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In vitro efficacy of fungicides and bioagents against alternaria blight of pigeonpea caused by *Alternaria alternata*

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Abstract
Alternaria blight disease caused by *Alternaria alternata* is devastating disease. In this experiment total eight seed dressing fungicides at their recommended dosages were evaluated *in-vitro* by poisoned food technique, against *Alternaria alternata* caused by alternaria blight. The result revealed that treatment with carboxin 37.5% + thiram 37.5% 75WP @ 0.25% (85.98%) was found effective fungicide followed by tebuconazole 25 % WG @ 0.2 % (83.57), captan 75% WP @ 0.3% (66.42%), carbendazim 12% + mancozeb 63% 75WP @ 0.25% (63.33%), pyroclostrobin 20% WG @ 0.1% (45.10%), carbendazim 50% WP @ 0.1% (21.02%) and thiophanate methyl 70% WP @ 0.1% (14.33%), against *Alternaria alternata*. The eight bioagents evaluated against *alternaria alternata* by dual culture technique. The most effective bioagent was found *T. hamatum* (85.88%) followed by *A. niger* (77.00%), *T. asperellum* (74.77%), *T. harzianum* (74.00%), *T. koningii* (54.21%), *T. longibrachatum* (44.80%) and *P. fluorescens* (43.44%).

Keywords: Pigeonpea, bioagents, fungicides, *Alternaria alternata*, alternaria blight

Introduction
Pigeonpea (*Cajanus cajan*) is an important legume crop of rainfed agriculture in the tropics and subtropics. It is a versatile crop grown primarily as a vegetable and a multi-use green crop (dhal) in India. Pigeonpea seed is composed of cotyledons (85%), embryo (1%) and seed coat (14%) (Faris and Singh, 1990) [8]. Pigeonpea provides high quality vegetable proteins (21%) and some important amino acids like metheonine, lysine and tryptophan to human beings and is one of the sources of animal feed and fire wood. It is also a valuable source of carbohydrates (62.7g/100 g mature raw seed). In Maharashtra, pigeonpea is cultivated on an area of 12.29 Lakh/ha with the production of 10.59 Lakh/tones. The fungi associated with seeds at the stage of harvest, transport, processing and under storage bring about several undesirable changes, making them unfit for human consumption and sowing. Seedborne pathogens may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage (Khanzada *et al.*, 2002) [11].
The literature revealed that more than hundred pathogens are known to affect the pigeonpea crop. Amongst them *Fusarium* wilt, *Alternaria* blight, *Phytophthora* blight, *Alternaria* leaf spot, *Rhizoctonia* root rot and *Cercospora* leaf spot are the most common fungal pathogens associated with stored seeds, mainly responsible for seed deterioration and reduction in the germination potential and also seedling vigour. Among all mycoflora of pigeonpea, *Aspergillus niger, A. flavus and Alternaria alternata* reduced germination and seedling vigour to a greater extent compared to other and they can also spoil the quality of grains during storage (Agrawal, 2003) [1]. Pigeonpea seeds therefore, need to be protected against these fungi to achieve a uniform plant stand and vigorous seedling. Seed treatment for controlling plant diseases has been termed as the “pain less method” for farmers. Seed treatment with fungicide application can minimize disease and thus increase genetic potential and ultimately yield. Biological agents *viz. Trichoderma* sp., *Bacillus* sp. and *Pseudomonas* sp. manage wide range of seed borne fungi and there is no risk to produced resistance. Present investigation was carried out with in vitro evaluation of fungicides and bioagents for control of *Alternaria alternata* casing alternaria blight of pigeonpea.

Materials and Methods
The experiments (*in vitro*) was conducted at Department of Plant Pathology, College of Agriculture, Latur during 2018-2019. Efficacy of various seed dressing fungicides were evaluated at their recommended dosages against *Alternaria alternata*, by applying Poisoned

food technique (Nene and Thapliyal, 1993) [13] and using Potato Dextrose Agar (PDA) as a basal culture medium. Based on active ingredient, requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (45^{0}C) PDA medium separately in conical flasks to obtain desired concentrations of the test fungicides. Fungicide amended PDA medium was then poured (20 ml / plate) separately and aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature. After solidification of the medium, all the plates were inoculated aseptically by putting in the center 5 mm culture disc obtained from a week old actively growing pure culture of *Alternaria alternata.* Each of the test fungicides and its concentration was replicated three times. Test pathogens were assessed separately. Petri plates filled with plain PDA (without fungicide) and inoculated with the culture disc of *Alternaria alternate*. Fungal and bacterial biocontrol agents were evaluated *in-vitro* against *Alternaria alternata* fungi pathogenic to pigeonpea, applying dual culture technique (Dennis and Webster, 1971) [5]. Seven days old cultures of the test bio-agents and the pathogens were used for the study. Discs of 5 mm diameter of culture growth of *Alternaria alternata* the test bioagents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and test bio-agent were placed at equidistance and exactly opposite to each other on autoclaved and cooled PDA medium in petri plates and incubated at 26±2^{0}C. Test pathogens were assessed separately. PDA plates inoculated separately with culture disc of *Alternaria alternata* were maintained as untreated control. The colony diameter of the fungus pathogens on medium was recorded and per cent inhibition was calculated by using following formula (Vincent, 1927) [15].

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,
C = growth of the test fungus in untreated control plate
T = growth of the test fungus in treated plate

Fungicide Treatments details

Tr. No.	Treatments	Conc. (%)	Tr. No.	Treatments	Conc. (%)
T_1	Carbendazim 50%WP	0.1	T_5	Pyraclostrobin 20% WG	0.1
T_2	Thiophanate methyl 70%WP	0.1	T_6	Carboxin 37.5% +thiram37.5% WP	0.25
T_3	Tebuconazole 25%WG	0.2	T_7	Carbendazim12%+mancozeb 63% WP	0.25
T_4	Captan 75%WP	0.3	T_8	Control (Untreated)	-

Bioagent Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Trichoderma asperllum*	T_5	*T. longibrachitum*
T_2	*T. harzianum*	T_6	*Aspergillus niger*
T_3	*T. hamatum*	T_7	*Pseudomonas fluorescens*

Result and Discussion

A total of eight seed dressing fungicides at their recommended field dosages were evaluated *in vitro* by Poisoned food technique, against *Alternaria alternata* of pigeonpea which were detected in seed health testing methods and the results obtained on their colony diameter (mm) and per cent inhibition of mycelial growth are presented in Table No. 1.

The results revealed that, all of the test fungicides exhibited significant mycelial growth inhibition of the *Alternaria alternata*, over untreated control. However, the effective fungicide was carboxin 37.5% + thiram 37.5% 75WP @ 0.25% (85.98%), followed by tebuconazole 25 % WG @ 0.2 % (83.57), captan 75% WP @ 0.3% (66.42%), carbendazim 12% + mancozeb 63% 75WP @ 0.25% (63.33%), pyroclostrobin 20% WG @ 0.1% (45.10%), carbendazim 50% WP @ 0.1% (21.02%) and thiophanate methyl 70% WP @ 0.1% (14.33%), against *Alternaria alternata*. Thus, except the fungicides, carbendazim 50% WP and thiophanate methyl 70% WP at their recommended dosages, rest of the five seed dressing fungicides tested were found highly effective against *Alternaria alternata*.

A total of seven bioagents were evaluated *in vitro* by dual culture technique, against *Alternaria alternata* of pigeonpea and the result obtained on colony diameter (mm) and per cent inhibition of mycelia growth of these fungi are presented in Table No. 2. For Alternaria alternata significantly highest mycelial growth inhibition was with *T. hamatum* (85.88%), followed by *A. niger* (77.00%), *T. asperellum* (74.77%), *T. harzianum* (74.00%), *T. koningii* (54.21%), *T. longibrachatum* (44.80%) and *P. fluorescens* (43.44%).

Therefore, in present study, various *Trichoderma* spp., followed by *Aspergillus niger* and *P. fluorescens* were also found effective against seedborne pathogenic fungi of pigeonpea and also reported as most effective against pigeonpea seedborne diseases by several earliear workers Pandey and Upadhay1999 [14], Dhar *et al.*,2006 [7], Gade *et al.*, 2007 [9]; Lokesha and Benagi, 2007 [12]; Barde *et al.*, 2016 [3];Athira, 2017 [2]; Chaudhari *et al.*, 2017 [4]; Devamani *et al.*, 2017 [6] and Kadam *et al*., 2018 [10].

Table 1: *In vitro* efficacy of various fungicides against *Alternaria alternata* associated with pigeonpea seeds

Sr. No.	Treatments	*Alternaria alternata*	
		Colony diameter (mm)	Inhibition (%)
T_1	Carbendazim 50% WP	71.08	21.02 (27.28)
T_2	Thiophanate methyl 70 % WP	77.10	14.33 (22.24)
T_3	Tebuconazole 25 % WP	14.78	83.57 (66.08)
T_4	Captan 75 WP	30.22	66.42 (54.58)
T_5	Pyroclostrobin 20% WG	49.41	45.10 (42.18)
T_6	Carboxin 37.5 % + Thiram 37.5 % WP	12.61	85.98 (68.01)
T_7	Carbendazim12%+ Mancozeb 63% WP	33.00	66.33 (52.73)
T_8	Control (untreated)	90	0.00 (00)
SE ±		0.81	0.79
CD (P=s0.01%)		2.38	2.31

Table 2: *In vitro* efficacy of various bioagents against *Alternaria alternata* associated with pigeonpea seeds

Sr. No.	Treatments	*Alternaria alternata*	
		Colony Diameter (mm)	Inhibition (%)
T_1	*T. asperellum*	22.70	74.77(59.84)
T_2	*T. harzianum*	23.40	74.00(59.34)
T_3	*T. hamatum*	13.60	85.88(67.11)
T_4	*T. koningii*	41.21	54.21 (47.41)
T_5	*T. longibrachitum*	49.68	44.80 (42.01)
T_6	*Aspergillus niger*	20.70	77.00 (61.34)
T_7	*Pseudomonas fluorescens*	50.90	43.44(41.23)
T_8	Control (Untreated)	90	0.00(00)
SE±		0.93	1.28
CD (P=s0.01%)		2.71	3.74

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