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Prevalence of seed borne fungi associated with soybean

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Abstract

Soybean (*Glycine max*) crop is attacked by a variety of seed and soil borne fungal pathogen as well as other pathogens. Therefore, the investigation was carried out in the laboratory of plant pathology section, college of agriculture, Nagpur to study the Association of seed borne fungi of soybean. The seed samples of four different varieties were collected to study the seed borne mycoflora. Eight different types of fungi were associated with seed were detected by following standard blotter paper method, 2, 4-D blotter method and agar plate method. All together, eight fungi comprising *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Colletotrichum dematium*, *Rhizoctonia bataticola*, *Curvularia lunata*, *Alternaria alternata* and *Rhizopus stolonifer* were observed common in all seed detection method. The maximum percentage association of seed borne fungi was noted on local variety. Highest percentage association of fungi with seeds was obtained in fungi *Aspergillus niger* followed by *Aspergillus flavus* and *Fusarium oxysporum*. Standard blotter paper method was found superior in recording more number of fungal colonies than 2, 4-D blotter method and agar plate method.

Keywords: Soybean, pathogens seed borne mycoflora, standard blotter paper method, agar plate method

Introduction

Soybean (*Glycine max* (L.) Merrill) is referred as “Golden bean” and “Miracle crop” of 21 century. It is one of the important oilseed as well as legume crop. It contributes more than 50% to the global production of edible oil.

Soybean contains 20% oil and 40% protein. Its protein is rich in all essential amino acids vitamin A, B and D, health promoting phytochemicals like is flavones. The soya protein stands unique by supplying all sixteen essential amino acids. Soybean oil is used as edible oil in Indian diet. It contains low level of saturated fatty acids. Therefore, soybean oil is better for human health.

Soybean is highly self-pollinated crop. Taxonomically soybean belongs to the order Fabales and family “Leguminosae” and subfamily “Papilionidae” and the genus *Glycine*. It is annual leguminous herbaceous plant. The genus *Glycine* is divided in two subgenera, *Glycine* (16 perennial species) and *Soja* having two annual species, *Glycine soja* (2n=40) and *Glycine max* (L.) Merrill (2n=40). *Glycine soja* is the wild ancestor of *Glycine max* and grows widely in China, Japan, Korea, Taiwan and Russia. Significantly highest soybean seed yield, stover yield and biological yield were recorded with broad bed furrow method of cultivation which proved as the most suitable method for cultivating soybean crop (Parlawar *et al.*, 2017) [14]. Soybean seed samples may contain various kind of microflora and they can affect the vigour of crop and ultimately the yield. The detection methods are very useful to find the association of different microbial population so that the management practices can be undertaken right from the seed sowing to the harvesting.

Material and Methods

Detection of seed borne mycoflora

The seed samples of soybean varieties JS-335, JS-93-05, JS-95-60 and TAMS-38, were collected from Regional Research Station Amravati. Detection of seed borne mycoflora from soybean seeds was carried by using standard blotter paper method, 2-4, D blotter method and agar plate method. During the detection work by using above noted three methods the observations are taken with untreated seeds and treated seeds with HgCl₂ (0.1%) solution,

To detect seed borne fungi, seeds were surface disinfected by dipping in mercuric chloride HgCl_2 solution at 0.1 per cent concentration for one minute and washed subsequently in four changes of sterile distilled water in order to remove traces of mercuric chloride. The seeds were dried and plated. The seeds as such without surface disinfection were also used for detection of seed borne mycoflora. Soybean seeds were transferred to the plates. Plates containing three layered moist blotter papers. Ten seeds per plate were placed at equal distance and the plates were incubated at $28 \pm 2^\circ\text{C}$ under alternate cycles of 12 hour near UV light and darkness for 7 days. Four hundred such seeds from each cultivar were tested. After seventh day of incubation, the seeds were examined under stereoscopic binocular microscope for associated fungi and they were identified based on habit and colony characters (Anonymous 2016)^[4]. The per cent occurrence of each fungus associated with the seed was recorded.

B. 2, 4-D blotter paper method

2, 4-D is a plant growth regulators retards seed germination and seedling growth, due to which the seeds are not displaced and the examination of fungi becomes easy. The blotters were soaked in 0.1 per cent 2, 4-D suspension and then placed in sterilized plates. Hundred seeds were incubated for seven days as in blotter method and examined for fungi under stereoscopic microscope. The incidence of fungi on seed under blotter paper method was recorded and the per cent occurrence of fungal species was calculated.

C. Agar plate method

Northern Ireland first used this method for seed health management. In this method, pre sterilized petri plates were poured with 15 ml of autoclave potato dextrose agar. On cooling the medium, the seeds were equidistantly placed aseptically.

Ten seeds of test sample petri plate⁻¹ were then placed four hundred seeds were used test⁻¹. The plates were incubated at $28 \pm 2^\circ\text{C}$ under diurnal conditions. On seventh day of incubation, seeds were examined under stereoscopic microscope for determining the fungal growth. The identification and further confirmation of seed borne fungi was made by preparing slides of the fungi.

Results and Discussion

It is clear from the table 1, 2 and 3 that total number of ten fungi viz., *Aspergillus niger*, *A. flavus*, *Rhizoctonia bataticola*, *Fusarium oxysporum*, *Curvularia lunata*, *Colletotrichum dematium*, *Alternaria alternata* and, *Rhizopus stolonifer* were isolated from four varieties of soybean seeds by standard blotter paper, 2, 4 – D blotter and agar plate method. It is interesting to note that per cent incidence of mycoflora was more on standard blotter paper method than 2, 4 – D blotter paper and agar plate method.

The range of infection (Table 1) was from 6.50% to 53% in untreated and in treated it was 0.50% to 32.85% using standard blotter paper method. Among the different fungi *Aspergillus niger* was found predominant in all varieties that the range from 35.50% to 53.00% in untreated and 1.28% to 32.85% in treated, followed by fungi *Aspergillus flavus* where infection was from in the range of 38.50% to 48.25% in untreated and in treated it was 8.50% to 23.50% and minimum frequency of fungi was found in the *Rhizopus stolonifer* in the range of 5.81% to 15.50% in untreated seeds and in treated seeds it was 0.00% to 2.25%. Occurrence of higher number of *Aspergillus niger*, *A. flavus* has been earlier

reported by Abbas *et al.* (2013)^[1] and Khade (2016)^[11], who noted higher fungal population was obtained in untreated seeds than that of treated seeds. In treated seeds fungal population significantly reduced by using HgCl_2 (0.1%). Similar results were reported by Jogdand and Talekar (2010), they obtained higher number of fungi 80% in untreated and it was 25% in treated, it might be due to reduction of seed mycoflora in treated seeds. The results of present investigation are more or less similar to Nagpure and Patwari (2014)^[13]. They reported that standard blotter paper method gave higher per cent of mycoflora population as compared to agar plate method, Al-Almod (2015)^[3] and Khade (2016)^[11] also found that standard blotter paper method gave higher per cent fungi as compared to agar plate method.

The range of infection (Table 2) was 2% to 47.00% in untreated and in treated it was 0.4% to 22% using 2,4-D blotter paper method. Among the different fungi growth of *Aspergillus niger* was in the range of 30% to 47% in untreated and it was 11.75% to 22% in treated seeds of all varieties seed samples and it was followed by *Aspergillus flavus* i.e. 23.52% to 37.25% in untreated and 8.50% to 12.50% in treated and minimum frequency was recorded in *Rhizopus stolonifer* fungi i.e. 0.00% to 8.75% in untreated and 0.00% to 2% in treated.

The range of infection (Table 3) was from 1.31% to 33.75% in untreated and in treated it was 0% to 20.0% using agar plate method. Among the different fungi *Aspergillus niger* was found dominantly in all varieties seed samples i.e. in range of 18.50% to 33.75% in untreated and in the range of 12.25% to 20.00% in treated followed by *Aspergillus flavus* i.e. 17.75% to 28.50% in untreated and 9.5% to 17.75% in treated and minimum frequency of fungi occurred in *Rhizopus stolonifer* fungi and it was in the range of 0.00% to 1.31% in untreated and 0% in treated.

The results of present investigation corroborates with findings of Ahmed (2016)^[2]. They reported that standard blotter paper method gave higher per cent of mycoflora population as compared to agar plate method. Dawar and Ghaffar (1991)^[5] also found higher per cent fungi in standard blotter paper method when compared with agar plate method.

The three seed health testing methods viz., standard blotter paper method, 2,4-D blotter paper method, agar plate method (Table 1, 2 and 3) represent the total per cent incidence of fungi. It was found that highest total fungi was noted in JS-335 variety seed samples i.e. 198.2%, 153.5% and 107.6% followed by JS-95-60 i.e. 192%, 131% and 103.75%, JS-95-50 i.e. 188%, 126.5% and 94.6%, TAMS-38 i.e. 141.9%, 126.02% and 86.00% respectively. Least mycoflora occurred on variety TAMS-38 i.e. 141.9%, 126.02% and 86.00% respectively.

Data tabulated in (Table 4) clearly indicated that the highest number of fungal species were obtained by without surface disinfectant of seeds as compared to the kind of fungi on treated seeds with HgCl_2 (0.01%), it yielded lesser number of fungi than from seeds without sterilization in all three detection methods. The range of mycoflora isolated by using three different methods were 0% to 43.37%.

Three seed health testing methods viz., standard blotter paper method, 2, 4-D blotter method and agar plate method were compared to know the efficacy of different detection methods. The experimental result indicated that, among three methods employed for detection of seed mycoflora standard blotter paper method was found superior and recorded maximum total fungal colonies. *Aspergillus niger* found predominant in untreated i.e. 1.31% to 43.375% and in treated i.e. 0.4% to

22.71%. The highest number of fungal species was obtained on standard blotter paper method with or without treatment, followed by number of fungal species on 2, 4-D blotter method and gradually diminishing least in number on agar plate method.

The maximum percentage association of seed borne fungi was

noted on local variety. Highest percentage association of fungi with seeds was obtained in fungi *Aspergillus niger* followed by *Aspergillus flavus* and *Fusarium oxysporum*. Standard blotter paper method was found superior in recording more number of fungal colonies than 2, 4-D blotter method and agar plate method.

Table 1: Per cent association of seed borne mycoflora soybean by standard blotter paper method in untreated and treated seeds

Seed borne fungi	Per cent association of seed borne fungi								Average seed borne mycoflora (%)	
	JS-335		JS-93-05		JS-95-60		TAMS-38		UN	T
	UN	T	UN	T	UN	T	UN	T		
<i>Aspergillus niger</i>	53	1.28	40.5	18.25	44.50	32.85	35.50	11.75	43.37	22.71
<i>Aspergillus flavus</i>	38.50	8.50	24.75	11.50	45	14.35	48.25	23.50	39.12	14.43
<i>Rhizoctonia bataticola</i>	30	27	35.50	11.50	37	18.25	11.75	2.75	28.56	14.87
<i>Fusarium oxysporum</i>	41.5	33.75	41.5	6	20.25	23.50	13.15	1.75	29.1	16.25
<i>Alternaria alternata</i>	11	10	16.50	3	19.50	9.50	17.50	8.75	17.62	7.81
<i>Colletotrichum dematium</i>	11.75	3.75	20	0.50	16.25	2.00	8	1.25	14	7.5
<i>Curvularia lunata</i>	6.50	5.75	9.25	-	-	1.25	-	-	3.93	1.75
<i>Rhizopus stolonifer</i>	13.75	-	-	2	15.50	-	7.75	2.25	5.81	1.06
Total	198.2	116	188	52.75	192	101.7	141.9	51.95	181.2	86.39

Where, UN- Untreated, T- Treated

Table 2: Per cent association of seed borne mycoflora of soybean by 2, 4-D blotter paper method in untreated and treated seeds

Seed borne fungi	Per cent association of seed borne fungi								Average seed borne mycoflora (%)	
	JS-335		JS-93-05		JS-95-60		TAMS-38		UN	T
	UN	T	UN	T	UN	T	UN	T		
<i>Aspergillus niger</i>	47	11.75	35.50	22	30	20	40.25	13.25	38.18	16.75
<i>Aspergillus flavus</i>	37.25	12.50	37.25	9.5	24.75	12.50	23.52	8.50	30.69	10.75
<i>Rhizoctonia bataticola</i>	30.25	3.25	14.75	6.25	39.25	13.25	14.50	14.75	24.68	9.375
<i>Fusarium oxysporum</i>	17.25	10.75	16	11.50	15.50	5.75	23.75	3.75	18.12	7.93
<i>Alternaria alternata</i>	20	3.00	11.50	4.25	12.25	3.75	16.25	3.75	15	3.81
<i>Colletotrichum dematium</i>	4.50	8	11.50	2.25	-	1.75	7.75	-	5.93	3
<i>Curvularia lunata</i>	6.50	-	-	-	9.25	-	-	5.75	39.3	1.437
<i>Rhizopus stolonifer</i>	8.75	-	-	-	-	2	-	-	2.18	0.4
Total	153.5	49.25	126.5	55.75	131	59	126.0	49.75	174.1	53.47

Where, UN- Untreated, T- Treated

Table 3: Per cent association of seed borne mycoflora of soybean by agar plate method in untreated and treated seeds

Seed borne fungi	Per cent association of seed borne mycoflora								Average seed borne mycoflora (%)	
	JS-335		JS-93-05		JS-95-60		TAMS-38		UN	T
	UN	T	UN	T	UN	T	UN	T		
<i>Aspergillus niger</i>	33.75	20.00	25.50	12.75	27	14	18.50	12.25	26.18	14.75
<i>Aspergillus flavus</i>	28.50	17.75	20.75	9.50	20	9.5	17.75	10	21.75	11.68
<i>Rhizoctonia bataticola</i>	15.75	-	-	-	17	6.25	11.50	-	11.06	1.56
<i>Fusarium oxysporum</i>	14.50	1.75	14	2.75	15.50	1.25	9.00	-	13.25	1.43
<i>Alternaria alternata</i>	10.75	2.25	17	3	11.75	-	10.00	-	12.375	1.31
<i>Colletotrichum dematium</i>	1.38	-	9.25	-	4.25	-	10.75	-	6.40	-
<i>Curvularia lunata</i>	3.00	-	8.1	-	8.25	-	3.25	-	5.65	-
<i>Rhizopus stolonifer</i>	-	-	-	-	-	-	5.25	-	1.31	-
Total	107.6	41.75	94.6	28	103.75	30.95	86	22.5	97.98	30.75

Where, UN- Untreated, T- Treated

Table 4: Comparison between different seed borne mycoflora detection method of soybean

Seed borne fungi	Per cent association of seed borne fungi						Average fungal colony	
	Blotter paper method		2,4-D blotter method		Agar plate method		UN	T
	UN	T	UN	T	UN	T		
<i>Aspergillus niger</i>	43.375	22.71	38.18	16.75	26.18	14.75	35.91	18.07
<i>Aspergillus flavus</i>	39.12	14.43	30.69	10.75	21.75	11.68	30.52	12.28
<i>Rhizoctonia bataticola</i>	28.56	14.87	24.68	9.375	11.06	1.56	21.43	8.60
<i>Fusarium oxysporum</i>	29.1	16.25	18.125	7.937	13.25	1.43	20.15	8.539
<i>Alternaria alternata</i>	17.625	7.81	15	3.81	12.375	1.31	15	4.31
<i>Colletotrichum dematium</i>	14	7.5	5.93	3	6.40	-	8.59	3.5
<i>Curvularia lunata</i>	3.93	1.75	39.3	1.437	5.65	-	16.22	1.06
<i>Rhizopus stolonifer</i>	5.81	1.06	2.18	0.4	1.31	-	3.1	0.48
Total	181.2	86.39	174.09	53.47	97.98	30.75	150.92	56.839

Where, UN- Untreated, T- Treated

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