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Antimicrobial activity of medicinal plant to control seed borne pathogen of soybean

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Abstract
Several species of fungi belonging to 12 genera viz. *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Mucor mucedo, Penicillium chrysogenum, Fusarium oxysporum, Rhizopus stolonifer, Cephalosporium acromonium, Rhizopus leguminicola, Alternaria alternata, Colletotrichum dematium, Macrophomina phaseolina, Phoma sp., Sclerotium rolfsii and Curvularia lunata* were isolated from seeds of soybean cultivars. Among these fungi *Aspergillus flavus, Fusarium oxysporum,* and *Alternaria alternata* were found to dominant. Seed borne fungi of soybean can be controlled by using leaf extract of medicinal plant and biocontrol agent. The seeds were treated with leaf extracts of plants like, *Azadirachta indica* A.Juss., *Acacia nilotica* (L.) Del., *Datura stramonium* L., *Polyalthia longifolia* (Sooner.)Thw., *Allium sativum* L. and *Annona squamosa.* An attempt has made to know the efficacy of leaf extract by food poisoning technique. Among these plants *Polyalthia longifolia* Thw., *Allium sativum* L. and *Azadirachta indica* A. Juss. Were more effective than other plants. All these plant extracts showed inhibitory effect on linear growth of dominant fungi.

Keywords: Soybean seeds, Medicinal plants, and seed borne fungi

INTRODUCTION

Indiscriminate use of chemical pesticides all over the world deteriorated the soil mycoflora and damaged our environment beyond repairs. From last four decades reckless use of non-target pesticides to kill the pest, we lost lot of biodiversity and disturbed our ecosystem.

Many efforts have been done to discover new antimicrobial compounds from various kinds of sources. Such pesticide activity has been explored in order to make available pesticides which are easily biodegradable, which can be locally produced, for farmers who cannot afford expensive synthetic pesticides.

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. [1]. The beneficial effects of plant materials typically results from the combination of secondary products present in the plant.

India is striving hard to increase agricultural production with a view to accelerate food and oil production to feed the ever increasing population through an integrated approach towards the application of farm technology. Soybean seeds are generally associated with certain saprophytic or parasitic microorganism which perpetuate in the oil seed lost on the advent of favorable conditions. Pathogens present in almost any oil seed lot of economically important crops which may be disastrous. Therefore, oil seeds must be substantially free from inoculums with high level of germinations and purity before sowing.

Seed borne fungi causes losses in terms of seed quality and quantity in all oil seed crops. These fungi also reduce the germination and storability of the oil seed. They are responsible for seed rot, seedling blight, root/shoot rot, foliar infection as well as pod blight diseases [2 and 3].

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MATERIALS AND METHODS

Collection of oil seed samples (Cultivars).

The method described by Neergard 1973 [4] has been adopted for the collection of seed samples. Accordingly, three random samples of seeds (half Kg each) were collected from oil mills, market place and oil seed research station, Latur.

Detection of seed mycoflora.

The seed mycoflora was isolated by using different methods such as standard blotter paper method, agar plate method and seed washates as recommended by international seed testing association ISTA (1966) [5], Neergaard (1973) [4] and Agrawal (1976) [6].

Observations were recorded in present incidence of seed borne fungi associated with unsterilized seeds. The fungi which appeared on seed were isolated in pure culture for identification and for further study. Three different methods of isolation techniques for assessment of seed mycoflora were used.

Standard blotter method

The standard blotter method was developed by Doyer in 1938 [7] which was later included in the International Seed Testing Association Rules of 1966. Four hundred seed ofeach variety were tested by employing standard blotter method in 3 replications. Three pieces of blotting paper of 90 mm size were moistened with distilled

water and placed in 90 mm sterilized Petri plates after draining excess water. Untreated seeds were placed at the rate of 25 seeds per Petri plate at equal distance. The plates were incubated at room temperature (20± 2º C) under alternate cycles of 12 hours NUV light and darkness. After eight day of incubation the seeds were examined under stereoscopic –binocular microscope for the associated fungi and they were identified based on "habit and colony characters [8].

Agar plate method.

In Northern Ireland, first used the method for seed health management. In this method, pre sterilized petri plates were poured with 15 ml of autoclave potato dextrose agar (PDA). On cooling the medium, the seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described for blotter method.

Seed washates method.

The washing test is a seed health testing method, employed to test seeds for seed borne pathogens, the inoculum of which is present loosely on the seed surface. Two grams of chick pea seed samples were taken in a test tube with 10 ml of sterilized water and shaken for 10 minutes on a mechanical shaker. The suspended spores were concentrated by centrifugation at 3000 rpm for 20 minutes. The supernatant was discarded and the spore were again suspended in two ml of lacto phenol and the suspension was then examined under the microscope for the presence of fungal spores

Collection of medicinal plants.

Seasonal surveys were made to collect plant from Marathwada. Plant materials were collected from the region and identified after critical examination in P.G. Department of Botany, Shivaji Mahavidyalaya, Udgir. Herbarium sheet were prepared and voucher specimens are deposited in the herbarium of P.G. Department of Botany, Shivaji Mahavidyalaya, Udgir, Dist. Latur.

Seventy plant species were screened out of which twenty plants belonging to different families were selected for their antimicrobial properties.

Efficacy of selected six medicinal plant extracts against *Fusarium oxysporium.*

From above six medicinal plant leaf extracts, the most effective leaf extracts of *Azadirachta indica* A.Juss., *Acacia nilotica* (L.) Del., *Datura stramonium* L., *Polyalthia longifolia* (Sooner.)Thw., *Allium sativum* L. and *Annona squamosa.* Against the test pathogens (*Fusarium oxysporum*) were evaluated for their efficacy. Antifungal activity of leaf extract was tested by poisoned food technique. Potato dextrose agar was prepared in flask and required concentration of the leaf extract was added and then sterilized. The plant extract along with medium was poured in to Petri plates, then disc of 0.5 cm of the fungus was cut with help of sterile cork borer and transferred aseptically in the culture of petridish containing the medium with the certain amount of plant extract. Suitable checks were maintained. The fungal colony diameter was determined every 24 hours of interval for 5 days. Observations were recorded on the mycelial growth of the test pathogens. Each treatment was replicated five times.

RESULT AND DISCUSSION

Table 1.Fungi associated with seeds of Soybean (*Glycine max* L.) Cv. MAUS -2

Sr. No.	Name of Fungi	Percent(%)incidence of Mycoflora		
		Standard blotter paper	Agar plate	Seed Washates.
1	*Rhizopus stolonifer*	40	43	35
2	*Mucor mucedo*	38	40	33
3	*Absidia corymbifera*	36	38	31
4	*Aspergillus flavus*	30	31	26
5	*Aspergillus niger*	27	28	22
6	*Aspergillus fumigates*	24	25	19
7	*Penicillium chrysogenum*	21	22	00
8	*Cephalosporium acromonium*	18	19	13
9	*Fusarium oxysporum*	16	18	11
10	*Rhizopus leguminicola*	12	17	08
	S.E +	2.87	2.91	2.89
	C.D.at 5 %	6.48	6.57	6.54

Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Mucor mucedo, Penicillium chrysogenum, Fusarium oxysporum, Rhizopus stolonifer, Cephalosporium acromonium, Rhizopus leguminicola, Alternaria alternata, Colletotrichum dematium, Macrophomina phaseolina, Phoma sp., Sclerotium rolfsii and Curvularia lunata were isolated from seeds of soybean cultivars (Table 1).

It is clear from table 2 that linear growth of *Fusarium oxysporum* was 19 mm on 8th day of incubation, when treatment of *Allium sativum* L. was given at 8.0% concentration showing the maximum inhibition. On the other hand, the growth of *Fusarium oxysporum* on control plate on 8th day of incubation was 65 mm. At different concentrations ranging from 2.0% to 8.0%, the linear growths of fungus were 60 mm, 55 mm, 45 mm and 19 mm

respectively. At 10.0% concentration there was complete inhibition of the all tested fungi.

Table 2. Effect of *Allium sativum L.* on linear growth of *Fusarium oxysporum* Schlecht.

Leaf extract Conc. (%)	Linear growth (mm) Incubation period (Days)							
	1	2	3	4	5	6	7	8
0.0 (Control)	20	25	30	35	40	45	55	70
2.0	18	19	23	28	30	35	45	60
4.0	17	20	22	24	27	32	40	55
6.0	16	18	20	22	24	28	30	45
8.0	00	00	00	00	00	10	16	19
10.0	00	00	00	00	00	00	00	00
S.E ±	3.34	3.72	4.38	5.13	5.75	5.86	7.13	9.62
C.D. at p=0.01	13.46	14.99	17.65	20.67	23.17	23.61	28.73	38.76
C.D. at p=0.05	8.58	9.56	11.25	13.18	14.77	15.06	18.32	24.72

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