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Association of seed borne mycoflora of groundnut

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Abstract
Groundnut (*Arachis hypogaea* L.) crop is attacked by a variety of seed and soil borne fungal pathogen as well as other pathogens, under field condition as well as during storage causing economical losses. Therefore, present investigation was planned to study the Association of seed borne fungi of groundnut. The seed samples of six different varieties were collected to study the seed borne mycoflora. Ten 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, ten fungi comprising *Aspergillus flavus, A. niger, A. fumigatus, A. nidulans*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia bataticola*, *Curvularia lunata*, *Alternaria alternata* and *Rhizopus stolonifer* were observed common in all seed detection method. The difference in methods of recording fungi was also significant and standard blotter paper method was most efficient in recording more number of fungal colonies.

Keywords: Mycoflora, groundnut, economical, *Arachis hypogaea*

Introduction
Groundnut (*Arachis hypogaea* L.) or peanut is a self-pollinated annual legume crop and it originated from South America. It is also called as "King of oil seeds", widely grown for its high quality edible oil and food use in the tropical and warm temperate regions of the world. The crop is grown in more than 100 countries. The major groundnut producers are China, India, Nigeria, USA, Senegal, Myanmar, Indonesia, Burma and Sudan. Groundnut is adapted to varying agro-climatic conditions and soils. It is primarily grown in rainy (*Kharif*) under rainfed conditions in India, which accounts for 83% of the total area under the crop in the country. The remaining 17% of the area is cultivated mostly in the post rainy (*Rabi/* summer) season with irrigation or on residual soil moisture.

Groundnut contain in an average 45-50 per cent oil, 40.1 per cent of fat and 26-28 per cent of protein and it is the rich source of calcium, iron, and vitamin B complex like thiamine, riboflavin, niacin and vitamin A. It is used not only as a major cooking medium for various food items but also for manufacture of soaps, cosmetics, shaving creams and lubricants. The groundnut finds extensive use as cooking medium both as refine oil and vanaspati ghee. Kernels are also eaten raw, roasted or sweetened. They are rich in protein and vitamins A, B and some member of B_2 group. Their calorific value is 349 per 100g. The residual oil cake contains 7 to 8 percent of N, 1.5 percent of P_2O_5 and 1.2 percent of K_2O and is use as a fertilizer. It is an important protein supplement in cattle and poultry rations. It is also consumed as confectionary product. The cake can be used for manufacturing coarse board, cork substitutes etc. Groundnut is also of value as rotation crop. Being a legume with root nodules, it can synthesis atmospheric nitrogen and therefore improve soil fertility.

Material and Methods
Detection of seed borne mycoflora
The seed samples of groundnut varieties TAG-24, AK-159, AK-335, AK-303, Kopargaon and Local were collected from different places i.e. Oilseed Research Station, Dr. PDKV, Akola, College of Agriculture, Nagpur and from local market. Detection of seed borne mycoflora from groundnut seed was carried by standard blotter paper method, 2-4, D blotter method and agar plate method under two heads.

a. Untreated seed

b. Seed treatment with $HgCl_2$ (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. Groundnut seeds were transferred to the plates containing three layer moist blotter papers. Ten seeds per plate were placed at equal distance and the plates were incubated at 28±2^{0}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 (ISTA, 1976) [6]. 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 blotter 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.

Methods of Detection

A. Standard blotter paper method

Three layers of sterilized blotter paper disc of 90 mm diameter were placed in sterilized petri plates (90 mm diameter) moistened with sterile distilled water. The excess water was drained out from the plates.

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 per petri plate were then placed four hundred seeds were used per test. The plates were incubated at 28± 2^{0}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 was isolated namely. *Aspergillus flavus, A. niger, A. fumigatus, A. nidulans, Fusarium oxysporum, Sclerotium rolfsii, Rhizoctonia bataticola, Curvularia lunata, Alternaria alternata* and *Rhizopus stolonifer* were isolated from six varieties of groundnut seeds by standard blotter paper, 2, 4 – D blotter and agar plate method it is interesting to note that per cent incidence of mycoflora more on standard blotter paper method than 2, 4 – D blotter paper and agar plate method.

(Table 1) The range of infection was from 1.29% to 48.25% in untreated and in treated it was 0.58% to 22.95%. Among the different fungi *Aspergillus flavus* was found predominant in all varieties that range from 42.50% to 52.50% in untreated and in treated 17.75% to 29.75, followed by *Aspergillus niger* 30.00% to 40.25% in untreated and in treated 15.00% to 24.25% and minimum frequency of fungi occurred that *Rhizopus stolonifer* ranged from 0.00% to 7.75% in untreated and in treated 0.00% to 3.50%. Occurrence of higher number of *Aspergillus flavus*, *A. niger* has been earlier reported by Abbas *et al.* 2013 [1] and Khade 2016 [8, 9, 10]. The higher fungal population obtained in untreated seed than in treated seed fungal population significantly reduced by using $HgCl_2$ (0.1%). Similar result reported by Jogdand and Talekar (2010) [7], they obtained higher number of fungi 80% in untreated and 25% in treated, it was because treating seed reduces in seed mycoflora. The result of present investigation are more or less similar to Nagpure and Patwari, 2014 [11], reported standard blotter paper method gave higher per cent of mycoflora population as compared to agar plate method, Al-Almod, 2015 [3] and Khade, 2016 [8, 9, 10], also find out the standard blotter paper method give higher percent fungi as compared to agar plate method.

(Table 2) The range of infection was from 7.37% to 41.75% in untreated and in treated it was 0.25% to 17.45%. Among the different fungi *Aspergillusflavus* 37.75% to 45.25% in untreated and in treated 13.50% to 22.25%, was found dominantly in all varieties seed sample and followed by *Aspergillusniger* 21.50% to 57.25% in untreated and in treated 10.75% to 19.25% and minimum frequency of fungi occurred that *Rhizopus stolonifer* 0.00% to 22.25% in untreated and in treated 0.00% to 9.50% recorded during the study. The result of present investigation more or less similar found by Jogdand and Talekar, 2010 [7] reported that standard blotter paper method gave higher per cent of mycoflora population as compared to agar plate method. Elwalkil, 2001 and Rasheed, 2004 [5, 12] and also find out the standard blotter paper method give higher percent fungi as compared to agar plate method.

(Table 3) The range of infection was from 6.25% to 37.37% in untreated and in treated it was 0.12% to 17.79%. Among the different fungi *Aspergillus flavus* was found dominantly in all varieties seed sample in range 29.50% to 46.25% in untreated and in treated 11.75% to 23.50% followed by *Aspergillus niger* 24.75% to 39.25% in untreated and in treated 8.50% to 16.25%, and minimum fungi occurred that *Rhizopus stolonifer* 0.00% to 11.75% in untreated and in treated 0.00% to 7.75%.

The result of present investigation corroborates with finding Ahmed, 2016 reported that standard blotter paper method give higher per cent of mycoflora population as compare to agar plate method. Dawar and Ghaffar, 1991 [12] also find out the standard blotter paper method gave higher percent fungi as compared to agar plate method.

Experimental result from the (Table 1, 2 and 3) represent the total per cent incidence of fungi, it was found that highest total fungi was noted in local variety seed sample 217.0%,192.75% and 192.75% followed by AK-303 189.5%,174.43% and 174.43%, TAG-24 173.25%,155.25% and 155.25%, Kopargaon 172.75%,155.00% and 155.00%, AK-335 171.0%, 151.00% and 125.08 % least mycoflora occurred on variety AK-159 168.7%, 147.50% and 147.50%).

Data tabulated in table 4, clearly indicated that the highest number of fungal species were obtained by without surface disinfection of seed 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 that were 5.23% to 44.91%.

Three seed health testing methods viz., standard blotter paper method, 2, 4-D blotter method and agar plate method were compare to know the efficacy of different detection methods. The experimental results 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 flavus* found predominant in untreated 41.75% to 46.75% and in treated 17.29% to 22.95%. 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 in number and it is least on agar plate method.

Conclusion

The maximum percentage association of seed borne fungi was noted on local variety. Highest percentage association was of *Aspergillus flavus* followed by *Aspergillus niger* and it was followed by *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 of groundnut by standard blotter paper in untreated and treated seeds

Seed borne fungi	Association of seed borne mycoflora (%												Average fugal colonies	
	AK-159		AK-303		AK-335		TAG-24		KOPARGAON		LOCAL			
	UN	T	UN	T	UN	T	UN	T	UN	T	UN	T	UN	T
Aspergillus flavus	44.50	22.00	48.75	29.75	42.50	19.75	48.75	17.75	45.50	24.50	54.50	24.00	48.25	22.95
Aspergillus niger	30.50	17.75	33.50	24.25	40.25	15.25	39.50	23.00	38.75	15.00	36.00	22.50	36.41	19.29
Aspergillus nidulans	10.75	2.25	7.00	-	13.75	9.25	12.50	-	10.50	7.75	13.50	10.75	11.33	5.08
Aspergillus fumigatus	9.75	1.25	14.50	2.25	11.75	2.50	7.50	3.75	4.50	-	12.75	2.00	10.12	2.68
Fusarium oxysporum	21.25	5.00	23.00	3.00	14.50	1.75	19.00	7.50	17.75	3.00	28.50	6.25	20.66	4.41
Sclerotium rolfsii	18.00	7.25	19.75	-	13,75	-	14.25	1.50	19.00	2.00	22.25	3.75	17.83	2.33
Rhizoctonia bataticola	14.50	2.00	16.25	-	13.25	-	12.00	3.75	14.75	3.25	20.00	-	15.12	1.50
Curvularia lunata	4.50	-	9.75	-	8.50	-	8.25	-	11.25	2.00	8.25	4.75	8.41	1.12
Alternaria alternata	15.00	4.00	17.00	3.25	12.75	-	11.50	8.00	10.75	2.50	13.50	3.00	13.41	3.45
Rhizopus stolonifer	-	-	-	-	-	-	-	-	-	-	7.75	3.50	1.29	0.58
Total	168.7	61.5	189.5	62.50	171.0	48.5	173.25	65.25	172.75	60.00	217.0	80.5	182.83	63.39

Where, UN- Untreated, T- Treated

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

Seed borne fungi	Association of seed borne fungi (%)													Average fugal colonies
	AK-159		AK-303		AK-335		TAG-24		KOPARGAON		LOCAL			
	UN	T	UN	T	UN	T	UN	T	UN	T	UN	T	UN	T
Aspergillus flavus	39.50	19.25	45.25	15.50	37.75	16.00	43.50	13.50	40.00	22.25	44.50	18.25	41.75	17.45
Aspergillus niger	21.50	11.75	32.25	16.50	45.00	19.25	33.25	16.00	30.75	10.75	57.25	12.50	33.33	14.45
Aspergillus nidulans	8.25	-	12.25	-	11.25	3.75	8.50	-	7.00	-	9.50	2.50	9.45	1.29
Aspergillus fumigatus	8.75	-	13.50	-	9.50	-	4.50	1.00	-	-	8.00	0.50	7.37	0.25
Fusarium oxysporum	18.50	3.50	20.50	12.50	11.50	-	15.50	3.50	14.75	3.00	20.25	6.00	16.91	4.75
Sclerotium rolfsii	16.25	4.75	18.25	-	10.00	-	12.50	3.00	15.25	-	20.00	3.75	15.37	1.91
Rhizoctonia bataticola	11.75	2.00	13.00	-	9.50	5.00	7.50	-	13.50	2.75	16.25	1.50	11.91	1.54
Curvularia lunata	11.00	-	6.75	-	8.50	3.25	5.75	1.00	7.00	1.75	-	-	7.58	1.00
Alternaria alternata	12.25	-	14.00	6.50	8.25	-	8.75	6.50	4.50	1.50	9.50	2.50	9.54	2.83
Rhizopus stolonifer	-	-	-	-	-	-	15.50	2.75	22.25	9.50	7.50	3.00	7.54	2.54
Total	147.5	41.25	174.43	51.00	151.2	47.25	155.25	47.25	155.0	51.5	192.75	52.0	160.75	48.01

Where, UN- Untreated, T- Treated

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

Seed borne fungi	Association of seed borne fungi (%)												Average fugal colonies	
	AK-159		AK-303		AK-335		TAG-24		KOPARGAON		LOCAL			
	UN	T	UN	T	UN	T	UN	T	UN	T	UN	T	UN	T
Aspergillus flavus	33.50	11.75	35.50	20.25	37.50	13.50	42.00	17.75	29.50	17.00	46.25	23.50	37.37	17.29
Aspergillus niger	24.75	8.50	31.25	9.25	35.50	11.50	30.25	9.00	28.50	12.75	39.25	16.25	31.58	11.20
Aspergillus nidulans	4.50	1.25	9.75	-	7.50	-	6.75	2.25	8.50	-	9.50	3.50	8.25	1.08
Aspergillus fumigatus	6.50	-	9.50	-	-	-	5.00	0.75	3.75	-	12.75	-	6.25	0.12
Fusarium oxysporum	13.15	1.75	16.00	4.25	10.00	2.50	13.25	2.00	12.25	-	17.25	5.75	13.65	2.70
Sclerotium rolfsii	12.50	2.00	14.50	-	7.50	2.00	10.25	1.00	14.50	-	15.50	3.75	12.45	1.45
Rhizoctonia bataticola	8.00	-	11.50	2.00	11.00	1.00	6.50	-	13.00	2.75	11.50	1.25	10.25	1.16
Curvularia lunata	9.25	-	5.75	-	8.58	-	4.50	1.75	6.25	-	7.75	1.75	7.00	0.58
Alternaria alternata	10.75	2.50	11.75	-	7.50	-	6.75	3.25	5.50	-	8.50	4.50	8.45	1.70
Rhizopus stolonifer	-	-	11.75	7.75	-	-	10.50	5.75	8.25	5.50	10.75	6.50	6.87	4.25
Total	122.9	27.75	157.25	45.5	125.08	30.50	138.7	43.50	130.0	39.0	179.0	66.25	142.12	41.53

Where, UN- Untreated, T- Treated

Table 4: Comparison between different seed borne mycoflora detection methods of groundnut

Seed borne fungi	Association of seed borne fungi (%)						Average fugal colonies	
	Blotter paper method		2, 4-D blotter method		Agar plate method			
	UN	T	UN	T	UN	T	UN	T
Aspergillus flavus	48.25	22.95	41.75	17.45	37.37	17.29	42.45	19.23
Aspergillus niger	36.41	19.29	33.33	14.45	31.58	11.20	33.77	14.94
Aspergillus nidulans	11.33	5.08	9.45	1.29	8.25	1.08	9.67	2.48
Aspergillus fumigatus	10.12	2.68	7.37	0.25	6.25	0.12	7.91	1.01
Fusarium oxysporum	20.66	4.41	16.91	4.75	13.65	2.70	17.07	3.95
Sclerotium rolfsii	17.83	2.33	15.37	1.91	12.45	1.45	15.21	1.89
Rhizoctonia bataticola	15.12	1.50	11.91	1.54	10.25	1.16	12.42	1.40
Curvularia lunata	8.41	1.12	7.58	1.00	7.00	0.58	7.66	0.90
Alternaria alternata	12.91	3.45	9.54	2.83	8.45	1.70	10.30	2.66
Rhizopus stolonifer	1.29	0.58	7.54	2.54	6.87	4.25	5.23	2.45
Total	182.33	63.39	160.75	48.01	142.12	41.53	161.69	50.91

Where, UN- Untreated, T- Treated

References

1. Abbas N, Mubashir W, Bhat NA, Uzameen W, Dar MS, Tak A. Effect of seed borne mycoflora on quality of three varieties of *Arachis hypogea.* Int. J Agril. Sci. Res. 2013; 3(1):35-42.
2. Ahmed O, Balogun OS, Fawole OB, Fabiyi OA, Kassoun KO. Seed borne fungi of soybean (*Glycine max*) in the guinea savannah agro ecology of Nigeria. J Agril. Sci. 2016; 61(1):57-68.
3. Al-Almod MO. Seed borne fungi of some peanut varieties from Abyan Governorates in Yemen. J Argil. Tech. 2015; 11(6):1359-1370.
4. Dawar S, Gaffar A. Detection of seed borne mycoflora of sunflower. Pakistan J Bot. 1991; 23(2):173-178.
5. Elwalkil MA, El. Mitwally MA, Seed borne fungi of peanut in Egypt, pathogenicity and transmission. Pakistan Bio. Sci. 2001; 4:63-68.
6. ISTA, International seed testing association. Seed Sci. Tech. 1976; 4:51-77.
7. Jogdand SK, Talekar SM. Fugal population on seed of *Arachis hypogea* L. J Eco. 2010; 2(6):11-13.
8. Khade KA. Examination of seed borne mycoflora of groundnut. Int. J Res. Pharma. Life Sci. 2016; 1(1):2456-3889.
9. Khade KA. Examination of seed borne mycoflora of groundnut. Int. J Res. Pharma. Life Sci. 2016; 1(1):2456-3889.
10. Khade KA. Examination of seed borne mycoflora of groundnut. Int. J Res. Pharma. Life Sci. 2016; 1(1):2456-3889.
11. Nagpure VS, Ptwari JM. Seed borne mycoflora of groundnut. J Sci. 2014; 2(1):43-45.
12. Rasheed S, Dawar S, Ghaffar A, Shaukat S. Seed borne mycoflora of groundnut. Pakistan J Bot. 2004; 36(1):199-202.