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ANTIFUNGAL POTENTIAL OF BOTANICAL LEAF EXTRACTS AGAINST SEED BORNE FUNGAL PATHOGENS ASSOCIATED WITH SOME GOURD SEEDS.

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ABSTRACT
Nutritionally gourd vegetables are excellent source of folate, iron, vitamins, calcium and proteins. In Marathwada region of Maharashtra state, these vegetable plants are commonly cultivated. It is evident from the literature that vegetables and their seed carries large number of mycoflora, both in field as well as during storage. Standard blotter paper and glucose nutrient agar method used in the present study. selected gourd seeds (Bottle gourd, Bitter gourd and Ridge guord) shown twelve mycoflora. *Aspergillus niger, Aspergillus flavus, Alternaria alternata* and *Fusarium oxyporum* were found dominant. Seed borne pathogens causes diseases of seed, seedling and at various growth stages of plant. Biological method should be preferred as one of better alternative as they have minimum environmental impact in contrast to synthetic pesticides. In the view of this, present investigation has been undertaken to screen some plant extracts against seed borne pathogens. Main aim of the study was to search an alternative approach for preventing bio-deterioration of seeds and enhance rate of germination in an eco-friendly way.

KEYWORDS: Leaf extract, Seeds, Seed-borne fungi,

INTRODUCTION

The seed is a miniature plant and is considered as a basic input for production. About 90% of the worlds food crops are sown using seed. Seed plays vital role in the total biological yield per unit time and per unit plant surface. Crops are attacked by number of pathogens, majority of which are seed borne. Seed borne pathogens are carriers of some important seed-borne diseases caused by biotic agents, which results in considerable losses in yields (Anwar *et al.*, 2012). Seed borne pathogens are of two types: Those adhere to the outer covering of seed and those borne inside the seed. About 1500, seed borne microorganism are known and they have been recorded an about 600 genera of crop plants. (Agarwal & Sinclair, 1993.) Many time seed borne pathogen may be destructive agents of diseases in the field levels. Every seed has distinct micro flora and they are present in seeds may play important role in appearance of disease from one season to another or for several seasons.

The association of various fungi with vegetable seeds has been reported all over the world (Ismail *et al.*, 2012; Idrees *et al*, Al Kassim and Monawar, 2000; Esuruoso *et al.* 1975; Karwasra and Singh 1982). Most of survey conducted by various scientists indicated that common seed borne organism on spices, condiments and vegetables seeds are *Alternaria, Aspergillus, Rhizopus, Penicillium , Fusarium , Curvularia, Humicola , Cladosporium* etc.

Seed health testing for the presence of seed borne pathogens and their control by various means is an important step in the management of crop disease. The present study was carried on Gourd seeds *viz*. Bottle gourd, Bitter gourd and Ridge gourd to identify the seed borne fungi and search an alternative approach for preventing bio-deterioration of seeds and enhance rate of germination in an ecofriendly way.

MATERIALS AND METHODS

Present work is divided in to two Stages:

A) Assessment of seed borne pathogens
B) Assessment of effectiveness of medicinal plants against seed borne pathogens

A) Assessment of seed borne pathogens:

Selection of seeds: Seed samples of Gourd seeds were obtained from local market of Udgir town.

Table – 1 Gourd seeds

Sr. No.	Vernacular Name	Scientific Name
01	Bitter gourd	*Momordica charantia* L.
02	Bottle gourd	*Lagenaria siceraria* MoL
03	Ridge gourd	*Luffa acutangula*

Study of mycoflora:
The study of seed mycoflora was carried out by two important commonly used methods which are standard blotter paper method and glucose nutrient agar method.

Blotter paper method:
In Blotter test method seeds were placed equidistance in petridish and incubated for 8 days and fungal growth was observed.

Glucose Nutrient Agar method (GNA):
In GNA method, extract of GNA media is transferred into petriplates in sterile condition and then seeds were placed and incubated for 8 days. After the 8 days of incubation period, each Petri dish was examined for the presence of seed-borne fungal pathogens.

Percentage of incidence of mycoflora was calculated as:

$$\% \text{ incidence} = \frac{\text{No. of seeds on which a species appeared appeared}}{\text{Total number of seeds observed}} \times 100$$

Percentage of seed germination was calculated as:

$$\% \text{ germination} = \frac{\text{No. of germinating seeds}}{\text{Total number of seeds observed}} \times 100$$

B) Assessment of effectiveness of medicinal plants:

Collection of plants:
The plants used in present study were collected from diffrent regions of Udgir town. After pressing and drying herbarium sheets of these plants, their identification was confirmed through consultation with Department of Botany, Shivaji Mahavidyalaya, Udgir by reffering 'Flora of Marathwada'.

Table – 2: List of selected medicinal plants

Sr. No.	Scientific Name	Family
01	*Azadiracta indica A. Juss*	Meliaceae
02	*Adhatoda vesica L.*	Acanthaceae
03	*Calotropis gigantea R.Br*	Apocunaceae
04	*Datara metal L.*	Solanaceae
05	*Ocimum sanctum*	Lomiaceae

Preparation of Leaf extracts:
The selected plant leaves were chopped after cleaning in running tap water and then distilled water. The extracts were prepared by crushing these leaves in morter and pestle with distilled water in 1:1 ratio and 100% leaf extract of each plant were prepared. The leaf extract were filtered through muslin cloth and then Whatmans filter paper No. 1.

Seed Treatment: selected seed sample of Gourd vegetables were treated by following dipping method. The seeds were dipped in 1:1 and 1:2 (100% and 50%) dilution for 1 hour in previously prepared leaf extracts. The treated seeds were

allowed to dried up on filter paper for some time and were tested on Standard blotter paper for incidence of mycoflora and seed germination.

RESULTS AND DISCUSSION

The results are Shown in Table 3 to 5.

Table – 3: Mycoflora associated with untreated Gourd seed on Blotter paper:

Sr. No.	Seed sample	% incidence of mycoflora	% of seed germination	Fungi associated
01	Bitter gourd	86.7	43.3	*Aspergillus niger, A. flavous, Alternaria altarnata, Fusarium oxysporum.*
02	Bottle gourd	73.3	40.0	*Aspergillus niger, A. flavous, Fusarium oxysporum, Curvularia, Alternaria altarnata, Mucor sp.*
03	Ridge gourd	83.3	53.3	*Aspergillus niger, A. flavous, Fusarium oxysporum, Alternaria altarnata, Chaetonium sp. Rhizoctonia.*
	SE (+/-)	**2.01**	**3.12**	

Table – 4: Mycoflora associated with untreated Gourd seed on GNA:

Sr. No.	Seed sample	% incidence of mycoflora	% of seed germination	Fungi associated
01	Bitter gourd	86.7	40.0	*Aspergillus niger, A. flavous, Fusarium oxyporum, Alternaria sp., Penicillium, Rhizopus stolonifer.*
02	Bottle gourd	80.0	46.7	*Aspergillus flavous, Fusarium oxyporum., Helimanthosporium, Curvularia, Rhizopus stolonifer.*
03	Ridge gourd	86.7	56.7	*Aspergillus niger, Fusarium oxyporum, Alternaria alternate, Chaetonium sp. Rhizopus stolonifer.*
	SE (+/-)	**1.01**	**2.34**	

Table – 5 : Effect of various leaf extract on seed mycoflora and % of germination:

A	Bitter gourd **(on Blotter paper)**			
Sr. No.	**Leaf Extract**	**Concentration used**	**% of seed mycoflora**	**% of seed germination**
01	Azadiracta indica A. Juss	1:1	13.3	86.7
		1:2	16.7	80.0
02	*Adhatoda vesica L.*	1:1	16.7	83.3
		1:2	20.0	83.3
03	*Calotropis gigantea R.Br*	1:1	20.0	76.7
		1:2	23.3	70.0
04	*Datara metal L.*	1:1	16.7	76.7
		1:2	20.0	73.3
05	*Ocimum sanctum*	1:1	13.3	90.0
		1:2	16.7	80.0
		SE (+/-)	**0.12**	**0.17**

B	Bottle gourd **(on Blotter paper)**			
Sr. No.	**Leaf Extract**	**Concentration used**	**% of seed mycoflora**	**% of seed germination**
01	Azadiracta indica A. Juss	1:1	10.0	86.7
		1:2	10.0	83.3
02	*Adhatoda vesica L.*	1:1	13.3	73.3
		1:2	20.0	70.0
03	*Calotropis gigantea R.Br*	1:1	16.7	70.0
		1:2	16.7	66.7
04	*Datara metal L.*	1:1	16.7	83.3
		1:2	23.3	73.3
05	*Ocimum sanctum*	1:1	13.3	86.7
		1:2	20.0	70.0
		SE (+/-)	**0.21**	**0.37**

B	Ridge gourd **(on Blotter paper)**			
Sr. No.	**Leaf Extract**	**Concentration used**	**% of seed mycoflora**	**% of seed germination**
01	*Azadiracta indica* A. Juss	1:1	16.7	86.7
		1:2	16.7	80.0
02	*Adhatoda vesica L.*	1:1	20.0	73.3
		1:2	20.0	66.7
03	*Calotropis gigantea R.Br*	1:1	23.3	80.0
		1:2	26.7	76.7
04	*Datara meles L.*	1:1	16.7	83.3
		1:2	20.0	80.0
05	*Ocimum sanctum*	1:1	13.7	86.7
		1:2	16.7	73.3
		SE (+/-)	**0.14**	**0.21**

Seeds of three different Gourd vegetable were screened for incidence of Fungi for eight days by Glucose Nutrient Agar plate and Blotter paper methods. It is clear from the result presented in table–3 and table–4 that in all twelve fungi were recorded from seeds of Bitter gourd, Bottle gourd and Ridge gourd. Out of the total twelve fungi, more or less same fungi were recorded on seeds of different vegetables. Bitter gourd showed maximum incidence of fungi followed by Bottle gourd and Ridge gourd. More incidence of mycoflora was recorded on Glucose Agar plates than the Blotter paper method. The common fungi recorded were *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Alternaria sp, Rhizopus, Curvularia, Chaetonium sp., Hellimianthosporium, Mucor, Penecillium and Rhizopus stolanifer. The dominant fungi recorded were Aspergillus niger, Aspergillus flavus, Alternaria alternata* and *Fusarium oxyporum.* Similar studier were carried out by different researchers like Telang (2010) isolated mycoflora on seeds of Brinjal; Survase (2012) evaluated methi seeds.

These seed borne fungi were associated with deterioration of seeds and inhibit the percent of seed germination. Bottle gourd seeds had shown less germination followed by Ridge gourd and Bitter gourd.

It is evident from the result presented in Table–5 that the leaf extracts of all the test medicinal plants were found inhibitory in more or less percentage against incidence of seed mycoflora with higher percentage for seed germination. The leaf extract of *Azadiracta indica* were found more inhibitory for incidence of seed mycoflora in Bitter gourd, Ridge gourd and Bottle gourd seeds with 1:1 concentration (13.3%, 16.7%, and 10% respectively) followed by *Ocimum sanctum* (13.3% in each). Lowest inhibitory action for incidence of seed mycoflora was observed with *Calotropis gigantea* with 1:2 concentration (23.3%, 16.7 and 26.7 respectively). Similar study has been carried out by Mansur *et al.* (2008) and Nwangburuta (2013).

Azadiracta indica has shown more inhibitory activity against seed borne mycoflora. Kadam *et al.* (2008) also studied antifungal activity of *Azadiracta indica* on Groundnut seeds.

Ocimum sanctum shown maximum seed germination in Gourd seeds with 1:1 concentration level (90.0%, 86.7% and 86.7% respectively) followed by *Azadiracta indica* (86.7% each). *Adhatoda vesica, Calotropis gigantean* and *Datara metal* also showed inhibitory action with more or less percentage with increase in seed germination. Pre and post harvest bio-deterioration and spoilage of grains, vegetables, fruits and agriculture produce due infection of microorganisms may cause losses upto 100%. Seed associated with variety of fungi cause significant losses in seed quality (Satish *et al.*, 2007).

Thus there is an urgent need to search for an alternative method for prevention of bio-deterioration of seed during storage without any toxicity to the consumer. Exploitation of naturally available chemicals from plants which retards available chemicals from plants which interns reduces the reproduction of micro-organisms would be a more realistic and ecologically sound method for plant protection and will have a prominent role in development of future crop protection strategy. The findings of the present investigation would be found to be an important step for deciding crop protection strategies against antifungal activity of seed borne diseases.

CONCLUSION

The plants *Azadiracta indica, Adhatoda vesica, Calotropis gigantea, Datara metal and Ocimum sanctum* were found very effective against control of seed borne pathogens and shown increased seed germination.

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