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Association of MyCoFlora with Soybean Seed Their Significance and Management

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ABSTRACT

Seed mycoflora of Soyabean *(Glycine max* L.) seeds were examined in agar plate, blotter method and found the association of fungi *i.e., Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Macrophomina phaseolina,Sclerotium rolfsii* and *Alternaria alternata.* Seed mycoflora and their culture filtrate caused considered reduction in seed germination and seedling growth. Effect of fungicides, bio-agents and Phyto-extracts on seed mycoflora, germination and vigour index of Soyabean was also evaluated. Seed treatments improved seed germination, vigour index and reduced seed borne mycoflora.

Keywords: Soybean, seed mycoflora, seed germination, vigor index.

I. INTRODUCTION

Soybean (Glycine max L) is native of eastern asia. Soybean belongs to the family Fabaceae. It contains 40-44% protein, 20% oil, 8.77% fats and 5.6% fibers. It is also rich in both major and minor minerals.

Major fungal diseases of Soybean (Glycine max L) include Leaf spot, Blight, seedling rot, collar rot, charcoal rot, downy mildew (Mukharjee et.al. 1986). Studies on the mycoflora associated with soya bean seeds and their significance have been made by different researchers and they revealed that more than hundred pathogens were known to affect the Soyabean crop(Sinclair, 1982). The fungal pathogens associated with seeds are responsible for several undesirable changes, making them unfit for human consumption as well as sowing (Patil et.al. 2012).The continuous use of chemicals to control diseases results in accumulation of harmful residues of chemicals in the soil, water and seed.

Thus, in recent years, considerable success has been achieved byintroducing antagonists to control seed borne fungalpathogens. A notable work has been done formanagement of seedling diseases of many cropscaused by Rhizoctonia solani and Sclerotium rolfsiiboth in vitro and in pot culture experiments by usingTrichoderma (Akhter 1999, Pradeep et al. 2000,Raihan et al. 2003, Haider 2005).Plant extracts also show antifungalactivity against wide range of fungi (Abd-Allaet.al 2001).Present investigation wasundertaken to find out the fungi associated withthe seeds of Soybean, and effect offungicides, bio-agents and plant-extracts onseed mycoflora, seed germination, seedlinglength and vigor index.

II. MATERIALS AND METHODS

Collection of seed samples: (Source of cultivars)

Seeds of Soybean (Glycine max L)-Cv. PK-472, Cv. MAUS-30, Cv. MAUS-38.were obtained from the pulses Researchstation, Marathwada Agricultural University,Badnapur, Jalna, Maharashtra, local farmers,local dealer, etc. The seeds were stored at22°C in cloth bags and used whenever

Plating of the seed component

Standard blotter paper method and Agar plate method as described by the International Seed Testing Association (ISTA 1996), was used for the isolation of the seed-borne fungi associated with the Soybean seed samples.

Standard blotter test:

Seeds were equidistantly spaced on moist sterile blotters in Petri plate moist chambers.10 Petri plates of 9" diameter each containing 10 seeds were incubated at 27+2°C for eight days. Observations were made for fungi appearing on seeds every 24 hours and growth was carefully transferred to PDA slants for further studies.

Agar plating:

Seeds were equidistantly plated on GNA plates aseptically. Colonies which developed during three days were picked up and maintained on PDA/GNA slants. Untreated seeds disinfected externally by treating with10% sodium hypochlorite solution for 10 minutes were used for internally seed mycoflora.

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. These treated seeds were evaluated by paper towel method(Khare, M.N.1996)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, 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 pigeon pea were soaked in spore suspension of each of the bio-agents and phytoextracts 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 107 - 108 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 (Khare, M.N.1996). Seeds soaked in sterilized distilled water served as control treatment.

Identification of Fungi:

The various fungi were identified to their generic and specific taxon on the basis of gross colonial and microscopic morphology as per the Key given in "Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and Key to species" (Tsuneo Watanabe, 2002). The fungi were identified on the basis of the shape, measurement and size of the conidiophores, sporangiophores, vesicles, sterigmata, conidia, hyphae, conidial head morphology by using binocular microscope (LABO Bioplan XL). Morphological studies were usually made from the material mounted on slides in lacto phenol and cotton blue. Certain fungi, however particularly those imperfect species which from chains of spores that were easily displaced. Such imperfect fungi were studied without disrupting their growth pattern.

III. RESULTS AND DISCUSSION

Eight fungi belonging to six genera were isolatedby standard blotter paper and agar plate method. Those were Alternaria alternata,Fusarium oxysporum, Fusarium moniliforme, Sclerotium rolfsii, Curvularia lunata, Macrophomina phaseolina, Aspergillus niger and Aspergillus flavus.

Effect of seed infecting fungi on seed health status

Assessment by artificially Inoculation of pigeon pea seeds with fungi significantly reduced seed germination,shoot and root length, and thereby seedling vigour index (Table 1). Seeds inoculated withFusarium oxysporumshowed lowest seed germination (56%) which was at par with Fusarium moniliforme(58%) as earlier reported. All treatments resulted in reduced shoot length, root length and seedling vigor index. Fusarium oxysporumrecorded minimum shoot length (4.00 cm), root length (5.25 cm) and seedling vigor index (518.20). Treatment with Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Alternaria alternata, Sclerotium rolfsii, Curvularia lunata and Macrophomina phaseolina. recorded similarly.

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

Seeds treated with culture filtrates of Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme, Alternaria alternata, Sclerotium rolfsii., Curvularia lunata and Macrophomina phaseolina recorded 43, 45, 54, 58,62, 66, 69, 73and 78 % germination,respectively (Table, 2). Aspergillus niger recorded minimum shoot length (3.30 cm), root length (4.70 cm) and seedling vigour index (344.20) which wasat par with Aspergillus flavus 3.45 cm, 4.83 cm and 372.72, respectively. Similarly, Fusarium oxysporum, Fusarium moniliforme, Alternaria alternata, Sclerotium rolfsii,Curvularia lunata and Macrophomina phaseolina, also recorded less shoot length, root length and seedling vigor index. Incomparison, significantly highest seed germination (96.00%), shoot length (8.15 cm), root length (12.80 cm) and seedling vigor index(2011.00) were obtained in healthy seeds(Lokesh, M.S. et.al 1992).

Management Effect of seed treatment with fungicides on soybean seed

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) alsoincreased root length over control.

Different bio-agents and phyto-extracts were tested to check their effect on seed germination and seedling health of pigeon pea 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, 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.

IV. CONCLUSION

Results of this work show that the three varieties of soybean seeds studied are infected are Alternaria alternata,Fusarium oxysporum, Fusarium moniliforme, Sclerotium rolfsiiCurvularia lunata, Macrophomina phaseolina. and saprophytic fungi are Aspergillus flavus, Aspergillus niger. The higher seed germination, seedling dry weight, vigour index and lower seed infection are found in fungicides than in bioagent and phyto extracts. From the above discussion it is clearthat the studies on seed mycoflora of food cropslike Soybean is an important aspect of the plantprotection because without seed health tests wecannot touch the target of food security as thehealthy seeds are the pre-required of the healthyagriculture.

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	SVI
Alternaria alternata	76.00	21.65	7.68	18.98	9.78	26.63	1326.30
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

Sclerotium rolfsii,	69.00	28.12	6.68	18.03	9.65	24.60	1127.10
Curvularia lunata	73.00	73.00	23.96	6.20	23.92	9.15	1121.10
Macrophomina phaseolina	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	344.2
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

Table 2. Effect of culture filtrate of isolated seed infecting fungi on seed germination, shoot length, and 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**	**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
Sclerotium rolfsii	62.00	35.42	5.30	34.96	6.48	49.37	729.90
Curvularia lunata	69.00	28.12	6.68	18.03	9.65	24.60	1127.10
Macrophomina phaseolina	73.00	23.96	6.20	23.92	9.15	28.51	1121.10
Aspergillus niger	43.00	55.20	3.30	59.50	4.70	63.28	344.20
Aspergillus flavus	43.00	55.20	3.30	59.50	4.70	63.28	344.20
Control (Healthy seed)	96.00	-	8.15	-	12.80	-	2011.00

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

Treatment	Conc.	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	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% + Metiram 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
CD 0.05%		3.71		0.19		0.20		65.24

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