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Isolation of seed-borne fungi from stored groundnut seeds and their role on seed spoilage

by

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Several fungi have been reported to cause diseases on groundnut. Noble *et al* (1958) reported the following fungi, *Cercospora arachidicola, C personata, Macrphomina phaseoli, Sclerotium rolfsii, Thielaviopsis basicola, Diplodia* spp., *Fusarium* spp. and *Aspergillus niger* as pathogenic on groundnut. Norton *et al.* (1954) found *A. flavus* as a pathogen on groundnut. Besides these, numerous fungi have been isolated from the stored groundnut seeds. Joffee and Shiray (1966) isolated 71 fungal species from groundnut kernels stored at 90 percent RH. The present studies aim at the isolation of the groundnut seed-borne fungi, testing their pathogenicity, determining their role on stored seeds and suggesting the possible means of overcoming the adverse effect of the seed-borne fungi.

Materials and Methods: Groundnut seeds were collected from different places in Tamil Nadu and the seed-borne fungi were isolated by the International Seed Testing Method (Anon., 1959). The groundnut seeds were deshelled and both kernel and pods were used for the isolation of fungi. Both external and internal seed-borne infections were detected by plating the surface sterilized (sterilized with 0.1% mercuric chloride) and unsterilized pods and kernels.

The pathogenicity of the most frequently encountered seed-borne fungi was tested by treating the surface sterilized, healthy, groundnut seeds with the heavy spore suspensions of the fungi. Seed germination percentage and the plumule and radicle elongations of 30 day old seedlings were assessed.

The effect of the seed-borne fungi on the stored groundnut seeds was tested by storing the groundnut kernels treated with different seed-borne fungi in desiccators maintaining low (32.3 per cent RH) and high (95.0 per cent RH) humidity levels. Saturated solutions of calcium chloride and sodium sulphite were used to keep up 32.3 per cent and 95.0 per cent RH respectively at 20°C (International Critical Tables, 1926). Seed germination percentage was assessed after 6 months of storage.

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To control the seed-borne fungal damage, groundnut seeds were treated with TMTD, (tetramethyl thiuram disulphide), captan (N-trichloromethyl thio tetra hydrophthalimide) and ceresan (Methoxy ethyl mercuric chloride) and stored in different storage containers viz., wooden boxes, paper bags, polythene bags and gunny bags. After 6 months of storage, seed viability was tested in each treatment.

Results: Groundnut pods and kernels were used for the isolation of various seed-borne fungi and the results obtained are presnted in Table 1.

TABLE 1. *Percentage of groundnut seeds yielding different seed-borne fungi*

Sl. No.	Fungi isolated	Pod		Kernel	
		Unsterilized	Sterilized	Unsterilized	Sterilized
1.	*Rhizopus nigricans* Ehrenberg	90	52	80	30
2.	*Rhizoctonia bataticola* Manbl.	15	10	60	45
3.	*Aspergillus flavus* Link.	40	30	62	50
4.	*A niger* van Tieghem.	30	10	35	25
5.	*A. nidulans* var. latus.	20	10	10	5
6.	*A ustus* Bainier.	30	10	15	5
7.	*A fumigatus* Fresenius.	0	0	15	5
8.	*A. terreus* Thom.	35	20	35	0
9.	*A. tamari* Kita.	0	0	20	10
10.	*Emericella variecolor* Berk. and Br.	10	0	10	0
11.	*Helminthosporium tetramera* McKinney	25	10	10	15
12.	*Phomopsis* sp. Sacc.	20	10	10	15
13.	*Cunninghamella bertholletiae* Stadel	40	10	30	15
14.	*Thielavia terricola* (Gilman and Abbot) Emmons	0	0	15	10
15.	*Fusarium solani* (Martius) Appel and Wollenweber var. minus Wollenweber.	0	0	30	15
16.	*F. moniliforme* Sheldon var. minus Wollenweber	10	5	0	0
17.	*Sporotrichum roseolum* Oindemans and Bei	20	15	35	20
18.	*Neocosmospora vasinfecta* Smith	50	10	0	0
19.	*Cladosporium herbarum* (Persoon) Link.	40	40	0	0
20.	*Chaetomium globosum* Kunze	30	5	10	2
21.	*Botryodiplodia* sp. Sacc.	5	5	50	30
22.	*Syncephalastum racemosum* (Cohn) Schroeter	20	5	5	0
23.	*Alternaria brassicola* (Schw.) Wiltshire	2	0	0	0
24.	*Penicillium expansum* (Link) Thom.	10	0	0	0
25.	*Mucor hiemalis* Wehmer	5	0	0	0
26.	*Curvularia lunata* (Wakker) Boedijn.	0	0	5	0
27.	*Absidia hyalospora* (Saito) Lendner	10	0	10	0

Twenty seven fungal species were identified on groundnut seeds. Both pods and kernels were equally infected and both internal and external infections occurred widely. Pathogenicity of some of the predominant fungi was tested and the results are presented in Table 2.

TABLE 2. *Effect of various seed-borne fungi on seed germination and seedling vigour*

Sl. No.	Fungi	Seed germination percentage	% reduction over control	Shoot length in cm	% reduction over control	Root length in cm	% reduction over control
1.	*Rhizopus nigricans*	100.0	0.0	27.5	3.0	16.7	3.4
2	*Rhizoctonia bataticola*	70.3	29.7	7.8	72.5	6.3	63.0
3.	*Aspergillus flavus*	82.3	17.7	14.4	49.0	1.2	93.0
4.	*A. niger*	68.3	31.7	15.3	46.0	0.8	95.0
5.	*Helminthosporium tetramera*	83.0	16.7	20.8	26.7	9.6	44.0
6.	*Fusarium moniliforme*	100.0	0.0	20.3	28.0	9.4	45.0
7.	*Sporotrichum roseolum*	88.3	11.7	23.8	19.0	15.8	8.6
8.	*Neocosmospora vasinfecta*	100.0	0.0	26.6	6.3	11.5	34.0
9.	*Cladosporium herbarum*	82.3	17.7	21.6	23.0	15.1	12.0
10.	*Chaetomium globosum*	100.0	0.0	24.8	12.0	16.2	6.3
11.	*Botryodiplodia* sp.	100.0	0.0	23.2	18.0	11.2	35.0
12.	*Alternaria brassicola*	91.6	8.4	23.0	19.0	15.0	13.0
13.	*Mucor hiemalis*	96.6	3.4	28.7	0.0	15.9	8.0
14.	*Curvularia lunata*	100.0	0.0	26.2	7.7	15.9	8.0
15.	Control (uninoculated)	100.0	—	28.4	—	17.3	—

Seed germination was not much affected by the seed-borne fungi. However, *Aspergillus niger* and *Rhizoctonia bataticola* infected seeds showed about 30 per cent reduction in germination. But seedling vigour was greatly affected by the seed-borne fungi. Root development was more reduced than shoot development. *A. flavus* and *A. niger* caused very severe damage on the root development while *Rhizoctonia bataticola* inflicted more damage on shoots. It is interesting to note that although *Rhizopus nigricans* predominated fungal flora of groundnut seeds, it did not cause any damage on seed germination and seedling vigour. The other fungi which do not cause any appreciable damage are *Mucor hiemalis* and *Curvularia lunata.*

The effect of seed-borne fungi on the groundnut seeds during storage was studied and the results are presented in Table 3.

TABLE 3. *Effect of seed-borne fungi on germination of stored groundnut seeds*

Sl. No.	Fungi	Germination percentage	
		Stored at low humidity (32.3% RH)	Stored at high humidity (95.0% RH)
1.	*Rhizoctonia bataticola*	30.0	2.6
2.	*Aspergillus flavus*	16.9	0.1
3.	*Cladosporium herbarum*	36.6	12.3
4.	*Helminthosporium tetramera*	54.0	44.0
5.	*Neocosmospora vasinfecta*	50.0	50.0
6.	Control	90.8	92.0

All the fungi tested caused seed germination failures. *A. flavus, Rhizoctonia bataticola* and *Cladosporium herbarum* inflicted considerable damages. Seeds stored at high humidity level suffered more of fungal attack.

Three fungicides were tested for their efficacy to protect the seeds against the seed-borne fungi and the results (Table 4) indicated T.M.T.D. as the best fungicide affording maximum protection.

TABLE 4. *Effect of seed treatment on seed viability*

Sl. No.	Fungicides	Germination Percentage	
		After 3 months storage	After 6 months storage
1.	T. M. T. D.	93.3	92.5
2.	Captan	70.8	70.3
3.	Ceresan	44.2	37.5
4.	Control	23.8	13.8

Seeds stored in gunny bags were found to lose the viability quickly (Table 5) indicating the unsuitability of the gunny bags for the storage of groundnut seeds.

TABLE 5. *Effect of storage containers on seed viability*

Sl. No.	Storage containers	Germination percentage	
		After 3 months storage	After 6 months storage
1,	Wooden box	70.8	57.9
2.	Paper bag	61.7	53.3
3.	Polythene bag	50.4	50.0
4.	Gunny bag	43.8	39.6

Discussion: The present studies revealed the presence of 27 fungal species on groundnut. Some of the species have already been reported. Wilson (1947) reported the occurrence of *Rhizoctonia* (*Sclerotium*) *bataticola, Rhizopus nigricans,*

Aspergillus spp. and *Fusarium* spp. Prince (1944) indicated the presence of *Alternaria* spp. and *Penicillium* sp. on groundnut. Norton *et al.* (1954) observed the damage caused by *Aspergillus niger* and *A. flavus* on peanuts. Noble *et al.* (1958) reported the occurrence of *Thielaviopsis* sp. on groundnut. Barbosa (1965) isolated *Aspergillus tamarii* and *Syncephalastum nigricans* from stored groundnut. *Chaetomium* and *Neocosmospora* have also been isolated from groundnut seeds (Kang and Chohan, 1966; Barbosa, 1965).

Helminthosporium tetramera, *Phomopsis* sp, *Cunninghamella bertholletiae*, *Sporotrichum roseolum*, *Cladosporium globosum*, *Botryodiplodia* sp, *Mucor hiemalis*, *Curvularia lunata* and *Absidia hyalospora* have been recorded for the first time on groundnut. *Diplodia* spp and *Sclerotrium rolfsii* which were frequently isolated from groundnut by many workers (Prince, 1944; Wilson, 1947; Garren and Higgins, 1947; Norton *et al*, 1954) were not encountered in the present studies.

Most of the fungi isolated affected the seedling vigour and not seed germination. Vidhyasekaran *et al* (1970) reported the toxin production by seed-borne fungi and the toxin did not inhibit seed germination but affected the seedling vigour.

When the seeds were stored for 6 months, after treating with the seed-borne fungi, seed germination was much affected. Since toxin produced by the seed-borne fungi affected only seedling vigour, the seed germination failure during storage may be due to a completely different phenomenon. Vidhyasekaran and Govindaswamy (1968) attributed the paddy seed germination failure due to depletion of reserve starch and fatty substances. Schenik and Kennedy (1955) reported that most of the reserved food materials in wheat seeds were lost with concomitant increase in the production of Co_2 due to the seed-borne fungal invasion. Thus depletion of seed reserves due to the fungal invasion might have caused seed germination failures.

Control of seed-borne pathogens by chemical treatments has been reported by many workers. Ceresan (Gould, 1943), agrosan (Tarr, 1958), captan and thiram (Jackson, 1965) have been reported to be good protectants for groundnut seed-borne pathogens. But all these studies aimed at the eradicatin of the field pathogens only and no detailed studies have been made to increase the seed viability during the storage period. The present studies indicate that T.M.T.D. is the best fungicide to keep up the seed viability. Storage of seeds in gunny bags should be discouraged as it leads to loss of seed viability.

Summary: Twenty seven fungal species were isolated from stored groundnut seeds. Both pods and kernels were equally and heavily contaminated. Some of the fungi isolated were found to be pathogenic, causing seed germination failures and reduction in seedling vigour. *Aspergillus flavus* and

A. niger affected the root development while *Rhizoctonia bataticola* affected the shoot development. Although *Rhizopus nigricans* was the most predominant fungus on the groundnut seeds, it was not pathogenic. The seed-borne furgi infiicted heavy damage on seed viability during storage particularly, when stored at high humidity level. T.M.T.D. was observed to be the best fungicide affording protection to the seeds. Seeds stored in gunny bags were severely damaged.

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