International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 3591-3595

Received: 14-03-2018
Accepted: 18-04-2018

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Effect of seed mycoflora on seed germination and seedling vigour of cowpea cultivars

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Abstract
The seed germination, seedlings length and Seedling Vigour Index (SVI) of cowpea cultivars was drastically reduced by all the seed mycoflora except *Macrophomina phaseolina* and *Alternaria alternata. Fusarium oxysporum* showed maximum detrimental effects on seed germination, seedling length and SVI in all cultivars. *Aspergillus terreus* and *Macrophomina phaseolina* showed relatively less inhibitory effect on seed germination in cultivar GDVC 1 and seedlings length of GC 3 and GDVC 1 as compared to other cultivars. *Rhizopus* sp., *Fusarium oxysporum*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata* and *Macrophomina phaseolina* showed seed germination, seedlings length and SVI between 27.50 to 85.75 per cent, 6.00 to 7.92 cm and 185.62 to 671.22, respectively in all the cultivars. Discoloration on cowpea seedling increased by seed mycoflora infection in all cultivars. The highest per cent discoloration intensity (PDI) was recorded in *Fusarium oxysporum* treatment. On the basis of per cent seed germination of the cultivar GDVC 1 and AVCP 1, seedling length of the cultivar GC 3 and GC 4, SVI of the cultivars GDVC 1 and AVCP 1 and PDI of the cultivars GC 3 and GDVC 1 appeared to be least tolerant to seed mycoflora.

Keywords: cowpea cultivars, seed mycoflora, seed germination, seedlings length, seedling vigour, discoloration intensity

Introduction
Cowpea (*Vigna unguiculata* (L.) Walp.), an annual legume, is also commonly referred to as southern pea, blackeye pea, crowder pea, lubia, niebe, coupe or frijole. Cowpea originated in Africa and is widely grown in Africa, Latin America, South east Asia and in the southern United States. On an average cowpea grains contain 23-25% protein and 50-67% starch in dry bases (Quin, 1997) [7].

In India, total area under pulses is 23.47 million hectares, having total production of 18.32 million tonnes with the productivity of 781 kg per hectare. (Anon., 2016) [1] and in Gujarat total area under pulses is 0.813 million hectares having total production of 0.738 million tonnes with the productivity of 908 kg per hectare (Anon., 2016) [1]. In Gujarat area under cowpea is 0.52 million hectares and the production 0.35 million tonnes with the productivity of 665 kg per hectare (Anon., 2016) [1].

In India, cowpea is mainly grown in the states of Karnataka, Kerala, Maharastra, and Tamilnadu in kharif season for seed, green pods, animal fodder and organic green manure purpose.

In Gujarat, cowpea is mainly grown in the districts of Kachchh, Banaskantha, Mehsana and Panchmahal in *kharif* season under inadequate and erratic rainfall. However, it is grown in very large area during summer season in Kheda, Baroda and Panchmahal districts. It is cultivated during *kharif* in Saurashtra.

Gowda and Sullia (1987) [4] reported that fungi infected 96-98 % of seeds of cowpea and soybean were infected by fungi. Cowpeas are susceptible to attacks by several fungal organisms at all stages of their growth (Enyiukwu and Awurum, 2013) [3]. Reduced germinability of seeds may be attributed to damaged embryos from deep seated infection of seeds. All the fungi used in this study induced disease symptoms on germinating seedlings.

Materials and Methods
The experiment was undertaken at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand during 2016-17. Seed of cowpea cultivars received from Centre of Excellence for Research on Pulses, SDAU, Sardarkrushinagar

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Effect of seed mycoflora on seed health

Total five cultivars viz., GC 3, GC 4, GC 5, GDVC 1 and AVCP 1 were used for further study. Following methods were used to study the influence of seed mycoflora on seed health in respect to seed germination and seedling vigour index of cowpea.

Seed inoculation with seed mycoflora

Cowpea seeds of five cultivars were artificially inoculated with each of the seed mycoflora separately. Seeds moistened by sterilized water were mixed thoroughly with 10 days old respective fungal culture obtained on PDA at 25 + 2 ˚C. Such treated seeds were kept in Petri plates for overnight at 25 + 2 ˚C, and then these seeds were used for seed germination, seedling vigour index study and assessment of seedling abnormalities.

Effect on seed germinability

Effect of seed mycoflora on seed germination was carried out by paper towel method (Fig. 1). One sheet of germination paper was wetted by distilled water. Twenty five seeds of respective cowpea cultivars inoculated with respective seed mycoflora were placed on first sheet evenly. Second sheet of germination paper was placed on first sheet. Second sheet was wetted carefully. Both sheets were rolled along with wax coated paper. The rolled papers were incubated in seed germinator at 25 ˚C for 7 days. At the end of incubation, rolled towel papers were opened carefully. Germinated and ungerminated seeds were counted treatment-wise and variety-wise, healthy seeds without inoculation of seed mycoflora was kept as control. Four replication each of 100 seeds were maintained for each of the treatments. Seedlings length of germinated seeds were recorded.

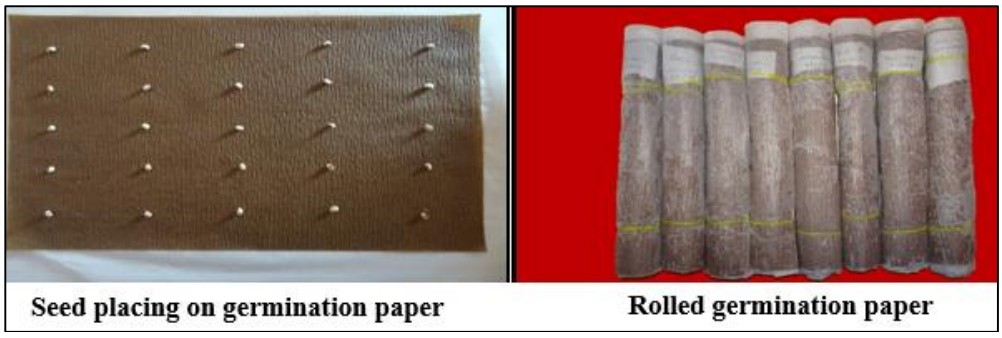

Fig 1: Germination test by Paper towel method

Seedling vigour index

Seedling vigour index was calculated on the basis of seed germination and seedling length after seven days of incubation. (Thippeswamy and Lokesh, 1977).

Vigour Index (VI) = (Mean root length + Mean shoot length) × Seed Germination (%)

Assessment of seedlings by symptoms grade

The seedlings symptoms were observed after ten days of incubation of inoculated seeds of respective cultivars by using paper towel method. Each of the developing seedlings was observed visually and if required with the help of magnifying hand lens for seedlings discolouration due to seed mycoflora infection on developing seedlings. Seedlings discoloration were categorized into 0-4 rating scale as 0= Healthy seedling (no visible symptoms), 1= Discolouration of 1-10 per cent part of seedling, 2= Discolouration of 11-25 per cent part of seedling, 3= Discolouration of 26-50 per cent part of seedling and 4= Discolouration of >50 per cent part of seedling. Four repetition each of 25 seeds were evaluated for each of the treatments. The per cent discoloration intensity was calculated as per formula given by Kotastahane and Agarwal, 1976.

$$PDI = \frac{\text{Sum of all rating}}{\text{No. of seedling examined} \times \text{Maximum rating scale}} \times 100$$

(PDI = Per cent Discoloration Intensity)

Results and Discussion

Effect of seed mycoflora on seed germination

Seed germination was influenced by seed mycoflora revealed significant differences (Table 1). All the test fungi showed pronounced inhibitory effects on seed germination of cowpea cultivars as evident from reduced germination percentage as compared to control treatment. Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Alternaria alternata, Rhizopus sp., Aspergillus fumigatus, Macrophomina phaseolina, Aspergillus terreus and control (Untreated) showed germination with an average of 48.70, 56.70, 65.65, 66.35, 68.30, 68.40, 68.65, 70.25 and 90.75 per cent, respectively. Significantly lowest seed germination per cent was found in GC 3 (56.75%) which was followed by GC 4 (60.47%) and GC 5 (63.30%) cultivars. Maximun per cent reduction in seed germination was recorded in Fusarium oxysporum (48.70%) inoculated seeds, which was followed by Aspergillus niger (56.70%) and Aspergillus flavus (65.65%).

Sadhu (2014) [8] studied the effect of six dominant seed-borne fungi on seedling emergence shoot and root length of green gram. The fungi Aspergillus niger and Drechslera tetramera affected most adversely to seedling emergence (40 % each, control 90 %). Thavaranjit (2016) reported different fungal genera exhibited different effects on a particular plant seed variety. After 24 hours of incubation for germination, highest growth rate was observed in cowpea which was produced by Trichoderma spp.(10.30 mm), Alternaria spp.(10.20 mm) and Colletotrichum spp. (9.83 mm) and no significant difference was observed in their activity on seed germination.

Table 1: Seed germination of cowpea cultivars as influenced by seed mycoflora

Cultivars	Seed germination (%)									Mean (C)
	Fungi									
	Rhizopus sp.	*Fusarium oxysporum*	*Aspergillus Terreus*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Alternaria alternata*	*Macrophomina phaseolina*	Control	
GC 3	61.75	27.50	63.25	36.00	58.25	61.00	54.75	61.75	86.50	56.75
GC 4	62.25	33.50	63.50	46.75	60.50	64.50	62.75	60.75	89.75	60.47
GC 5	64.50	52.25	66.25	57.00	60.75	57.75	59.75	60.00	91.50	63.30
GDVC 1	80.50	71.50	85.75	76.75	78.75	81.50	80.75	84.75	93.75	81.55
AVCP 1	72.50	58.75	72.50	67.00	70.00	77.25	73.75	76.00	92.25	73.33
Mean (F)	68.30	48.70	70.25	56.70	65.65	68.40	66.35	68.65	90.75	
	C	F	C X F							
S. Em.±	0.60	0.80	1.80							
C. D. at 5 %	1.68	2.25	5.04							
C.V%	5.38									

Note: C = Cultivar, F = Fungi

Effect of seed mycoflora on seedling length

Assessment of seedling length of cowpea cultivars influenced by seed mycoflora was carried out by paper towel method. Results showed that all test fungi significantly reduced seedling length as compared to control (without inoculation of fungi).

Sadhu (2014) [8] studied the effect of six dominant seed-borne fungi on seedling emergence, shoot and root length of green gram. The fungi Aspergillus niger and Drechslera tetramera affected most adversely seedling emergence (40 % each, control 90 %), shoot length (5 cm, control 14 cm), and root length (5 cm, control 10 cm), respectively. The fungus Rhizopus stolonifer affected less adversely the seedling emergence compared to rest of the fungi (seedling emergence 80 %, control 90 %), shoot length (12 cm, control 14 cm) and root length (10 cm, control 10 cm). Shoot length was less affected in case of seeds infested with Fusarium moniliforme (13 cm, control 14 cm). Root length was not affected in case of seeds treated with Rhizopus stolonifer and Aspergillus fumigatus but more root length (12 cm) was recorded in case of seeds infested with Fusarium moniliforme over control.

Fusarium oxysporum showed maximum detrimental effect thereby recorded minimum seedling length in all cultivars viz., GC 3 (6.75 cm), GC 4 (6.38 cm), GC 5 (6.00 cm), GDVC 1 (6.53 cm) and AVCP 1 (6.65 cm). Macrophomina phaseolina and Alternaria alternata showed minimum inhibitory effect in average seedling length in 7.63 cm and 7.49 cm, respectively. Overall, Rhizopus sp., Fusarium oxysporum, Aspergiilus terreus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata, Macrophomina phaseolina and control revealed the seedling length in all cultivars ranged between 7.05 to 7.28, 6.00 to 6.75, 7.15 to 7.78, 6.18 to 6.89, 6.10 to 7.12, 6.23 to 7.65, 7.21 to 7.89, 7.37 to 7.92 and 8.40 to 9.04 cm, respectively (Table 2, Fig. 2).

Kandhare (2015) studied on effect of fungal metabolites on seed health of pulses and result showed that, mycotoxins obtained from all common and dominant seed-borne fungi affected adversely seed germination, shoot and root length.

Fig 2: Seedlings length

Table 2: Seedling length of cowpea cultivars as influenced by seed mycoflora

Cultivars	Seedling length (cm)									Mean (C)
	Fungi									
	Rhizopus sp.	*Fusarium oxysporum*	*Aspergillus terreus*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Alternaria alternata*	*Macrophomina phaseolina*	Control	
GC 3	7.05	6.75	7.78	6.89	7.12	7.65	7.89	7.87	8.95	7.54
GC 4	7.16	6.38	7.68	6.55	7.07	7.14	7.21	7.47	9.04	7.29
GC 5	7.28	6.00	7.15	6.18	6.10	6.23	7.35	7.58	8.85	6.96
GDVC 1	7.17	6.53	7.52	6.61	6.76	6.88	7.64	7.92	8.40	7.27
AVCP 1	7.06	6.65	7.68	6.78	6.80	6.87	7.39	7.37	8.96	7.28
Mean (F)	7.14	6.46	7.56	6.59	6.77	6.95	7.49	7.63	8.84	
	C	F	C X F							
S. Em.±	0.080	0.10	0.24							
C. D. at 5 %	0.22	0.30	7.57							
C.V%	6.67									

Note: C = Cultivar, F = Fungi

Effect of seed mycoflora on seedling vigour index

Seedling vigour index (SVI) was influenced by individual seed mycoflora. Seedliing vigour index was worked out by multiplying the seedling length with germination per cent revealed significant differences. Each of seed mycoflora significantly reduced SVI of all cultivars over the control, Table 3.

In respect of average SVI of five cultivars, Fusarium oxysporum showed the maximum detrimental effect thereby recorded minimum SVI (314.08) followed by Aspergillus niger (373.61) and Aspergillus flavus (444.27), while Aspergillus terreus (531.01) revealed the lowest adverse effect on SVI among eight seed mycoflora evaluated. Remaining seed mycoflora viz., Rhizopus sp., Aspergillus fumigatus, Alternaria alternata and Macrophomina phaseolina showed SVI in between 359.78 to 671.22. Seedling vigour index of cowpea cultivars GC 3, GC 4, GC 5, GDVC 1, and AVCP 1 as influenced by various mycoflora ranged between 185.62 to 492.08, 213.73 to 487.68, 313.50 to 473.68, 466.89 to 671.22 and 390.68 to 560.12, respectively. Cultivar GC 3 revealed lowest SVI (185.62) due to Fusarium oxysporum treatment, which was followed by GC 4 (213.73) and GC 5 (313.50).

Shetty (1990) [9] reported that all the fungi tested reduced percentage seed germination. Among seed borne organisms, fungi caused maximum seed damage, which include reduced germination and vigour. Reduced germinability of seeds was attributed to damaged embryos from deep seated infection of seeds. It was further observed that fungi such as Aspergillus flavus and Fusarium solani were associated with damage to plumule, radicle and hypocotyl of germinating seedlings.

Table 3: Seedling vigour index of cowpea cultivars as influenced by seed mycoflora

Cultivars	Seedling vigour index									Mean (C)
	Fungi									
	Rhizopus sp.	*Fusarium oxysporum*	*Aspergillus terreus*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Alternaria alternata*	*Macrophomina phaseolina*	Control	
GC 3	435.33	185.62	492.08	248.04	414.74	466.65	431.97	485.97	774.17	437.17
GC 4	445.71	213.73	487.68	306.21	427.73	460.53	452.42	453.80	811.34	451.01
GC 5	469.56	313.50	473.68	352.26	370.57	359.78	439.16	452.90	809.77	449.01
GDVC 1	577.18	466.89	644.84	507.31	532.35	560.72	616.93	671.22	787.50	596.10
AVCP 1	511.85	390.68	556.80	454.26	476.00	530.70	545.01	560.12	826.56	539.10
Mean (F)	487.92	314.08	531.01	373.61	444.27	475.67	497.09	524.80	801.86	
	C	F	C X F							
S. Em.±	3.89	5.22	11.68							
C. D. at 5 %	10.90	14.63	32.71							
C.V%	4.73									

Note: C = Cultivar, F = Fungi

Per cent discolouration intensity of cowpea seedlings

Per cent Discolouration intensity was influenced by individual seed mycoflora. Results showed significant differences in per cent discolouration intensity in all cowpea cultivars. Rhizopus sp., Fusarium oxysporum, Aspergillus terreus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata and Macrophomina phaseolina showed PDI between 47.78 to 54.80, 63.90 to 75.83, 47.25 to 56.18, 61.08 to 73.13, 59.60 to 71.50, 56.18 to 64.15, 51.63 to 61.64 and 53.00 to 59.50, respectively (Table 4, Fig. 3).

Fusarium oxysporum showed highest PDI in cultivars viz., GC 5 (75.83) and GC 4 (73.20). Rhizopus sp. showed significantly lowest per cent discoloration intensity in cultivar GDVC 1 (47.78), followed by AVCP 1 (48.13). In respect of average PDI showing overall effects on seedling irrespective of cowpea cultivars Rhizopus sp., Fusarium oxysporum, Aspergillus terreus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Alternaria alternata and Macrophomina phaseolina showed PDI in between 51.37 to 69.36.

Chavan and Kakde (2008) [2] reported that groundnut seeds were highly susceptible to diseases, as they serve as a source of stored nutrients for fungi such as Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, C. lunata, C. pellescens, Fusarium oxysporum, F. equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum and P. chrysogenum causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity.

Table 4: Per cent discoloration intensity of cowpea seedling as influenced by seed mycoflora

Cultivars	Per cent discoloration intensity									Mean (C)
	Fungi									
	Rhizopus sp.	*Fusarium oxysporum*	*Aspergillus terreus*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Alternaria alternata*	*Macrophomina phaseolina*	Control	
GC 3	53.75	65.50	53.50	65.00	60.00	59.50	54.00	56.00	6.33	52.62
GC 4	52.43	73.20	53.75	70.68	65.08	61.63	60.08	53.00	5.50	55.03
GC 5	54.80	75.83	56.18	73.13	71.50	64.15	61.64	59.50	4.75	57.94
GDVC 1	47.78	68.38	50.25	65.38	62.11	60.83	56.29	54.75	5.25	52.33
AVCP 1	48.13	63.90	47.25	61.08	59.60	56.18	51.63	54.08	4.33	49.57
Mean (F)	51.37	69.36	52.18	67.05	63.65	60.45	56.72	55.46	5.23	
	C	F	C X F							
S. Em.±	0.59	0.79	1.77							
C. D. at 5 %	1.65	2.22	4.97							
C.V%	6.65									

Note: C = Cultivar, F = Fungi

Conclusion

All the test fungi significantly reduced seed germination in all cultivars as compared to control treatment. Significantly highest inhibitory effect on seed germination was obtained in Fusarium oxysporum (48.70%) inoculated seeds, which was followed by Aspergillus niger (56.70%), Aspergillus flavus (65.65%) and other seed mycoflora. Cultivar GDVC 1 and AVCP 1 were less affected by Fusarium oxysporum in respect to percent seed germination as compared to other cultivars.

Similar detrimental effect on seedlings length of all cultivars was observed due to seed mycoflora infection except Macrophomina phaseolina. Macrophomina phaseolina induced seedlings length in all cultivars. Macrophomina phaseolina and Alternaria alternata showed minimum inhitory effect in seedling length in GDVC 1 (7.92 cm) and GC 3 (7.89 cm) as compared to other cultivars. Remaining seed mycoflora reduced seedling length (6.46 to 7.63 cm) as compared to control treatment (8.84 cm).

Seedling vigour index (SVI) also reflected the adverse effect of seed mycoflora on the vigour of tiny seedlings of cowpea cultivars. Among the eight fungi, Fusarium oxysporum showed the maximum (314.08) detrimental effect on SVI, while Aspergillus terreus revealed least adverse effect on SVI (531.01). Cultivar GC 3 revealed lowest SVI (185.62) due to Fusarium oxysporum treatment, which was followed by GC 4 (213.73) and GC 5 (313.50).

Discoloration on cowpea seedlings increased due to infection of seed mycoflora except Rhizopus sp., in all cultivars as compared to control treatment. The highest PDI (69.36) was recorded in Fusarium oxysporum treatment as compared to control treatment (5.23). Remaining seed mycoflora showed PDI in between 51.37 to 69.36. Seed germinability, seedling length, SVI and PDI study revealed that cultivar GDVC 1 and AVCP 1, GC 3 and AVCP 1, GDVC 1 and AVCP 1, GC 3 and AVCP 1 showed least detrimental effect of Fusarium oxysporum.

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