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RESEARCH ARTICLE

DETECTION AND TRANSMISSION OF SEED BORNE MYCOFLORA IN GREEN GRAM AND EFFECT OF DIFFERENT FUNGICIDES

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Abstract

An experiment was undertaken to identify the seed borne fungi in green gram and subsequently determine their effect on seed germination in laboratory condition. Seed samples were examined in blotter method showed association of ten fungi belonging to eight genera viz., *Acremonium strictum, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Fusarium semitectum, Fusarium solani, Macrophomina phaseolina, Phoma medicaginis, Penicillium* sp. and *Rhizopus* sp. In component plating maximum colonization of *M. phaseolina* was observed in seed coat. Under seedlings symptom test *M. phaseolina* was found transmissible from seed to plant causing seedling blight. Seed treatment with thiram+carbendazim (2:1) 3g/kg of seed was increasing the seed germination (85.00%), shoot length (11.17 cm), root length (9.27 cm) and seedling vigour index (1734).

INTRODUCTION

Green gram [Vigna radiata (L.) Wilckzek] is the important pulse crop of India next to Pigeon pea. The seed-borne pathogens associated with seeds externally or internally may cause seed rot, seedling blight and resulting into low germination. Some fungi are associated with testa and cotyledonary of seeds infected in form of mycelium, pycnidium and conidia spores, after germination the infection translation to hypocotyls and stem bases as well as dicotyledonary leaves of seedling. Some fungal seed borne pathogens having ability to kill the seedling or plants and substantially reduce the productive capacity. (Shamsur Rahman et al.1999). Seed mycoflora play an important role in determining the quality and longevity of seed. Hence, in the present investigations the detection, location, transmission of seed borne fungi of green gram and their management with seed dressing fungicides was studied.

MATERIAL AND METHODS

Green gram seed samples of five varieties viz., Kopergaon, Green gold, AKM-8802, AKM-4 and TARM-18 collected from Pulse Research Unit, Akola and nine fungicides viz., Thiram 0.3% Carbendazim 0.1%, Thiram + carbendazim (2:1) (0.3%), Captan 0.3%, Thiophanate methyl 0.1%, Propineb 0.3%, Tebuconazole 0.1%, Hexaconazole 0.1%, mancozeb 0.3% collected from Department of Plant Pathology, Akola.

Blotter paper method

Two hundred seeds of each sample were sown on three layers of pre soaked moist blotter paper having 9 cm diameter. According to ISTA In each plate 25 seed were arranged, 16 seeds in the outer ring 8 in middle ring and one in the centre of plastic Petri plates. The plated seeds were then incubated at 27 ± 2^0C, under alternate cycle of 12 hrs light and 12hrs darkness for seven days by using two 40W white fluorescent tubes. After seven days of incubation, seeds were examined under stereoscopic microscope by using a magnification of 6X to

50X. To record the per cent infection of reduction in seed mycoflora the seeds were treated with requisite quantity of fungicides and untreated seed served as control.

Component plating method

The method was performed to know the site of infection of seed borne fungi. The seeds were soaked in petri dish containing sterilized distilled water for 4hrs. Individual seeds were aseptically dissected into three components viz., seed coat, cotyledon and plumule radical axis by using sterilized blade every time. Thus, out of each sample 10 seeds were dissected. Component of each seed were placed with sterilized forceps on solidified PDA medium in plates. Plated components were incubated under alternate cycle of light and darkness for 7 days at room temperature and then examined for presence of fungi by preparing slides and observed under compound microscope.

Test tube agar method

Symptoms can easily be studied being visible on roots as well as green parts. In each water agar stab, one seed was incubated at 27 ± 2^0C under alternate cycle of 12hrs light and 12hrs darkness. To retain moisture, they were cover individually by aluminium foil which was removed when the seedlings has reached to cover. Thirty seeds of each variety were tested for detection of seed borne fungi. The seedlings were examined after 14 days for typical symptoms of disease on the various parts of seedlings.

Rolled paper method

Treated seeds 200 were placed between paper rolls in four replicates of 50 seeds each for germination. The rolls were kept at 23 ± 2^0C in seed germinator. The first count of normal seedlings was taken on the 3rd day and the second count on the 7th day. The germination per cent was calculated. Normal seedlings were evaluated for seedling vigour index.

Seedling vigour index = [Mean root length (cm) + mean shoot length (cm)] × percentage germination

RESULTS AND DISCUSSION

Seed samples of Kopergaon variety was recorded highest 98.50 per cent fungal association among all varieties tested by blotter method. The total ten fungi belonging to eight genera were recorded viz., *M. phaseolina* (36.50%), followed by *F. oxysporum* (22.50%), *F. semitectum* (10.50%), *A. flavus* (9.50%), *Penicillium* sp. (7.00%), *C. lunata* (4.50%) *P. medicaginis* (3.50%), *Rhizopus* spp. (3.50%) and *A. niger* (1.50%). Seed borne fungal association in other varieties of green gram i.e. AKM-8802, AKM-4, TARM-18, Greengold were in the range of (95-73.50%) Maximum association recorded were *M. phaseolina* (32-22.00%) followed by *Fusarium oxysporum* (19-15.00%), *F. semitectum* (16-10.50%), *A. flavus* (13-5.50%), *Penicillium* sp. (11-4.50%), *P. medicaginis* (3.50-2.00%), *C. lunata* (4.50-1.50%), *Rhizopus* sp. (4.50-2.00%) and *A. niger* (3.50-1.50%). Among the seed borne fungi of green gram *M. phaseolina* observed to be a pre dominant fungi (22-36.50%) followed *by F. oxysporum* (22.50-15.00%). (Table 1). The association of seed borne fungi of present investigation are in agreement with Brau et al. (2007) who recorded the association of ten fungi belonging to eight genera viz., *Acremonium strictum, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Fusarium semitectum, Macrophomina phaseolina, Phoma medicaginis, Penicillium* spp. and *Rhizopus* spp. Similar seed borne fungi of green gram were also observed by Ali et al. (2010).

Pre treatment of seeds was made to avoid over growth of saprophytic fungi and to allow slow growing pathogenic fungi to colonize on seed. Pre treatment of seeds was done with Sodium hypochlorite (Naocl) solution. Result indicated that pre treatment not only reduced saprophytic fungi viz., *Aspergillus niger, Penicillium* spp, *Rhizopus* spp. *Aspergillus flavus* (0-3%) and *Fusarium semitectum* (0-2.1%), but also reduced the count of pathogenic fungi *Curvularia lunata, Phoma medicaginis, Fusarium oxysporum* (0-3.6%) and *Macrophomina phaseolina* (2-5.00%).

Maximum colonization of seed borne fungi was observed in seed coat followed by cotyledon. Among all seed component, seed coat carried maximum frequency of seed borne fungi (5.50%) followed by cotyledon (3.50%) and plumule radical axis (1.50%). *M. phaseolina* pre dominantly located in all parts of seeds (Table 2). These results confirmed the finding of Jaiman and Jain (2011) observed the presence of *M. phaseolina* in seed coat, cotyledon and embryo of Cluster bean seeds.

Under seedling symptom test fungi responsible for seed rot and seedling blight were *M. phaseolina, F. moniliforme, F. oxysporum, F. semitectum, A. flavus and C. globosum.* However, *C. graminicola, M. phaseolina* were found transmissible from seed to plant causing seedling blight (Table 3). Shamsur Rahman (1999) noted heavy infection of *M. phaseolina* causing pre and post emergence mortality and seed rot in mung bean.

In the Table 4 result showed under rolled paper method seed samples of Kopregaon treatment with thiram + carbendazim (2:1) 3 g/kg of seed was increasing the seed germination (85.00%), shoot length (11.17 cm), root length (9.27 cm) and seedling vigour index (1734) followed by propineb seed germination (78.00%) shoot length (10.70 cm), root length (9.97 cm) and seedling vigour index(1596). Similar result recorded by Koche et al. (2009) seed treatment with thiram + carbendazim (2:1) 3 g/kg increases the seed germination (85.00 to 79.00%)

Table 2: Association Frequency of seed borne fungi with different parts (component plating method)

Variety	Seed borne fungi associated	Per cent association of seed borne fungi		
		Seed coat	Cotyledon	Plumule radical axis
AKM-8802	*M. phaseolina*	0.50	1.00	-
	F. oxysporum	0.50	-	-
	A. flavus	0.50	-	-
AKM-4	*M. phaseolina*	0.50	-	0.50
	F. semitectum	-	0.50	-
Green gold	*M. phaseolina*	-	1.00	-
	P. medicaginis	1.00	-	-
	C. globosum	-	0.50	-
Kopergaon	*M. phaseolina*	-	0.50	0.50
	P. medicaginis	1.00	-	-
	A. flavus	0.50	-	-
TARM-18	*M. phaseolina*	0.50		0.50
	A. niger	0.50	-	-
Total fungi		5.50	3.50	1.50

Table 3: Effect of seed borne fungi on seeds and seedlings (Test tube agar method)

Variety	No. of seed tested	Per cent emergence	Seed rot (%)	Seedling blight (%)	Fungi associated	
					Seed rot	Seedling blight
AKM-8802	**30**	**73**	**16**	**10**	*M. phaseolina* *F. semitectum* *F. oxysporum*	*M. phaseolina*
Koper gaon	**30**	**66**	**20**	**13**	*A. niger* *F. oxysporum* *M. phaseolina*	*M. phaseolina*
AKM-4	**30**	**74**	**14**	**12**	*F. oxysporum* *F. moniliforme* *M. phaseolina*	*M. phaseolina*
TARM-18	**30**	**84**	**16**	**-**	*A. flavus* *F. semitectum* *M. phaseolina*	**-**
Greengold	**30**	**72**	**12**	**16**	*A. flavus* *C. globosum* *F. semitectum* *M. phaseolina*	*C. graminicola* *M. phaseolina*

Table 4: Effect of fungicides seed treatment on seed germination, shoot root length and seedling vigour index

Seed treatments	Dose (g/kg seed)	Green gram			
		Germination (%)	Shoot length (cm)	Root length (cm)	Seedling Vigour index
Thiram	3.00	76.33 (60.90)*	9.96	8.45	1405
Carbendazim	1.00	72.66 (58.48)*	8.55	7.26	1148
Thiram + Carbendazim(2:1)	3.00	85 (67.21)*	11.17	9.27	1734
Thiophanate methyl	1.00	66.33 (54.54)*	8.46	7.58	1063
Captan	3.00	70.00 (56.79)*	9.33	8.06	1217
Mancozeb	3.00	64.66 (53.53)*	8.11	7.55	1012
Propineb	3.00	78 (62.03)*	10.7	9.77	1596
Tebuconazole	1.00	60 (50.77)*	8.01	7.38	923
Hexaconazole	1.00	56 (48.45)*	7.68	6.79	810
Control		50 (45.00)*			
'F test		Sig.			
SE(m)±		0.25			
CD (P=0.01)		0.98			

*Arc sin value

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Table 1. Association of seed borne fungi with untreated and treated Green gram seeds (Blotter paper method)

Seed borne fungi	Kopergaon		AKM-8802		AKM-4		TARM-18		Green gold		Average untreated	Average treated
	UT	PT	UT	PT	UT	PT	UT	PT	UT	PT		
Acremonium strictum	-	-	-	-	2.50	-	-	-	-	-	2.50	-
Aspergillus flavus	9.50	-	13.00	1.00	8.50	2.00	5.50	-	5.50	-	9.40	3.00
Aspergillus niger	1.50	-	2.00	-	2.50	-	3.50	-	1.50	-	2.20	-
Curvularia lunata	4.50	-	2.50	-	-	-	-	-	1.50	-	2.83	-
Fusarium semitectum	10.50	1.50	16.00	3.00	10.50	2.50	11.00	1.50	11.50	2.00	12.90	2.10
Fusarium solani	-	-	-	-	6.00		-	-	4.50	-	10.50	-
Fusarium oxysporum	22.50	3.50	19.00	4.50	18.00	3.50	16.50	3.00	15.50	3.50	18.30	3.60
Macrophomina phaseolina	36.50	5.00	32.00	5.00	26.50	3.00	22.00	2.00	28.00	3.50	29.00	3.70
Penicillium sp.	7.00	-	5.50	-	4.50	-	11.00	-	-	-	7.00	-
Phoma medicaginis	3.50	-	3.00	-	-	-	2.00	-	3.00	-	2.87	-
Rhizopus sp.	3.00	-	2.00	-	4.50	-	2.50	-	2.50	-	3.00	-
Total fungi	98.50	10.00	95.00	13.50	83.50	11.00	74.00	6.50	73.50	8.00	90.00	12.40

UT-Untreated, PT-Pre treated