SUSCEPTIBILITY OF MUSHROOMS TO *APHELENCHOIDES SWARUPI* AND *APHELENCHUS AVENAE*

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Summary. An experiment was conducted to assess the relative susceptibility of the mushrooms *Agaricus bisporus*, *A. bitorquis*, *Pleurotus sajor-caju* and *Calocybe indica* to *Aphelenchoides swarupi* and *Aphelenchus avenae in vitro*. The sex ratio of *A. swarupi* multiplying on *A. bisporus* was also assessed. *Agaricus bisporus* was the most susceptible mushroom followed by *A. bitorquis* and *C. indica*. Neither nematode affected the growth of *P. sajor caju* or reproduced on this mushroom. Twenty days after nematode inoculation, per cent mycelial depletion in *A. bisporus*, *A. bitorquis* and *Calocybe indica* was 41.8, 38.4 and 23.7, respectively. Of the nematodes, *A. swarupi* was the more destructive, causing mycelia depletion of 22.3%, larger than the 13.5% depletion caused by *A. avenae.* The largest population of *A. swarupi* (5,197) was recorded on *A. bisporus*, closely followed by 5,042 on *A. bitorquis*. With *A. swarupi* on *A. bisporus*, the average male to female ratio of the progeny developed from a single gravid female was 1:2.

Key words: *Agaricus bisporus, A. bitorquis, Calocybe indica*, pathogenicity, *Pleurotus sajor-caju*.

Mushrooms are the macrofungi with distinct fruiting bodies, representing transformation of inedible waste into edible biomass. They are accepted as highly nutritive food with royal flavour and palatability. Due to their excellent culinary characteristics, they have been recognized as a delicacy in royal kitchens over hundreds of years. Among the edible cultivated mushrooms, white button mushroom *Agaricus bisporus* (Lange) Imbach has received the most attention by growers and consumers due to its impressive shape and mouth-watering taste. One third of global mushroom production is of this species (Chang, 1996). In India, 85% of production is of button mushroom, even though specialty mushrooms have great scope in the country (Tewari, 2005). Rodman's Agaricus, i.e. *Agaricus bitorquis* (Quél.) Sacc., is a closely related species, suitable for commercial cultivation at lower altitudes having a warm climate (Dayal and Gupta, 2005). Oyster mushroom, *Pleurotus sajor-caju* Singer is preferred by growers for its easy production technology and by consumers for its flavour and high nutritive value. Milky mushroom, *Calocybe indica* Purkayastha *et* Chandra is fast gaining acceptance as a specialty mushroom because of its drying and rehydration capability and its milky white colour.

Edible mushrooms are extremely delicate and are highly susceptible to various pests and pathogens, which may attack the crop at any stage, from mixing of substrate ingredients to cropping. The severity of the problem increases greatly when cultivation is under unhygienic conditions in makeshift houses lacking controlled conditions. Various nematode, insect and mite pests cause significant damage to mushrooms under such unhygienic conditions (Gill, 1987; Brar and Sandhu, 1989; Sandhu, 1995; Lewandowski *et al*., 1999; Khanna and Chandran, 2002; Khanna and Jandaik, 2002). Total crop failure is not uncommon when these pests occur from the initial phase of mushroom cultivation (Seth, 1984; Khanna and Sharma, 2001; Deepthi *et al*., 2004).

Himachal Pradesh is the leading state in the cultivation of these mushrooms in India. However, only a few growers undertake the production in purpose-built mushroom houses. Most of the farms run by marginal farmers are severely infested by one or more pests, which adversely affect crop production initially and result in complete crop failure if continuous cropping is undertaken without taking proper cook out measures after every crop. Myceliophagous nematodes are highly destructive pests and are known to cause damage ranging from 41 to 100% in button mushrooms, depending on the nematode species involved, their initial density and cropping stage at the time of infestation (Sharma *et al*., 1984; Khanna, 1991, 1993). Although information regarding these pests and their threat to *A. bisporus* and *Pleurotus* spp. is available, no research has so far been conducted on other cultivated mushrooms, including newly introduced specialty mushrooms. Questions regarding the most destructive pests (i.e. nematodes), their pathogenicity, damage potential and management remain unanswered. Therefore, investigations were undertaken to ascertain the susceptibility of the most important mushrooms cultivated in India to the most damaging nematodes.

MATERIALS AND METHODS

Isolation and raising of pure cultures of the nematodes. Suspensions of *Aphelenchoides swarupi* Seth *et* Sharma and/or *Aphelenchus avenae* Bastian were isolated from mushroom compost samples collected from mushroom farms of the area. Samples were processed by Cobb's

decanting and sieving technique (Cobb, 1918). Specimens of both species of nematodes were hand picked separately under a stereoscopic microscope and gravid females (larger in size) were inoculated singly onto Petri plates already impregnated with fully grown *A. bisporus* mycelium developed on malt extract agar medium. Plates showing multiplication of the respective nematodes were considered as pure cultures. The cultures thus raised were maintained, multiplied and used in further experiments.

Preparation of the medium. The medium was prepared by melting 25 g of malt extract in 500 ml of distilled water by heating. Twenty grams of agar-2 were added with continuous stirring until all the constituents were thoroughly mixed, and the final volume was made upto 1,000 ml with distilled water. The pH of the medium was adjusted to 6.5 by adding HCl or NaOH. The medium so prepared was poured into conical flasks and these were plugged tightly with non-absorbent cotton and autoclaved at 121.6 °C (1.5 kg/cm^2) for 20 minutes. The medium thus prepared was poured into sterilized Petri plates (9 cm diameter) under aseptic conditions in a sterilized laminar flow cabinet and allowed to solidify.

Susceptibility of the mushrooms to the nematodes. Pure cultures of the mushrooms *Agaricus bisporus*, *A. bitorquis, Pleurotus sajor-caju* and *Calocybe indica* were obtained from the Mushroom Research Laboratory, Department of Mycology and Plant Pathology, UHF, Nauni and NRCM, Chambaghat, Solan. Small uniform pieces of mycelium were cut with a cork borer and single pieces were placed in the centres of Petri plates containing solidified medium, under aseptic conditions. After inoculation, the Petri plates were incubated at respective temperatures of 25 ± 1, 28 ± 1, 27 ± 1 and 33 ± 1 °C for 14 days to favour the complete spread of the mycelium.

The susceptibility of the mushrooms was tested against the nematodes *A. swarupi* and *A. avenae*. For this, 27 Petri plates (9.5 cm diameter) of each test mushroom with fully-grown mycelium were used. Nine of the 27 Petri plates of each test mushroom were inoculated with pure cultures of surface sterilized *A. swarupi* at ten individuals per plate. Another nine plates were inoculated with similar inoculum of *A. avenae* and the remaining nine were maintained as controls. Before inoculation, the nematodes were hand picked, surface sterilized with mercuric chloride (0.01%) + streptomycin sulfate (1%) solution for two minutes, washed three times with sterilized water and finally transferred aseptically into the Petri plates. The Petri plates were incubated at 25 ± 1 °C.

Mycelial depletion was recorded 10 and 20 days after inoculation by calculating the area of the Petri plate. Per cent mycelial depletion was assessed by using the formula

$$\% \text{ mycelial depletion} = \frac{\text{Area depleted (cm}^2\text{)}}{\text{Total area of the Petri plate (cm}^2\text{)}} \times 100$$

Nematode population per Petri plate was recorded 20 days after inoculation by removing the upper layer of the medium and extracting the nematodes in clean water. Aliquots of the nematode water suspension were observed under a stereoscopic microscope and the nematodes counted.

The experiment was laid out in Completely Randomized Design (CRD).

Male to female sex ratio of A. swarupi. To determine the sex ratio in the *A. swarupi* populations, two sets of Petri plates were laid out. In the first set, ten juveniles were inoculated into each of 24 Petri plates fully impregnated with *A. bisporus* mycelium grown on malt extract agar medium. In the second set, one gravid female was inoculated onto each of 24 plates. The Petri plates were incubated at 25 ± 1 °C. Six plates from each set were washed out at each observation. The numbers of females and males produced were recorded at 10, 20, 30 and 40 days of inoculation by extracting nematodes from the upper layer of the medium in water. Mycelial depletion was also recorded at each observation using the formula referred to above to record the role of availability of food on sex ratio, if any.

Table I. Effect of *Aphelenchoides swarupi* and *Aphelenchus avenae* on the mycelium of *Agaricus bisporus*, *A. bitorquis*, *Pleurotus sajor caju* and *Calocybe indica* 10 days after inoculation.

Nematode species	% mycelial depletion in				Mean
	A. bisporus	*A. bitorquis*	*P. sajor caju*	*C. indica*	
A. swarupi	36.0 (36.9)	31.9 (34.4)	0.0 (0.0)	21.4 (27.6)	22.3 (24.7)
A. avenae	21.1 (27.3)	19.1 (25.9)	0.0 (0.0)	13.8 (21.8)	13.5 (18.8)
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean	19.1 (21.4)	17.0 (20.1)	0.0 (0.0)	11.7 (16.4)	
$CD_{0.05}$:	Nematode (N)		0.49		
	Mushroom (M)		0.57		
	N × M		0.98		

Figures in parentheses are arc sine transformed values.

Table II. Effect of *A. swarupi* and *A. avenae* on the mycelium of *A. bisporus*, *A. bitorquis*, *P. sajor caju* and *C. indica* 20 days after inoculation.

Nematode species	% mycelial depletion in				Mean
	A. bisporus	*A. bitorquis*	*P. sajor caju*	*C. indica*	
A. swarupi	84.3 (66.9)	76.9 (61.3)	0.0 (0.0)	45.6 (42.5)	51.7 (42.7)
A. avenae	41.0 (39.8)	38.4 (38.3)	0.0 (0.0)	25.6 (30.4)	26.3 (27.1)
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean	41.8 (35.6)	38.4 (33.2)	0.0 (0.0)	23.70 (24.27)	
$CD_{0.05}$:	Nematode (N)		0.69		
	Mushroom (M)		0.80		
	N × M		1.39		

Figures in parentheses are arc sine transformed values.

Table III. Multiplication potential of *A. swarupi* and *A. avenae* on the mycelia of *A. bisporus*, *A. bitorquis*, *P. sajor caju* and *C. indica* 20 days after inoculation.

Nematode species	Population per plate				Mean
	A. bisporus	*A. bitorquis*	*P. sajor caju*	*C. indica*	
A. swarupi	5,197 (2.487)	5,042 (2.455)	000 (1.000)	4,218 (2.278)	3,614 (2.055)
A. avenae	928 (1.388)	869 (1.367)	000 (1.000)	603 (1.265)	600 (1.255)
Control	000 (1.000)	000 (1.000)	000 (1.000)	000 (1.000)	000 (1.000)
Mean	2,041 (1.625)	1,970 (1.607)	000 (1.000)	1,607 (1.514)	
$CD_{0.05}$:	Nematode (N)		0.035		
	Mushroom (M)		0.041		
	N × M		0.071		

Figures in parentheses are square root (x+1) transformed values.

RESULTS

Effect on mycelial depletion. The susceptibility of the test mushrooms to *A. swarupi* and *A. avenae* was evaluated separately (Figs 1 and 2). Per cent mycelial depletion 10 days after inoculation (Table I) revealed that *A. bisporus* was the most susceptible mushroom species with a mean mycelial depletion of 19%, followed by the significantly lower (17%) depletion in *A. bitorquis*. Mycelial depletion in *C. indica* was 11.7%, significantly lower than the former species of mushroom, and no mycelial depletion of *P. sajor caju* was observed. Of the nematodes, *A. swarupi* was the more destructive, causing a mean mycelial depletion of 22.3%, significantly greater than the 13.5% caused by *A. avenae*. Thus, mycelial depletion was 36% in *A. bisporus* inoculated with *A. swarupi*, significantly more than the 31.9% in *A. bitorquis*. Mycelial depletion caused by *A. swarupi* in *C. indica* and by *A. avenae* in *A. bisporus* was similar (21.4 and 21.1%, respectively). The least mycelium depletion of 13.8% was observed in *C. indica* inoculated with *A. avenae*.

Twenty days after nematode inoculation (Table II), mycelial damage increased to 41.8% in *A. bisporus* and was greater than the 38.4% depletion recorded for *A. bitorquis* and 23.7% for *C. indica*. No damage was recorded in *P. sajor caju*. Again, *A. swarupi* caused the greater mycelial damage of 51.7%, nearly double that caused by *A. avenae* (26.3%). A depletion of 84.3% was observed in *A. bisporus* inoculated with *A. swarupi,* which was greater than the 79.9% in *A. bitorquis. Aphelenchus avenae* caused significantly less damage, 41 and 38.4% respectively, to *A. bisporus* and *A. bitorquis*. *Calocybe indica* suffered the least mycelial depletion of 45.6% and 25.6% by *A. swarupi* and *A. avenae*, respectively. These observations implied that *P. sajor caju* was resistant to both species of test nematodes as neither caused mycelial depletion in this mushroom.

Effect of the mushrooms on nematode population. Aphelenchoides swarupi and *A. avenae* (Table III) multiplied greatly in 20 days and mean populations of 2,040, 1,970 and 1,607 were observed in *A. bisporus*, *A. bitorquis* and *C. indica,* respectively. The reproductive potential of *A. swarupi*, with a mean population of 3,614, was significantly greater than that of the 600 specimens of *A. avenae*.

The largest population of *A. swarupi* (5,197) was recorded in plates impregnated with *A. bisporus*, closely followed by those with *A. bitorquis* (5,042). *Calocybe indica* supported 4,218 *A. swarupi*. The reproduction potential of *A. avenae* was significantly lower than that of

A. swarupi on all the test mushrooms, with nematode populations of 928, 869 and 603 recorded in the plates impregnated with *A. bisporus, A. bitorquis* and *C. indica*, respectively. *Pleurotus sajor caju* showed complete resistance against both species of test nematodes as the initial inoculum did not survive when added to the fully developed mycelium of this mushroom.

Male to female sex ratio of A. swarupi. The average number of females was always greater than that of males.

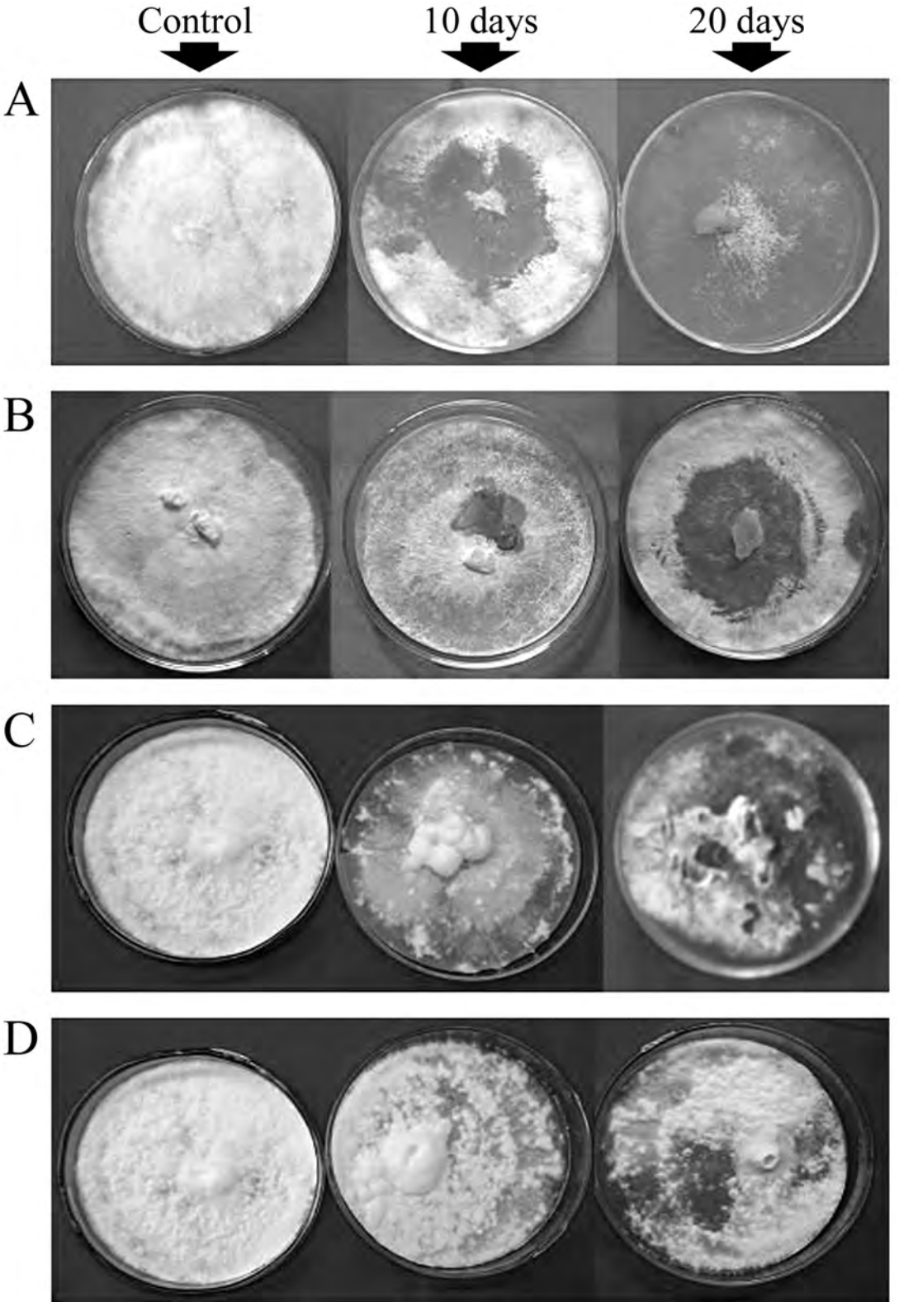

Fig. 1. Relative susceptibility of test mushrooms against *Aphelenchoides swarupi* and *Aphelenchus avenae* 10 and 20 days after inoculation, compared with control. A: *Agaricus bisporus* mycelium depleted by *A. swarupi*; B: *A. bisporus* mycelium depleted by *A. avenae*; C: *A. bitorquis* mycelium depleted by *A. swarupi*; D: *A. bitorquis* mycelium depleted by *A. avenae*.

When ten juveniles were inoculated, the average male to female ratio was 1:1.91, 1:1.95 and 1:1.94 at 10, 20 and 30 days after inoculation, respectively. The corresponding mycelial decline due to nematode feeding was 30.0, 88.0 and 100.0% at 10, 20 and 30 days after inoculation, respectively (Table IV). The whiteness of the mycelial mat completely disappeared at 30 days. Up to this time, the population of *A. swarupi* grew normally as sufficient mycelium was available for them to feed upon. The overall male to female ratio up to 30 days, with sufficient food

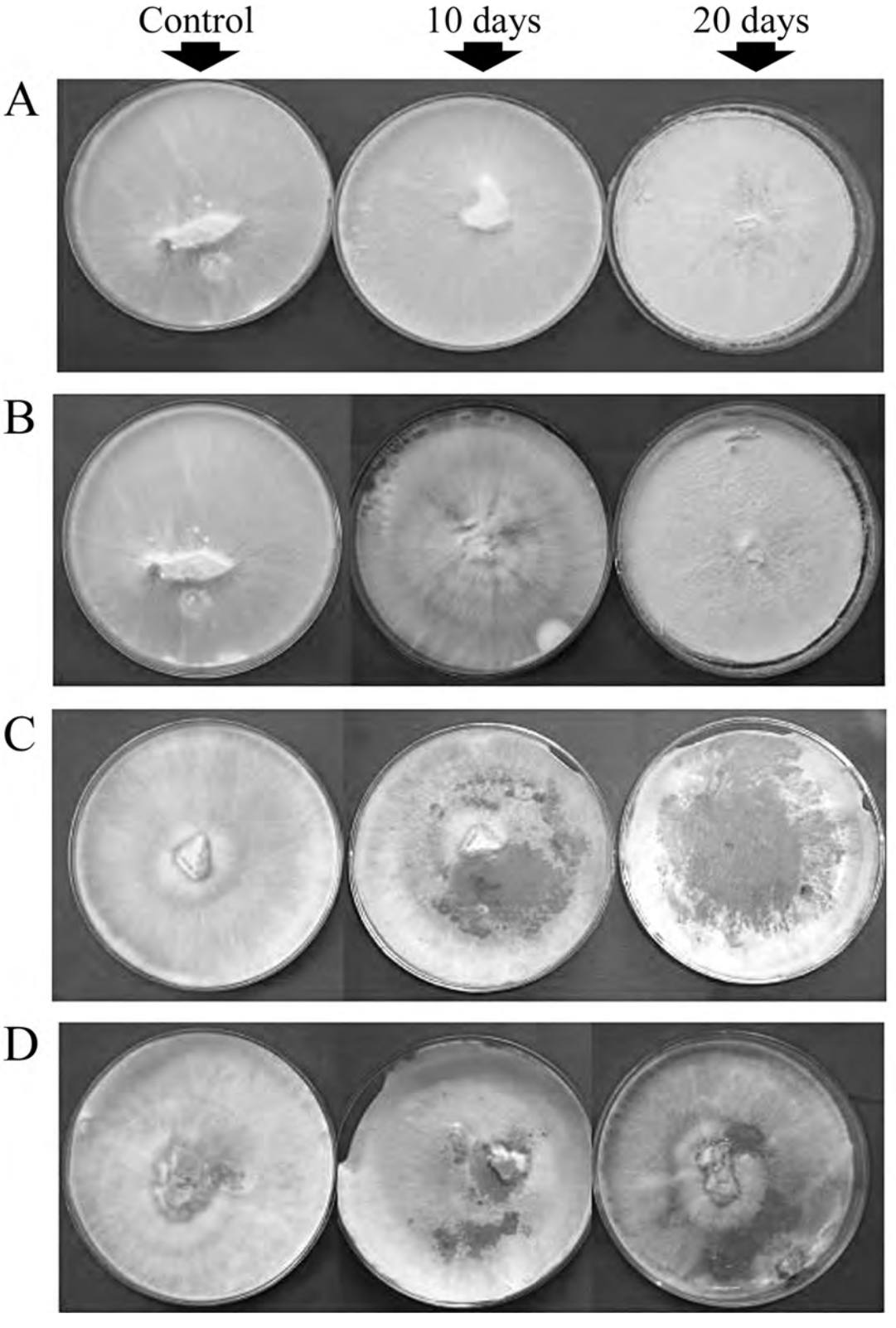

Fig. 2. Relative susceptibility of test mushrooms against *Aphelenchoides swarupi* and *Aphelenchus avenae* 10 and 20 days after inoculation, compared with control. A: *Pleurotus sajor caju* mycelium inoculated with *A. swarupi*; B: *P. sajor caju* mycelium inoculated with *A. avenae*; C: *Calocybe indica* mycelium depleted by *A. swarupi*; D: *C. indica* mycelium depleted by *A. avenae*.

Table IV. Male to female sex ratio in populations of *Aphelenchoides swarupi* reared on the mycelium of *Agaricus bisporus*.

Inoculum	Number of adult nematodes and mycelial depletion after (average of six replicates)											
	10 days				20 days				30 days			
	M	F	Ratio	Depletion (%)	M	F	Ratio	Depletion (%)	M	F	Ratio	Depletion (%)
10 juveniles/ plate	33	64	1:1.93	30.0	155	304	1:1.96	88.0	1,668	3,242	1:1.94	100.0
One gravid female	17	33	1:1.94	20.0	785	1578	1:2.01	62.0	801	1604	1:2.00	88.0

Inoculum	40 days			
	M	F	Ratio	Depletion (%)
10 juveniles/ plate	526	923	1:1.75	100.0
One gravid female	1,576	3,080	1:1.95	100.0

F = Female
M = Male

available, was 1:1.94. On the other hand, the male to female ratio dropped noticeably to 1:1.75 when the *A. swarupi* population was left without food for 10 days following complete consumption of the mycelium by 30 days. This suggests that females survive less well than males during food shortage. The male to female ratio developed from a single gravid female was 1:1.97, 1:2.01, 1:2.00, 1:1.95 at 10, 20, 30 and 40 days after inoculation, respectively. The corresponding mycelial consumption was 20.0, 62.0, 88.0 and 100%. The overall male : female sex ratio in this experiment was 1:1.98.

DISCUSSION

Earlier studies on resistance to nematodes in some *Agaricus* spp. revealed the wild mushroom *Agaricus edulis* Bull. = *Agaricus bitorquis* (Quél.) Sacc. to be resistant to *Ditylenchus myceliophagus* Goodey (Cayrol and Ritter, 1971) and *A. silvicola* (Vittad.) Peck to be a poor host for this nematode (Cayrol and Combettes, 1973). The resistance in *A. sylvicola* against *D. myceliophagus* was attributed to the chemical composition of the mycelial sap. Similar observations were also recorded by Thapa *et al.* (1987) on the resistance of *P. sajor caju* to *D. myceliophagus* and *A. sacchari* Hooper. Khanna and Sharma (1989) reported resistance in oyster mushrooms (*P. sajor caju*) against the myceliophagous nematode, *Aphelencoides agarici* Seth *et* Sharma. The presence of the nematotoxic principle muscarine in *P. sajor caju* was thought to be responsible for mortality in nematodes (Nath *et al.*, 1999). As *P. sajor caju* was found resistant to *A. swarupi* and *A. avenae*, this mushroom was excluded from further studies concerning nemic associations in mushrooms.

Seinhorst (1960) stated that measurable damage could occur only if the population density exceeded a certain limit in the case of fungal feeders damaging mushrooms. Arrold and Blake (1968) observed that the total basidiomata yield declined by 26% from the initial inoculum of one individual of *A. composticola* per 100 g of compost, and the loss increased to 46% when the initial inoculum was increased to 50 nematode specimens per 100 g of compost. Khanna and Sharma (1988) reported that, in addition to population level, the time of nematode inoculation also played a significant role in determining mycelial growth. *Aphelenchoides agarici* at an initial inoculum of ten nematodes per 1,000 g of compost at spawning time caused mycelial depletion of 45% by 45 days after inoculation, and this increased to 100% when 1,000 nematodes were inoculated. Similarly, ten nematodes inoculated at casing depleted only 26.7% of mycelium by 40 days, a figure that increased to 50% with an initial inoculum of 1,000 individuals.

In natural populations of *A. avenae*, males were not recovered from any of the localities surveyed, suggesting a parthenogenetic mode of reproduction in this species. On the other hand, in natural populations of *Aphelenchoides swarupi* recovered from various localities, a good

number of males were observed. On mycelium of *Agaricus bisporus*, the male to female ratio of *A. agarici* was 1:1.5 (Khanna and Sharma, 2000), while males were altogether absent in *A. dactylocercus* Hooper and *A. asterocaudatus* (Hooper) Bahl *et* Prasad. These observations indicate that variations in male to female ratios of different *Aphelenchoides* spp. feeding on the same host may occur.

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