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Original Article

Report on Mycoflora associated with infected stems and capsules of *Nigella sativa* L. (Black Cumin)

[*]Shamim Shamsi and [1]Sarowar Hosen

Department of Botany, University of Dhaka, Dhaka-1000

ABSTRACT: A total of five species of fungi were found to be associated with infected stems and capsules of *Nigella sativa* L. The fungi associated with infected stems were *Aspergillus niger* Van Tieghem, *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire, *Fusarium moniliforme* var. *subglutinans* Wr. & Reink and *Penicillium digitatum* Sacc. Infected capsules showed the association with *A. tenuissima*, *F. moniliforme* var. *subglutinan*s and *Periconia byssoides* (Mangin) Sacc. This is the first record about association of fungi with black cumin.

KEYWORDS: Report, Mycoflora, infected stems and capsules, *Nigella sativa*

CORRESPONDENCE: Shamim Shamsi. Email Address: prof.shamsi@gmail.com

INTRODUCTION

Nigella sativa L. (black cumin) is an annual flowering plant that belongs to the family Ranunculaceae[1]. It is native to south and southwest Asia. The plant is known as a "life elixir for all illness except death". Black cumin is also called Habbatul barakah, meaning "seed of blessing." Black cumin seeds (*Nigella sativa)* have been used therapeutically for over 3,300 years. Many studies, both in the East and the West, have been conducted to investigate the positive effects of the herb on diseases. Black cumin is used against more than hundreds of worst diseases. Black cumin has been shown to contain the following properties, which help to define its powerful medicinal values such as, Analgesic (pain-killing), Antibacterial, Anti-inflammatory, Anti-ulcer, Anti-cholinergic, Anti-fungal, Anti-hypertensive, Antioxidant, Antispasmodic, Antiviral, Bronchodilator, Gluconeogenesis inhibitor (anti-diabetic), Hepato-protective (liver protecting), Hypotensive, Insulin sensitizing, Interferon inducing, Reno-protective (kidney protecting), Tumor necrosis factor alpha inhibiting effects, etc[2] . Like all other commercially important crops production of the disease free *N. sativa* plants is essential for its immense medicinal and economical point of view. So far no report is available regarding fungal diseases of *N. sativa*. Recently severe damage of *N. sativa* was recorded with blight symptom in a field plot of Bajitpur union, Rajoir upazilla, Madaripur district in Bangladesh. With the advance of disease entire crop in the field was infected. Present investigation was undertaken to find out the association of fungi with infected stems and capsules of *N. sativa*.

MATERIALS AND METHODS

Infected stems and capsules of *Nigella sativa* with blight symptoms were collected from selected fields of Rajoir upazilla, Madaripur district to record the prevalence of fungi during the tenure of December 2015 to April 2016. Samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolating associated fungi following "Tissue planting method" on PDA medium[3]. From infected stem and capsule samples of 30 inocula, each measuring 2^2 mm sized were cut separately with a pair of sterilized scissors and kept in a separate sterilized Petri plate. The inocula were washed with sterile water and then the surface was

sterilized by dipping in 10% chlorox solution for three minutes, after that washing was done with sterile water. Surface sterilized inocula were placed separately on 10 sterilized Petri plates containing 15 ml of PDA medium with an addition of 1 drop (ca 0.03ml) of lactic acid to check the bacterial growth. Three inocula were placed in each per plate and incubated in an incubator (25±2˚C) for 7 days. Percentage frequency of the occurrence of the fungal isolated was calculated by adopting the following formula[4]:

$$\% \text{ frequency} = \frac{\text{Total number of inocula from which a fungal isolate was observed}}{\text{Total number of inocula}} \times 100$$

Detail morphological studies of the fungal isolates were made in order to determine their identity. The microscopic structural view of the fungi was taken by a digital camera. Species identification was done by Camera Lucida drawing[5]. All specimens, included in the present study were preserved in Mycology and Plant Pathology section, Department of Botany, University of Dhaka, Bangladesh. Identities of the isolates were determined following the standard literatures[6-10].

RESULTS AND DISCUSSION

Severe blight symptom was recorded on stems and capsules of *Nigella sativa* (**Plate 1**). A total of five species of fungi were associated with infected stems and capsules of *Nigella sativa*. The fungi associated with infected stems were *Aspergillus niger* Van Tieghem, *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire, *Fusarium moniliforme* var. *subglutinans* Wr. & Reink and *Penicillium digitatum* Sacc. Infected capsules showed the association with *A. tenuissima, F. moniliforme* var. *subglutinan*s Wr. & Reink and *Periconia byssoides* (Mangin) Sacc. Table 1 showed that for infected stems the percent frequency of *F. moniliforme* var. *subglutinan*s (46.67) was highest followed by *A. tenuissima* (26.67), *P. digitatum* (20) and *A. niger* (6.67). Table 1 also showed that for infected capsules the percent frequency of *Fusarium moniliforme* var. *subglutinan*s (60) was highest (33.45) followed by *A. tenuissima* (33.33) and *P. byssoides* (6.67).

Taxonomic descriptions of fungal taxa associated with stems and capsules of *Nigella sativa*

1. Alternaria tenuissima (Kunze ex Pers.) Wiltshire, Trans. Br. Mycol. Soc., **18**: 157 (1933). (**Plate 2A**).

Colonies greyish black, cottony, reverse blackish. Hyphae brown, septate, profusely branched. Conidiophores mostly solitary, simple or branched, straight or flexuous, more or less cylindrical, septate, pale or mid brown, smooth, with one or several conidial scars, up to 115 µm long, 4−6 µm thick. Conidia deep brown, solitary or in short chain, straight or curverd, obclavate, tapering gradually to the beak. Beak mostly short, pale or golden brown, 4-7 transverse and several longitudinally or oblique septate, slightly or not constricted at the septa, 27−67 × 8−20 µm. Beak 2−4 µm thick, swollen apex 4−5 µm wide.
Specimen examined: Isolated from infected stem and capsule of *Nigella sativa*, Bajitpur union, Rajoir upazilla, Madaripur district, S. Hosen 20, 6 December 2015.

2. Aspergillus niger Van Tieghem, Ann. Sci. Nat. Bot., Ser. 5, **8**: 240 (1867). (**Plate 2B**).

Colonies effuse, black. Mycelium well-developed, septate, profusely branched and brownish. Cells are multinucleate. Conidiophores brown 200−400 × 7−10 µm. Vesicles globose or subglobose, thick walled commonly 20−50 µm, occasionally up to 100 µm in diameter. Sterigmata 20−30 × 6−8 µm. Conidia dark brown, one celled globose, spinose 2−4 (5) µm in diameter. Cattenulate.
Specimen examined: Isolated from infected stem, Bajitpur union, Rajoir upazilla, Madaripur district S. Hosen 21, 6 December 2015.

3. Fusarium moniliforme var. subglutinans Wr. & Reink., Phytopathology **15**: 163 (1925) **(Plate 2C).**

Colonies white, cottony, reverse dark purple. Conidiophores hyaline, 0−2 septate. Microconidia hyaline, oval to obclavate, begin to form 2−3 days on simple lateral phialides, 7−12 × 2.7−4.5 µm. Phialides measure 15−25 × 2.5−3 µm. Macroconidia hyaline, canoe shaped, 3−5 septate, 28−52 × 3−5 µm.

Specimen examined: Isolated from infected stem and capsule of *Nigella sativa*, Bajitpur union, Rajoir upazilla, Madaripur district, S. Hosen 22, 6 December 2015.

4. Penicillium digitatum Sacc., Bur. Anim. Ind., Bul. **118**: 31−33 **(**1910). (**Plate 2D**).

Colony small, cottony, greenish, reverse creamy. Hyphae septate, branched, hyaline. Conidiophores hyaline, septate. Sterigmata equally variable, 15−28 µm long and 3.5−5.0 µm wide. Conidia elliptical to subglobose, smooth aceptate with greenish, tinge commonly 3.5−5.0 µm and occasionally up to 10−12 µm in diameter. Cattenulate.
Specimen examined: Isolated from infected stem and capsule of *Nigella sativa*, Bajitpur union, Rajoir upazilla, Madaripur district, S. Hosen 23, 17 December 2015.

5. Periconia byssoides (Mangin) Sacc., Syll. Fung. **18**: 569 (1906). **(Plate 2E).**

Colonies effuse, small and blackish. Mycellium mostly immersed but sometimes partly superficial. Styroma frequently present, mid to dark brown. Conidiophores dark brown, 200-400 µm, 12-23 µm thick at the base, 9-18 µm immediately below the head. Apical cell subhyaline, 12-26 × 11-28 µm. Heads 44-120 µm in diameter. Conidia spherical, brown, verrucose, 9-17 µm in diameter.
Specimen examined: Isolated from infected capsule of *Nigella sativa*, Bajitpur union, Rajoir upazilla, Madaripur district, S. Hosen 24, 6 December 2015.

Table 1. Frequency percentage of association of fungi with infected stem and capsule of *N. sativa*.

Name of the Fungi	**Frequency percentage of isolated fungi**	
	Stem	Capsule
Alternaria tenuissima	26.67	33.33
Aspergillus niger	6.67	-
Fusarium moniliforme* var. *subglutinans	46.67	60.00
Periconia byssoides	-	6.67
Penicillium digitatum	20.00	-

" - " = respective fungus did not show mycelial growth.

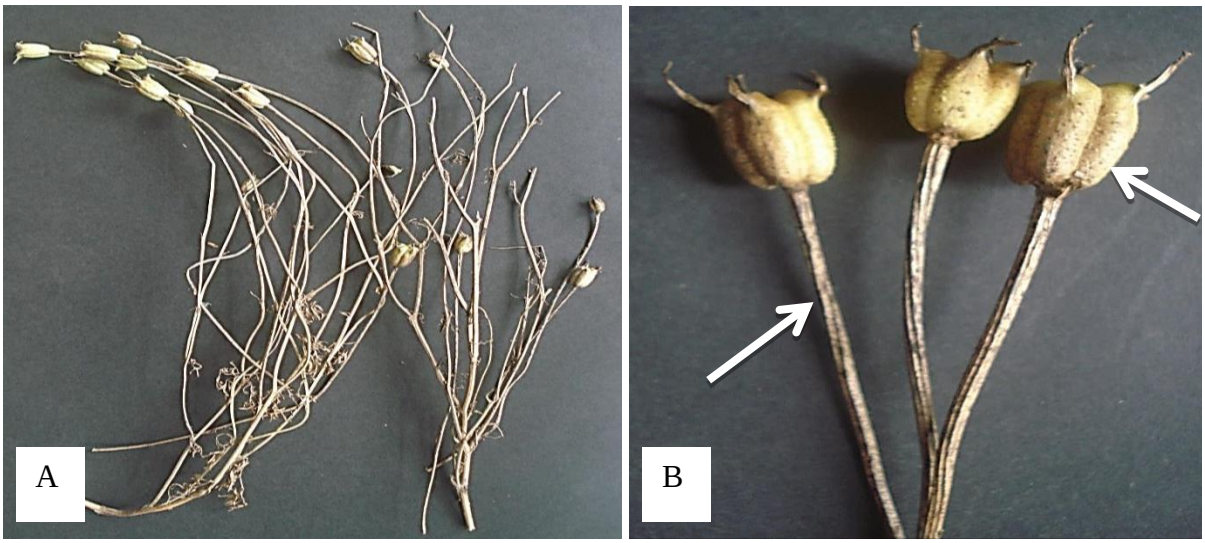

Figure 1: *Nigella sativa.* **A**. Dried plants showing blight symptom and **B**. Infected stems with capsules.

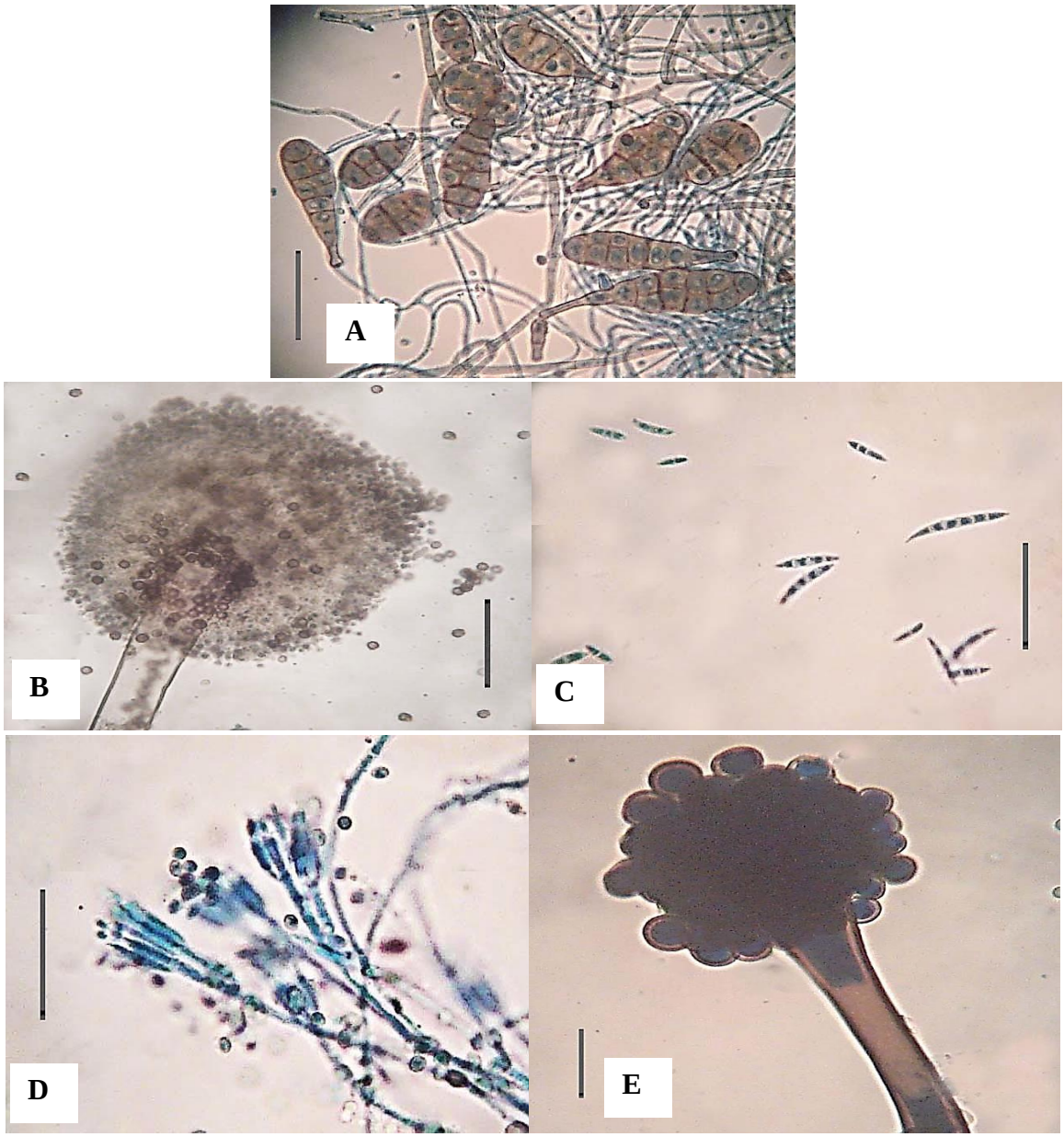

Figure 2: *Nigella sativa.* **A.** *Alternaria tenuissima*, **B.** *Aspergillus niger*, **C.** *Fusarium moniliforme* var. *subglutinans*, **D.** *Penicillium digitatum* and **E.** *Periconia byssoides* (Bar = 50 µm).

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