Fungitoxic properties of some leaf extracts against oilseed-borne fungi

Jat J G and H R Agalave

Department of Botany, Swami Ramanand Teerth Marathwada University, Nanded-431602
jjat023@gmail.com

ABSTRACT

Pathogenic fungi were isolated from oilseeds like groundnut, soybean, sesame, sunflower and safflower on PDA medium. Antifungal activity of aqueous and methanolic leaf extract of medicinal plants was evaluated against oilseed-borne fungi. For this study 50% and 100% concentration of aqueous and methanolic extracts was used. 50% and 100% aqueous leaf extract of ten medicinal plants was screened against ten oilseed-borne fungi. Methanolic extract was found to be effective against oilseed-borne fungi as compared to aqueous extract. Antifungal activity was maximum as concentration of methanolic and aqueous extract of leaf was increased. It was found that methanolic extract of *Azadirachta indica, Eucalyptus* sp., and *Withania somnifera* was found to be more fungitoxic against seed-borne fungi as compared to other medicinal plants. *Aspergillus niger, Aspergillus flavus* showed minimum growth in presence of methanolic leaf extract of *Azadirachta indica, Eucalyptus* sp., and *Withania somnifera.*

Key words: Antifungal activity, aqueous and methanolic leaf extract

INTRODUCTION

Plants are the richest resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.,* 1999). Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Swaminathan (1978) found that green plants are reservoir of biotoxicants and inexhaustible source of number of pesticides. Kumar *et al.,* (1995) found 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. Similarly, 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). The extensive use of agrochemicals especially fungicides, which leads more carcinogenic risk than other pesticides which may give rise to undesirable biological effects on animals and human beings (Osman and Abdulrahman, 2003). Badar *et al.,* (2012) found that medicinal plants gums showed antifungal activity against *Alternaria alternata, Aspergillus niger, Curvularia lunata, Curvularia pellescence, Fusarium equiseti, Fusarium oxysporum, Macrophomina phaseolina, Penicillium digitatum, Penicillium chrysogenum and Rhizopus stolonifer.* Koche *et al.,* (2012) screened ethanolic extract of the leaves of *Ocimum gratissimum* was screened for its phytochemical and antibacterial properties on *E. coli* and *Listeria monocytogenes* at different concentrations. They found that growth of *E. coli* and *Listeria monocytogenes* at concentration of 250mg/ml was inhibited with highest diameter zone of inhibition ranging from 22mm to 25mm. Gaikwad *et al.,* (2012) reported that crude extract of *Jatropha* scpecies proved to be inhibitory against plant pathogenic fungi. Therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. One source of potential new pesticides is natural products produced by plants. Considering this fact, present investigation has been emphasized to study the fungitoxic properties of aqueous and methanolic leaf extract of some medicinal plants against oilseed-borne fungi.

MATERIALS AND METHODS

Different varieties samples of groundnut, soybean, sesame, safflower and sunflower seeds 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 oilseed mycoflora associated with oilseed samples like groundnut, soybean, sesame, sunflower and safflower, the methods recommended by ISTA (1966) and Neergaard (1973) 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. After seven days plates were observed for fungal growth. Several fungi were found to occur on different media. Ten dominant fungi viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Fusarium moniliforme, Macrophomina phaseolina, Penicillium digitatum, Penicillium chrysogenum* and *Rhizopus nigricans* were selected for the antifungal test with ten medicinal plants viz. *Azadirachta indica, Polyalthia longifolia, Jatropha curcus, Santalum album, Withania somnifera, Dhatura strominum, Eucalyptus angophoroides, Vitex nigundo, Annona squamosa* and *Murraya koenigii.* Leaves of selected medicinal plants were thoroughly washed with tap water followed by distilled water, shade dried, crushed in a homogenizer to fine powder and stored in air tight bottles.

Preparation of aqueous leaf extract of some medicinal plants

100g leaf powder of each plant was dissolved in 100ml of distilled water to make 100% concentration. The plant extract was prepared in distilled water. By stirring the plant extract was thoroughly mixed.

Preparation of methanol leaf extract of some medicinal plants

20g of shade dried powder of each plant was filled in the thimble and extracted successively with methanol solvent in Soxhlet extractor for 48 hours. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in air tight bottles until further use.

Antifungal activity of botanicals

10ml of aqueous and methanolic extract was added in Glucose nitrate medium separately. 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 in gram after harvesting. The mycelial weight of the fungi compared with check, was taken as a measure of the fungal toxicity (Nene and Thapliyal, 1993).

RESULTS AND DISCUSSION

Antifungal properties of aqueous leaf extract (50%) of medicinal plants

Selected fungi were previously isolated from abnormal oilseeds like groundnut, soybean, sesame, sunflower and safflower on PDA medium and were screened against leaf extracts of some medicinal plants. Leaf extract of *Eucalyptus angophoroides* hampered the growth of all oilseed-borne fungi except *Alternaria alternata* and *Aspergillus niger*. Growth of *Fusarium moniliforme* was reduced in leaf extract of all the medicinal plants except *Santalum album, Vitex nigundo* and *Annona squamosa.* Leaf extract of *Azadirachta indica* and *Polyalthia longifolia* was found to be responsible for reduction in growth of *Fusarium moniliforme, Macrophomina phaseolina* and *Rhizopus nigricans.* Leaf extract of *Eucalyptus angophoroides, Vitex nigundo* and *Annona squamosa* hampered the growth of *Alternaria alternata.* Growth of *Penicillium notatum* and *Penicillium chrysogenum* was hampered due to leaf extract of *Withania somnifera.* On the other hand, leaf extracts of all the medicinal plants favoured the growth of *Aspergillus niger* and *Aspergillus flavus* (Table 1).

Antifungal properties of aqueous leaf extract (100%) of medicinal plants

Antifungal properties of some medicinal plants were screened and results are given in table 2. Leaf extract of *Eucalyptus angophoroides* and *Withania somnifera* was found to be inhibitory for the growth of all oilseed-borne fungi. Growth of *Alternaria alternata* was hampered due to leaf

extract of *Eucalyptus angophoroides, Vitex nigundo* and *Annona squamosa*, whereas, leaf extract of *Jatropha curcus* found to be inhibitory for the growth of *Aspergillus niger, Aspergillus flavus, Macrophomina phaseolina.*

On the other hand, growth of *Alternaria alternata* was low due to leaf extract of *Eucalyptus angophoroides, Vitex nigundo* and *Annona squamosa.* Growth of *Fusarium moniliforme* was decreased due to leaf extract of *Santalum album* and *Jatropha curcus.* Leaf extract of *Dhatura strominum* and *Eucalyptus angophoroides* was found to be inhibitory for the growth of *Penicillium notatum* and *Penicillium chrysogenum.*

Table 1: Antifungal properties of aqueous leaf extract (50%) 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***	***Mur koe***
	Mycelium dry weight (g)										
Alternaria alternata	0.096	0.144	0.143	0.207	0.192	0.155	0.162	0.082	0.070	0.051	0.174
Aspergillus niger	0.073	0.170	0.174	0.151	0.209	0.191	0.154	0.122	0.076	0.122	0.182
Aspergillus flavus	0.085	0.238	0.186	0.143	0.184	0.172	0.264	0.067	0.282	0.231	0.226
Curvularia lunata	0.079	0.164	0.205	0.193	0.235	0.140	0.244	0.058	0.232	0.212	0.216
Fusarium oxysporum	0.078	0.174	0.158	0.251	0.170	0.149	0.204	0.049	0.111	0.058	0.134
Fusarium moniliforme	0.105	0.073	0.056	0.050	0.112	0.061	0.081	0.091	0.121	0.129	0.050
Macrophomina phaseolina	0.159	0.064	0.083	0.156	0.167	0.173	0.191	0.090	0.168	0.136	0.114
Rhizopus nigricans	0.074	0.043	0.072	0.139	0.145	0.169	0.123	0.061	0.071	0.030	0.133
Penicillium notatum	0.067	0.128	0.196	0.190	0.208	0.061	0.128	0.060	0.129	0.133	0.108
Penicillium chrysogenum	0.101	0.152	0.180	0.155	0.146	0.069	0.136	0.120	0.188	0.164	0.180

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

Antifungal properties of methanolic leaf extract (50%) of medicinal plants

Methanolic leaf extract of some medicinal plants was tested against selected fungi and results are summarized in table 3. Growth of *Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, Macrophomina phaseolina, Rhizopus nigricans* and *Penicillium notatum* was hampered due to methanolic leaf extract of *Eucalyptus angophoroides* and *Vitex nigundo*. Methanolic leaf extract of *Annona squamosa* and *Withania somnifera* was found to be fungitoxic for the growth of *Aspergillus niger*. Growth of *Fusarium moniliforme* was drastically hampered due to methanolic extract of *Azadirachta indica, Polyalthia longifolia, Jatropha curcus, Santalum album, Withania somnifera, Dhatura strominum, Eucalyptus angophoroides, Vitex nigundo, Annona squamosa* and *Murraya koenigii.*

Antifungal properties of methanolic leaf extract (100%) of medicinal plants

Selected fungi were tested with methanolic extract of some medicinal plants and results are given in table 4. Methanolic extract of all the medicinal plants were found to be fungitoxic for the growth of *Alternaria alternata, Fusarium*

Table 2: Antifungal properties of aqueous leaf extract (100%) 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***	***Mur koe***
	Mycelium dry weight (g)										
Alternaria alternata	0.096	0.078	0.072	0.107	0.092	0.090	0.086	0.062	0.036	0.051	0.074
Aspergillus niger	0.073	0.090	0.078	0.051	0.109	0.091	0.074	0.066	0.046	0.068	0.080
Aspergillus flavus	0.085	0.048	0.086	0.053	0.084	0.072	0.154	0.038	0.156	0.101	0.120
Curvularia lunata	0.079	0.080	0.105	0.083	0.135	0.040	0.144	0.038	0.168	0.074	0.118
Fusarium oxysporum	0.078	0.074	0.058	0.146	0.070	0.049	0.188	0.028	0.076	0.038	0.080
Fusarium moniliforme	0.105	0.040	0.036	0.025	0.012	0.038	0.056	0.046	0.079	0.066	0.030
Macrophomina phaseolina	0.159	0.060	0.043	0.058	0.067	0.073	0.096	0.046	0.088	0.070	0.074
Rhizopus nigricans	0.074	0.028	0.032	0.059	0.045	0.069	0.086	0.036	0.041	0.020	0.078
Penicillium notatum	0.067	0.080	0.096	0.080	0.108	0.041	0.036	0.038	0.076	0.088	0.060
Penicillium chrysogenum	0.101	0.095	0.080	0.085	0.101	0.039	0.068	0.078	0.076	0.098	0.081

Table 3: Antifungal properties of methanol leaf extract (50%) 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*	*Mur koe*
	Mycelium dry weight (g)										
Alternaria alternata	0.094	0.140	0.136	0.203	0.183	0.145	0.152	0.072	0.060	0.071	0.164
Aspergillus niger	0.072	0.166	0.169	0.146	0.199	0.063	0.144	0.112	0.066	0.062	0.172
Aspergillus flavus	0.081	0.234	0.181	0.138	0.174	0.162	0.214	0.057	0.257	0.221	0.216
Curvularia lunata	0.078	0.158	0.200	0.188	0.215	0.130	0.234	0.048	0.211	0.202	0.206
Fusarium oxysporum	0.075	0.170	0.154	0.245	0.160	0.139	0.194	0.039	0.104	0.048	0.124
Fusarium moniliforme	0.103	0.066	0.051	0.046	0.102	0.052	0.071	0.081	0.090	0.119	0.040
Macrophomina phaseolina	0.155	0.060	0.079	0.149	0.157	0.164	0.181	0.080	0.088	0.126	0.104
Rhizopus nigricans	0.071	0.039	0.068	0.130	0.135	0.156	0.113	0.051	0.051	0.020	0.123
Penicillium notatum	0.064	0.123	0.190	0.180	0.198	0.052	0.118	0.050	0.090	0.123	0.098
Penicillium chrysogenum	0.108	0.146	0.175	0.145	0.136	0.058	0.126	0.110	0.178	0.154	0.170

Table 4: Antifungal properties of methanol leaf extract (100%) 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*	*Mur koe*
	Mycelium dry weight (g)										
Alternaria alternata	0.096	0.058	0.052	0.087	0.072	0.070	0.066	0.042	0.016	0.031	0.054
Aspergillus niger	0.073	0.070	0.058	0.031	0.089	0.071	0.054	0.046	0.026	0.048	0.060
Aspergillus flavus	0.085	0.028	0.086	0.033	0.084	0.052	0.134	0.018	0.136	0.081	0.100
Curvularia lunata	0.079	0.060	0.085	0.063	0.115	0.020	0.124	0.018	0.148	0.054	0.098
Fusarium oxysporum	0.078	0.054	0.038	0.126	0.050	0.029	0.068	0.008	0.056	0.018	0.060
Fusarium moniliforme	0.105	0.021	0.016	0.008	0.005	0.018	0.036	0.026	0.059	0.046	0.010
Macrophomina phaseolina	0.159	0.041	0.023	0.038	0.047	0.053	0.076	0.026	0.068	0.050	0.054
Rhizopus nigricans	0.074	0.018	0.012	0.039	0.025	0.049	0.066	0.016	0.021	0.007	0.058
Penicillium notatum	0.067	0.060	0.076	0.060	0.088	0.021	0.016	0.018	0.056	0.068	0.040
Penicillium chrysogenum	0.101	0.075	0.060	0.065	0.081	0.019	0.048	0.058	0.056	0.078	0.061

oxysporum, Fusarium moniliforme Macrophomina phaseolina, Rhizopus nigricans and *Penicillium chrysogenum*. On the other hand, methanolic extract of *Polyalthia longifolia, Santalum album, Dhatura strominum, Eucalyptus angophoroides, Vitex nigundo* and *Murraya koenigii* was found to be stimulatory for the growth of *Aspergillus flavus* and *Curvularia lunata.*

Singh and Prasada (1993) 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*. Kakde *et al.,* (2011) found that 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 leaf 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*. Mogle and Maske (2012) found that 10%, 20% and 30% plant extracts of *Argemone mexicana* extract was more effective followed by *Semecarpus anacardium, Cassia fistula* and *Tephrosia purpurea* against *Collectotrichum destructivum* which is a harmful pathogen of cowpea plant. In the next year, Mogle (2013) found that 10% leaf extract of *Eucalyptus globulus, Argemone mexicana, Tridax procumbens* and *Parthenium hysterophorus* were highly inhibitory for the growth of *Aspergillus niger, Penicillium digitatum, Botrytis cinera, Rhizopus arrhizus, Aspergillus flavus, Chaetomium brasiliense* and *Rhizoctonia solani.*

Leaf extract of some medicinal plants did not showed antifungal activity in aqueous medium but proved to be fungitoxic in methanolic extract. This might be due to some active compounds which are less soluble in water and not separated in the medium. Whereas, such compounds are soluble in methanol and separated in the medium, showed antifungal activity. Therefore, while fungicide formulation methanolic extract of above medicinal plants at 100% concentration is recommended.

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