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Fungi Associated with Cysts of *Globodera rostochiensis*, *Heterodera cruciferae* and *Heterodera schachii* (Nematoda: Heteroderidae)

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Abstract Cysts of *Globodera rostochiensis* (Wollenweber) Behrens from potato (*Solanum tuberosum* L.) fields and *Heterodera cruciferae* Franklin from cabbage (*Brassica oleracea* L. var. *capitata* subvar. *rubra* L.) fields and *Heterodera schachtii* Schmidt from sugar-beet (*Beta vulgaris* L.) fields in Turkey were collected and examined for the presence of fungi. Of the total of 196 cysts of *G. rostochiensis*, 39.7% were colonized by one or more of 7 different species of fungi, all of which were from the genera *Cylindrocarpon*, *Fusarium*, *Gliocladium*, *Verticillium* and *Alternaria*. Of the total of 136 cysts of *H. cruciferae*, 37.5% were colonized by one or more of 7 different species of fungi, all of which were from the genera *Cylindrocarpon*, *Fusarium*, *Nematophthora*, *Periconia* and *Verticillium*, and 38.9% of the 154 cysts of *H. schachtii* were colonized by one or more of 7 different species from the same genera.

Keywords: Nematophagous fungi, *Globodera rostochiensis, Heterodera cruciferae, Heterodera schachtii*, biological control

Globodera rostochiensis, Heterodera cruciferae ve *Heterodera schachtii* (Nematoda: Heteroderidae)'nin Kistleri ile İlişkili Mantarlar

Özet: Türkiye'de patates (*Solanum tuberosum* L.) tarlalarından *Globodera rostochiensis* (Wollenweber) Behrens, lahana (*Brassica oleracea* L. var. *capitata* subvar. *rubra* L.) tarlalarından *Heterodera cruciferae* Franklin ve şeker pancarı (*Beta vulgaris* L.) tarlalarından *Heterodera schachtii* Schmidt kistleri toplanmış ve mantar mevcudiyeti bakımından incelenmiştir. Toplam 196 *G. rostochiensis* kistinin 39.7% si 7 farklı türe ait bir ya da daha fazla mantar türü ile beraber bulunmuştur. Bu mantar türleri, *Cylindrocarpon, Fusarium, Gliocladium, Verticillium* ve *Alternaria* cinslerine aittir. Toplam 136 *H. cruciferae* kistinin 37.5% i 7 farklı türe ait bir ya da daha fazla mantar türü ile beraber bulunmuş olup bunların hepside *Cylindrocarpon, Fusarium, Nematophthora, Periconia* ve *Verticillum* cinslerine ait mantar türleridir. *H. schachtii* ye ait toplam 154 kistin 38.9% u ise yine aynı şekilde bir önceki cinslere ait olan mantarlar ile ilişkili olarak bulunmuştur.

Anahtar Kelimeler: Nematofaj mantarlar, *Globodera rostochiensis, Heterodera cruciferae, Heterodera schachtii*, biyolojik kontrol

1. INTRODUCTION

Nematophagous (nematode-destroying) fungi are natural enemies of nematodes. Nematophagous fungi have been found in all regions of the world, from tropics to Antarctica. They have been reported from agricultural, garden and forest soils, and are especially abundant in soils rich in organic material [1]. They comprise three main groups of fungi; the nematode-trapping and the endoparasitic fungi that attack vermiform living nematodes by using specialized structures, and the egg and cyst parasitic fungi that attack these stages with their hyphal tips [2, 3]. The reason for the continuing interest in these fungi is, in part, their potential as biocontrol agents against plant and animal parasitic nematodes. From this point of view especially, the egg and cyst parasitic fungi have been investigated in depth because of the promise of these fungi as biocontrol agents [4, 5].

Biological control of plant parasitic nematodes using nematophagous fungi has received considerable attention recently, because of the urgent need for alternatives to replace synthetic nematicides that are being phased out due to environmental concerns [6, 7]. The potato cyst nematode (PCN), *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and the cabbage cyst nematode (CCN), *Heterodera cruciferae* Franklin, 1945 and the beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, 1871 are some of the most important plant parasitic nematodes in the world. Since nematophagous fungi were first discovered in soil in 1852 [8], more than 200 species of fungi have been identified as colonizers of cysts, eggs and females of eight species of cyst nematodes in soil, including PCN and BCN [9,10]. The percentages of cysts, eggs and females of cysts nematodes colonized by fungi in agricultural soil ranged from 10-90%, with about 50% being the most common [11, 12]. Two possible routes for biological management of plant parasitic nematodes have been proposed. One is mass produce an effective nematode destroying fungus in the laboratory, and then apply it to soil [13] and the other is enhance the natural nematophagous fungal populations in soil by altering their surrounding conditions. But commercial success of these approaches has been limited; however, there are encouraging reports on reducing nematode populations by adding certain kinds of amendments, such as chitin and green manure crops to soil [14, 15, 16, 17].

The objective of this study was to investigate the species and frequencies of fungi colonizing cysts of PCN, CCN and BCN collected from Central Anatolia of Turkey.

2. MATERIAL and METHODS

Fungal isolation from cysts of nematodes: Soil samples were collected from potato (*Solanum tuberosum* L.) fields (Nevşehir: 38° 37,2'N ; 34° 43,2'E) naturally infested with PCN, and from cabbage (*Brassica oleracea* L. var. *capitata* subvar. *rubra* L.) fields (Çorum: 40° 33,0'N ; 34° 57,0'E) naturally infested with CCN, and from sugar-beet (*Beta vulgaris* L.) fields (Eskişehir: 39° 46,2N ; 30° 30,0'E) infested with BCN in several areas of Central Anatolia in Turkey. The soil was air-dried overnight and the cysts were extracted by the Fenwick Can Method [18]. A total of 196 cysts of PCN, 136 of CCN and 154 of BCN were collected. Cysts were handpicked under a stereoscopic microscope (Meade model 8300), at 15 x magnification and transferred consecutively into a 10% sodium hypochlorite (NaClO) solution for 5 min, 100 $\mu L\ L^{-1}$ streptomycin for 15 min, 20 $\mu L\ L^{-1}$ malachite green for 10 min, and sterilized water for surface disinfestations. The cysts were partially air-dried afterwards. Five surface-dried disinfested cysts were placed onto the corners at a sterilized square cover glass which was on potato dextrose agar in a Petri dish (9 cm diameter) under sterile conditions. The Petri dishes were sealed with paraffin film and incubated at 23 °C. Fungi growing from the cysts were examined visually or with a light microscope (Olympus model CX21) at low magnification (x 40) to determine the sites from which the fungi grew. The fungal colonies emerging from cysts were transferred once they reached the agar under the cover glass. Identifications of fungi were made from these subcultures. Identification of the nematophagous fungi was based on the morphological characteristics of conidiophores and conidia [19, 20, 21, 22]. If needed, nematodes were added to fungal cultures to induce sporulation for identification. Sporulation was also induced in some cultures by exposing fungal mycelium to a black light lamp (Model X-15B 115 volts 60Hz).

3. RESULTS AND DISCUSSION

3.1. Fungi associated with cysts of PCN: Of the 196 cysts of PCN examined, 78 or 39.7% were colonized by fungi (Table 1). Seven species of fungi were identified, representing 5 different genera. Of the fungi identified, most were species of *Fusarium*. *Fusarium oxysporum* Schlechtendahl was found to be associated with 33 cysts or 16.8%. *Gliocladium roseum* Bainier, *Verticillium coccosporum* W. Gams, *Alternaria alternata* (Fr.) Keissl, *Cylindrocarpon destructans* (Zinser) Scholten were infrequently associated with cysts (Table 1; Figure 1). Most fungi isolated emerged from anywhere on the cysts surface whereas *C. destructans* emerged only from the vulva of the cysts. This difference is important for identifications of fungi.

3.2. Fungi associated with cysts of CCN: Of the 136 cysts of CCN examined, 51 (37.5%) were colonized by fungi (Table 2; Figure 2). Seven species of fungi were isolated and identified. Most fungi associated with cysts of PCN were species of *Fusarium*. *Fusarium oxysporum* was associated with 26 cysts (19.1%), and *C. destructans* associated with 11 cysts (8.0%). *Fusarium solani* and *F. tabacinum* each colonized 2.9% of the cysts. All other species from other genera occurred at relatively low frequency (1.5 - 2.5%).

3.3 Fungi associated with cysts of BCN: For this research, of the 154 cysts of BCN examined, 60 (38.9%) were colonized by fungi (Table 3; Figure 3). Seven species of fungi were isolated and identified. All of them were the same species of fungi that were isolated from cysts of BCN. The frequencies of association of these fungi with cysts of BCN were similar to those of CCN. Most fungi associated with cysts of BCN were species of *Fusarium*. *Fusarium oxysporum* was associated with 30 cysts (19.4%) but *C. destructans* was associated with 16 cysts (10.3%). All other species from other genera occurred at relatively low frequency for BCN (0.5 – 2.5%).

This research showed that numerous fungi were associated with cysts of cyst nematodes. The fungal genera from PCN, CCN and BCN were similar, especially those found in CCN and BCN. These fungi associated with nematodes may represent a distinct mycoflora in the soil. Agricultural soils generally contain hundreds of species of fungi belonging to 170 genera [20]. At the species level, 10 fungal species were isolated from cysts of PCN, CCN and BCN. *Fusarium oxysporum, C. destructans* and *V. coccosporium* were associated with these cyst nematodes whereas *F. heterosporium, G. roseum* and *A. alternata* were associated with only PCN. This suggests that the mycofloras of the cyst nematodes may be different. Because biological life cycle, hosts and nutritional needs of cyst nematodes are generally different.

Cylindrocarpon destructans has been reported as an egg parasite of several cyst nematodes [23, 24]. A *Fusarium* species associated with egg masses but not females has been reported to be an egg parasite [25]. All the fungi isolated from PCN, CCN and BCN have been reported to be associated with plant-parasitic nematodes, especially cyst-forming nematodes. A few of them have been proven obligate parasites of nematodes but most of them are opportunistic parasites and saprophytes. For those obligate parasites, their effectiveness in destroying nematodes in vitro has not led them to be successful bio-control agents of plant parasitic nematodes. However, there are reports which indicate that viability of nematodes was greatly reduced after being colonized by some of these opportunistic fungal parasites in laboratory [23]. In soil, the populations of these opportunistic fungi associated with nematodes can be significantly greater than populations of obligate parasites [26, 27, 28]. Many nematode trapping fungi have been found to occur more frequently in the rhizospheres of several plants, especially leguminous plants, e.g. soybean and pea, than in root free soil [29, 30, 31]. This effect could possibly be due to increased or changed root exudation in these plants. The suppressiveness of suppressive soils against plant parasitic nematodes has been reported to be positively related to the population of all the fungal parasites, including the opportunists [9]. Although a great deal of knowledge is lacking on the mode of action and population dynamics of these opportunistic fungal parasites, their importance in future integrated management of plant parasitic nematodes should not be underestimated.

4. CONCLUSION

One important aspect of nematophagous fungi is the possibility of using them for biological control of plant- and animal- parasitic nematodes. Plant parasitic nematodes, e.g. root knot and cyst nematodes, are global pests in agriculture and horticulture, causing severe yield losses.

Owing to the ban of many nematicides, e.g. methyl bromide, because of health and environmental concerns, new alternatives for nematode control are therefore needed. Biological control may be such an alternative.

Mostly, plant parasitic nematodes attack plant roots and, therefore, the ability of the nematophagous fungi to grow in the rhizosphere is of great importance for their capacity to control these nematodes.

Table 1. Fungal species associated with cysts of *Globodera rostochiensis*

	Cysts colonized by fungi	
Fungal species	**Number (n)**	**Percentage (%)**
Fusarium heterosporum	10	5.1
Fusarium oxysporum	33	16.8
Fusarium solani	4	2.0
Gliocladium roseum	5	2.5
Verticillium coccosporum	7	3.5
Alternaria alternate	8	4.0
Cylindrocarpon destructans	11	5.6
Total cysts colonized *	78	39.7
PSD: 9.21		

* A total of 196 cysts were examined.
PSD: Population Standart Deviation

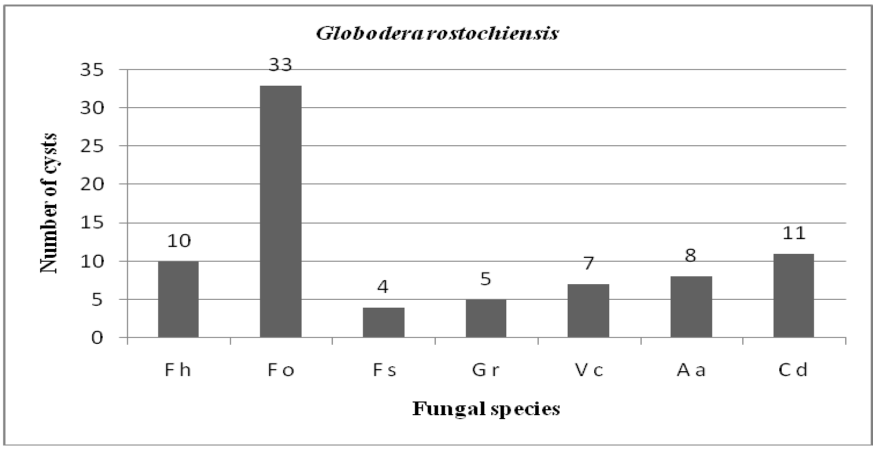

Figure 1. Fungal species associated with cysts of *Globodera rostochiensis* (F h. *Fusarium heterosporum*, F o: *Fusarium oxysporum*, F s: *Fusarium solani*, G r: *Gliocladium roseum*, V c: *Verticillium coccosporum*, A a: *Alternaria alternate*, C d: *Cylindrocarpon destructans*).

Table 2. Fungal species associated with cysts of *Heterodera cruciferae*

	Cysts colonized by fungi	
Fungal species	**Number (n)**	**Percentage (%)**
Cylindrocarpon destructans	11	8.0
Fusarium oxysporum	26	19.1
Fusarium solani	4	2.9
Fusarium tabacinum	4	2.9
Nematophthora gynophila	2	1.4
Periconia macrospinosa	1	0.7
Verticillium coccosporium	3	2.2
Total cysts colonized *	51	37.5
PSD: 8.20		

* A total of 136 cysts were examined.
PSD: Population Standart Deviation

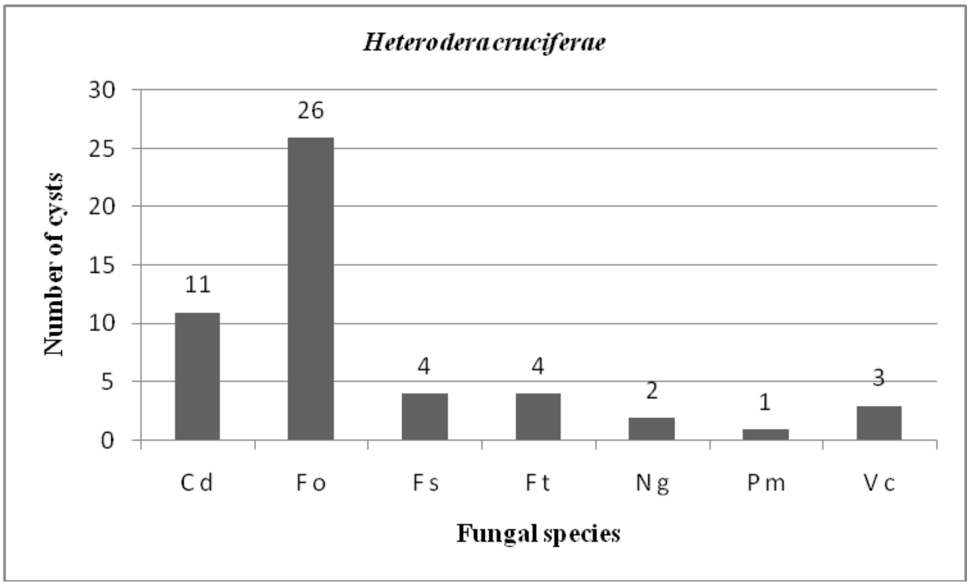

Figure 2. Fungal species associated with cysts of *Heterodera cruciferae* (C d: *Cylindrocarpon destructans,* F o: *Fusarium oxysporum*, F s: *Fusarium solani*, F t: *Fusarium tabacinum*, N g: *Nematophthora gynophila*, P m: *Periconia macrospinosa*, V c. *Verticillium coccosporium*).

Table 3. Fungal species associated with cysts of *Heterodera schachtii*

	Cysts colonized by fungi	
Fungal species	Number (n)	Percentage (%)
Cylindrocarpon destructans	16	10.3
Fusarium oxysporum	30	19.4
Fusarium solani	4	2.5
Fusarium tabacinum	2	1.2
Nematophthora gynophila	4	2.5
Periconia macrospinosa	3	1.9
Verticillium coccosporium	1	0.6
Total cysts colonized *	60	38.9
PSD: 9.91		

* A total of 154 cysts were examined.
PSD: Population Standart Deviation

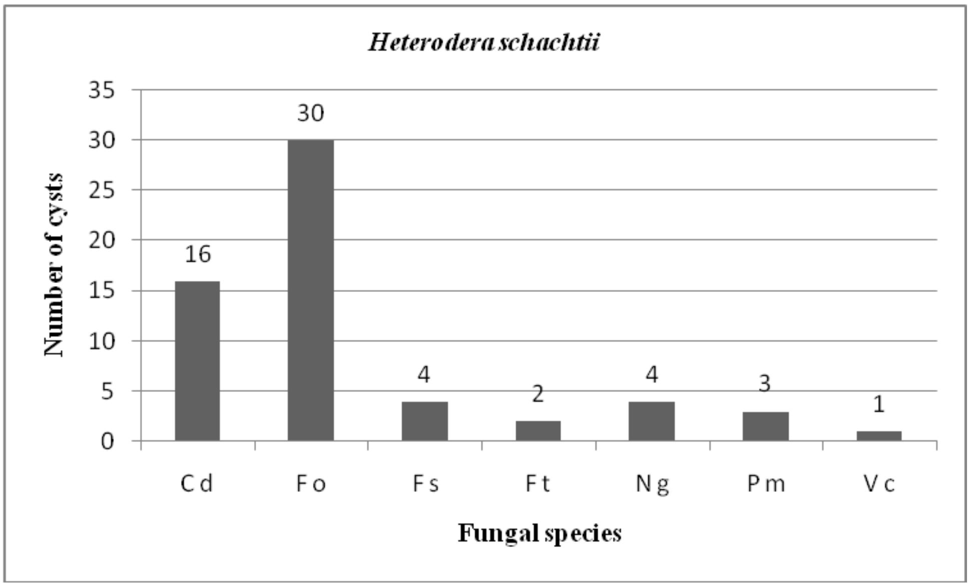

Figure 3. Fungal species associated with cysts of *Heterodera schachtii* (C d: *Cylindrocarpon destructans,* F o: *Fusarium oxysporum,* F s: *Fusarium solani,* F t: *Fusarium tabacinum,* N g: *Nematophthora gynophila,* P m: *Periconia macrospinosa,* V c. *Verticillium coccosporium*).

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