Studies on Seedborne Nature and Management of *Alternaria alternata* and *A. brassicae* in Pigeonpea

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Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the major grain legumes of the tropics and subtropics of the world, and is widely grown in many Indian states (Nene and Sheila, 1990). Most of the *Alternaria* spp. are primarily saprophytic; however, some species are true pathogens causing diseases of a wide range of crops (Bart Thomma, 2003). Leaf spot/blight of pigeonpea caused by *Alternaria alternata* Keissler has been observed occasionally in pigeonpea growing areas of eastern Indo-Gangetic plains of Bihar and Uttar Pradesh, and also in Andhra Pradesh, Gujarat and Rajasthan (Mehta and Sinha, 1982). Infected plants produce discolored and wrinkled seeds that carry 31-35% infection of *A. alternata* (Khare *et al.*, 2003). The seed-borne nature and seed transmission of *A. brassicae* (Berk) Sacc. in rape seed and Mustard has been demonstrated (Shrestha *et al.*, 2000), but no such report is available for pigeonpea. In the present investigation, the seedborne nature of *Alternaria* spp. was studied and new formulations with a combination of contact and systemic fungicides were evaluated against seedborne infection of *Alternaria* spp. affecting pigeonpea. This would greatly facilitate the long-term conservation and safe exchange of pigeonpea germplasm.

Four pigeonpea accessions (ICP 12108, ICP 13446, ICP 13448 and ICP 13271) that had shown >90 % seed infection by *Alternaria* spp. during the seed health testing in 2005 at Plant Quarantine Laboratory, ICRISAT, Patancheru were used for detecting the seedborne nature of *Alternaria* spp. Seed of pigeonpea accession, ICPL 87051 was used as resistant check (<3 % infection) in all the experiments.

The selected accessions were assayed for fungal colonization by blotter method (ISTA, 1993), in which a total of 300 seeds from each accession in 10 replications (30 seeds/rep) were plated and incubated at 22 ± 2°C with 12 h photoperiod for seven days in a completely randomized fashion. Prior to plating, seeds were surface sterilized with clorax (NaOCl, 2.2%) for 2 min and washed twice with distilled sterilized water (dsw). Seed infection by *A. alternata* can be easily detected on incubated seed using blotter method wherein the pathogen produces wooly or powdery chains of dark brown conidia of variable lengths and shapes (Ahmed and Ravinder Reddy, 1993).

The component plating method (Siddiqui *et al.*, 1983) was used to determine the nature of seed infection. Twenty-five seed from each of the four accessions (ICP 12108, ICP 13446, ICP 13448, ICP 13271) and ICPL 87051 were surface sterilized with 2.2% clorax, thoroughly washed with distilled water and then soaked in 3% potassium hydroxide (KOH) solution for 14 h for the separation of seed coat, cotyledon and embryo. As the seed components of ICP 13448 could not be separated with this treatment, the component plating was done only for three accessions. The seed components were washed in dsw, dried for a while and transferred on to the potato dextrose agar (PDA) plates, and incubated at 22± 2°C for five days with 12 h photoperiod. Data were recorded seven days after incubation for the number of seed components colonized with the species of *Alternaria*.

Iprodione 25 % + carbendazim 25 % (Quintal™) a combination formulation of contact and systemic fungicides (Bayer Cropscience Company, India); tricyclazole 75% (Beam®), a systemic fungicide (Indofil Chemicals Company, India); benomyl (Coromandel Fertilizers Limited, India) and thiram, (Chemet Chemicals Ltd. India); either alone or in combination were used for seed treatment. Tricyclazole 75% ® (2 g a.i.) kg seed^{-1}, iprodione 25 % + carbendazim 25 %, thiram and benomyl + thiram (1:1) each @ 3 g a.i. kg seed^{-1} were used for seed dressing.

Twenty-five seeds of each accession (5 seeds/rep) were treated with each fungicide separately and tested for seed colonization using blotter method. Untreated seed of each accession served as control. Data were recorded on the number of seed colonized by *Alternaria* spp. and seed germination at seven days after incubation.

Two species of *Alternaria, A. alternata* and *A. brassicae* were detected (using the identification key as described by Ellis, 1971) in seed of all the four accessions. *A. alternata* was identified by the appearance of long conidial chain on

incubated seed under stereo-binocular microscope and the dark-brown conidia with short beak under Compound microscope. Similarly, the presence of *A. brassicae* was confirmed by the presence of distinct, solitary big pale brown conidia with long beak on incubated seed. Seed colonization ranged from 76 to 85% by *A. alternata* and from 15-23% by *A. brassicae* across the susceptible accessions, whereas infection in resistant control was only 3% by *A. alternata* and there was no infection of *A. brassicae* (Table 1). The overall seed germination ranged from 18-29 % in infected seeds across the genotypes, whereas it was 81% in resistant control (Table1). There was a significant negative correlation between germination and infection (r = 0.90).

Component plating revealed that seed coat recorded 100 % infection by both the species of *Alternaria* in all three genotypes. *A. alternata* infection in cotyledons varied from 76 to 86 %, and in embryos from 3 to 8 % except in ICP 12108 and resistant check ICPL 87051 (Table 2). *A. brassicae* infection in seed cotyledon ranged from 3 to 9% whereas in embryo it was 2 % only in ICP 13446.

Seed colonization by both *Alternaria* species was significantly inhibited in all the accessions by benomyl + thiram, iprodione 25%+carbendazim25% and thiram treatments compared to tricyclazole® and control (Table 3). There was no seed colonization with *Alternaria* spp. in the treatment of benomyl +thiram in ICP 13446, ICP 13448 and ICP 13271, and only 4 % seed colonization occurred in ICP 13446. It was observed that growth of *Alternaria* spp. was suppressed at initial stages in tricyclazole® treatment in all genotypes, but the effect did not last long as evidenced by heavy colonization. The results indicated that the iprodione 25%+carbendazim25% and mixture of benomyl and thiram proved most effective in reducing the internal seedborne infection by *Alternaria* spp.

Fungicide seed treatment showed significantly increased seed germination compared to untreated control. Germination of treated seed with benomyl+thiram, iprodione 25%+ carbendazim 25% and thiram ranged from 56 to 100%, whereas it was 24-36% in untreated seed and 84% in resistant check ICPL 87051 (Table 3). Among the fungicides, iprodione 25%+carbendazim25% showed the maximum seed germination. Seed treated with tricyclazole although showed germination could not contain the seed infection as effectively as the other fungicides. The benomyl and thiram (1:1) mixture as suggested by Kannaiyan *et al.*, (1980) has been routinely used for pigeonpea seed treatment (3 g kg^{-1}) for the germplasm exchange at Plant Quarantine laboratory at ICRISAT, Patancheru. Seed dressing with benomyl (at 0.1 lb a.i./100lb seed) was also reported to be effective in controlling *A. brassicae* in rape seed (Ansari *et al.*, 1990).

This study provided information on the internally seedborne nature of both *A. alternata* and *A. brassicae,* although embryo infection frequency was low in the pigeonpea accessions evaluated. Most of *A. alternata* infection appeared to have been limited to seed coat and cotyledon and of *A. brassicae* to seed coat. However, the limited infection in embryo is of great significance from plant quarantine perspective in transmitting the pathogen from one location to another through germplasm exchange. *A. alternata* has already been known as a pathogen of pigeonpea (Ahmed and Ravinder Reddy, 1993) but the detection of seedborne nature of *A. brassicae* in pigeonpea is probably reported for the first time, although it is well known in rape seed and mustard (Shrestha *et al.*, 2000).

Based on the results of this study, it is suggested that iprodione 25%+carbendazim 25% that have both contact and

Table 1. Seed colonization and germination of pigeonpea genotypes by *Alternaria* using blotter method

Genotypes	Seed coloniztion (%)[a]		Seed germination (%)[a]
	A. alternata	*A. brassicae*	
ICP 12108	78	20	25
ICP 13271	85	15	18
ICP 13446	76	23	29
ICP 13448	79	20	27
ICPL 87051 (Res. control)	3	00	81
LSD (P=5%)	3.5	4.2	12.91

a. Data are the mean of 10 replications (30 seed /replication), after 7 days of incubation

Table 2. Detection of *Alternaria* species infection in seed components of pigeonpea genotypes

Genotype	Infection (%)[a]					
	A. alternata			*A. brassicae*		
	SC	CO	EM	SC	CO	EM
ICP 12108	100	76	0	100	6	0
ICP 13271	100	86	8	100	3	0
ICP 13446	100	84	3	100	9	2
ICPL 87051 (Control)	00	00	0	0	0	0
SE±	0.0	8.8	2.7	0.0	0.8	0.0

SC = Seed coat; CO = Cotyledon; EM = Embryo
a. Data are the mean of five replications (five seed/replication)

Table 3. Effect of fungicidal seed treatment on infection and seed germination in pigeonpea genotypes infected with *Alternaria* spp.

	Infection (%)[a] and germination (%)[a] in different accessions									
	ICP 12108		ICP 13271		ICP 13446		ICP 13448		ICPL 87051 (RC)	
Treatments	Inf	Ger	Inf	Ger	Inf	Ger	Inf	Ger	Inf	Ger
Bn + T	0	96	0	89	4	92	0	76	0	96
Iprodione 25%+carbendazim25%	0	96	0	92	0	100	0	92	0	96
Tricyclazole®	100	80	92	56	100	80	100	56	0	92
Thiram	0	96	16	60	4	100	8	80	0	96
Control (Untreated)	100	24	100	28	100	24	100	36	0	84
LSD (P=5%)	3.8	11.3	7.4	14.2	5.1	11.9	4.2	13.1	0.0	9.2

a.Data are the mean of 10 replications/accession
Inf = Infection; Ger = Germination; Bn +T = Benomyl + Thiram; RC = Resistant control

systemic actions could now be used to treat pigeonpea seed @ 2 g a.i. kg^{-1} for regeneration for long-term conservation and for export.

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References

Ahmed K M and Ravinder Reddy Ch 1993. A pictorial guide to the identification of seed borne fungi of Sorgum, Pearl Millet, Finger Millet, Chickpea, Pigeonpea and Groundnut. Information Bulletin No. 34. Patancheru, Andhra Pradesh 502324, India. International Crops Research Institute for the Semi-Arid Tropics. pp. 200.

Ansari N A, Wajid Kahn M and Muheet A 1990. Evaluation of some fungicides for seed treatment and foliar application in management of damping-off of seedlings and blight of rapeseed caused by *Alternaria brassicae*. *Mycopathologia* **110 :** 163-167.

Bart Thomma P H J 2003. Pathogen profile, *Alternaria* spp. from general saprophyte to specific parasite. *Molecular Plant Pathology* **4 :** 1-225.

Ellis M B 1971. *Alternaria.* **In**: Ellis, M.B. (ed.) Dematiaceous Hyphyomycetes, CAB International. pp.608.

ISTA 1993. International rules for seed testing. *Seed Science and Technology* **21 :** supplement 333p.

Kannaiyan J, Nene Y L and Sheila V K 1980. Control of microflora associated with pigeonpea seeds. *Indian Journal of Plant Protection* **8 :** 93-98.

Khare A, Srivastava M, Kumar Kumud and Kumar Vijay. 2003. In vitro studies on associative and antagonistic effects between seedborne fungi and *Alternaria tenuissima* causing leaf blight in pigeonpea. *Farm Science Journal* **12 :** 153-154.

Mehta P P and Sinha R K P 1982. A new leaf spot disease of arhar from India. *Science and Culture* **48 :** 44.

Nene, Y L and **Sheila, V K 1990.** Pigeonpea: geography and importance. The pigeonpea. (eds. Nene, Y. L., Susan, D. Hall and Sheila, V. K.) CAB International, ICRISAT, Patancheru, Andhra Pradesh, India. pp. 1-14.

Shrestha S K, Mathur S B and Munk L 2000. *Alternaria brassicae* in seeds of rapeseed and mustard, its location in seeds, transmission from seeds to seedlings and control. *Seed Science and Technology* **28 :** 75-84.

Siddiqui M R, Mathur S B and Neergaard R 1983. Longevity and pathogenicity of *Colletotrichum* spp. in seed stored at 5°C. *Seed Science and Technology* **11 :** 353-36.

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