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Management of seed mycoflora of cowpea

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Abstract
In vitro evaluation of three fungicides, two phytoextracts, *Trichoderma viride* and cow urine as seed dresser against seed mycoflora of cowpea revealed that Thiram, Carbendazim and Carboxin were superior. Thiram proved to be most effective with least number of fungal species followed by Carbendazim. *Trichoderma viride* and phytoextracts proved its potential as promising approach against seed mycoflora. Cow urine appeared ineffective.

Keywords: cowpea, fungicides, phytoextracts, bioagent and cow urine

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) [8].

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]. The fungi associated with cowpea seeds are externally or internally seedborne. The infected and/or infested seeds increase the inoculum potential in soil and also serve as primary foci for infection. *Ascochyta phaseolorum, Cladosporium vignae, Corynespora cassiicola, Diaporthe phaseolorum, Fusarium oxysporum* and *Macrophomina phaseolina* are major seed-borne pathogens on cowpea seeds.

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 (Dist: Banaskantha) the trial was laid out in completely randomized design under laboratory condition.

Management of seed mycoflora
Evaluation of fungicides, phytoextracts, bioagent and cow urine for the management of seed mycoflora of cowpea was carried out by Agar plate method.

Treatment details

1. Carbendazim @0.1%..... T_1
2. Carboxin @0.1%.....T_2
3. Thiram @0.3%..... T_3
4. Onion (*Allium cepa* L.) bulb extract @10%..... T_4
5. Nilgiri (*Eucalyptus globulus* L.) leaf extract @10%..... T_5
6. Cow urine @100%..... T_6
7. *Trichoderma viride* Pers @10^8 CFU/ml…… T_7
8. Untreated check (Control)…… …..T_8

Preparation of fungicidal solutions

Required concentration of fungicidal solutions for seed treatment of each fungicide under the study were prepared on the basis of active ingredient available in the formulation.

Preparation of Phytoextracts

Fresh and healthy leaves of eucalyptus were collected and washed thoroughly in running tap water. These leaves were cut into small pieces and macerated in sterilized distilled water (1:1 w/v basis) by blender. Similarly, healthy bulbs of red onion, after removing the outermost first thin layer, were macerated in sterilized distilled water by blender. Resulting crude extract of each plant material was filtered through single layer of sterilized muslin cloth. Filtered extract was considered as stock (100 %) solutions. Stock solutions were further diluted to the desired concentration (10 %) by adding required quantity of water, and were used for presoaking for 5 minutes.

Mass culturing of *Trichoderma viride*

The pure culture of *T. viride* available in the Department of Plant Pathology, AAU, Anand was used. It was mass cultured on PDA for one week at 28 $\pm$ 2 ˚C and suspension of fungal growth containing spores was prepared in distilled water so as to obtain 10^8 CFU/ml for seed treatment. The counting of spore was done by following procedure.

Spore Counting

- Drop of conidial suspension that was prepared from the pure culture of *T. viride,* was placed on the engraved grid of haemocytometer and kept for two minutes to allow the conidia to settle at bottom.
- Cover slip was kept over it with care, so that no air bubble enter between the slide and cover slip.
- Counting of spore was carried out by microscopic observation of spore present in the squares.
- Calculation of the spore/ml was carried out by following formula. Average number of spores in large square × 10^4 per cm^3 *i.e.*

$$\frac{A+B+C+D+E}{5} \times 10^4 \text{ per cm}^3$$

Collection of cow urine

Fresh cow urine of morning hours was collected from Livestock Research Station, AAU, Anand. Collected urine was considered as standard suspension (100 %). Cow urine was used for treating the seeds by dipping for 10 minutes.

Results and Discussion

In vitro evaluation of three fungicides (Carbendazim, Carboxin and thiram), two phytoextracts (Onion and Nilgiri), bioagnt (*Trichoderma viride* Pers.) and cow urine as seed treatment at their respective concentrations against seed mycoflora revealed significant differences in per cent seeds mycoflora (Table 1). None of the treatments gave complete control of all fungi. However, Thiram @ 0.3 per cent (T_3) revealed minimum number of fungal species (3) and minimum per cent seeds showed mycoflroa was 0.75 followed by Carbendazim @ 0.1 per cent (T_1) and Carboxin @ 0.1 per cent (T_2).

Less number of seed mycoflora associated was found in the cowpea seed treated with the fungicides *viz*., Benomyl, Dithane M-45 (75% WP) @ 0.2 per cent (Mogle and Maske, 2012) [7]. Singh *et al.* (1990) [10] studied the effect of ten fungicides against stem and root rot of cowpea caused by *Macrophomina phaseolina* (Tassi) Goid in Rajasthan. Seed treatment with Bavistin (3 g/Kg seed) was found to be most effective for controlling the disease followed by Thiram, Carboxin and Topsin-M.

Ashwini and Giri (2014) [2] reported that seed treatment with thiram + carbendazim (2:1) 3g/kg of seed increased the seed germination (85.00%), shoot length (11.17cm), root length (9.27 cm) and seedling vigour index (1734) in green gram**.**

Rhizopus sp., *Aspergillus terreus*, *Aspergillus fumigatus*, *Alternaria alternata* and *Macrophomina phaseolina* did not grow on seeds treated with Thiram (T_3). In respect of per cent seeds showing mycoflora, control (T_8) recorded significantly highest per cent (88.50), followed by cow urine treatment (T_6) and Onion bulb extract treatment (T_4), which showed seed mycoflora 71.25 and 64.75 per cent, respectively.

Thaware *et al.* (2010) [11] evaluated plant extracts which showed antifungal activity against *A. alternata.* The bulb extract of garlic (*Allium sativum*) recorded maximum inhibition (63.33%) of mycelial growth of test fungus and was significantly superior to rest of the treatments. This was followed by sadaphuli (*Catharanthus roseus*), glyricidia (*Glyricidia maculata*), neem (*Azadirachta indica*), karanj (*Pongamia pinnata*), tulsi (*Ocimum sanctum*), and ashok (*Polyalthia longifolia*).

Seed treatment with *Trichoderma viride* Pers., (10^8 CFU/ml) and Nilgiri leaf extracts (10%) were found effective for reducing seed mycoflora in cowpea as compared to cow urine (100%). Similar results have been shown by Sangli and Bambawale (2004) [9] who reported *in vitro* evaluation of the antagonists against *F. oxysporum* f. sp. *sesami* in which *T. viride* reduced the growth of pathogen 83.18 per cent and *T. harzianum* reduced the growth upto 79.54 per cent.

Thaware *et al.* (2010) [11] reported that the bioagents *T. harzianum*, *T. viride* and *T. koningii* significantly inhibited the mycelial growth of the test fungus. Maximum inhibition (85.88% and 80.00%) was observed in *T. harzianum* when it was placed at periphery and at the centre, respectively. *T. viride* and *T. koningii* were also effective in inhibiting the growth of the test fungus (77.44 to 81.88 and 73.33 to 78.11 per cent inhibition, respectively). Kumari *et al.* (2012) [6] found that *Trichoderma harzianum* was more effective as compared to other bio-control agents and inhibited maximum fungal growth (23.20%) of *Macrophomina phaseolina* followed by *Trichoderma viride*, while *P. fluorescens* was the least effective in growth inhibition of the fungus. Under pot conditions, *Trichoderma harzianum* was found most effective in reducing pre and post emergence mortality (13.50 and 9.09%) in green gram.

Kakde *et al.* (2011) [5] studied on antifungal activity of leaf extracts of medicinal plants and showed that *Azadirachta indica* and *Polyalthia longifolia* found antifungal against *Macrophomina phaseolina, Rhizopus stolonifer* and *Penicillium digitatum.*

Table 1: *In vitro* management of seed mycoflora of cowpea

S. No	Treatment	Conc. (%)/Dose	Per cent seeds sowing mycoflora									
			Rhizopus sp.	*Fusarium oxysporum*	*Aspergillus terreus*	*Aspergillus Niger*	*Aspergillus flavus*	*Aspergillus Fumigatus*	*Alternaria Alternate*	*Macrophomina phaseolina*	Total fungal species	Total (%)
1.	Carbendazim	0.1	0.75	3.25	2.25	2.75	1.75	0.00	0.75	0.00	6	11.50
2.	Carboxin	0.1	1.50	8.25	3.00	4.00	3.25	1.75	1.00	1.00	8	23.75
3.	Thiram	0.3	0.00	0.25	0.00	0.25	0.25	0.00	0.00	0.00	3	0.75
4.	Onion (*Allium cepa* L.) bulb extract	10	9.00	12.50	6.50	11.25	9.75	6.75	5.00	4.00	8	64.75
5.	Nilgiri (*Eucalyptus globulus* L.) leaf extract	10	7.75	14.75	5.00	9.50	7.25	6.00	4.00	3.00	8	57.25
6.	Cow urine	100	9.75	16.00	7.00	10.25	11.00	8.25	6.00	3.00	8	71.25
7.	*Trichoderma viride* Pers	10^8 CFU/ml	6.25	10.25	4.25	5.75	5.50	4.00	3.00	2.00	8	41.00
8.	Untreated check (Control)	-	11.75	18.75	8.00	15.50	13.25	9.25	6.75	5.25	8	88.50
	S. Em. ±		0.22	0.34	0.16	0.22	0.24	0.17	0.12	0.08		
	C. D. at 5 %		0.64	1.01	0.47	0.64	0.71	0.51	0.36	0.25		
	C. V %		7.61	6.59	7.17	8.13	7.21	7.07	6.89	7.16		

T_1 = Carbendazim
T_2 = Carboxin
T_3 = Thiram
T_4 = Onion (*Allium cepa L.*) bulb extract
T_5 = Nilgiri (*Eucalyptus globulens L.*) leaf extract
T_6 = Cow urine
T_7 = *Trichoderma viride* Pers
T_8 = Untreated check (Control)

Fig 1: Management of seed mycoflora

Conclusion

Three fungicides, two phytoextracts, *T. viride* and cow urine evaluated *in vitro* condition as seed dresser revealed that all fungicides, *T. viride* and phytoextracts reduced seed mycoflora load over control as well as cow urine. Thiram @ 0.3 per cent showed the least number of fungal species (3) with 0.75 per cent seed mycoflora followed by Carbendazim @ 0.3 per cent (6) with 11.50 per cent seed mycoflora. *Trichoderma viride* (41.00 %), Nilgiri leaf extracts (57.25 %) and *Allium cepa* bulb extract (64.75 %) reduced seed mycoflora as compared to control (88.50 %) and proved its promising potential as eco-friendly approach for the management of seed mycoflora. Cow urine failed to control seed mycoflora over control treatment significantly.

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