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Seed Mycoflora Associated with Pigeonpea [*Cajanus cajan* (L.) Millsp.], their Significance and the Management

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Pigeon pea *(Cajanus cajan* L.) seeds mycoflora was studied and subsequently determine their effect on seed germination and seedling growth in pot condition. Seed sample were examined in agar plate and blotter method showed association of nine fungi belonging six genera *i.e., Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Fusarium udum* and *Alternaria alternata*. Seed mycoflora and their culture filtrate caused considered reduction in germination per cent and seedling growth as compared to untreated check. Lowest seed germination recorded by *Aspergillus niger* in both culture filtrate (43.00%) and seed inoculated (56.00%).The effect of different chemical fungicides, bio-agents and phyto-extracts on seed mycoflora, germination and vigour index of pigeonpea was evaluated. The seed treatments improved seed germination, vigour index and reducing seed borne mycoflora of pigeonpea seeds. Different fungicides, bio-agents and phyto-extracts were evaluated as seed treatment. In fungicides, Matalaxyl 8% + Mancozeb 64% @ 0.2% was found superior in seed germination (93.33%), shoot length (11.23cm), root length (13.67cm) and vigour index (2323.73) whereas, in bio-agents *Trichoderma viride* was found most effective in seed germination (88.00%), shoot length (9.03cm), root length (11.17cm) and vigour index (1777.46). seed treatment with phyto-extracts Neem seed extract gave more seed germination (78.67%), shoot length (7.97cm), root length (9.20cm) and vigour index (1350.00) compared to control.

Keywords: Seed borne fungi, Pigeonpea, Culture filtrate, Chemical, Bio-agent, Phyto-extract.

Pigeonpea (*Cajanus cajan* (L.) Millsp.) belongs to family Fabaceae, a valuable *Kharif* pulse crop, is cultivated under 3.90 M ha with production of 3.17 mt and 1230 kg/ha productivity[5] in India and 2.1 M ha cultivated in Gujarat with the production of 2, 09, 000 tonnes[2]. It is both a food crop and a forage/cover crop having 21 per cent of protein content. Studies on the mycoflora associated with pigeonpea seeds and their significance have been made by different researchers and they revealed that more than hundred pathogens were known to affect the pigeonpea crop. Among them *Fusarium, Alternaria, Phytophthora, Alternaria, Rhizoctonia* and *Cercospora* are the most common fungal pathogen associated with stored seed and are mainly responsible for seed deterioration, reduction in germination potential and seedling

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vigour[4]. 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[16]. The seeds are passive carriers of pathogens that are transmitted when seeds germinated under suitable environmental conditions. It held that progressive reduction in the concomitant loss of viability due to seed borne fungal spores. Seed treatment for controlling plant diseases has been termed as the "pain less method" for farmers. Seed treatment with fungicides, bio-agents and phyto extracts application can minimize disease and thus increase genetic potential and ultimately yield. Chemical fungicides widely use and easily apply for control of fungal diseases. Biological agents *viz; Trichoderma viride, T. harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* are manage wide range of seed borne fungi, there is no risk to produced resistance. Another, one source of potential new pesticides is natural products produced by plants. Plant extracts and essential oils show antifungal activity against a wide range of fungi[1]. The present investigation was undertaken to find out the seed borne fungi associated with the seeds of pigeonpea and the effect of fungicides, bio-agents and plant-extracts on seed mycoflora, seed germination, seedling length and vigour index of pigeonpea.

MATERIALS AND METHODS

Experimental location

The experiment was conducted in the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari in 2014-15. Navsari has an average temperature of 37 °C and annual rainfall of 1959 mm (last 10 years), 2420 mm in 2013 and 1475 mm in 2014, it's Latitude 20^0 57' 07.05" N, Longitude 72^0 55'16.50" E and altitude is 12.33 meter above sea level.

Sources of experimental materials

Seed samples of pigeonpea were collected for isolation and identification of seed-borne fungi from ten villages (Karankhat, Manpur, Abrama, Simalgam, Dandi, Sadakpor, Khergam, Kesali, Khambhala, and Dandi) of Navsari district and also from Navsari Agricultural University's farm during month April, 2014. Farmers were selected randomly for sampling. From each seed sample, an amount of 250g seeds were taken and kept separately in labeled, pre-sterilized polythene bags.

Plating of the seed component

Standard blotter paper method and Agar plate method as described by the International Seed Testing Association[6], was used for the isolation of the seed-borne fungi associated with the pigeonpea seed samples.

Standard blotter paper method

In the blotter paper method, pair of sterile white blotter papers of 8.5 cm diameter was soaked in sterile distilled water and were placed in pre-sterilized petri plates of 90 mm diameter. Ten seeds per Petri plates, in order to isolate only internal seed mycoflora, were surface sterilized for 2 minutes with 1% sodium hypochlorite solution followed by three subsequent washings in sterilized distilled water to remove sodium hypochlorite from seed and non-surface sterilized, were placed at equal distance on three layers of properly moistened sterilized blotters. 400 seeds were used in each experiment. These plates were incubated at a temperature of 25±2 ^{0}C for 12 hrs in alternating cycles of light and darkness. The seeds were examined regularly for the growth of fungi over the seed.

Agar plate method

In Agar plate method, pre-sterilized Petri plate were poured with 20 ml of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described in blotter test method.

Examination of incubated seeds

Sampling for identification of fungi was done at seventh days. The Petri dishes were brought to the examination area in the laboratory, where each seed was examined under a microscope for growth habits of the various fungi growing in the Petri plates. Slide preparations of the various fruiting structures of the fungi were made and identified under the stereozoom compound microscope. The samples of fungus were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi. The binocular compound microscope was used to determine the

type of fungus in each plate. The seed-borne fungi were identified using identification keys and cross-checked for each seed plates to identify the type of fungus growing on each seed. After seven days of incubation, fungal species found growing on the surface of seeds, were identified and their percentage frequency of occurrence of fungal was calculated by applying the following formula: PF = (No. of seeds on which fungus appear / Total number of seeds) X 100

Inoculation

Impact of seed infecting fungi and effect of cultural filtrate on seed health status was studied in respect of seed germinability and seed vigour from artificially inoculated seeds with fungi isolated from naturally infected pigeonpea seeds.

Fungal species used for artificial inoculation of seeds

Healthy seeds of pigeonpea cv. Vaishali were artificially inoculated with each of nine fungal species separately. For artificially inoculation, seeds moistened by sterilized distilled water were mixed thoroughly with 10 days old respective fungal culture growth were obtained at 25 ± 2 ^{0}C on PDA plates. One sheet of germination paper was wetted by sterilized distilled water. Twenty five seeds of respective treatment were placed on first sheet evenly. Second sheet of germination paper was placed on first sheet followed by wetting it carefully. Both sheets were rolled along with wax coated paper. The rolled papers were incubated in seed germinator at 25 ^{0}C for 7 days. At the end of incubation period, rolled towel papers were carefully opened. Germinated and un-germinated seeds were counted from each of the treatments. Emergence of seedling from the seeds was considered as successful germination. Three replications each of 25 seeds were maintained for each of the treatments. After end of incubation period, these seeds were used for study of seed germination and seedling vigour index. Un-inoculated seeds served as control treatment for comparison.

Effect of culture filtrates of seed-infecting fungi on seed germination and seedling growth

All different fungi were separately cultured on modified Richards's liquid medium at 25± 2 ^{0}C for 10 days. Liquid medium along with fungal growth of each fungus was filtered through Watman filter No. 42. Resulting filtrates were used to evaluate their effect on seed germination and seedling growth. Healthy seeds of pigeonpea cv. Vaishali were treated by soaking the seeds for 8 hr into culture filtrate of respective fungus obtained from the 15 days old fungal culture grown on modified Richard's liquid medium at 25± 2 ^{0}C. Then, influence of culture filtrate of respective fungus was evaluated by Paper towel method[9]. Seeds soaked in sterilized distilled water served as control treatment. Seventy five treated seeds in each of the treatments with three replications were tested.

Observations were recorded on seed germination, discolouration of radicals and plumules if any, and seedling length after 10 days of incubation at room temperature. Emergence of seedling from the seeds was considered as successful germination of seeds.

Seed treatments Treatment with fungicides

Seven chemical fungicides used for seed treatment in different concentration *viz;* mancozeb 75% WP, carbendazim 50% WP, metalaxyl 8% + mancozeb 64%, pyraclostrobin 5% + metiram 55%, carbendazim 12% + mancozeb 63%, carboxin 75% WP & chlorothalonil 75% WP were evaluated to check their efficacy on germination and vigour index of seeds inoculated with isolated fungi. For this healthy seeds of pigeonpea were inoculated with the mixture of all isolated fungus by soaking the seeds into mixed spore suspension of fungi and then, treated with all respective chemicals. These treated seeds were evaluated by paper towel method[9] and incubated at 27 ± 2 ^{0}C for 7 days. After end of incubation period observations were recorded as number of germinated seeds, shoot length and root length to calculating vigour index and germination percentage.

Treatment with antagonists and phyto-extracts as bio-priming

Bio-priming was done to study the effect of different bio-agents *viz; Trichoderma viride* @ 0.4%, *Trichoderma harzianum* @ 0.4%, *Pseudomonas fluorescens* @ 5.0 ml, *Bacillus subtilis* @ 5.0 ml and phyto-extracts *viz;* Cumin seed extract @ 0.2%, Neem seed extract @ 1.0% & Garlic clove extract @ 1.0% used in different concentration on germination and seedling vigour. Seeds of pigeonpea were soaked in spore suspension of each of the bio-agents and phyto-extracts for 24 hours. 2% sugar solution was added into the suspension as sticky material and to

provide nutrition to bio-agents which were used for inoculation. Concentration of each bio-agent was adjusted at 10^7-10^8 cfu/ml with the help hemocytometer and serial dilution technique. Then, effect of respective bio-agents and phyto-extracts were evaluated by Paper towel method[9]. Seeds soaked in sterilized distilled water served as control treatment. Seventy five treated seeds in each of the treatments with three replications were tested.

After incubation period, observation was recorded as no. of seed germinated, root length and shoot length and with these observation, vigour index was calculated with the help of formula; Vigour index (VI) = Germination (%) X Mean seedling length (cm)[12].

Statistical analysis

The data, collected under study were subjected to the statistical analysis for proper interpretation. The standard method of analysis of variance technique appropriate to the Complete Randomized Design (C. R. D.) as described by[15] was used. The data were analyzed with the technical help received from computer center of N. M. College of Agriculture, N. A. U., Navsari. The treatment differences were tested by employing 'F' test at five per cent level of significance on the basis of null hypothesis. The appropriate standard error of mean (S. Em. ±) were calculated in each case and the Critical Difference (C. D.) at five per cent level of probability were worked out to compare the two treatment means, where the treatment effects were found significant under 'F' test. The co-efficient of variation percentage (C. V. %) was also worked out for all the cases.

RESULTS AND DISCUSSION

Isolation

Totally nine fungi with six genera were isolated by standard blotter paper and agar plate method i.e. *Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, Fusarium udum, Drechslera sp., Curvularia lunata, Rhizoctonia sp., Aspergillus niger* and *Aspergillus flavus*. Such similar results were observed by[15]. He found sixteen fungal species belonging to genera namely, *Alternaria alternata, Aspergillus flavus, A. niger, Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum, F. moniliforme, F. roseum, Pythium sp., Rhizoctonia solani, Rhizopus stolonifer, Botrytis cineria, Macrophomina phaseolina, Penicillium notatum* and *Phytophthora cinnamomi* were associate with seeds of pigeonpea by agar plate method. Fourteen fungal species viz; *Alternaria alternata, A. longissimi, Aspergillus niger, A. flavus, Botrytis cineria, Cladosporium cladosporides, Colletotrichum dematium, Curvularia lunata, Fusarium moniliforme, F. semitectum, Phyllostictacajani, Rhizoctonia bataticola, R. solani* and *Trichothecium roseum* were isolate from pigeonpea seeds by blotter paper method[21]. Kandhare reported total seventeen fungi from different categories of pigeonpea seeds viz; *Alternaria alternata, A. tenis, Aspergillus flavus, A. carbonarius, A. fumigatus, A. nidulans, A. niger, Chaetomium globosum, Cladosporium spp., Colletotrichum truncatum, Curvularia lunata, Drechslera tetramera, Fusarium moniliforme, F. oxysporum, Macrophomina phaseolina, Penicillium spp.* and *Rhizopus stolonifer* by standard blotter and agar plate method[8].

Effect of seed infecting fungi on seed health status

Assessment by artificially inoculation of pigeonpea seeds separately by nine different fungi revealed significant effect on seed germination, shoot and root length, and thereby seedling vigour index (Table 1). Each of the fungi exhibited significant adverse effects on seed germination, shoot and root length.

Seed inoculated by *Aspergillus niger* showed lowest seed germination (56.00%) which was at par with Aspergillus flavus (58.00%). Whereas, in *Fusarium oxysporum, Fusarium moniliforme, Fusarium udum, Alternaria alternata, Drechslera sp., Curvularia lunata* and *Rhizoctonia* sp. recorded 63.00, 68.00, 71.00, 76.00, 81.00, 86.00 and 91.00 per cent germination, respectively. The result in terms of shoot and root length with seedling vigour index, all the treatments showed smaller shoot length, root length and seedling vigour index as compared to control. *Aspergillus niger* recorded minimum shoot length (4.00 cm), root length (5.25 cm) and seedling vigour index (518.20) which was at par with *Aspergillus flavus* (4.15 cm, 5.43 cm and 555.70, respectively). Similarly, *Fusarium oxysporum, Fusarium moniliforme, Fusarium udum, Alternaria alternata, Drechslera sp., Curvularia lunata* and *Rhizoctonia* sp. also recorded less shoot length,

root length and seedling vigour index. On the contrary, significantly highest seed germination (97.00%), shoot length (9.48 cm), root length (13.33 cm) and seedling vigour index (2212.00) were obtained in healthy seeds.

The present results are similar as reported by Lokesh and Hiremath who found the 68.00 per

Table 1. Effect of seed inoculation with different fungi on seed germination, shoot length, root length and seedling vigour index in pigeonpea

Fungi	Seed germination (%)*	Decrease in seed germination over healthy seed (%)	Shoot length (cm)*	Decrease in shoot length over healthy seed (%)	Root length (cm)*	Decrease in root length over healthy seed (%)	Seedling vigour index (SVI)
Alternaria alternata	76.00	21.65	7.68	18.98	9.78	26.63	1326.30
Fusarium oxysporum	63.00	35.05	6.30	33.54	7.65	42.61	878.58
Fusarium moniliforme	68.00	29.89	5.48	42.19	7.03	47.26	850.74
Fusarium udum	71.00	26.80	6.85	27.74	8.43	36.75	1084.70
Drechslera sp.	81.00	16.49	7.30	22.99	9.45	29.10	1356.30
Curvularia lunata	86.00	11.34	8.05	15.08	10.30	22.73	1578.00
Rhizoctonia sp.	91.00	6.18	8.60	9.28	10.60	20.48	1746.20
Aspergillus niger	56.00	42.26	4.00	57.80	5.25	60.61	518.20
Aspergillus flavus	58.00	40.20	4.15	56.22	5.43	59.27	555.70
Control (Healthy seed)	97.00	-	9.48	-	13.33	-	2212.00
S. Em ±	1.13		0.06		0.07		18.30
CD 0.05%	3.25		0.17		0.20		52.85
CV %	4.76		2.79		2.52		4.78

length and seedling vigour index in pigeonpea
*Average of four repetitions and 25 seeds each repetition

Table 2. Effect of culture filtrate of isolated seed infecting fungi on seed germination, shoot length, root length

Fungi	Seed germination (%)*	Decrease in seed germination over healthy seed (%)	Shoot length (cm)*	Decrease in shoot length over healthy seed (%)	Root length (cm)*	Decrease in root length over healthy seed (%)	Seedling vigour index (SVI)
Alternaria alternata	66.00	31.25	5.80	28.83	8.73	31.79	958.50
Fusarium oxysporum	54.00	43.75	4.73	41.96	5.83	54.45	569.70
Fusarium moniliforme	58.00	39.58	5.60	31.28	7.10	44.53	736.90
Fusarium udum	62.00	35.42	5.30	34.96	6.48	49.37	729.90
Drechslera sp.	69.00	28.12	6.68	18.03	9.65	24.60	1127.10
Curvularia lunata	73.00	23.96	6.20	23.92	9.15	28.51	1121.10
Rhizoctonia sp.	78.00	18.75	7.03	13.74	10.33	19.29	1352.90
Aspergillus niger	43.00	55.20	3.30	59.50	4.70	63.28	344.20
Aspergillus flavus	45.00	53.12	3.45	57.67	4.83	62.27	372.72
Control (Healthy seed)	96.00	-	8.15	-	12.80	-	2011.00
S. Em ±	1.03		0.07		0.05		15.80
CD 0.05%	2.98		0.19		0.15		45.62
CV %	5.07		3.79		2.07		5.36

length and seedling vigour index in pigeonpea
*Average of four repetitions and 25 seeds each repetition

cent reduction in seed germination, 35.00 per cent reduction in shoot elongation and 38.91 per cent reduction in root elongation over control in seed tested with Aspergillus niger in pigeonpea crop[11]. Whereas, highest per cent decrease seed germination, shoot length and root length recorded 62.00, 61.01 and 59.49 per cent, respectively in *Fusarium* sp. and 21.00, 32.59 and 28.77 per cent, respectively in *Aspergillus niger* by Khayum *et al*. in soybean seeds[10].

Effect of culture filtrate of isolated seed infecting fungi on seed health

Results on seed germination, shoot and root length and seedling vigour index (SVI) of pigeonpea as influenced by culture filtrates of nine different isolated fungi, presented in Table-2,

Table 3. Effect of seed treatment with fungicides on pigeonpea seed germination, shoot length, root length and seedling vigour index *in vitro*

Treatment	Conc.	Seed germination (%)*	Increase in seed germination over control (%)	Shoot length (cm)*	Increase in shoot length over control (%)	Root length (cm)*	Increase in root length over control (%)	Seedling vigour index (SVI)
Mancozeb 75% WP	0.3%	84.00	57.51	9.20	48.39	11.87	61.93	1769.20
Carbendazim 50% WP	0.1%	74.67	40.01	8.30	33.87	10.57	44.20	1409.20
Metalaxyl 8% + Mancozeb 64%	0.2%	93.33	75.00	11.23	81.12	13.67	86.49	2323.73
Pyraclostrobin 5% + Mitiram 55%	0.2%	90.67	70.02	9.93	60.16	13.50	84.17	2124.53
Carbendazim 12% + Mancozeb 63%	0.2%	92.00	72.51	11.07	78.55	12.87	75.57	2201.07
Carboxin 75% WP	0.3%	80.00	50.00	8.13	31.13	9.45	28.92	1406.36
Chlorothalonil 75% WP	0.3%	77.33	45.00	7.73	24.68	9.17	25.10	1306.80
Control	-	53.33	-	6.20	-	7.33	-	721.07
SEm ±		1.24		0.06		0.07		21.76
CD 0.05%		3.71		0.19		0.20		65.24
CV %		4.85		2.18		1.94		04.15

*Average of three repetitions and 25 seeds each repetition

Table 4. Screening of known antagonists as bio-priming agents and phyto-extracts to control of pigeonpea seed borne fungi *in vitro*

Treatment	Conc.	Seed germination (%)*	Increase in seed germination over control (%)	Shoot length (cm)*	Increase in shoot length over control (%)	Root length (cm)*	Increase in root length over control (%)	Seedling vigour index (SVI)
Trichoderma viride	0.4%	88.00	73.67	9.03	72.99	11.17	81.03	1777.46
Trichoderma harzianum	0.4%	85.33	68.40	8.90	70.49	11.07	79.41	1703.73
Pseudomonas fluorescens	5.0ml	77.33	52.61	6.20	18.78	7.17	16.20	1033.47
Bacillus subtilis	5.0ml	73.33	44.72	7.60	45.60	8.63	39.87	1190.27
Neem seed extract	0.2%	78.67	55.26	7.97	52.68	9.20	49.10	1350.00
Garlic clove extract	1.0%	74.67	47.37	6.10	16.86	6.87	11.35	967.73
Cumin seed extract	1.0%	72.00	42.09	6.95	33.14	7.63	23.67	1049.13
Control	-	50.67	-	5.22	-	6.17	-	576.60
S. Em ±		1.00		0.05		0.04		13.57
CD 0.05%		3.00		0.15		0.12		40.69
CV %		4.22		2.22		1.46		3.56

*Average of three repetitions and 25 seeds each repetition

revealed significant effects on seed germination, shoot and root length, and thereby SVI.

Seed inoculated by culture filtrate of *Aspergillus niger* showed lowest seed germination (43.00%) which was at par with *Aspergillus flavus* (45.00%). Whereas, in *Fusarium oxysporum, Fusarium moniliforme, Fusarium udum, Alternaria alternata, Drechslera* sp., *Curvularia lunata* and *Rhizoctonia* sp. recorded 54.00, 58.00, 62.00, 66.00, 69.00, 73.00 and 78.00 per cent germination, respectively. The result in terms of shoot and root length with seedling vigour index, all the treatments showed smaller shoot length, root length and seedling vigour index as compared to control. *Aspergillus niger* recorded minimum shoot length (3.30 cm), root length (4.70 cm) and seedling vigour index (344.20) which was at par with *Aspergillus flavus* 3.45 cm, 4.83 cm and 372.72, respectively. Similarly, *Fusarium oxysporum, Fusarium moniliforme, Fusarium udum, Alternaria alternata, Drechslera* sp., *Curvularia lunata* and *Rhizoctonia* sp. also recorded less shoot length, root length and seedling vigour index. On the contrary, significantly highest seed germination (96.00%), shoot length (8.15 cm), root length (12.80 cm) and seedling vigour index (2011.00) were obtained in healthy seeds.

The similar result was observed by Lokesh and Hiremath[11]. They found that 64.37 per cent reduction in seed germination, 90.26 per cent reduction in shoot elongation and 90.25 per cent reduction in root elongation over control in seed tested with *Aspergillus niger* in pigeonpea crop. Jalander and Gachande also found that cultural filtrates of *Aspergillus niger* caused reduction in seed germination and root-shoot elongation in different pulse crops[7].

Management Effect of seed treatment with fungicides on pigeonpea seed

The results pertaining to germination of seeds under pot condition revealed that all the fungicides were superior over control. Seven different fungicides were tested to check their effect on seed germination and seedling health of pigeonpea seeds inoculated with mixture of all isolated fungi.

Data presented in the Table 3 revealed significant effect of all fungicides on seed germination, shoot length, root length and seedling vigour index. Seed treated with metalaxyl 8% + mancozeb 64% recorded highest seed germination (93.33%) which was at par with carbendazim 12% + mancozeb 63% (92.00%) and pyraclostrobin 5% + mitiram 55% (90.67%). Whereas, mancozeb 75% WP, carbendazim 50% WP, carboxin 75% WP and chlorothalonil 75% WP recorded 84.00, 74.67, 80.00 and 77.33 per cent seed germination, respectively.

Significantly maximum shoot length (11.23 cm) was observed in seed treated with metalaxyl 8% + mancozeb 64% which was at par with carbendazim 12% + mancozeb 63% (11.07 cm). Seeds treated with mancozeb 75% WP (9.20 cm), carbendazim 50% WP (8.30 cm), pyraclostrobin 5% + metiram 55% (9.93 cm), carboxin 75% WP (8.13 cm) and chlorothalonil 75% WP (7.73 cm) also increased shoot length over control. Significantly maximum root length (13.67 cm) was observed in metalaxyl 8% + mancozeb 64% which was at par with pyraclostrobin 5% + mitiram 55% (13.50 cm). Seeds treated with mancozeb 75% WP (11.87 cm), carbendazim 50% WP (10.57 cm), carbendazim 12% + mancozeb 63% (12.87 cm), carboxin 75% WP (9.45 cm) and chlorothalonil 75% WP (9.17 cm) also increased root length over control.

Maximum seedling vigour index recorded by seed treated with metalaxyl 8% + mancozeb 64% (2323.73) followed by carbendazim 12% + Mancozeb 63% (2201.07), pyraclostrobin 5% + mitiram 55% (2124.53), mancozeb 75% WP (1769.20), carbendazim 50% WP (1409.20), carboxin 75% WP (1406.36) and chlorothalonil 75% WP (1306.80). On the contrary, significantly lowest seed germination (53.33%), shoot length (6.20 cm), root length (7.33 cm) and seedling vigour index (721.07) were obtained in untreated seeds.

Similar results were obtain in seed treated with Cabria top (pyraclostrobin 5% + metiram 55%) @ 2 g/Kg recorded higher seed germination (76.6%) and disease control (61.1%) by Singh *et al*. in chickpea[23], while seed treated with Ridomil MZ (metalaxyl 8% + mancozeb 64%) gave higher seed germination (88.77%), plant height (56.78 cm) and decrease disease severity (23.63%) in sorghum by Singh and Singh[22]. Singh *et al*. observed that seed treated with Ridomil MZ @ 0.1% and Saaf @ 0.2% gave lowest disease incidence (8.56% and 9.55%) and higher disease control (78.03% and 75.49%) against root rot in pea crop[20]. Mallesh *et al*. recorded seed treated with mancozeb @ 2 g/Kg gave higher seed germination (93.00%) and

seedling vigour index (2273) at 30 DAS in pigeonpea[12]. Seed treated with carboxin recorded 84.00% seed germination in groundnut by Singh *et al*.[24]. Saroja also recorded slurry seed treatment with mancozeb @ 3 g/Kg gave maximum seed germination (85.00%) in chickpea[19]. Seed treated with vitavax gave 80.06 per cent seed germination and seedling vigour index is 6183.87 observed in pigeonpea by Singh *et al*.[23]. Mogle and Maske recorded seed treated with Dithane M-45 gave maximum seed germination (90.00%), shoot length (19.2 cm), root length (21.1 cm) and vigour index (3627) in cowpea seed[13]. Ram and Pandey found significantly minimum incidence of Fusarium wilt of pigeonpea (13.81%) in combined seed treatment of metiram @ 0.1% + Trichoderma viride[18]. Screening of known antagonists as bio-priming agents and phyto-extracts to control of pigeonpea seed borne fungi in vitro

Different bio-agents and phyto-extracts were tested to check their effect on seed germination and seedling health of pigeonpea seeds inoculated with mixture of all isolated fungi. Data presented in the Table 4 revealed significant effect of all bio-agents and phyto-extracts on seed germination shoot length, root length and seedling vigour index. Seed treated with Trichoderma viride recorded highest seed germination (88.00%) which was at par with Trichoderma harzianum (85.33%). Whereas, in Pseudomonas fluorescens, Bacillus subtilis, Neem seed extract, Garlic clove extract and Cumin seed extract recorded 77.33, 73.33, 78.67, 74.67 and 72.00 per cent seed germination, respectively. The results in terms of shoot and root length with seedling vigour index, all the treatments showed larger shoot length, root length and seedling vigour index as compared to control. Seed bio-priming with T. viride recorded maximum shoot length (9.30 cm), root length (11.17 cm) and seedling vigour index (1777.46) which was at par with T. harzianum (8.90 cm, 11.07 cm and 1703.73), respectively. Similarly, Neem seed extract (7.97 cm, 9.20 cm and 1350.00), B. subtilis (7.60 cm, 8.63 cm and 1190.27), Cumin seed extract (6.95 cm, 7.63 cm and 1049.13), P. fluorescens (6.20 cm, 7.17 cm and 1033.47) and Garlic clove extract (6.10 cm, 6.87 cm and 967.73) also recorded more shoot length, root length and seedling vigour index, respectively as compared to control (5.22 cm, 6.17 cm and 576.60).

Similarly, Mallesh *et al*. recorded maximum 92.00% seed germination in seed bio-priming with Trichoderma viride and T. harzianum and seedling vigour index 2240 and 2235 by Trichoderma viride and T. harzianum, respectively at 30 DAS in pigeonpea[12]. Purushothaman observed seed treated with Neem seed oil recorded highest seed germination (83.25%) and least seed mycoflora incidence (6.00%) in cowpea[17]. Seeds treated with Trichoderma harzianum gave highest per cent germination in cowpea was reported by Banyal and Ashlesha[3]. Murthy *et al*. was observed that seed treatment with Trichoderma harzianum @ 8 X 10^8 cfu/ml gave significant reduction of seed borne mycoflora up to 90 per cent with 84 per cent germination in different pulses[14]. Minimum incidence of Fusarium wilt of pigeonpea (13.81%) in combined seed treatment of metiram @ 0.1% + Trichoderma viride was found by Ram and Pandey[18].

CONCLUSION

Seed borne fungi cause qualitative and quantitative losses during storage seeds, results in huge economic losses. Present study was undertaken to identify of different fungi associated with pigeonpea during storage and deteriorated the seed germination, shoot length and root length. Study revealed that Aspergillus niger, in general, outnumbered all the other fungal species and were widely distributed in the seed samples of different categories. From the above discussion it is clear that the studies on seed mycoflora of food crops like chickpea is an important aspect of the plant protection because without seed health tests we cannot touch the target of food security as the healthy seeds are the pre-required of the healthy agriculture.

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