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STUDIES ON FUNGAL POPULATION OF CUMIN (*NIGELLA SATIVA* L.) FROM DIFFERENT PARTS OF MARATHWADA.

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Abstract

Cumin (Nigella sativa) seed samples were collected from different markets, shops, go downs, farms, farmers and seed shops of whole sale market. 35 seed samples were tested by different methods and 25 forms of fungi were isolated. Their frequency and percentage relative density in seeds were recorded. Only 7 fungi showed common occurrence in all the samples. Aspergillus niger showed maximum percentage relative density in seeds. In the second set of experiments metabolites of all the seven fungi showing 100% frequency were tested for the effect of their metabolite on seed germination and seedling growth. All the 7 fungal metabolites affect seed germination and seedling growth with various degree of inhibition. Maximum inhibition in seed germination was observed in Aspergillus niger and maximum reduction in seedling growth was observed in Fusarium solani.

Key words: Cumin (*Nigella sativa*), seed fungi, seed germination, growth of seedling.

Introduction:

Seed deterioration during storage in a major problem throughout the world, which affected the yield of crop. Due to transfer of pathogen through seed the post-harvest losses are numerous .storage of moisture free seeds invites the moulds and a great loss of stores seeds due to growth of fungi. Hal and Harman (1991) estimated approximately 30% - 80% loss of legumes due to moulds growth, in Africa(Turner, 1971). A significant portion of the agricultural produce becomes unfit for human consumption due to mycotoxins contamination ofseedsespecially thoseproduced by*Aspergillus*, *Fusarium* etc. (Chaudhary, (2000)).

Most of the worldwide seriousdisease of plants is caused by seed – borne fungi. The fungus survives in the forms of mycelium or conidia in or onto the seeds surface (Bateman and Kwasna, 1999). Chaudhary, (2000) estimated, more than 25% of the world's cereals are contaminated with known mycotoxins andmore than 300 fungal metabolites are reported to be toxic to man and animals. The moulds to mycotoxins after consumption cause carcinogenicity, genotoxicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immune suppression (Tiwari, (1993). The fungi many attack the seeds which result in the form of seed abortion, shrunken seed, reduced seed size, seed rot, discoloration of seed, low germination or to complete loss of germination and biochemical alteration (Seghal, et.

al,1965). Considerable loss of yield of crop occurs due to pathogenic fungi transfer through seeds (Seghal, et. al, 1965).

Major failure problem of seed germination is due to fungus. The infected seed may retain fungus in the seeds endosperm and in the tissue of embryo. The infected seed produce diseased seedling, (Prasad, 2004). A large number of fungi grow onseed dueto presence of moisture. Most of the fungi produce toxic substances which alter the physical and nutritive quality of seeds, so that such seeds become unfit for consumption.

Material and Method:

Collection of seeds- The seeds of black cumin (*Nigella sativa*) were collected from different shops of Maharashtra region; total 40 seed samples were collected. Each sample was collected in a separate sterile polythine bags and brought to the laboratory for further studies.

1. **Washing test:** The mechanical seed shaker was used. Thee grams of cumin seeds were taken and added 10 ml of sterile distilled water. Kept it on mechanical shaker for 10 minutes. The suspension was than separated and centrifuged at 3000 rpm for half an hour. The supernatant was removed and discarded and the fungal spores were again suspended in 2 ml lacto phenol mountant. The quantitative estimation was performed by the use of haemocytometer.

2. **Blotter method:** The circles of blotting paper were kept in sterile petriplate. Three layers of blotting paper were used in each plate. The blotting paper was made wet by adding few drops of sterile distilled water. 20 seeds of black cumin kept on wet paper amended petridishes at equal distance. The plates were kept at room temperature at 25+-1 ^{O} C . After 8 days of incubation period the fungi associated with seeds were examined under microscope and identified on the basis on spore types and growth pattern of fungus.
3. **Agar plate method:** The sterile petriplate were added with sterile PDA (Potato Dextrose Agar) medium in sterile condition. Before plating 120 mg sodium chloride was added to 1000 ml PDA medium. The seeds selected for study were washed with 0.1% HgCl2 solution and then placed on media amended petriplate. 12 seeds were placed in each plate at equal distance. The plates were incubated at 20 ^{O} Ctemperatures in incubation for growth of endogenous fungi grown on seeds recorded and identified by using different manuals.

Table: Mycoflora of black cumin (*Nigella sativa L.*)

Sr. no.	Seed mycoflora	No. of samples showing %	% Frequency in sample (40)
1.	*Fusarium solani*	37	92.5
2.	*Rhizopus nigricans*	38	95.0
3.	*Cladosporium cladosporiodes*	26	65.0
4.	*Mucor*	36	90.0
5.	*Trichothecium roseum*	20	50.0
6.	*Trichoderma viride*	24	60.0
7.	*Rhizoctonia solani*	21	52.5
8.	*Nigrosporaviride*	27	04.4
9.	*Alternaria alternata*	16	40.0
10.	*Fusarium oxysporum*	35	87.5
11.	*Alternaria solani*	14	35.0
12.	*Aspergillus niger*	38	95.0
13.	*Aspergillus flavus*	36	90.0
14.	*Penicillium citrinum*	23	57.5
15.	*Curvularia lunata*	15	37.5
16.	*Helminthosporium graminis*	11	27.5
17.	*Gliocladiumroseum*	05	04.5

4. **Preparation of fungal metabolites:** The fungal forms were grown in liquid potato dextrose medium. A 4mm disc of growing colony of each type of fungus was removed with sterile borer and inoculated in sterile liquid medium. The inoculate flasks were incubated for 8 days at room temperature 25 +- ^{O}C. After 8 days filtered through Whatman filter paper no. 1. The filtrate was centrifuged at 10000 rpm for 10 minutes. The filtrate was taken in sterile beaker. The seeds of black cumin soaked in filtrate for half an hour. The soaked seeds were than used for study of germination test. The percent germination and seedling growth were recorded.

Table: 2. Effect of fungal metabolites on seed germination.

Sr. no.	Mycoflora	% of seed germination	% of seed inhibition	% reduction in length of radicle
1.	*Fusarium oxysporum*	70.8	25.2	5.6
2.	*Aspergillus niger*	31.5	68.5	2.2
3.	*Curvularia lunata*	72.0	14.0	4.0
4.	*Penicillium citrinum*	65.2	34.8	5.2
5/	*Rhizopus nigricans*	75.0	21.0	6.5
6.	*Helminthosporium graminis*	87.5	08.5	6.8
7.	*Rhizoctonia solani*	30.5	69.5	3.4
8.	Control	96.0		8.5

Result: There are17 seed borne fungi were isolated from 40 seed samples of black cumin (*Nigella sativa*) collected from different parts of Maharashtra. The frequency of isolated fungi was ranged from 4.5 percent (present only in one sample) in *Gliocladiumroseum*, *Nigrosporaviride* and *Rhizopus nigricans* was 95% (present in all 40 samples). The *Fusarium solani*, *Mucor, Cladosporium Cladosporiodes*, *Fusarium oxysporum*, *Aspergillus niger and Aspergillus flavus* were commonly present in all 40 samples of black cumin (*Nigella sativa*). The Alternaria solani showed its presence in 24 samples. Out of 40 samples 38 samples showing presence of *Rhizopus nigricans*and *Aspergillus niger*.The Aspergillus flavus, Fusarium solani and Mucor found in 36 samples.

The metabolites of all the 8 fungi reduced seed germination. Percent seed germination due to treatment of metabolites of *Fusarium oxysporum* was 70.8. The *Penicillium citrinum* metabolite showed 65.2 % seed germination. The effect of metabolites of *Rhizopus nigricans* and *Helminthosporium graminis* was 75.0% and 87.5% respectively. Percent seed inhibition was highest in *Aspergillus niger* and *Rhizoctonia solani* i.e. 68.5% and 69.5% respectively. The *Aspergillus niger* and *Rhizoctonia solani* metabolites highly reduced seed germination to 31.5 % and 30.5% respectively. The length of radical highly reduced due to *Aspergillus niger* and *Rhizoctonia solani*. Hashim, and Thrane, (1990), isolated 16 fungi from seeds of black cumin (*Nigella sativa*), The seed borne mycoflora makes seeds unfit for consumption by producing mycotoxins. Mycotoxins are secondary metabolites of mold

fungi identified in many agricultural products screened for toxigenic molds (Chaudhary, (2000). Mycotoxins have been reported to be carcinogenic teratogenic, tremorogenic, hemorrhagic and dermatitis to a wide range of organisms and to cause hepatic carcinoma in man (Seghal, et. al,1965).

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