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EFFECT OF STORAGE FUNGI OF OIL YIELDING SEEDS ON GERMINATION AND SEEDLING DISEASES

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Altogether 29 spp of fungi with varying frequencies were isolated from stored sesame seeds, 33 spp from groundnut, 35 spp from sunflower and 29 spp from castor seedlot. The frequncy of *Aspergillus flavus* was maximum (81-100%). *Aspergillus* spp outnumbered the other spp. *Alternaria, Curvularia* and *Cladosporium* spp were common to all the seeds. It is evident that significant difference ($P<0.5$) exists between the germination of the control and fungus stored seedlot and the loss of germination is considerably high in sesame and groundnut followed by sunflower. Least loss was recorded in castor. Many storage fungi cause single type of symptom of disease. Considerable per cent of seedlings has been found to be affected. The common seedling deseases are stunting, cotyledonary/first leave smaller in size and pale, roots smaller and curved, root and foot rotting, water soaked and brown lesions in the foot, drying of the whole seedling and apical bud. Maximum *in vitro* lipolytic activity was observed in *A. flavus* followed in succession by *F. moniliforme, A. niger* and *A. tenuissima*. Maximum loss of oil content of the seed was cuased by *A. flavus* followed in succession by *A. niger, F. maniliforme* and *A. tenuissima*.

Keywords : Germination; Oil yielding seeds; Seedling diseases; Storage fungi.

Introduction

Much has been documented on the significance of storage fungi of the seeds of crop plants[1-9]. As regards the oily seeds, most of the work has centred around the mustard seeds[7, 10] that includes the seed decay, loss of germination, seedling diseases, change in the seed and seedling physiology etc. The present paper deals with the isolation of fungi of stored oily seeds, other than mustard, their lipolytic activity and effect on oil content of the seeds, seed germination and diseases of the seedling.

Materials and methods

Collection of seeds and isolation of fungi- Seeds of sesame (*Sesamum indicum* L) var. Kanke white, dehusked groundnut *(Arachis hypogea* L) var BGI, sunflower (*Helianthus annuus L)* var Armavir Kij (EC 68415) and castor (*Ricinus communis L)* var. EB 6-A stored with the farmers of different parts of Bihar state were collected in sterilized polyethylene pockets and stored at 5°C in a refregerator. The fungi were isolated adopting blotter technique[11] and using four hundred seeds. They were cultured on potato dextrose agar medium to purify them and their frequency was calculated.

Observation of seed germination and seedling diseases- For this purpose, 14 spp of fungi isolated from the seed were selected based on their frequency and grown on potato dextrose agar medium for 7 days at 28±1°C. *Chaetomium globosum* was cultured on Leonian agar for hastening the formation of large number of perithecia. The spore suspension was prepared and seeds mentioned above were infested[12] besides uninfested control and stored over saturated solution of $(NH_4)_2 SO_4$ to maintained (80% RH) in sealed desiccators at 30±1°C for 30 days. The seeds were germinated in garden soil previously autoclaved at 15 psi for 20 min for two consecutive days. The soil treated as above was filled in earthen pots of the dimension 25 cm top diameter, 15 cm base diameter and 25 cm depth. 25 seeds of each crop stored as above were sown in July 2002 nearly 1 cm deep in the soil. Four pots for each seed and fungus besides uninfested control lots were useds. The seeds after sowing were lightly watered with sterlized water and coverd with sterilized cotton wool nearly 1 cm thick. The pots were kept in the garden for 7 days with alternate day watering as above. The germination was recorded and per cent germination was calculated. After removing the cotton wool the seedlings were permitted to grow for 15 days more to observe the symptoms of diseases in the

seedlings, if any.

For observing the diseases in the foot and root region of the seedlings, the infested and stored seeds were germinated in sterilized moist towels for 7 days after surface sterilization with 0.1% $HgCl_2$ for 1 min and thorough washing with sterilized tap water and finally with sterilized distilled water. The replicates were maintained as stated for garden soil.

Lipolytic activity of storage fungi and loss of oil from the seeds- In vitro lipolytic activity of *Aspergillus flavus, A. niger, Alternaria tenuissima* and *Fusarium moniliforme* was assayed originally based on the method of Sierra[13] and modified by Prasad[14]. A basal medium consisting of Difco peptone 1 g, NaCl 0.05g, $CaCl_2.2H_2O$ 0.01g, agar 2 g and distilled water 100 ml. pH was maintained at 6.0. The medium was autoclaved at 15 psi for 15 min. Sarbitol monolaureate (Tween 20) as substrate was sterilized separately at 15 psi for 15 min. 1ml of Tween 20 per 100 ml of basal medium was mixed, coooled and poured in sterilized petri dishes of 10 cm diameter. The fungi noted above were inoculated in the centre with inoculating needle and cultured at 25±1°C for 7 dyas. A zone of white crystals round the colony indicates the presence of lipolytic activity of the fungus. The mean of radii was recorded in mm. More the length of radius, more the activity. The oil content of the infested and stored seedlot besides the control was extracted with Soxhlet extractor using 5g dry seed powder with 50 ml of diethylether at 60°C for 1 hr. The ether was removed at 60°C and the oil was weighed.

Results and Discussion

In all 29 spp of fungi with varying frequencies were isolated from sesame seeds, 33 spp from groundnut, 35 spp from sunflower and 29 spp from castor seed. The frequncy of *Aspergillus flavus* was maximum (81-100%). *Aspergillus* spp outnumbered the other spp. *Alternaria, Curvularia* and *Cladosporium* spp were common to all the seeds. It is evident that significant ($p<0.5$) difference exists between the germination of control seed and the fungus stored lots (Table 1) and the loss of germination is considerably high in sesame and groundnut followed by sunflower. Least loss was recorded in castor. Table 2 indicates that many fungi cause a single type of symptom of disease in seedlings. Of the common diseases in the seedlings stunting, leaves smaller and pale, roots smaller and curved, root and foot rotting, water soaked and brown lessions in the foot, drying of the whole seedlings and apical bud were observed. Maximum *in vitro* lipolytic activity was observed in *A. flavus* followed in succession by *F. moniliforme, A. niger* and *A. tenuissima* (Table 3). The oil content of the seed due to storage as recorded in Table 3 indicates considerable loss, maximum by *A. flavus* followed in succession by *A. niger, F. moniliforme* and *A. tenuissima.*

The variation in the frequency of storage fungi of the seeds might depend upon cumulative effect of agricultural operations and physico-chemical characteristics of the seeds behaving themselves as ecological niche[15]. Varying coat colour, thickness and chemical constituents of the seed in question might also play role in colonisation of the fungi. The suppression of seed germination due to storage fungi has been a general phenomenon[1,14-16] which might be due to toxic principles secreted by storage fungi inflicting ultrastructural[13] and biochemical change[6,8,9]. The extract of the seeds of coriander stored with *Aspergillus flavus* has been found to impede the activity of amylase, protease and lipase of the seeds[17,18] afflicting unavailability of hydrolytic products that would be utilized in various syntheses unavoidably needed for the seed germination. The metabolite in Richard solution of *A. flavus, A. niger* and *F. moniliforme* suppressed the seed germination of crop plants considerably[19].

As regards the infliction of diseases in the seedlings, recent authors have

Table 1. Per cent germination of oil yielding seeds due to storage fungi (figures were rounded to their whole number).

Fungus isolates	Oil yielding seeds			
	Sesame	Groundnut	Sunflower	Castor
Aspergillus flavus	36	46	51	75
A. niger	44	51	61	79
A. candidus	74	78	77	86
A. sydowi	64	76	78	87
A. nidulans	58	69	68	81
A. terreus	85	72	70	86
Chaetomium globosum	71	86	79	88
Alternaria tenuissima	50	58	61	79
A. alternata	53	54	64	79
Curvularia lunata	62	68	69	89
C. pallescens	65	70	72	89
C. herbarum	63	72	73	88
C. oxysporum	64	75	74	89
F. moniliforme	51	62	63	78
Control	89	92	85	92

CD for seed = 3.09, CD for fungus = 5.36

Table 2. Arrangement of seedbonre storage fungi isolated from sesame seeds based on the symptoms of disease produced by them in the seedlings.

Symptoms of disease	Seedlings affected (%)	Fungus isolates
1. Seedling stunted in growth	12-21	*Aspergillus flavus, A. niger, A. candidus, A. sydowi, A. terreus, A. nidulans, Alternaria tenuissima, A. alternata, Curvularia lunata, C. pallescens, Cladosporium herbarum, C. oxysporum, Fusarium moniliforme*
2. Leaves smaller and pale	7-16	*A. flavus, A. niger, A. candidus, A. sydowi, A. terreus, A. nidulans, A. tenuissima, A. alternata. C. lunata, C. pallescense*
3. Root smaller and curved	13-25	*A. flavus, A. niger*
4. Foot rotting	11	*A. nidulans, A. terreus, Chaetomium globosum, C. herbarum, C. oxysporum*
5. Root rotting	15	*F. moniliforme*
6. Water soaked lesion in the foot	12-17	*A. flavus, A. niger, A. candidus, A. terreus, C.globosum, A. tenuissima, A. alternata, C. lunata.*
7. Brown lesions in the foot	8-13	*A. flavus. A. niger, A. candidus, A. terreus, C. globosum, A. tenuissim, A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme*
8. Drying of seedlings	5-10	*A. flavus, A. niger, A. sydowi, A. nidulans, A. tenuissiuma, A. alternata*
9. Drying of the apical bud	9-12	*A. flavus, A. niger, A. tenuissima, A. alternata*

Table 2(contd). Arrangement of seedborne storage fungi isolated from groundnut seeds based on the symptoms of disease produced by them in the seedlings.

Symptoms of disease	Seedlings affected (%)	Fungus isolates
1. Seedling stunted	7-21	*A. flavus, A. niger, A. candidus, A. sydowi A. terreus, A. nidulans*
2. Cotyledonary leaves smaller	9-13	*A. flavus, A. niger, A. candidus, A. sydowi, A. terreus,A. nidulans, A. tenuissiuma, A. alternata, C. lunata, C. pallescense, C. herbarum, C. oxysporum, F. moniliforme.*
3. Water soaked lesions in the foot	7-15	*A. flavus, A. niger, A. candidus, A. sydowi, A. terreus, A. nidulans, A. tenuissiuma, A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme.*
4. Brown lession in the foot	6-12	*A. flavus, A. niger, A. candidus, A. sydowi, A. terreus, A. nidulans, A. tenuissiuma, A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme.*
5. Root rotting	7-11	*A. flavus, A. niger*
6. Drying of seedlings	5-9	*A. flavus, A. tenuissima, A. alternata*
7. Brown lesions in the foot	6-14	*A. flavus. A. niger, A. nidulans, A. tenuissima, A. alternata, C. lunata.*
8. Root rotting	5-10	*A. candidus, A. sydowi, F. moniliforme*
9. Foot rotting	4-9	*A. nidulans, C. globosum, F. moniliforme*

Table 2(contd). Arrangement of seedbonre storage fungi isolated from sunflower seed based on the symptom of disease produced by them in the seedlings.

Symptoms of disease	Seedlings affected (%)	Fungus isolates
1. Seedling stunted and somewhat pale	7-14	*A. flavus, A. niger, A. nidulans, A. tenuissima, A. alternata, F. moniliforme*
2. Water soaked lesions in the foot	6-12	*A. flavus, A. niger, A. candidus, A. sydowi, A. nidulans, A. tenuissima, C. globosum. A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme*
3. Brown lession in the foot	5-15	*A. flavus, A. niger, A. candidus, A. sydowi, A. nidulans, A. tenuissiuma, C. globosum. A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme*
4. Root rotting	4-9	*A. flavus, F. moniliforme*
5. Foot rotting	3-8	*A. flavus, A. niger, A. candidus, A. sydowi, A. nidulans, C. lunata, C. pallescens, F. moniliforme*
6. Drying of the apical bud	8-12	*A. flavus, A. niger*
7. Leaves smaller and somewhat pale	11-8	*A. tenuissima, A. alternata*

Table 2(contd). Arrangement of seedborne storage fungi isolated from castor seed based on the symptom of disease produced by them in the seedlings.

Symptoms of disease	Seedlings affected (%)	Fungus isolates
1. Cotyledonary leaves smaller and pale	4-9	*A. flavus, A. niger, A. tenuissima, A. alternata*
2. Brown lesion in the foot	6-10	*A. flavus, A. niger, A. candidus, A. sydowi, A. nidulans, A. terreus, C. globosum, A. tenuissima, A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme*
3. Brown lesion in the root	7-15	*A. flavus, A. niger, A. candidus, A. sydowi, A. nidulans, A. terreus, C. globosum, A. tenuissima, A. alternata, C. lunata, C. pallescens, C. herbarum, C. oxysporum, F. moniliforme*
4. Foot rotting	6-8	*A. flavus, A. nidulans, F. moniliforme*
5. Root rotting	7	*F. moniliforme*
6. Junction of the root and the shoot showing mycelial growth	8	*C. globosum*
7. Drying of apical bud	7-9	*A. tenuissima, A. alternata*
8. Seedling stunted	8-13	*A. tenuissima, A. alternata*

Table 3. Oil content (%) of the fungus stored seeds and lipolytic activity of the fungi.

Fungus isolates	Oil yielding seed				Lipolytic activity of fungi (In mm)
	Sesame	Groundnut	sunflower	Castor	
A. flavus	35.81	38.12	29.81	34.26	22.0
A. niger	38.97	41.38	31.93	34.75	16.0
A. tenuissima	39.27	42.47	31.76	34.83	12.0
F. moniliforme	39.42	41.83	30.76	34.64	10.0
Control	43.43	46.85	33.76	36.28	00.0

CD for seed = 3.63, CD for fungus = 3.63

documented instances in crop plants due to storage fungi. The effect of *A. flavus, A. niger* nd *F. moniliforme* occurring with fairly high frequency in seeds has been paid more attention still the effect of other fungi cannot be overlooked. The seedling diseases due to storage fungi have been reported earlier[12,20-22]. The stunting of the seedlings possibly results due to destruction of IAA through its rapid oxidation[22] and suppressed expansion of leaves can be seen in the same light. The effect of toxic principle secreted by the fungi cannot be ignored that restricts not only the growth but distrub the chlorophyll synthesis and consequently diminishing quantity of sugars. The synthesis of amino acids has been found to be disturbed due to sluggish activity of nitrate reductase and urease activities[23] and their excited oxidation, decarboxylation and deamination as observed in lablab bean seed[9]. The toxic principle also restricted cell division and produced abnormally smaller and big sized nuclei[19]. The water soaked lesions in the foot, foot and root rotting all seem possibly due to high activity of pectolytic and cellulolytic enzymes of the fungi[14] growing over the radicle[20]. Brown lesions in the foot appear due to dissolution of cutin[24] and oxidation of phenolic compounds at the exposed part. The drying of seedlings and the apical bud might be the result of the toxic principle causing necrosis of immature tissue[25].

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