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Studies on Seed Mycoflora of *Nigella Sativa* L During Pre Harvest, Post Harvest and Storage Conditions

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Abstract: ***Nigella sativa L is the herbaceous annual medicinal plant belonging to the family Ranunculaceae. In order to collect the succession of seed mycoflora during different stages, seeds of Nigella sativa were obtained at three stages of maturity, i.e. pre harvest, post harvest and the storage stage. Total 32 fungal species were isolated by using ISTA (agar plate, standard blotter paper method and deep freezing method) techniques. The result clearly show quantitative and qualitative differences in the mycoflora associated with three different stages . A significant changes in the mycoflora was recorded with the advancement of age. In general, number of fungal species associated was found to be more on storage seeds as compared to the pre and post harvest seeds.***

Keywords: *Nigella sativa*, Seed mycoflora, Black seed, Pre harvest, Post harvest

1. Introduction

Nigella sativa L is a spice plant of family Ranunculacea, commonly known as black cumin or black seed. It is an erect herbaceous annual plant. It grows in Mediterranean countries and Asian countries including India, Pakistan, Indonesia, Italy and Afghanistan. In India it is called as Kalonji or Kalajeera.

The seeds of *N. sativa* are used by the Indian people in pickles as spice and food preservative. For centuries, the seeds have been used for medicinal purpose. In old Latin it is called as 'Panacea' meaning 'cure all'. Ayurveda appreciates *N. sativa* L for many qualities and bitter, warming, stimulant nature. In Islam, it is regarded as one of the greatest forms of healing medicine available. **The Islamic prophet Muhammad once stated that the black seed can heal every disease except death.** It is also included in the list of natural drugs of 'Tibb-e-Nabavi', or "Medicine of the Prophet (Muhammad)", In the Unani Tibb system of medicine, *N. sativa* L is regarded as a valuable remedy for a number of diseases. Black cumin seeds have been widely used in traditional medicine as diuretic and antihypertensive (Zaoui et al., 2000), digestive and appetite stimulant (Gilani et al., 2004), antidiarrheal (Gilani *et al*., 2001), analgesic (Khanna *et al*., 1993; Khan *et al*., 1999), anthelmintic (Agarwal *et al*., 1979a; Chowdhury *et al*., 1998) and antibacterial agents (Ferdous et al., 1992; El-Kamali et al., 1998). Additionally, recent studies have shown black cumin to be antidiabetic (Meral *et a*l., 2001), anticancer (Abuharfeil *et al*., 2001; Farah and Begum, 2003), anti-inflammatory (Al-Ghamdi, 2001), spasmolytic and bronchodilatory (Gilani *et al*., 2001), hepatoprotective (Janbaz et al., 2003), renal protective (Badary et al., 2000) and possessing antioxidant properties (Mansour *et al., 2002).*
Practically, all seed lots are known to carry a wide range of micro-organisms on their surface which become active at the advent of favorable conditions and causing a considerable damage to seed. The distribution of these inoculums on seed may be either within the seed tissue or superficial, being confined to the surface of the seed or present as common contaminant. Amongst these microorganisms fungi play the most significant part in determining the quality and longevity of seed **(Christensen & Lopaz, 1963; Mirocha, 1976; Dennis, 1977).**

Depending upon the behavior and place of appearance, the fungi colonizing seeds have been classified into 2 groups-field fungi and storage fungi **(Christensen and Kaufmann, 1965, 1969).** Field fungi are those which invade seeds on the developing plants in the field, often in the maturing seeds and after the crop is harvested and swathed but before it is threshed. They may be pathogens or saprophytes, Common field fungi are species *Alternaria, Cladosporium, Curvularia,, Epicoccum, Fusarium, Verticillium* **(Malone and Muskett, 1964).** Since these fungi require high relative humidity (above 95%) for growth hence their activity is usually arrested during storage. In contrast storage fungi come in association with the seeds during storage. They do not invade the seeds to any serious extent before harvest **(Christensen and Kaufmann, 1969; Christensen 1971).**

Since there is paucity information regarding mycoflora associated with seeds of N.sativa in relation to pre harvesting, post harvesting stages and stored condition. Hence an attempted is made here to work out on these aspect.

2. Material and Method

2.1 Collection of seed samples

Seed samples of *Nigella sativa* were collected from field at pre harvest and post harvest condition, and stored seeds were collected from farmers, preserved in plastic bags and stored in refrigerator until used.

2.2 Seed health testing

The fungi associated with seeds were detected by incubation method.

Incubation method - Three incubation methods i.e. agar plate method, blotter method and deep freezing method as proposed by **ISTA (1985)** with seed collected at pre and post harvest and storage condition .

2.2.1 Agar Plate Method - In case of agar plate method, Potato Dextrose Agar (PDA) was poured aseptically in the sterilized glass petridishes of 9.00 cm diameter at the rate of about 15 ml per petriplate. After solidification of the medium 10 seeds per petriplate were plated. The seeds prior to plating on PDA were surface sterilized by emerging mercuric chloride solution (0.1 %) for 30 sec and subsequently rinsing for 3 times in sterilized distilled water. The petriplates were later incubated at 25±1 ^{o}C for seven days under 12 hours alternating cycle of near ultra violet (NUV) light and darkness. The seeds were examined for the presence of fungal growth after five to eight days of incubation.

2.2.2 Blotter method- Three layers of sterilized blotter were jointly soaked in sterilized distilled water and were kept in sterilized petridishes of 9 cm diameter. The seeds were sterilized by emerging in mercuric chloride solution (0.1%) for 30 sec and subsequently rinsing for 3 times in sterilized distilled water. 10 seeds per petridish were placed on blotter paper at equal distance. The petriplates were incubated at 25±1^{o}C for 7 days under 12 hr alternating cycle of near ultra violet (NUV) light and darkness . The seeds were examined on eight day under sterio scopic binocular microscope for the presence of seed born fungi.

2.2.3 Deep freezing method- Three layers of sterilized blotter were jointly soaked in sterilized distilled water and were kept in sterilized petridishes of 9 cm diameter. The seeds were sterilized by emerging in mercuric chloride solution (0.1%) for 30 sec and subsequently rinsing for 3 times in sterilized distilled water. Seeds were placed at the rate of 10 seeds per plate on moistened blotter. Petridishes were incubated at 25±1^{o}C for 24 hr under 12hr alternating cycle of near ultraviolet (NUV) light and darkness, for next 24 hr then plates were incubated at -20^{0}C in dark and keep black under original condition for the next 6 days. After eight days of incubation seeds were examined with the help of stereoscopic binocular microscope.

Identification of the fungal species were done on the basis of sporulation, conidial characteristics, fruiting structures etc. developed on the surface of seeds with the help of identification manuals.

3. Result

3.1 Pre harvest seeds

A total of 11 species belonging to seven genera were recorded from pre harvest seeds of *N. sativa* L . *Alternaria alternate, A. tenuissima, A. solani, Aspergillus flavus A. fumigates, A. niger, Chaetomium globosum, Cladosporium hebarum, Curvularia lunata, Memnoniella echinata, Mucor spp.* by blotter, agar plate and deep freezing methods

3.2 Post harvest seeds

A total of 18 species belonging to ten genera were recorded from post harvest seeds of *N. sativa* L i. e. .*Alternaria alternate, A. longissima, A. tenuissima, A. solani, Aspergillus flavus, A. fumigates, A. niger, A. nidulans, A. ochraceus, Cheatomium globusum, Cladosporium herbarum, Curvularia lunata, Fusarium solani, Memnoniella echinata, Mucor spp, Penicillium restrictum, Rhizopus nigricans, Trichoteicium roseum* by blotter, agar plate and deep freezing methods.

3.3 Stored seed

A total of 28 species belonging to 17 genera were recorded from post stored seeds of *N. sativa* L i.e. *Alternaria alternate, A. longissima, A. tenuissima, A.solani, Aspergillus flavus, A. fumigates, A. luchuensis, A.niger, A. ochracius, A. tamari, A. sulplureus, A.vercicolor, Cheatomium globusum, Cheatomium spp,. Cladosporium herbarum, Curvularia lunata, Emerceslla nidulance, Epicoccum nigrum, Fusarium moniliforme , F. oxysporium, Fusarium solani , Memnoniella echinata, Mucor spp., Nigrospora viridea, Penicillium citrinum, P. restrictum,* Phoma spp., *Trichoteicium roseum , Rhizoctonia solani,Rhizopus nigricans, and Verticillium terrestre* by blotter, agar plate and deep freezing methods.

4. Discussion

In order to collect the succession of seed mycoflora during different stages seeds of *N. sativa* L were obtained at three stages of maturity, i.e. pre harvest, post harvest and the storage stage. Total 32 fungal species belonging to 17

Table: Fungi detected from pre harvest, post harvest & stored seeds of *Nigella sativa* L

	Fungi isolated	*Pre harvest seeds*			*Post harvest seeds*			*Stored seeds*		
		Ager plate	Blotter paper	Deep freez	Ager plate	Blotter plate	Deep freez	Ager plate	Bloteer paper	Deep freez
1	*Alternaria alternata*	+	+	+	+	+	+	+	+	+
2	*A. longissima*	-	+	-	-	+	-	-	+	-
3	*A. tenuissima*	+	-	-	-	+	-	-	+	-
4	*A. solani*	-	-	-	+	-	-	+	-	-
5	*Aspergillus flavus*	+	+	+	+	+	+	+	+	+
6	*A. fumigatus*	+	+	+	+	+	+	+	+	+
7	*A. luchuensi*	-	-	-	-	-	-	-	+	-

8	*A. nidulens*	-	-	-	+	-	-	-	-	-
9	*A.niger*	-	-	-	+	+	+	-	+	+
10	*A. ochracius*	-	-	-	-	-	-	+	-	-
11	*A. restictus*	-	-	-	-	-	-	-	+	-
12	*A. sulphureus*	-	-	-	-	-	-	-	+	-
13	*A. versicolor*	-	-	-	-	-	-	+	-	-
14	*Cheatomium globusum*	+	+	+	+	+	+	+	+	+
15	*Cheatomium spp.*	-	-	-	-	-	-	-	+	-
16	*Cladosporium herbarum*	-	+	+	-	+	+	-	+	-
17	*Curvularia lunata*	+	+	+	+	+	+	+	+	+
18	*Emercella nidulance*	-	-	-	-	-	-	-	+	-
19	*Epicoccum purpurenscens*	-	-	-	-	-	-	-	+	+
20	*Fusarium moniliforme*	-	-	-	-	+	-	-	-	-
21	*F. oxysporium*	-	-	-	-	-	-	+	-	+
22	*Fusarium solani*	-	-	-	+	+	-	-	+	-
23	*Memnoniella echinata*	+	+	+	+	+	+	-	+	-
24	*Mucor spp.*	+	-	-	+	-	-	+	+	-
25	*Nigrospora viridea*	-	-	-	-	-	-	-	+	-
26	*Penicillium citrinum*	-	-	-	-	-	+	+	-	+
27	*P. restrictum*	-	-	-	-	-	-	-	-	+
28	*Phoma spp.*	-	-	-	-	-	-	-	-	-
29	*Rhizoctonia solani*	-	-	-	-	-	-	-	+	+
30	*Rhizopus nigricans*	-	-	-	+	+	-	+	+	-
31	*Trichoteicium roseum*	-	-	-	-	-	-	+	+	-
32	*Verticillium terrestre*	-	-	-	-	-	-	+	-	+

*** + Present, - Absent**

genera were isolated by using Aagar plate method, standard blotter paper method and deep freezing method.

Some fungi for e.g. *Alternaria alternate, A. tenuissima, Aspergillus flavus, A. fumigates, Cheatomium globusum, Cladosporium herbarum, Curvularia lunata, Fusarium solani, Memnoniella echinata, Mucor spp* were found to be almost common occurrences in pre harvest, post harvest,and stored seeds.

Some fungi were specially isolated only from by specific stages for e.g. *A. longissima* from pre harvest, *Fusarium moniliforme, Cladosporium herbarum,* from post harvest stage, *A. luchuensi, A, nidulens, A. niger, A. ochracius, A. restictus, A.vercicolor, Cheatomium spp. Emercella nidulance, Epicoccum purpurenscens, Penicillium citrinum, P. restrictumm, Phoma spp, Rhizoctonia solani, Verticillium terrestre* from stored seeds *of N. sativa* **L**.

Result of the experiment reveals that not only variation is recorded with regard to number of fungal species but also there is a variation in the quality of fungal flora associated with pre and post harvest and stored seeds samples. Christensen and Kaufmann (1969) has reported that qualitative and quantitative differences exist in mycoflora of seeds from pre and post harvested stages and during storage. Similar kind of qualitative and quantitative variation were reported by Clark *et al*, (1967, 1969) , Prasad *et al* (1980), Daivasikamini *et al* (1986), Picco *et a*l (1994), Rao *et al.* (2000).

Out of three methods adopted for detection of seed mycoflora, standard blotter method was proved to be superior to other methods as the total fungal colonies were more in stand blotter method.

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