	
Control		70.33 (57.00)*		1.58	4.96	460.39	52.9	68.67 (55.96)*		2.81	3.71	61.1		0.700		2.81	3.71	471.7033	61.1	
SE(M)±		0.570				5.200		0.700						0.700					8.120	
CD (P=0.01)		1.698				15.497		2.086						2.086					24.197	

Table 5. Effect of seed fungicides and bioagents on germination and SVI of chilli by *Colletotrichum capsici*

Treatments	Conc. (%)	Seed* germination (%)	Increase of seed germination over control (%)	Shoot length (cm)	Root length (cm)	Seedling* vigour index (SVI)	Increase of SVI over control (%)
Captan 75% WP	0.2	82.33 (65.14)*	25.37	5.10	6.05	918.26	77.7
Mancozeb 75% WP	0.2	76.67 (61.12)*	16.75	3.49	5.22	689.38	33.4
Carbendazim 50% WP	0.1	80.33 (63.68)*	22.32	4.58	6.47	896.03	73.39
Benomyl 50% WP	0.3	78.00 (62.03)*	18.77	4.55	5.38	779.18	50.78
<i>Trichoderma viride</i> (10^7 CFU)	0.4	74.33 (59.56)*	13.18	4.04	5.93	741.15	43.42
<i>Pseudomonas fluorescens</i> (10^8 CFU)	1.0	77.67 (61.80)*	18.27	4.45	6.67	871.76	68.70
Uninoculated Control	-	70.00 (56.78)*	6.59	3.36	5.42	620.2	20.02
Control (Inoculated)	-	65.67 (56.78)*	-	2.73	5.03	516.76	77.7
SE(M)±	-	0.680	-	-	-	6.290	-
CD (P=0.01)	-	2.026	-	-	-	18.744	-

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