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Fungitoxic properties of leaf extract and latex against storage fungi

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ABSTRACT

In present investigation mycoflora from groundnut, soybean, sesame, safflower and sunflower was isolated by using different agar media. Total thirty fungi were isolated from these oilseeds and screened against different plant leaf extract and latex. It was found that, out of five oilseeds, soybean yielded ninteen fungi, fifteen fungi were found to be associated with sesame and safflower seed. On the other hand sunflower yielded only nine fungi. Aqueous extract of *Eucalyptus angophoroides* retarded the growth of *Alternaria dianthicola, Curvularia pellescens, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum.* The latex of *Calotropis gigantia* for *Alternaria dianticola*; *Aloe vera* for *Curvularia lunata* and *Penicillium digitatum* were found to be fungitoxic.

Introduction

Groundnut (*Arachis hypogaea* L.) contains on an average 40.1 per cent of fat and 25.3 per cent of protein and is a 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. Popularity of soybean [(*Glycin max* L.) Merrill] crop is due to abundance (43%) high quality protein and a rich source of oil with high unsaturated fatty acids and with no cholesterol (Sharma and Mehrotra, 1988). Sunflower (*Helianthus annus* L.) oil is derived from sunflower seed carrying nearly 45-50% oil content 25% protein content. In safflower (*Carthamus tinctorius* L.) oil content varies from 24 to 36 per cent. The oil obtained by dry hot distillation is black and sticky and is used only for greasing well ropes and leather goods exposed to water. Safflower oil has also good dying properties and therefore it is used in the manufacture of paints, varnishes and linoleum. Sesame (*Sesame indicum* L.) oil content generally varies from 46 to 52%. Its grains may be eaten fried, mixed with sugar or in the form of sweet meats. Sesame oil is used as a cooking-oil in southern India. It is also used for manufacturing perfumed oils and for medicinal purposes. After harvesting seeds are stored in different storage conditions and if these storage conditions are not proper various microbes like viruses, bacteria, fungi and nematode are interacted with these seeds. Among these microbes, fungi play a dominant role in decreasing quality and longetivity of the seeds. Fungi cause various abnormalities to the seeds like discolored seeds, damaged seeds, shrunken seeds, undersized, rotted seeds and reduced in germinability. Fungal organisms plays significant role in infection, altering quality and longevity of seeds during the storage (Christensen and Kaufman, 1969). Such seeds are not fit for human consumption and rejected at industrial level. This ultimately affect on the yield and economy of the country. Pesticides play a vital role in the stabilization and increase of agricultural yield. On the other hand, it is being a possible source of atmospheric pollution, with residual toxicity to mammals and wildlife. The knowledge of extent and mode of inhibition of specific compounds which are present in plant extracts, may contribute to the successful application of such natural compounds for treatment of infection disorder like fungal and bacterial diseases. In this regard, aqueous leaf extract of ten medicinal plants were screened for the antifungal activity against seed-borne fungi. Various workers have screened a large number of plants belonging to angiosperm and gymnosperms for their fungitoxic properties. Mostly the aqueous extract of plants has been used to evaluate their fungitoxic properties (Thapliyal et al., 2000 and Algesaboopathi and Balu, 2002). Considering this fact aqueous leaf extract and latex of some medicinal plants were tested against the storage fungi.

Materials and methods

Collection of oilseed samples

Oilseeds samples of groundnut, soybean, sesame, safflower and sunflower of different varieties were collected from market places, store houses and fields from different parts of Marathwada region of Maharashtra state.

Isolation of oilseed mycoflora

For detection of seed mycoflora associated with seed samples, the method recommended by ISTA (1966), Neergaard (1973) and Agarwal (1976) were adopted. 10 seeds per pre-sterilized petriplates were equispaced asceptically on autoclaved Potato Dextrose Agar (PDA), Czapek Dox Agar (CZA), Glucose Nitrate Agar (GNA) and Rose Bengal Agar (RBA) media. Plates were then allowed to incubate at room temperature for seven days.

Antifungal activity of botanicals

Fungitoxic properties of ten selected medicinal plants (10% aqueous leaf extract) screened against test fungi (Nene and Thapliyal, 1993). Glucose 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

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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 seven days at room temperature.

Suitable checks were kept where the fungi were grown under the same condition in glucose nitrate without plant extract. Mycelial growth of the test fungi was measured after harvesting. The mycelial weight of the fungi compared with check, was taken as a measure of the fungal toxicity.

Results and discussion

Incidence of fungi on different oilseeds

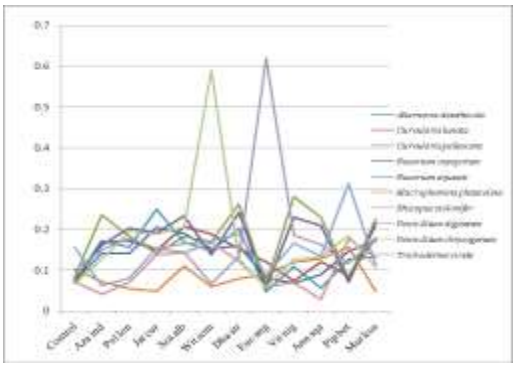
Five different oilseeds viz., groundnut, soybean, sesame, safflower and sunflower were screened to study the occurrence of mycoflora and the results are given in table 1. Out of five oilseeds, soybean yielded maximum fungi i.e. ninteen. *Macrophomina phaseolina* showed its quantitative dominance on soybean which is followed by *Aspergillus niger, A. flavus, Fusarium oxysporum, Fusarium solani, Rhizopus stolonifer* and *Penicillium chrysogenum*. It is interesting to note that *Cercospora kikuchii, Colletotrichum* sp. and *Helminthosporium* sp. showed their occurrence only on soybean seed. Groundnut showed occurrence of sixteen fungi. Out of this *Aspergillus* genera showed four fungi viz., *Aspergillus flavus, A. niger, A. fumigatus* and *A. terreus*. From *Fusarium* genera three fungi viz., *Fusarium chlamydosporum, F. equiseti, F. verticillioides* and *F. oxysporum* were occurred. Two species from *Penicillium* genera viz., *Penicillium digitatum* and *Penicillium chrysogenum* were yielded on groundnut. Only groundnut and soybean showed occurrence of *Aspergillus fumigatus, Alternaria alternata* and *Rhizoctonia solani*. Fifteen fungi were found to be associated with sesame seed. Sesame showed maximum incidence of *Aspergillus flavus, A. niger, Alternaria dianthicola, Fusarium equiseti, Penicillium digitatum* and *Trichoderma harzianum*. Sesame also showed association of two species from *Curvularia* genera i.e. *Curvularia lunata* and *Curvularia pellescens*. It is interesting to note that only sesame showed the occurrence of *Verticillium* sp. *Aspergillus terreus, Aspergillus niger, Fusarium equiseti, Fusarium oxysporum, Mucor sp.* and *Rhizopus stolonifer* found to be associated with both safflower and sunflower seeds. Among this *Fusarium oxysporum* and *Rhizopus stolonifer* showed the quantitative dominance. Safflower yielded four fungi from *Aspergillus* species viz., *Aspergillus flavus, Aspergillus terreus Aspergillus niger* and *Aspergillus ustus*. Sunflower showed occurrence of nine fungi. Sunflower showed the occurrence of two *Trichoderma* species i.e. *Trichoderma harzianum* and *Trichoderma viride*. It is interesting to note that fungi like *Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina* and *Rhizopus stolonifer* were found to be associated with all the oilseeds which indicate that these fungi are not host specific and can survive on a broad range of hosts.

On the other hand, some fungi like *Alternaria carthami, Cercospora kikuchii* and *Verticillium* sp. were present strictly on the safflower, soybean and sesame respectively suggesting their narrow range of survival or host specificity character. Similar types of variations in mycoflora in different oilseed crops have also been reported by various workers as in case of all the oilseed (Chavan and Kakde 2011), in case of groundnut (Reddy et al., 1991), soybean (Murthy and Raveesha, 1996), sunflower (Agarwal and Singh, 1974), safflower (Singh et al., 1987) and in sesame (Vyas et al., 1984).

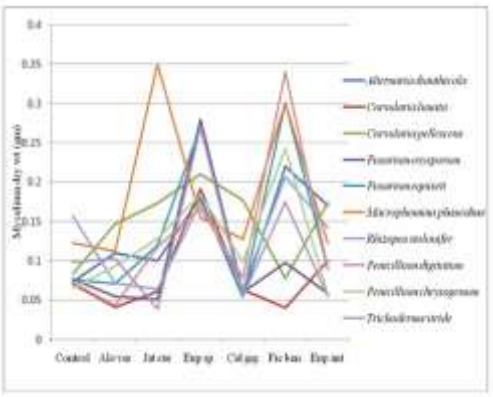
Antifungal activity of aqueous leaf extract of medicinal plants

Azadirachta indica and *Polyalthia longifolia* showed their antifungal activity 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, Curvularia lunata* and *Penicillium digitatum*. *Annona squamosa* hampered the growth of *Penicillium digitatum* and *Fusarium equiseti* (Table 2) (Fig.1). Similar results were reported by Singh and Prasada (1993). They found that leaf extract of *Azadirachta indica* and *Ocimum sanctum* inhibited the growth of *Fusarium oxysporum*. Similarly, 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*. Recently, Meena et al., (2010) tested leaf extract of ten medicinal plants against *Alternaria cucumerina*.



Graph 1: Antifungal properties of leaf extract of medicinal plants



Graph 2: Antifungal properties of latex

Antifungal activity of latex of some medicinal plants

Antifungal property of some latex was studied and results are summarized in table 3. The growth of *Rhizopus stolonifer* was hampered due to latex of *Aloe vera, Jatropha curcus, Calotropis gigantia* and *Ephorbia antiquorum*. Latex of *Ficus benghalnesis, Aloe vera, Jatropha curcus* and *Calotropis gigantia* were found to be inhibitory for the growth of *Curvularia lunata*.

Growth of *Fusarium oxysporum* was restricted due to latex of *Jatropha curcus, Aloe vera, Ephorbia antiquorum* and *Calotropis gigantia*. The latex of *Calotropis gigantia* for

Alternaria dianticola; *Aloe vera* for *Curvularia lunata* and *Penicillium digitatum* were found to be fungi toxic. It is interesting to mention that *Euphorbia* sp. latex was found to be stimulatory for the growth of storage fungi. Latex of *Jatropha curcus, Calotropis gigantia* and *Ephorbia antiquorum* yielded minimum growth of *Trichoderma viride* (Table 3) (Fig. 1).

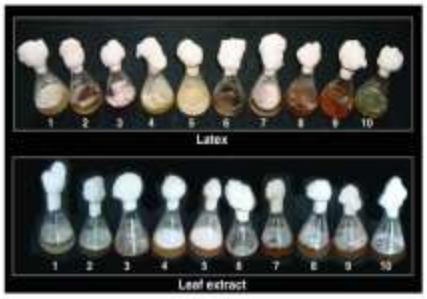


Fig 1: Antifungal properties of latex and leaf extract against storage fungi

Kareem et al. (2008) tested the latex of *Calotropis procera* against *Aspergillus niger, Aspergillus flavus, Microsporium boulardii* and one yeast *Candida albicans*. They reported that latex of *Calotropis procera* was found to be fungitoxic. On the other hand, Senugupta et al. (2008) found that latex of *Plumeria acutifolia* showed antifungal properties against *Penicillium expansum, Rhizoctonia solani* and *Myrothecium roridum*. Similarly, Subramanian and Saratha (2010) evaluated antibacterial activity of *Calotropis gigantea* latex extract on *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Shigella dysenteriae* and *Salmonella typhi* bacteria. The remarkable fungicidal effects of plant latex suggest that the latex may be a useful source for the development of novel antifungal agent against pathogenic fungi. From these results it can be concluded that, in comparision to synthetic compound, the pesticidal compounds of plant origin are more effective and have little or no side effects on human beings as suggested by Kumar et al., (1995).

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Table 1: Percent incidence of fungi on different oilseeds

Fungi	Groundnut	Soybean	Sesame	Safflower	Sunflower
Alternaria alternata	20	10	--	--	--
Alternaria carthami	--	--	--	20	--
Alternaria dianthicola	--	20	40	--	10
Alternaria tennuisima	--	10	--	--	--
Aspergillus flavus	90	30	80	10	--
Aspergillus fumigatus	30	20	--	--	--
Aspergillus terreus	20	--	--	10	30
Aspergillus ustus	--	--	10	10	--
Aspergillus niger	70	30	50	20	20
Cercospora kikuchii	--	20	--	--	--
Colletotrichum gloeosporioides	--	20	--	--	--
Curvularia lunata	30	20	20	10	--
Curvularia pellescens	--	20	20	10	--
Helminthosporium papulosum	--	20	--	--	--
Fusarium chlamydosporum	20	--	--	30	--
Fusarium culmorum	--	20	--	--	--
Fusarium equiseti	10	--	40	20	30
Fusarium solani	--	30	--	--	--
Fusarium roseum	--	--	20	10	--
Fusarium oxysporum	30	40	30	30	40
Fusarium verticillioides	--	10	--	--	--
Macrophomina phaseolina	30	50	10	20	--
Mucor indicus	30	--	--	10	20
Penicillium chrysogenum	20	30	20	--	--
Penicillium digitatum	20	--	30	20	--
Rhizoctonia solani	30	20	--	--	--
Rhizopus stolonifer	50	30	--	30	40
Trichoderma harzianum	--	--	30	--	20
Trichoderma viride	20	--	20	--	20
Verticillium tenerum	--	--	20	--	--

Table 2: Antifungal properties of leaf extract of medicinal plants

Fungi	Medicinal plants											
	Control	*Aza ind*	*Pol lon*	*Jat cur*	*Sca 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; Sca 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

Table 3: Antifungal properties of latex

Fungi	Latex						
	Control	*Alo ver*	*Jat cur*	*Eup sp.*	*Cal gig*	*Fic ben*	*Eup ant*
Alternaria dianthicola	0.073	0.110	0.100	0.181	0.054	0.220	0.170
Curvularia lunata	0.071	0.041	0.061	0.192	0.063	0.040	0.100
Curvularia pellescens	0.083	0.147	0.173	0.210	0.177	0.078	0.174
Fusarium oxysporum	0.077	0.055	0.051	0.280	0.060	0.098	0.059
Fusarium equiseti	0.076	0.071	0.120	0.270	0.058	0.301	0.089
Macrophomina phaseolina	0.123	0.112	0.350	0.155	0.127	0.300	0.122
Rhizopus stolonifer	0.157	0.072	0.064	0.175	0.065	0.207	0.142
Penicillium digitatum	0.072	0.043	0.117	0.167	0.078	0.340	0.090
Penicillium chrysogenum	0.065	0.093	0.128	0.184	0.098	0.243	0.056
Trichoderma viride	0.099	0.103	0.039	0.270	0.056	0.175	0.055

Alo ver- Aloe vera, Jat cur- Jatropha curcus, Eup sp.- *Euphorbia* sp. ***Cal gig- Calotropis gigantia, Fic ben- Ficus benghalnesis, Eup ant- Ephorbia antiquorum***