EFFICACY OF BIOAGENTS AND FUNGICIDES ON SEED MYCOFLORA, GERMINATION AND VIGOUR INDEX OF COWPEA

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ABSTRACT

The effect of different Leaf extract alone and in combination with *Trichoderma* and fungicides on seed mycoflora, germination and vigour index of Cowpea was evaluated. The seed treatments improved seed germination, vigour index and reducing seed borne mycoflora of Cowpea seeds. A total of 12 fungal species were isolated from cowpea seeds collected in Jalna district, Maharashtra, India. Isolated fungi were identified as *Rhizoctonia solani, Aspergillus flavus, Cladosporium* sp., *Aspergillus niger, Penicillium* sp., *Fusarium oxysporum, Fusarium solani, Fusarium semitectum, Trichoderma viridae, Curvularia lunata, Mucor sp.,* and *Verticillium sp. Rhizoctonia solani, Aspergillus niger, Fusarium oxysporum,* were the most dominant pathogen observed on treated and untreated seeds of Cowpea. It was effectively controlled in the in the treatment of *Trichoderma* + Leaf extract. Less number of mycoflora associated with the fungicides. *Fusarium oxysporum, F. semitectum, F. Solani, Penicillium* Sp*., A. niger, Cladosporium* sp., mostly occurred on the seeds and radicle becoming the weak in some cases decay. Seed germination of cowpea showed less correspondingly in the treatment of fungicides and control, incidence percentage of mycoflora was less recoded. Bioagents treated seeds showed beneficial effects on germination which resulted in increased root-shoot length and seedling vigour. Vigour Index was maximum in the treatment of Sa + Tv (4968) followed by Ph + Tv (4587), Am + Tv (4113) and lowest recoded in Leaf extract and fungicides.

Keywords: bioagents, leaf extract, Cowpea, Seed Borne diseases, Vigour Index, seed germination.

INTRODUCTION

Seed health testing for the presence of seed borne pathogens is an important step in the management of crop diseases. Cowpea is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al*., 1974). It is mainly consumed as a favorite foodstuff in the form of dried seeds, either as flour or split (Johnson and Raymond, 1964; van Wyk and Gericke, 2000). They are a good source of carbohydrates, vitamins, and protein, providing more than half of plant protein in human diets in some areas of the semiarid tropics (Singh *et al.,* 1997; Tuan and Phillips, 1992).

The fungus is seed borne, seed transmitted and causes reduced seed germination (Emechebe, 1981). The control of disease in cowpea has been sought through chemical means and the use of host plant resistance (Oladiran and Oso, 1983; Alabi *et al.,* 1986; Alabi and Emechebe, 1990).

However, the average Indian farmer cannot afford the increasing cost of synthetic chemicals. Furthermore, the use of fungicides has of late resulted in the build up of toxic chemicals potentially hazardous to man and environment and also in the build up of resistance by pathogens (Sinclair, 1971; Adesiyan, 1983).

Therefore the development of biopesticides has been focused as a viable pest control strategy in recent years. 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 (Masoko *et al*., 2007; Grane and Ahmad, 1988; Wilson *et al*., 1997; Abd-Alla *et al.,* 2001). Recently Alkhail (2005) showed that aqueous extracts of plants viz., *Allium sativum, Cymbopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia caryophyllus* had strong antifungal activity against fungi viz., *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani.* In the present study the antifungal activity of aqueous leaf extracts of four plants against *Collectotrichum destructivum* was investigated.

Plant extracts and essential oils show antifungal activity against a wide range of fungi [2]. Therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. The presence of antifungal compounds, in higher plants, has long been recognized as an important factor in disease resistance (Madhadevan, 1982). Such compounds, being biodegradable and selective in their toxicity are considered valuable for controlling some plant diseases eg., *Sclerotium rolfsii* root rot on barley (Singh and Dwivedi, 1987).

Datura is known as Jimsonweed, Thorn apple, Devil's trumpet. Among its 15 species, *D. alba* observes an important drug plants which is distributed through out the warmer portions of the world. The whole plant has medicinal value, but leaves and seeds alone are recognized as official (Sasthri, 1952).

The allelopathic and antifungal potential of *P. hysterophorus* is due to release of phytotoxic substances such as caffeic, ferulic, vanillic, chlorogenic, *p*- coumaric and parthenin, *p*-hydroxybenzoic acids, ambrosin and coronopilin (Jarvis *et al*., 1985). Shah *et al,* (1992), reported that *Argemone mexicana* seed extract was effective in eliminating most of the seed-borne fungi of cowpea but not against *Alternaria alternata*, *Curvularia lunata*, *Mucor* sp. and *Macrophomina phaseolina.* The present study is conducted in order to find out the effect of plant extracts, bioagents and fungicides on sed mycoflora, germination and vigour index of Cowpea.

MATERIALS AND METHODS

Plant extract for seed treatment: Plant materials such as fresh leaves of *Argemone mexicana, Parthenium hysterophorus and Solanum alba* were collected and thoroughly washed in tap water. 100g of leaves of each plant were macerated to thick paste with the help of a mortar and pestle. It was extracted with 100 ml of distilled water and filtered through the muslin cloth. The extract was then centrifuged at 5,000 rpm for 15 min. The supernatant obtained was collected and stored for further use. The plant extracts poisoned food technique was applied (Nene and Thapilyal, 2000) 10 % concentration plant extracts was used separately and amalgamation form with *Trichoderma*.

Seed treatment with Bioagents

Seeds of cowpea (*Vigna unguiculata* L.) were collected from local market of Jalna (MS) India. This experiment was conducted during the month of Dec. 2011. Seeds were first washed with distilled water then surface sterilized with 0.1% HgCl2 for three minutes while for, rinsed thoroughly in distlled water and dried aseptically. The seeds were treated with *T. viridae* fungi, cultures were agitated with sterilized distilled water using a soft camel hair-brush and spore suspension was collected and centrifuged at 10,000 rpm for 10 min. The sediment was collected, air-dried and mixed with the talcum powder in combination of leaf extract of *Argemone mexicana, Parthenium hysterophorus* and *Solanum alba* separately and in combination of *T. viridae*. The fungal spore load was adjusted to 78×106 spores/g seed using a spectrophotometer. The talcum formulation of each bioagent was used for the seed dusting at the rate of 15 g/kg seed. Untreated seeds served as control. There were three replicates of each treatment.

Treatment with fungicides

Chemical fungicides viz., Benomyl, Dithane M-45 75% WP (Manganese ethylene bis dithio carbamate plus zinc) and Bavistin 50% WP (Methyl-H-benzimidazole-2ylcarbamate) were collected from local market authorized agrochemical shops at Jalna in Maharashtra. Seeds were dressed separately with these chemicals at the rate of 0.2% concentration. In all cases, untreated seeds were served as control.

Determination of seed-borne fungi

Fungi were isolated from seeds using the standared blotter method as recommended by ISTA (2003). Three replications of 50 seeds each from each treatment were plated on three layers of well moistened blotter (Whatmann No.1 filter papers) by soaking in 1 % sodium hypochlorite solution for 2 min and washed in three successive changes of sterile distilled water. Three replications of 50 surface sterilized seed were again plated on moistened blotter. The seeded plated were incubated at 28 + /- 2C under incubation, the plated seed were examined under a stereoscopic binocular microscope for microflora association.

Subcultures were made on fresh PDA from fungi emerging on blotter and agar plates for proper identification and pathogenicity tests.

Seed Germination:

To know the effect of these chemicals and bioagents on the seed mycoflora, 50 seeds of each treatment were subjected to standard blotter method in which the seeds were incubated according to the standard procedures of ISTA (Anonymous, 1996). On the 8th day of incubation, seeds were evaluated for the incidence of mycoflora.

Vigour Index:

Radicle and Plumule length were recorded. Vigour index was calculated by using the formula of Baki and Anderson (1973) as shown below:

Vigour index (VI) *= (Mean shoot length + mean root length) x Germination (%)*

Recorded data were subjected to statistical analysis for mean values and test of significance. The variations among the respective data were compared following least significant difference (LSD) test.

Statistical analysis: All data were statistically analyzed by using the 'Analysis of variance'(ANOVA) test and treatments means were compared using the least significant differences (LSD) at P = 0.05 (Mungikar, 1997). The software SAS was also used to conduct all the statistical analyses.

RESULTS AND DISCUSSION

Effect of bioagents and fungicides on seed mycoflora, germination and vigour Index of cowpea was evaluated. Seed mycoflora of Cowpea showed variations in their composition depending on the treatments (Table 2). In blotter method total 12 fungi were isolated with the seeds of cowpea. Isolated fungi were identified as *Rhizoctonia solani, Aspergillus flavus , Cladosporium* sp., *Aspergillus niger, Penicillium* sp., *Fusarium oxysporum, Fusarium solani, Fusarium semitectum, Trichoderma viridae, Curvularia lunata, Mucor sp.,* and *Verticillium sp. Rhizoctonia solani, Aspergillus niger, Fusarium oxysporum,* were the most dominant pathogen observed on treated and untreated seeds of Cowpea. It was reduced in the treatment of *Trichoderma* + Leaf extract. Less number of mycoflora associated with the fungicides. *Fusarium oxysporum, F. semitectum, F. Solani, Penicillium* Sp*., A. niger, Cladosporium* sp., mostly occurred on the seeds and radicle becoming the weak in some cases decay. Seed germination of cowpea showed less correspondingly in the treatment of fungicides and high incidence of the seed borne fungus (Table 2).

Seed germination percentage (Table 1) recorded in the fungicides was 85, 87 and 90 % respectively. Germination percentages were high in the treatment of Leaf extract + *Trichoderma* as 90 to 95 %. The growth hormones which is present in the leaf extract, enhances the germination percentage and *Trichoderma* reduces the growth of pathogenic fungi. These results are in support of the findings of Jeyalakshmi *et al.,* (1998) who reported the biological control potential of *T. harzianum*. *Trichoderma* sp. and bacterial bioagents produce mycolytic enzymes, thus playing an important role in the degradation of target pathogens (Elad *et al.,* 1982; Aziz *et al.,* 1993; Baker and Dickman, 1993; Sivakumar and Narayanaswamy, 1998). Many plant extracts are reported to increase seed germination through decreasing *F. oxysporum* incidence (Bansal and Gupta, 2000).

Seed germination percentage of aqueous leaf extract alone was observed as 87, 90 and 92 but incidence of pathogenic fungi not effectively controlled. Fungicides acted possibly by inducing metabolic changes leading to development of toxic factors, resulting in the internal environment unfavorable for pathogens growth and activity, ultimately inducing the resistance and protection against infection. Fungicides not effectively increased the germination percentage Benomyl 85, Dithane (M-45) 90 and Bavistin 87 %. Fungicides decreased the percent incidence of mycoflora 11, 06 and 14 respectively. Dithane M-45 showed best results in germination as well as in mycoflora. It decreases the mycoflora and enhances the seed germination percentage. These results are confirmatory with the observations Parimala et al. (1998), Sandrou *et al.* (1998) and Kumar and Dubey (2001) in cowpea, blackgram, brinjal and sunflower, respectively.

Table 1: Effect of some leaf extract and fungicides on seed germination, vigour index of cowpea.

Treatment	Seed Germination %	Mean Root Length (cm)	Mean Shoot Length (cm)	Vigour Index
Control	80	18.1	16.8	2792
A) Leaf extract				
Am	90	23.0	20.7	3933
Ph	87	19.8	15.1	3036
Sa	92	20.1	18.2	3523
B) Leaf extract + bioagents				
Am + Tv	90	26.6	19.1	4113
Ph + Tv	94	28.2	20.6	4587
Sa + Tv	95	30.2	22.1	4968
C) Fungicides				
Benomyl	85	20.8	18.1	3306
Dithane M-45	90	21.1	19.2	3627
Bavistin	87	20.4	18.6	3393

Am = *Argemone Mexicana,* Ph= *Parthenium hysterophorus,* Sm = *Solanum alba* Nees and Tv = *Trichderma viridae*

Table 2: Effect of some leaf extract, *Trichoderma* + leaf extract and fungicides on seed germination, vigour index of cowpea

Treatment	Percent incidence of mycoflora												
	Rhizoctonia solani	*Aspergillus flavus*	*Cladosporium* sp.	*Aspergillus niger*	*Penicillium* sp.	*Fusarium oxysporum*	*Fusarium solani*	*Fusarium semitectum*	*Trichoderma viridae*	*Curvularia lunata*	*Mucor sps.*	*Verticillium sp.*	Total Mycoflora
Control	09	03	02	15	04	12	06	03	-	04	07	03	68
A) Leaf extract													
Am	02	01	-	03	01	-	-	-	-	01	06	-	14
Ph	07	02	01	05	03	05	03	01	-	02	05	01	35
Sa	03	01	-	06	04	03	03	-	-	-	02	-	22
B) Leaf extract + *Trichoderma*													
Am	01	-	-	01	01	-	-	-	10	-	06	-	19
Ph	03	-	01	06	04	02	-	-	12	-	01	01	30
Sa	03	01	-	06	04	03	-	-	06	-	-	-	23
C) Fungicides													
Benomyl	03	05	-	03	-	-	-	-	-	-	-	-	11
Dithane M-45	01	02	2	01	-	-	-	-	-	-	-	-	06
Bavistin	04	02	03	04	01	-	-	-	-	-	-	-	14

Reports of De and Chaudhary (1999) are also in confirmation with the present findings, who observed the minimization of wilt disease due to Bavistin, Mancozeb M-45 and Vitavax. Besides, the fungicide treated seeds enhanced the germination compared to control. Untreated seeds recorded the highest seed mycoflora percentage it is 68 and lowest seed germination percentage 80. Singh *et al.* (2002) have made a comparative *in vitro* study with some fungicides like Captan, Dithane M-45, Vitavax, Bavistin for their efficacy in controlling *Fusarium* species on mungbean.

Dubey and Patel (2001) also tested different concentrations of fungicides against various pathogens. Dithane M-45 and Bavistin were reported to be effective in reducing seed-borne infection of *Fusarium* sp. on maize seeds (Kumar and Agarwal, 1998).

Seed treatment improved the radicle and plumule length, highest radicle length was observed in the treatment of Sa + Tv, 30.2 cm followed by Ph + Tv, 28.2, Am + Tv, 26.6, Leaf extract of Am, 23.0 cm and decreasing level recorded in the fungicides and untreated seeds of cowpea. Highest plumule length was recorded in the treatment of bioagents, i. e. Sa + Tv (22.1 cm), followed by Ph + Tv (20.8), Am (20.7), Am + Tv (19.1) and minimum length recoded in the fungicides and untreated seeds of Cowpea.

Bioagents treated seeds showed beneficial effects on germination which resulted in increased root-shoot length and seedling vigour. Vigour Index was maximum in the treatment of Sa + Tv (4968) followed by Ph + Tv (4587), Am + Tv (4113) and lowest recoded in Leaf extract and fungicides. *T. viridae* treated seeds were more efficient than fungicides and leaf extract treated samples. Bunker and Mathur (2000) also reported the similar results with usage of *T. harzianum*. Present findings are in support of the reports of several workers (Dey *et al.,* 1989; Chakrabarty and Rao, 1992; Laxminaryan *et al.*, 1996).

Thus, the present study highlight the importance of seed treatment with most effective fungicides, bioagents and plant extracts in reduction of incidence mycoflora of cowpea seeds. It was recorded that seed treated with the fungicides effectively controlled the seed mycoflora but it is not ecofriendly, polluted our environment. If we can use bioagents and aqueous leaf extract of plant it increases the seed germination percentage as well as decreases the incidence of seed borne mycoflora.

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