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Seed-borne Infection, Pathogenic Importance and Control of *Epicoccum nigrum* Ehrenb, ex Schlecht. in Rape Seed (*Brassica napus* L.) in Kumaun Hills, U.P., India

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Epicoccum nigrum Ehrenb. ex Schlecht was recorded in 32 seed samples out of 42 seed samples of rape seed collected from warmer valleys of Kumaun Hills in 3 years study from 1987 to 1989. The pathogen was found to be responsible for seed rot and seedling infection. In the study of location, *E. nigrum* was found well established in seed coat and cotylendons with low embryonal infection. Pathogenicity tests and field study showed it's pathogenic and seed-plant transmitting nature in rape seed crop. Topsin and Vitavex at 0.3% concentration of seed weight proved to be significantly effective fungicides in controlling the seed-borne infection of *E.nigrum* by dry seed treatment.

Key Words: ***Epicoccum nigrum*, Seed-borne infection, Location, Pathogenicity, Seed transmission, control**

Introduction

Seed-borne infection of *Epicoccum nigrum* is not common. Several workers namely, Gilman (1957), Ellis (1971) and Subramanian (1971) found this fungus as seed and soil-borne. *E. nigrum* may live in soil as saprophytes or parasites (Ellis 1971).

The present paper deals with the seed-borne nature of *E. nigrum* in Lahi (*Brassica napus* L.) with special reference to its location in seed parts, pathogenic importance and control by the use of systemic and non-systemic fungicides.

Materials and Methods

Collection and Isolation

Forty two seed samples of rape seed belonging to Badsahi, Elephant ear, Varuna and Local varieties were collected from different warmer localities of Kumaun Hills in aseptic polythene bags. Infected seedlings, leaves and pods were also collected to find out the incidence of *E. nigrum* in different growth stages of *B. napus*.

Seed health testing was carried out according to the rules recommended by International Seed Testing Association (ISTA, 1966).

E. nigrum was isolated from small bits of infected seedlings, leaves and pods of *B. napus* by following standard isolation methods.

Location of *E. nigrum* in different seed parts of *B. napus* was determined showing higher infection. For this, 400 seeds were soaked in sterilized water for 48 hours and dissected aseptically to separate out seed coat, cotyledons and embryos. Each part was pretreated with NaOCl, washed by sterilized water, blotter dried and different parts of a seed

were plated in a single plate. After incubation, seed parts were examined for incidence of *E. nigrum* according to the method of Mathur et al. (1975).

Pathogenicity and Seed-Plant Transmission Tests

Pathogenicity test was conducted by artificial inoculation of seeds. For this study, healthy seeds of cultivars Elephant ear, Badsahi, Varuna and Local were selected. Seeds were pretreated by NaOCl solution and were soaked in the spore suspension of *E. nigrum* at room temperature for 48 hours. Seeds were then removed and planted in greenhouse in pots with sterilized peat soil and in field of warmer part on the line of Hyung Lee et al. (1984). Suitable checks were also maintained. Observations were made regularly for one month both in green house and fields. During experimental period average soil moisture and air temperature ranged from 35-50% and 25 ± 2°C.

Seed Treatment with Fungicides

To control *Epicoccum nigrum* in *B. napus*, an evaluation of some systemic and non-systemic chemicals was made in the laboratory in blotter plates and greenhouse pots.

Healthy and viable seed of *B. napus* were rolled over actively sporulating culture of *E.nigrum* and kept in a sterilized moist blotter plates for 48 hours at 15 ± 2°C for the infection. These seeds were treated by different chemicals i.e. Bavistin, Brassicol, Captafol, Dithane M-45, Thiram, Topsin and Vitavax at 0.3% concentration.The tested seeds were placed on blotter plates (5 replicates of 40 seeds) for 14 days at 20 ± 2 °C in an incubation chamber adopting the method of Kadian and Suryanarayana (1971).

In an another experiment, *E nigrum* infested seeds were treated by each fungicide separately sown in sterilized soil in a green house (5 pots with 40 seeds/pot). Suitable control were also maintained. Regular observations were made for symptoms and the effect of chemicals on the pathogen. Significance of data was calculated by the method of Kothari (1987).

Results and Discussion

Infection of Epicoccum Nigrum in Seed Samples of Rape Seed

A total of 19 fungal species viz., *Alternaria alternata, A. brassicae, A. brassicicola, Aspergillus flavus, A. fumigatus, Cladosporium cladosporioides, C. sphaerospermum, Curvularia lunata, Cordana musae, Epicoccum nigrum, Fusarium moniliforme, F. oxysporum, F. semitectum, Penicillium chrysogenum, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Verticillium albo-atrum* were isolated from the seeds of rape seed.

Out of 42 seed samples tested in 3 years study, 32 showed infection of *E. nigrum* which varied from 0.5% to 53% in the case of untreated seeds and 0.5% to 53% in case of pretreated seeds (table 1). Highest infection was found in local cultivar and lowest infection in

Table 1 *Percentage incidence of* ***Epicoccum nigrum*** *in* ***B. napus*** *seeds from seed sample collected from different warmer areas of Kumaun*

Year	No. of samples tested	No. of samples showed infection	Range of Infection (%)		Average of untreated and treated	Average of infection (%)		
			Untreated	Treated		Untreated	Treated	Total average of untreated and treated
1987	14	10	0.5-35.0	0.5-19.0	1.0-27.0	12.5	6.0	8.0
1988	14	12	1.0-45.0	0.5-30.0	2.5-38.0	17.0	9.0	11.5
1989	14	10	6.5-53.0	0.5-34.0	4.5-43.5	21.0	12.0	16.5

(Seeds were treated by NaOCl solution 1% available chlorine for 10 minutes)

Elephant ear cultivar. Chlorine pretreatment checked the incidence of the fungus significantly. These results revealed that *E. nigrum* known as a soil fungus (Gilman 1957 and Ellis 1971) is also seed-borne, infecting *B. napus* seeds in Kumaun hills.

Location of the Pathogen in Seed Parts

In the seed-samples of all the cultivars the fungus was generally found in seed coat with moderate invasion of cotyledons. However, embryo infection was found rarely (table 2).

This observation indicates that *E. nigrum* is capable of causing internal infection besides external infection. The study also reveals that this fungus is primarily extraembryonal and may be rarely intraembryonal. Mathur et al. (1975) also observed such type of occurrence of *Fusarium moniliforme* in sorghum seeds.

Table 2 *Infection of* ***Epicoccum nigrum*** *in different parts of* ***B.napus*** *seeds based on* 200 *seeds cultivar*

Cultivars	No. of Infected seeds	Seed Coat	Cotyledons	Embryo
Elephant ear	64	61	42	3
Badsahi	71	70	42	5
Varuna	75	75	51	5
Local	106	104	80	10

Symptoms

In the warmer parts of Kumaun Hills, seedling, leaf and pod infection incited by *E. nigrum* was recorded. In the field, infected seedlings showed rotting of stem near soil surface, roots, damping-off; and blighting of young leaves. Mature leaves showed small, angular necrotic black spots covered by chlorotic area. Infected pods showed black discoloration and necrosis containing black dotted sporodochial mass of the pathogen. The incidence of this pathogen in seeds, seedlings, leaves and pods of *B.napus* in different agroclimatic regions of Kumaun hills indicate its pathogenic behaviour and susceptibility of rape seed cultivars at different growth stages.

Pathogenicity and Seed-Transmission study in Field

In the pathogenicity experiment by showing artificially inoculated seeds in green house sterilized pot soil, moderate to heavy pre-and post-emergence damping-off, leaf blight and leaf spotting in all the tested cultivars were recorded. Highest infection was recorded in local cultivar and lowest infection in Elephant ear cultivar (table 3).

In the seed-plant transmission test under field condition, seeds of Elephant ear, Badsahi, Varuna and Local cultivars showed 25%, 31.5%, 38% and 49% seed rotting and 12.5%, 15%, 19.5% and 21% seedling mortality re-

Table 3 *Pathogenic effect of* ***Epicoccum nigrum*** *in* ***B. napus*** *by seed inoculation in green house sterilized pot soil*

Varieties tested	Seed germination (%)		Seed rot (%)		Healthy seedlings (%)		% Loss caused by the pathogen					
							Pre-emergence loss		Post-emergence loss		Total loss	
	T	C	T	C	T	C	T	C	T	C	T	C
Elephant ear	69.5	100.0	30.5	0.0	55.5	100.0	30.5	0.0	14.0	0.0	44.5	0.0
Badsahi	64.0	100.0	36.0	0.0	47.5	100.0	36.0	0.0	16.5	0.0	52.5	0.0
Varuna	63.0	100.0	37.0	0.0	47.0	100.0	37.0	0.0	16.0	0.0	53.0	0.0
Local	54.0	100.0	46.0	0.0	33.0	100.0	46.0	0.0	21.0	0.0	67.0	0.0

(Based on 400 seeds/cultivar); T = Tested; C = Control. Experiment was carried out till one month.

spectively. The seedlings were killed due to the rotting near collor region and blighting of leaves (table 4). These results reveal that *E. nigrum* is pathogenic and transmitted from seed to plant causing seed rotting and seedling mortality. The results also indicate that Elephant ear cultivar was slightly resistant than the other tested cultivars against seed-borne infection of this pathogen.

Seed Treatment with Fungicides

In chemical control against seed-borne *E. nigrum*, Captafol, Thiram, Topsin and Vita-

Table 4 *Seed-transmission of* ***Epicoccum nigrum*** *in different varieties of* ***B. napus*** *in field conditions*

Varieties	No. of Seeds sown	% Seed germination	Seed rotting	% Healthy seedling after one month	% Seedling mortality during experiments (seed transmission)
Elephant Ear	100	75.0	25.0	62.5	12.5
Badsahi	100	78.5	31.5	53.5	15.0
Varuna	100	62.0	38.0	42.5	19.5
Local	100	51.0	49.0	30.0	21.0

Table 5 *Efficacy of some fungicides against* ***Epicoccum nigrum*** *in* ***B. napus*** *seeds in petriplates under laboratory conditions*

Fungicides used	Total loss caused by pathogen	% Reduction in total loss after use of fungicides	Mean (%) Reduction	't' value in comparison to control seeds	Significance in comparison to control seeds	Difference between effect of particular fungicide and effect all fungicities used (M-μ)	't' value in comparison to computed effect of fungicides	Significance in computed effect of fungicides
Control	96.0	0.0	0.0	0.0	—	0.0	0.0	—
Bavistin	50.0	46.0	9.2	15.77	Significant	−5.29	9.45	Significantly less effective
Brassicol	55.0	40.5	8.1	20.25	Significant	−6.38	15.95	Significantly less effective
Captafol	5.0	91.0	18.2	54.87	Significant	+3.71	11.24	Significantly more effective
Dithane M-45	25.0	70.5	14.1	42.81	Significant	−0.39	1.18	Moderately effective
Thiram	3.0	93.0	18.6	45.11	Significant	+4.11	10.02	Significantly more effective
Topsin	11.0	85.0	17.0	29.81	Significant	+2.51	4.40	Significantly more effective
Vitavax	15.0	81.0	16.2	22.05	Significant	+1.71	2.33	Moderately effective

Note: 1. Significance in comparison to controlled seeds is determined by comparing the computed value of 't' with expected value of 't' at 5% level for 8 degrees of freedom i.e. 2.305.
2. Significance in comparison to computed effect of fungicides is determined by comparing the computed value of 't' with expected value of 't' at 5% level of significance for 4 degrees of freedom i.e. 2.776.

Table 6 *Efficacy of some fungicides against Epicoccum nigrum in B. napus seeds in greenhouse pot soil*

Fungicides used	Total loss caused by pathogen (%)	% Reduction in total loss after use of fungicides	Mean (%) Reduction	't' value| in comparison to control seeds	Significance in comparison to control seeds	Difference between effect of particular fungicide and effect of all fungicides used (M-μ)	't' value comparison to computed effect of fungicides	Significance in comparison to computed effect of fungicides
Control	83.0	0.0	0.0	0.0	—	0.0	0.0	—
Bavistin	43.0	40.0	8.0	15.38	Significant	−5.07	9.75	Significantly less effective
Brassicol	38.5	44.5	8.9	26.96	Significant	−4.17	12.63	Significantly less effective
Captafol	2.0	81.0	16.2	33.06	Significant	+3.13	6.38	Significantly more effective
Dithane M-45	20.0	63.0	12.6	24.70	Significant	−0.47	0.92	Moderately effective
Thiram	1.0	82.0	16.4	32.16	Significant	+3.32	6.50	Significantly more effective
Topsin	9.0	74.0	14.8	30.20	Significant	+1.73	3.53	Significantly more effective
Vitavax	10.0	73.0	14.6	38.93	Significant	+1.53	4.08	Significantly more effective

Note: 1. Significance in comparison to controlled seeds is determined by comparing the computed value of 't' with expected value of 't' at 5% level for 8 degrees of freedom i.e. 2.306.
2. Significance in comparison to computed effect of fungicides is determined by comparing the computed value of 't' with expected value of 't' at 5% level of significance for 4 degrees of freedom i.e. 2.776.

vax were found as the best seed dressing chemicals followed by Dithane M-45 at 0.3% concentration of seed weight both in laboratory blotter plates as well as in green house pots. These fungicides checked the seed-borne infection, improved the seed germination and seedling health. However, Bavistin and Brassicol were found significantly less effective (tables 5&6). But Captafol and Thiram are known to be dangerous for human health. So, Topsin and Vitavax can be used effectively against *E. nigrum* infected seeds of rape seed for dry seed treatment in storage or before seed sowing as protective and therapeutic means. These results agree with those of Grover and Bansal (1970), Dharam vir et al.(1971) and Neergaard (1977) who also found Thiram, Captafol and Vitavax as effective chemicals against several fungi Neergaard (1977) also proved fungicidal activity of these chemicals against a wide range of fungi.

This disease incited by *E. nigrum* appears to be a seed-borne and seed plant transmitting in rape seed crop. The available literature reveal that this is the first record of seed-borne and seed-plant transmitting disease of rape seed from India caused by *E. nigrum.*

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