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BLACK EGG DISEASE OF *HETERODERA AVENAE* IN HARYANA (INDIA) AND THE ROLE OF FUNGAL PARASITES IN THE NATURAL REGULATION OF NEMATODE POPULATIONS

by

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Summary. A survey of *Heterodera avenae* infested fields in Haryana (India) revealed the widespread prevalence of a black egg disease which infected up to 100% of the eggs in the old cysts. The fungus mycelium appeared dark with thick-walled, brownish, swollen and yeast-like cells. The fungus infected the embryos within the egg shell. Infection of the new progeny of cysts occurred during July to September and the black yeast fungus destroyed 13-14% of the eggs in a field study. Some other fungal parasites attacked newly emerged females of *H. avenae*; as many as 26.3% females/white cysts were destroyed by a zoosporic fungus in a naturally infested field soil.

The cereal cyst nematode, *Heterodera avenae* Woll. is one of the economically most important pests of wheat and barley in the southern belt of Haryana comprising districts of Bhiwani, Faridabad, Gurgaon, Mahendergarh and Rewari. Casual observations of populations of *H. avenae* revealed the presence of black eggs in some cysts.

Goffart (1932, c. f. Tribe, 1972) recorded a black egg condition in the cysts of *H. avenae* in Germany. He found that the fungus-filled eggs were frequently black and opaque and some of the embryos were destroyed. Rozsypal (1934, c. f. Tribe, 1977) considered this fungus to be the principal parasite of *Heterodera schachtii* cysts in Moravian soils. He observed that the infected eggs and embryos were black and filled with hyphae made up of rows of usually spherical, brown and thick-walled cells, each containing a large drop of oil. Bursnall and Tribe (1974) found that the eggs and juveniles of *H. schachtii* in samples from England were parasitized by four principal parasites, one of them being a "black yeast" similar to *Phialophora malorum* and *Rhinocladiella mansonii*.

Because of the apparent prevalence of the black egg disease of *H. avenae* in the Haryana samples, studies were conducted to assess the frequency of occurrence of the causative fungus in the nematode-endemic areas, and to ascertain the role of this and other fungi in the natural regulation of *H. avenae* populations.

Materials and methods

A total of 30 soil and root samples were collected in September and February from fields previously identified as infested with *H. avenae*. Each sample comprised five cores collected at random from an area of one acre, using a spade. The undried samples were mixed manually and 100 cc soil (and roots) were sieved through 840 and 150 µm pore screens. White and brown cysts were hand picked from the material on the 150 µm screen, crushed in wa-

ter, and the numbers of black eggs and apparently healthy eggs were counted with the aid of a microscope (40x).

A field in the village of Majra (District Mahendergarh) was selected for further studies because of the availability of the host crop (wheat) of *H. avenae* in the forthcoming seasons, frequency of occurrence of diseased eggs, and accessability. Monthly samples were taken from selected points in this field for two years (September, 1992 to September, 1994) and processed as described above, with three replicates for each sampling time.

In addition bulk soil was brought from the field in September, 1993 for a pot experiment. The soil was mixed manually, and thirty 10 cm pots were filled and planted with wheat, *Triticum aestivum* L. (cv. WH-147) in November. During April (when the plants matured), the shoots were removed but the pots were maintained and watered regularly. Three pots were removed at monthly intervals (February, 1994 to September, 1994), coinciding with the field sampling dates, and the soil processed for the extraction of cysts. Ten white/brown cysts were picked randomly from those in each pot, rinsed several times in sterile water and preserved at room temperature in sterile sand in glass vials. During October, 1994, cysts from all the vials were separated from sand, crushed in water, and the numbers of black and healthy eggs were recorded.

Part of the soil collected from Majra village was steam-sterilized and put into 20 paired plastic Petri dishes (10 cm diam) (Crump and Kerry, 1977). A second set of 20 Petri dishes was filled with naturally-infested field soil (containing 45 healthy and five black eggs per g soil), after determining the initial densities of black and healthy eggs in a representative sample. Wheat (cv. WH-147) seeds were placed through an opening created in the top of the Petri dish, and after germination one seedling was retained. One week after germination, seedlings in the Petri dishes containing sterilized soil were inoculated with apparently healthy eggs (ca. equal to number in the other set) in a water suspension pipetted around the roots. All the dishes were sealed with cellotape strips, covered with black paper, kept vertically in a glasshouse and watered regularly. The Petri dishes were examined periodically using a stereomicroscope (40x). From January onwards the plates were examined more frequently and when the J_4/white females emerged, these were marked on the lid/base of the Petri dishes. Any female showing sudden discolouration or loss of turgor pressure was removed from the dish and examined at higher magnification (400x) to ascertain if there was any microbial infection. Weekly counts of healthy and diseased females were recorded in both of the sets until the end of February when no more females were formed.

Results and discussion

Under north Indian conditions, wheat and barley are sown from mid-November to late December. White cysts of *H. avenae* are present during late January and turn brown from late March to April when the crop is harvested. These brown cysts that then remain in the soil can easily be distinguished by their full contents and elasticity from those of the previous season still present in the soil. Thus, the egg stage of the nematode is exposed to parasites/predators for as long as 7-8 months, although protected within the cyst wall. As our study was concerned with egg pathogens, soil samples were collected in September, when the eggs within the cysts had already been in the soil for several months, and in February, when new eggs were being laid.

Irrespective of the season, black eggs were recorded in all the field samples, although the number of diseased eggs varied. In September, 1991, the percentage of black eggs ranged from 4 to 40 but in February, 1992, the incidence of black eggs in Manhethi village was 100% and in Nilaheri village was 48.7%. In the later instances

host crop was absent and thus no new cysts with healthy eggs were formed. In other field sites the eggs contained in the old cysts were all black, whereas the immature new white cysts contained healthy eggs.

It is interesting to note that 100% infection of eggs was recorded only in the old cysts. In new cysts only a small proportion (up to 10%) of the eggs were infected by September/October. As the majority of the healthy eggs in the new cysts hatch by December, only the diseased eggs persist and thus nearly 100% black eggs persist in the old cysts.

Examination of the black eggs at high magnification revealed infection by a fungus, the mycelium of which appeared to be composed of thick-walled, yeast-like swollen and brownish cells. The fungus completely infected the J2 coiled inside the egg shell (Fig. 1A), except the conus portion of the stylet (Fig. 1B), and the infected juvenile upon release from the egg shell appeared entirely as a rope of intertwined hyphae (Fig. 1C). The fungal hyphal cells were also found attached to the inside of the cyst wall (Fig. 1D). The remnants of the contents of old cysts resembled a network of mycelia engulfing all the blackened eggs which were destroyed beyond recognition. The description of the black yeast given by Rozsypal (1934; see Tribe, 1977) and Tribe (1977) supported our observations, and hence the same term "black yeast" is retained for the causal fungus of black egg disease in this study. Black yeasts are a group of well-known soil fungi under the generic names – *Phialophora*, *Aureobasidium*, *Rhinocladiella* and *Exophiala* (Tribe, 1977).

The survey clearly depicts the widespread incidence of black egg disease in *H. avenae*-infested areas of Haryana. The other egg parasite encountered was perhaps a zoosporic fungus, the zoosporangia of which were recorded in two samples. In this case, all the eggs contained in the cysts were infected with no trace of the J2; and presumably the infection might have occurred at the time of the white cyst stage. The presence of VAM vesicles in the empty of partially-filled cysts was also observed but was not accounted for as these did not parasitize the eggs as such.

The field from which the bulk soil sample was obtained (Majra village) had a long history of *H. avenae* infestation. Black eggs comprised 13.4% of the total egg population in September 1992, when the study commenced. Natural biodegradation, and cultivations associated with wheat sowing might have led to a decrease in the overall egg counts until November, when hatching commenced. As the juveniles from healthy eggs emerged and started penetrating the wheat roots, the percentage of black eggs increased from 16.2 in December and peaked to 46.2 in January, by which time most of the eggs had hatched. In February, as the females started laying eggs, the population of healthy eggs increased from 13 to a peak level of 135 eggs per g soil in March. Consequently, the population of black eggs decreased from 16.1% in February to 0.7% in March (Table I).

The new population of eggs did not show any sign of black yeast fungus infection, but the black egg population in the old cysts persisted, although gradually decreasing (up to 1 egg per g soil) perhaps due to natural biodegradation. The gradual decline in the population of healthy eggs from March onwards also may be attributed to natural decline due to predators and/or parasites and ploughing the field following harvest in May, leading to a more even distribution of cysts. However, black egg disease was noticed for the first time in the new progeny of eggs from July onwards, and their population increased from 1.2 in June to 8.6 eggs per g soil in September. The availability of soil moisture and conducive temperature during this period may be responsible for the activity of the black yeast fungus. Kerry *et al.* (1980) also observed that the development and parasitism of fungal antagonists was greater on *H. avenae* during prolonged periods of higher rainfall.

The surveillance study clearly shows the contribution of the black yeast fungus towards natu-

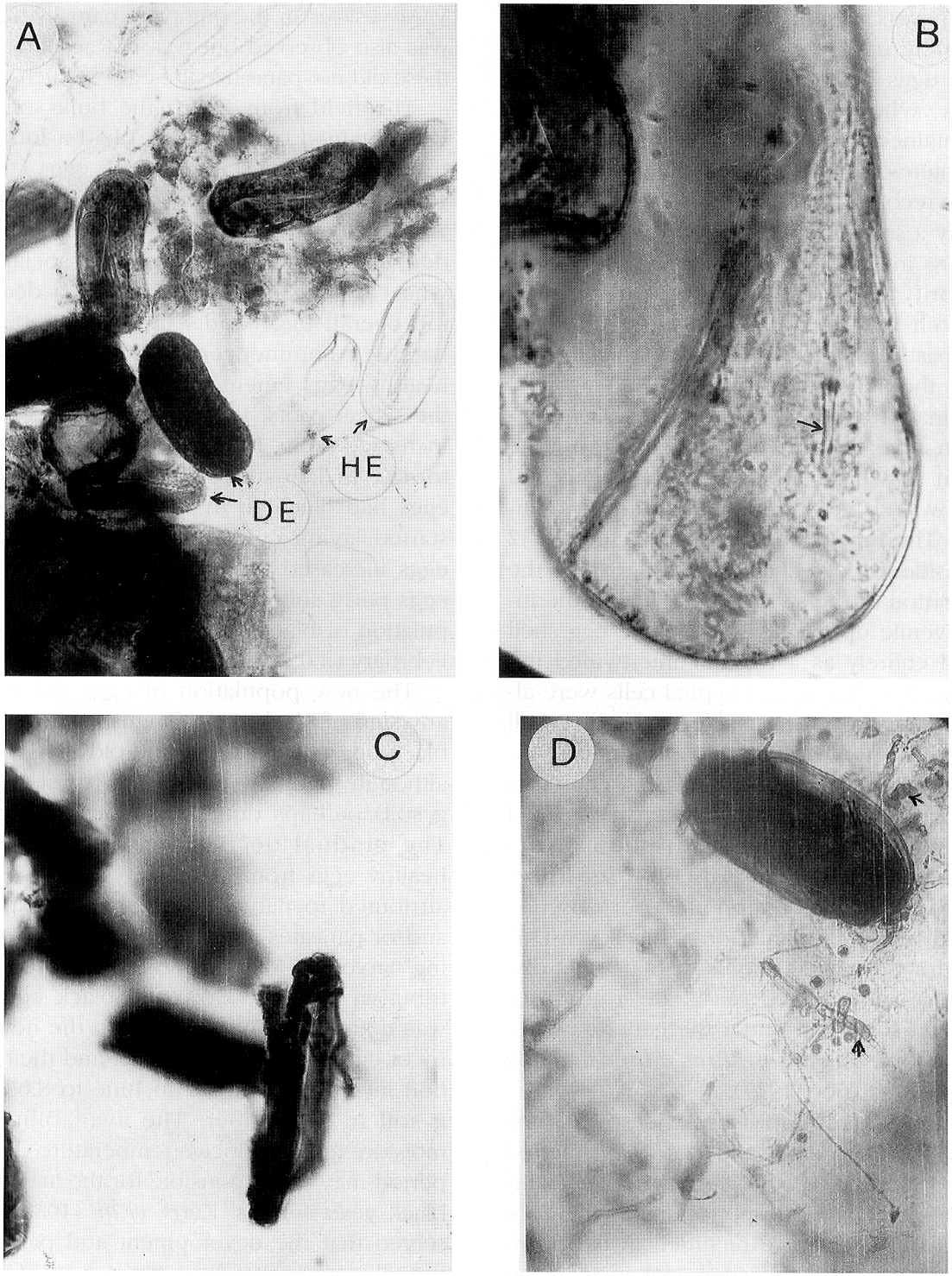

Fig. 1 - A, crushed cyst of *Heterodera avenae* showing a few healthy (HE) and several black eggs (DE) with infected J_2 coiled inside the egg shell (200x); B, magnified view of an infected egg, showing the only remnant i.e., conus part of the stylet (1250x); C, infected J_2 still coiled after release from egg shell (200x); D, swollen thick-walled cells attached to inner side of the cyst wall (200x).

TABLE I - *Surveillance on black egg disease of* Heterodera avenae *at Majra village.*

Month/year	Healthy eggs (Old + New cysts)	Black eggs per g soil in			% Black eggs
		Old cysts	New cysts	Total	
September, 1992	52.2	8.1	–	8.1	13.4
October	38.1	5.3	–	5.3	12.2
November	47.4	4.9	–	4.9	9.4
December	18.6	3.6	–	3.6	16.2
January, 1993	5.6	4.8	–	4.8	46.2
February	13.0	2.5	0	2.5	16.1
March	135.8	1.0	0	1.0	0.7
April	108.2	1.6	0	1.6	1.5
May	83.3	1.0	0	1.0	1.2
June	65.3	1.2	0	1.2	1.8
July	62.5	0.3	2.0	2.3	3.6
August	50.1	0.4	4.9	5.3	9.6
September, 1993	54.4	0.2	8.4	8.6	13.6

ral population balance of *H. avenae*. Between 13-14% eggs were destroyed by this fungus alone, the role of other parasites and/or predators notwithstanding. As a similar trend was observed during the second year (1993-94), only the data pertaining to 1992-93 are presented in Table I.

The pot experiment confirmed these findings. Newly formed cysts removed from pots containing naturally-infested soil during February to May, and preserved in sterile sand, had not developed black egg disease by October. Cysts removed from June/July onwards, however, contained 5.2 to 18.4% black eggs by October (Table II). Thus the black yeast fungus becomes active from July onwards until September/October, during which period the soil temperature and rainfall are perhaps most conducive for its activity. Notably, 21.2 and 8.7% of the eggs in cysts collected during February and March, respectively, developed infection of some other fungus, most likely a zoospooric fungus, since empty zoosporangia were present inside the egg shells.

In the Petri dish experiment females emerging on plants grown in naturally infested soil were attacked by fungal parasites and this was most pronounced during the second half of January (Table III). A sudden collapse/discolouration of some of the marked juvenile stages/females was noticed which could have been due to emergence of males or microbial infection of the females. Examination of parasitized females at higher magnifications revealed the presence of a fungus, the zoosporangia of which (ca.

TABLE II - *Development of fungal diseases in the cysts of* H. avenae *collected from naturally-infected soil at monthly intervals.*

Time of cyst collection	Percentage of diseased eggs during October	
	Black eggs	Others
February	0	21.2
March	0	8.7
April	0	0
May	0	0
June	5.2	0
July	18.4	0
August	15.4	0
September	16.0	0
October	18.1	0

TABLE III - *Fungal parasitism of females in the soil artificially/naturally infested with* H. avenae.

Weekly observations	No. of females/white cysts visible per Petri dish			
	Artificially infested soil		Naturally infested soil	
	Healthy	Diseased	Healthy	Diseased
January-1	2.3	–	–	–
January-2	8.6	–	–	–
January-3	14.1	–	6.4	1.2
January-4	22.0	–	13.7	4.2
February-1	21.5	0.1	15.0	4.2
February-2	25.4	0.2	12.8	5.8
February-3	26.2	0.2	16.1	5.8
February-4	26.2	0.2	14.3	5.8

100) filled the entire young female. In two cases, a different fungus having broad hyphae, filled the whole of female body.

A total of 26.3% of the females were destroyed due to fungal attack in naturally infested soil. However, in steam sterilized soil only 0.4% females were parasitized perhaps due to the introduction of fungus along with unsterilized nematode inoculum (Table III). Three species of fungi namely, *Catenaria auxiliaris* (Kuhn) Tribe, *Nematophthora gynophila* Kerry *et* Crump and a Lagendiaceous fungus have been reported to attack female cyst nematodes. All are zoosporic fungi which parasitize females on the root surface, cause break down of the nematode cuticle, and prevent cyst formation (Kerry, 1980). It was not possible to identify the fungus recorded parasitic on females since neither the catenate rhizomycelium (*Catenaria*) nor the resting sporangia (*N. gynophila*) were observed in this study.

Besides males of *H. avenae*, worm-like creatures (enchytraeids?) were also observed migrating through the soil spaces. Occasionally, these were seen probing and jabbing the females/white cysts. Similar observations were also made by Crump and Kerry (1977).

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