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Deteriorative changes in oilseeds due to storage fungi and efficacy of botanicals

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KEYWORDS

Oilseeds, storage fungi, nutritional changes, aqueous extract, fungitoxic

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ABSTRACT

Improper storage makes the oilseeds vulnerable to storage fungi which deteriorate the stored oilseeds both qualitatively and quantitatively. They bring about the variety of biochemical changes in the suitable conditions. Considering this fact, experiments were undertaken to understand nutritional changes like change in reducing sugar, change in crude fat content and change in crude fiber content of oilseeds due to artificial infestation of storage fungi. It was found that, *Alternaria dianthicola, Curvularia lunata, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina* and *Rhizopus stolonifer* causes decrease in reducing sugar of oilseeds. *Alternaria dianthicola, Curvularia pellescens, Macrophomina phaseolina, Penicillium digitatum* and *Penicillium chrysogenum* hampered the fat content of oilseeds. *Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus stolonifer* and *Penicillium digitatum* increased the fiber content in oilseeds. An attempt was also made to control the seed-borne fungi by using aqueous extract of ten medicinal plants. Aqueous extract of *Eucalyptus angophoroides* was found to be most fungitoxic.

Introduction

India is one of the largest producers of oilseeds in the world and this sector occupies an important position in the agricultural economy covering an area of 38 million hectares and accounting for the production of about 32 million tonnes of oilseeds annually. India contributes about eight per cent of the world oilseeds production and about six per cent of the global production of oils and fats and currently is the 4th largest edible oil economy in the world, after China, EU-15, and USA. India has a wide range of oilseeds crops grown due to the different agroclimatic zones. Groundnut, soybean, mustard/ rapeseed, sesame, safflower, linseed, castorseed are major traditionally cultivated oilseeds. Seeds in the field as well as in ill storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively (Christensen and Kaufman, 1969). The microorganisms thrive on the seeds at the expense of easily digestible components. The successful invasion or colonization, however, depends largely upon the efficiency of microorganisms to degrade complex molecules into simpler forms (Bilgrami and Verma, 1978). Damage to the oilseeds has been reported to be caused by fungi associated with them. Fungi like *Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum* causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oilseeds (Chavan and Kakde, 2008). As oilseeds are rich in oil content, which boost the vigor of pathogenic fungi resulting in biodeterioration by production of lipase. (Umatale, 1995; Waghmare, 1996; Kakde and Chavan, 2011a; Kakde and Chavan, 2011b). Fungi growing on stored grains, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces moisture content, free fatty acid content enhancing other biochemical changes of grains (Bhattacharya, 2002). Such seeds are not fit for human consumption and are also rejected at the industrial level. There fore, in first part of the study includes investigation of the biochemical changes like change in reducing sugar, crud fat and crude fiber content of groundnut, sesame, soybean, safflower and sunflower seeds due to some dominant seed borne fungi. In comparision to synthetic compound, the pesticidal compounds of plant origin are more effective and have little or no side effects on human beings (Kumar et. al. 1995). Green plants appear to be reservoir of biotoxicants and constitute inexhaustible source of number of pesticides (Swaminathan, 1978). Hooda and Srivastava (1998) have mentioned that natural fungicides are free from environmental toxicity as compared to synthetic compound. Natural compounds are less phytotoxic, easily biodegradable and more systematic (Saxena et. al., 2005). Therfore, second part includes ecofriendly management of these seed-borne fungi by aqueous leaf extract of some medicinal plants.

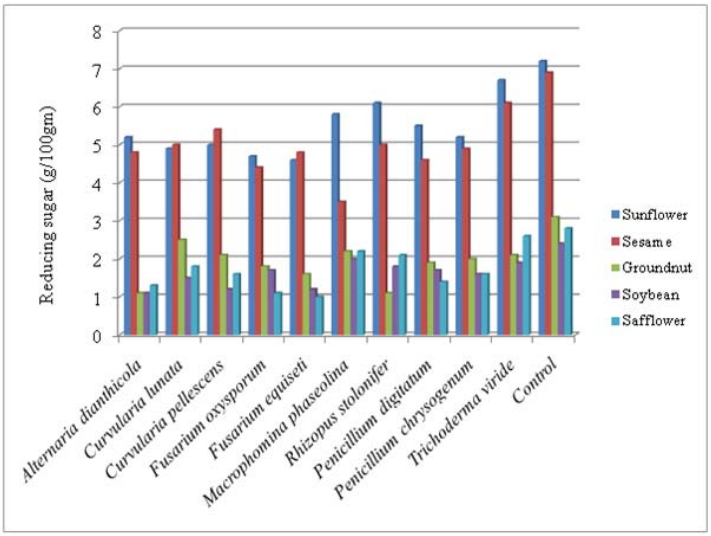
Materials and Methods

Ten dominant fungi which were isolated from abnormal oilseeds, selected to study its impact on nutritional status of oilseeds. Healthy seeds of groundnut, soybean, sesame, safflower and sunflower were surface sterilized with 0.1% mercuric chloride solution and subsequently washed and soaked in sterile distilled water for four hours. Excess water was decanted from the seeds. The seeds were distributed into flasks (l00g per flask) and were inoculated separately with 10ml spore suspension of the test fungi. The flasks were incubated at room temperature for 14 days. At the time of harvest, seeds were thoroughly washed under running tap water in order to remove complete mycelia mat from their surface. Subsequently, the seeds were dried at 60°C for 48 hours and crushed into fine powder for the estimation of chemical changes in the seeds. Seeds incubated in a similar manner but without inoculating spore suspensions of fungi served the control. The crude fat in the plant material was estimated by the

standard Soxhlet method (A.O.A.C., 1970). The sugar content in the plant material was estimated by the procedure recommended by Oser (1979). Crude fiber content was estimated by the method recommende by Sadashivam and Manickam (2008). Fungitoxic properties of ten selected medicinal plants (10% aqueous leaf extract) screened against test fungi (Nene and Thapliyal, 1993). Glocose nitrate medium was prepared in flasks and sterilized. To this medium, the requisite quantity of the plant extract was added. The plant extract was prepared by collecting fresh plant parts, washed thoroughly in distilled water and grinned in distilled water. The plant extract was thoroughly mixed by stirring. The medium was then autoclaved at 15 lbs pressure for 20 minutes. After cooling the medium, fungi were inoculated in asceptic condition and incubated for 6 days at room temperature, suitable checks were kept where the fungi were grown under the same condition on glucose nitrate without plant extract. Mycelial growth and sporulation of the test fungi was measured after harvesting. The mycelial growth of the fungi compared with check, was taken as a measure of the fungal toxicity.

Table 1. Change in reducing sugar (g/100gm) due to seed-borne fungi

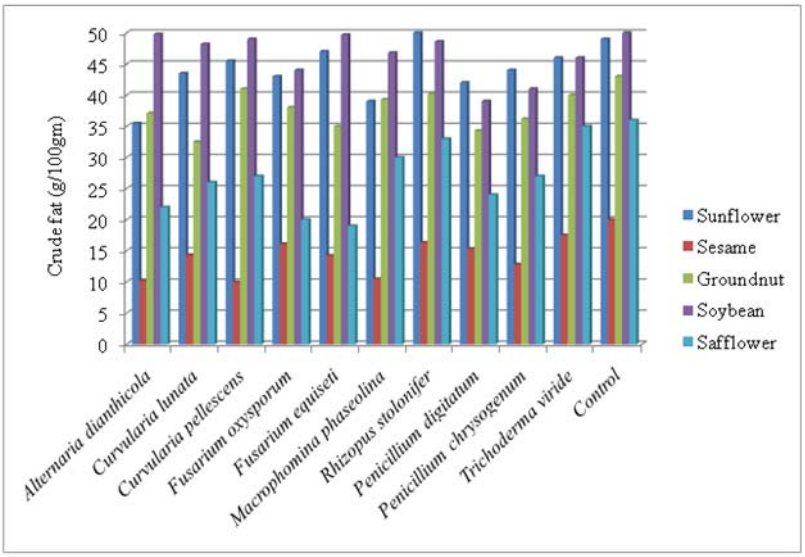
Fungi	Sunflower	Sesame	Groundnut	Soybean	Safflower
Alternaria dianthicola	5.2	4.8	1.1	1.1	1.3
Curvularia lunata	4.9	5.0	2.5	1.5	1.8
Curvularia pellescens	5.0	5.4	2.1	1.2	1.6
Fusarium oxysporum	4.7	4.4	1.8	1.7	1.1
Fusarium equiseti	4.6	4.8	1.6	1.2	1.0
Macrophomina phaseolina	5.8	3.5	2.2	2.0	2.2
Rhizopus stolonifer	6.1	5.0	1.1	1.8	2.1
Penicillium digitatum	5.5	4.6	1.9	1.7	1.4
Penicillium chrysogenum	5.2	4.9	2.0	1.6	1.6
Trichoderma viride	6.7	6.1	2.1	1.9	2.6
Control	7.2	6.9	3.1	2.4	2.8
C.D. at 0.05	0.52	0.62	1.21	0.24	0.21



Graph 1. Change in reducing sugar (g/100gm) due to seed-borne fungi

Table 2. Change in crude fat (g/100gm) due to seed-borne fungi

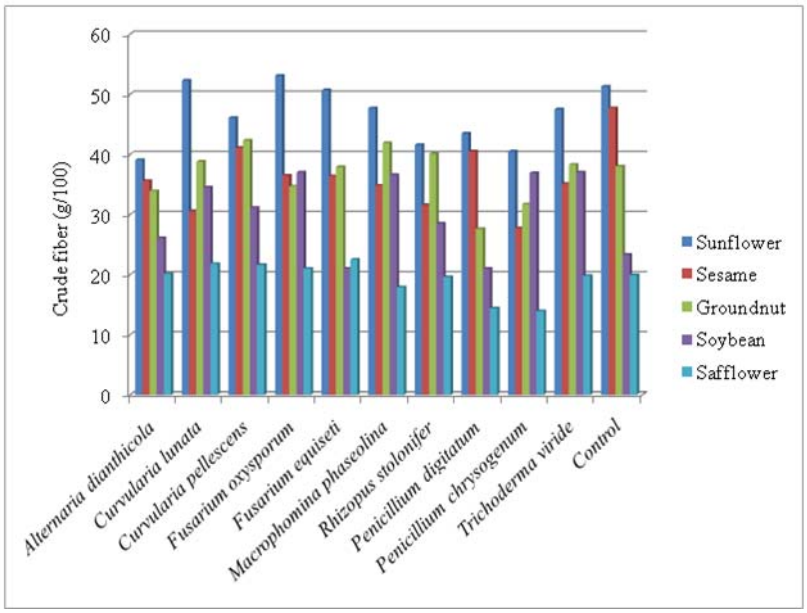
Fungi	Sunflower	Sesame	Groundnut	Soybean	Safflower
Alternaria dianthicola	35.5	10.2	37.1	49.8	22
Curvularia lunata	43.5	14.3	32.5	48.2	26
Curvularia pellescens	45.5	10	41	49	27
Fusarium oxysporum	43	16.1	38	44	20
Fusarium equiseti	47	14.2	35	49.7	19
Macrophomina phaseolina	39	10.5	39.3	46.8	30
Rhizopus stolonifer	50	16.3	40.2	48.6	33
Penicillium digitatum	42	15.3	34.3	39	24
Penicillium chrysogenum	44	12.8	36.2	41	27
Trichoderma viride	46	17.5	40	46	35
Control	49	20	43	50	36
C.D. at 0.05	2.71	2.05	2.04	2.4	3.7



Graph 2. Change in crude fat (g/100gm) due to seed-borne fungi

Table 3. Change in crude fiber (g/100) due to seed-borne fungi

Fungi	Sunflower	Sesame	Groundnut	Soybean	Safflower
Alternaria dianthicola	39.1	35.6	33.9	26.1	20.1
Curvularia lunata	52.3	30.6	38.8	34.5	21.8
Curvularia pellescens	46.1	41.1	42.3	31.1	21.6
Fusarium oxysporum	53.1	36.5	34.7	37.0	21.0
Fusarium equiseti	50.7	36.4	37.9	21.0	22.5
Macrophomina phaseolina	47.7	34.8	41.9	36.6	17.9
Rhizopus stolonifer	41.6	31.6	40.1	28.5	19.6
Penicillium digitatum	43.5	40.5	27.6	21.0	14.4
Penicillium chrysogenum	40.5	27.7	31.7	36.9	13.9
Trichoderma viride	47.5	35.1	38.3	37.0	19.8
Control	51.3	47.7	38.0	23.3	19.9
C.D. at 0.05	3.16	6.11	2.39	4.2	1.82

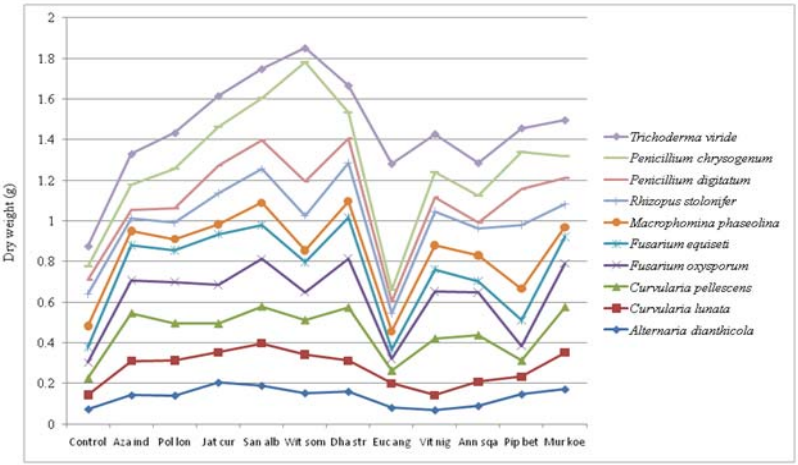


Graph 3. Change in crude fiber (g/100) due to seed-borne fungi

Table 4. Antifungal properties of leaf extract of medicinal plants

Fungi	Medicinal plants											
	Control	Aza ind	Pol lon	Jat cur	San alb	Wit som	Dha str	Euc ang	Vit nig	Ann sqa	Pip bet	Mur koe
Alternaria dianthicola	0.073	0.142	0.141	0.205	0.190	0.153	0.160	0.080	0.068	0.089	0.147	0.172
Curvularia lunata	0.071	0.168	0.172	0.149	0.207	0.189	0.152	0.120	0.074	0.120	0.087	0.180
Curvularia pellescens	0.083	0.236	0.184	0.141	0.182	0.170	0.262	0.065	0.280	0.229	0.080	0.224
Fusarium oxysporum	0.077	0.162	0.203	0.191	0.233	0.138	0.242	0.056	0.230	0.210	0.071	0.214
Fusarium equiseti	0.076	0.172	0.156	0.249	0.168	0.147	0.202	0.047	0.109	0.056	0.126	0.132
Macrophomina phaseolina	0.103	0.071	0.054	0.048	0.110	0.059	0.079	0.089	0.119	0.127	0.156	0.048
Rhizopus stolonifer	0.157	0.062	0.081	0.154	0.165	0.171	0.189	0.088	0.166	0.134	0.313	0.112
Penicillium digitatum	0.072	0.041	0.070	0.137	0.143	0.167	0.121	0.059	0.069	0.028	0.176	0.131
Penicillium chrysogenum	0.065	0.126	0.194	0.188	0.206	0.59	0.126	0.058	0.127	0.131	0.185	0.106
Trichoderma viride	0.099	0.150	0.178	0.153	0.144	0.067	0.134	0.620	0.186	0.162	0.116	0.178

Aza ind- *Azadirachta indica*; Pol lon - *Polyalthia longifolia*; Jat cur- *Jatropha curcus*; San alb- *Santalum album*; Wit som- *Withania somnifera*; Dha str- *Datura strominum*; Euc ang- *Eucalyptus angophoroides;* Vit nig- *Vitex nigundo;* Ann sqa- *Annona squamosa;* Pip bet- *Piper betel;* Mur koi- *Murraya koenigii*



Graph 4. Antifungal properties of leaf extract of medicinal plants

Results and Discussion

Reducing sugar

In case of sunflower, *Fusarium equiseti, Fusarium oxysporum* and *Curvularia lunata* showed maximum decrease in reducing sugar while, in sesame, reducing sugar is signficantly decreased due to infestation of *Macrophomina phaseolina* and *Fusarium oxysporum.* There is a considerable loss of reducing sugar in groundnut due to *Fusarium equiseti* and *Rhizopus stolonifer.* Soybean seeds showed signficant decrease in reducing sugar due to *Alternaria dianthicola, Curvularia pellescens* and *Fusarium equiseti.* There is signficant decrease in reducing sugar content in safflower due to *Fusarium equiseti, Fusarium oxysporum* and *Alternaria dianthicola* (Table 1).

Crude fat

Crude fat content in groundnut signficant reduced due to *Alternaria dianthicola* and *Macrophomina phaseolina.* It is interesting to note that fat content in sunflower is increased due to *Rhizopus stolonifer. Curvularia pellescens, Alternaria dianthicola* and *Macrophomina phaseolina* showed maximum decrease in fat content of sesame. In case of groundnut *Curvularia lunata, Fusarium equiseti* and *Penicillium digitatum* were found to be responsible for the maximum decrease in the fat content*. Penicillium digitatum, Penicillium chrysogenum* and *Fusarium oxysporum* signficantly depleted the fat content in soybean. Fat content in safflower is signficantly reduced due to *Fusarium oxysporum* and *Fusarium equseti* fungi in sunflower (Table 2).

Crude fiber

In sunflower increased in crude fiber was observed due to *Alternaria dianthicola, Penicillium chrysogenum* and *Rhizopus stolonifer. Penicillium chrysogenum* and *Curvularia lunata* were found to be responsible for the maximum decrease in the crude fiber content in sesame. In groundnut due to *Penicillium digitatum, Penicillium chrysogenum* and *Alternaria dianthicola*; in soybean, except *Fusarium equiseti* and *Penicillium notatum* all fungi showed increased in crude fiber. In safflower *Penicillium digitatum, Penicillium chrysogenum* and *Macrophomina phaseolina* showed increase in the fiber content of safflower (Table 3).

Several reports reveal that storage fungi cause nutritional changes in seeds. Biochemical changes in groundnut (Ward and Diener, 1961; Sing *et al.,* 1974) and other oilseeds (Sharma, 1977) due to storage fungi were found to damage or discolor the kernels which consequently affect fat and reducing sugar contents. The results indicate that there is a large variation in these compounds due to infection by fungal organisms. Production of lipase by fungal species might have reduced the fat content of oilseeds. Similarly other metabolic process of fungal organisms on the oilseeds might be responsible for the increase or decrease of fats and reducing sugars as has been suggested by Reddy and Rao (1975) and Inman (1965). Embaby et al., (2006) reported that, most reduction and loss percent were found with fat, carbohydrate due to *Fusarium oxysporum* in legume seeds.Decreased in reducing sugar may be due to fungi utilized sugar as a substrate for its growth. There is a decrease in crude fat, it is because of fungi might have degraded the lipids by lipase enzyme. Some of the fungi showed the increase in crude fiber as compared to control which means that mycelium of these fungi might be remained in the substrate.

Antifungal acivity of aqueous leaf extract of medicinal plants

Azadirachta indica and *Polyalthia longifolia* showed its antifungal acivity against *Macrophomina phaseolina, Rhizopus stolonifer* and *Penicillium digitatum.* The growth of *Macrophomina phaseolina* was hampered due to *Murraya koenigii, Jatropha curcus, Withania somnifera* and *Datura strominum.* Aqueous extract of *Eucalyptus angophoroides* found to be fungitoxic for the growth of *Alternaria dianthicola, Curvularia pellescens, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum.* Aqueous extract of *Vitex nigundo* reduced the growth of *Alternaria dianthicola* and *Penicillium*

digitatum. *Annona squamosa* hampered the growth of *Penicillium digitatum* and *Fusarium equiseti* (Table 4).

Various workers have screened large number of plants belonging to angiosperm and gymnosperms for their fungitoxic properties. Manoharachary and Gourinath (1991) found that aqueous leaf extract of *Eucalyptus lonceolatus* was inhibitory for the germination and growth of *Curvularia lunata, Cylindrocarpon lichenicola* and *Fusarium solani*. Singh and Prasada (1993) found that leaf extract of *Azadirachta indica* and *Ocimum sanctum* inhibited the growth of *Fusarium oxysporum*. Mostly the aqueous extract of plants has been used to evaluate their fungitoxic properties (Thapliyal et. al., 2000 and Algesaboopathi and Balu, 2002). Shafique et al., (2007) from experiment recommended that aqueous extracts of allelopathic trees especially those of *A. indica* and *M. indica* can be used to treat the wheat grains for 10 minutes before sowing or storage to reduce the fungal incidence. Similarly, Meena et al., (2010) tested leaf extract of ten medicinal plants against *Alternaria cucumerina*. They reported that, leaf extract of *Azadirachta indica, Calotropis gigantia* and *Aloe barbadensis* were found to be effective in controlling spread of *Alternaria cucumerina*. Aqueous extract of *Eucalyptus angophoroides* showed retardation of growth of storage fungi which reveals that it is most fungitoxic and can be used in biopesticides formulations.

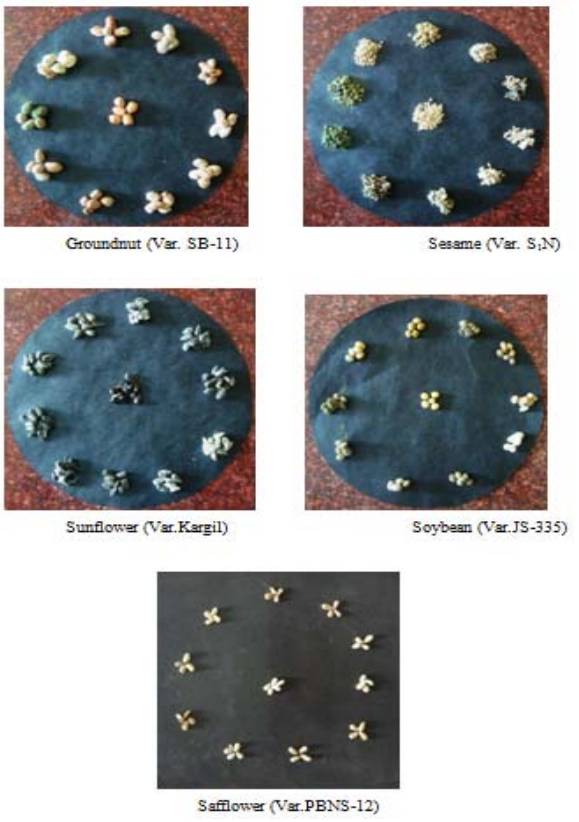


Fig. 1: Deteriorated oilseeds due to storage fungi

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