EFFECT OF POPULATION LEVELS OF *APHELENCHOIDES SWARUPI* AND *APHELENCHUS AVENAE* INOCULATED AT SPAWNING ON MYCELIAL GROWTH OF MUSHROOMS AND NEMATODE MULTIPLICATION

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Summary. An experiment was conducted to investigate the effects of population levels of *Aphelenchoides swarupi* and *Aphelenchus avenae*, inoculated at spawning time, on the mushrooms *Agaricus bisporus, Agaricus bitorquis* and *Calocybe indica*. Mycelial growth was reduced with time, depending upon nematode species and the population levels. After 40 days of spawning, mycelial growth of *A. bisporus* was reduced to 1.7% of that of the uninoculated control at an initial inoculum of 1,000 individuals of *A. swarupi*/1,000 g of compost, compared to 28.2% with 10 individuals/1,000 g. Although, *Aphelenchus avenae* restricted mycelial growth less than *A. swarupi* but the trend with population level was similar. Although *Agaricus bitorquis* showed comparatively better mycelial growth than *A. bisporus* when inoculated with both nematodes, the effect of initial population levels of the nematodes on mycelial growth was also severe. The mycelial growth of *C. indica* was only slightly affected by the nematodes and was of 99.5% at the initial inoculum of 10 individuals of either *A. swarupi* or *A. avenae* which declined to 96.1 and 96.7%, respectively, at the initial inoculum of 1,000 nematodes per 1,000 g compost. Both *A. swarupi* and *A. avenae* multiplied on *A. bisporus* and *A. bitorquis*, the rate of multiplication being higher at low initial inoculum and vice versa. Only negligible nematode population densities and no reproduction occurred on *C. indica*.

Key words: *Aphelenchoides swarupi*, *Aphelenchus avenae*, *Agaricus bisporus*, *A. bitorquis, Calocybe indica*.

Edible mushrooms are extremely delicate and are highly susceptible to various pests and pathogens, which may attack the crop at any stage, right from mixing of substrate ingredients to cropping. The severity of the problem increases greatly when cultivation is under unhygienic conditions in makeshift houses that lack environmental control (Khanna and Chandran, 2002). Various nematode pests cause significant damage to mushrooms under such unhygienic conditions (Lewandowski *et al.*, 1999; Khanna and Chandran, 2002; Khanna and Jandaik, 2002). Total crop failure is not uncommon when myceliophagous nematodes infect mushrooms at spawning time (Thapa *et al.*, 1977; Seth, 1984; Khanna and Sharma, 2001). In India, white button mushroom [*Agaricus bisporus* (Lange) Imbach], Rodman's Agaricus [*Agaricus bitorquis* (Quel.) Sacc.] and oyster mushroom (*Pleurotus sajor caju* Singer) represent a major portion of the total mushroom production of the country. Recently, the milky mushroom [*Calocybe indica* (Purkayastha *et* Chandra)] has gained popularity due to its taste and it is cultivated in the tropical region of the country. However, only a few growers undertake its cultivation 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 often cause virtual crop failure if continuous cropping is undertaken without taking proper 'cook out' measures after every crop.

Myceliophagous nematodes are highly destructive and are known to cause damage ranging from 41 to 100% crop loss in button mushrooms, depending on the nematode species involved, its population density and the cropping stage at the time of infestation (Sharma *et al.*, 1984; Khanna, 1991, 1993; Khanna *et* Kumar, 2005). Moreover, no information is available on the effect of nematodes on the new speciality mushroom, *C. indica*. Therefore, an investigation was undertaken to estimate the mycelial growth of the above-mentioned mushrooms as affected when nematode infestation occurs at spawning time.

MATERIALS AND METHODS

Isolation and raising of pure cultures of test myceliophagous nematode species

The populations of *Aphelenchoides swarupi* Seth *et* Sharma and/or *Aphelenchus avenae* Bastian were isolated from mushroom substrate samples collected from different localities of Himachal Pradesh. Individuals of both species of nematodes were hand picked separately under a stereoscopic microscope and surface sterilized with mercuric chloride at 0.1%, followed by two or three washings in distilled water to avoid toxicity. Gravid females of the nematodes were inoculated singly onto Petri plates of malt extract agar medium already impregnated with fully grown *A. bisporus* mycelium. The nematode cultures thus raised were maintained,

multiplied on the same mycelium and used in further experiments. The pure culture of *A. bisporus* was obtained from the National Research Centre for Mushrooms, Chambaghat, Solan (India). Small uniform pieces of mycelium were cut with a cork borer and one piece was placed, under aseptic conditions, at the centre of each Petri plate containing solidified malt extract agar. After inoculation, the Petri plates containing *A. bisporus* were incubated at 25 ± 1 °C temperature for 14 days to allow complete spread of the mycelium.

Substrate preparation and production of test mushrooms

White button mushroom, A. bisporus, *and Rodman's Agaricus*, A. bitorquis. Compost required for the experiments with *A. bisporus* and *A. bitorquis* was obtained from the Department of Horticulture, United Nation Development Project (UNDP), Chambaghat, Solan. The compost was made of 1,000 kg wheat straw, 400 kg poultry manure, 100 kg wheat bran, 14.5 kg urea, 30 kg gypsum and 800 kg lindane dust. Spawn (S-11) supplied by Spawn Laboratory, Chambaghat, Solan (HP) was used in the experiment. For both species of mushrooms, pasteurized compost (68-70% moisture content and pH 7.2) was spawned at 0.5% on a fresh weight basis (i.e. 50 g spawn/10 kg compost) and used to fill in 1 kg capacity polypropylene bags (12 × 8 inches). The spawn was thoroughly mixed with the compost by gently pressing and squeezing the bags by hand. Finally, the bags were covered with newspaper sheets dipped in 2% formalin (to avoid aerial contamination) and transferred to a spawn run room at the environmental conditions reported in Table I. When the compost had been uniformly colonised by mushroom mycelium after 25 days of spawning, casing soil (spent compost and farm yard manure in equal proportion) was spread over the colonised compost as a 4 cm deep layer, after removing newspaper from the top of the bag.

Preparation of substrate and spawning of milky mushroom, C. indica. Wheat straw, after chopping into small pieces, and wheat bran at 5% (w/w) of straw were soaked separately in clean tap water for 10-12 hours. After draining off the excess water, the substrates were mixed and steam sterilized at 121.6 °C at 1.5 kg/cm^2 pressure for one hour. The sterilized substrate was spawned with 4% (wet weight basis) wheat grain spawn and transferred to polypropylene bags of 1 kg capacity (12 × 8 inches). After spawning, the bags were kept in a spawn running chamber at 30-35 °C. After complete colonization of the substrate with mycelium, the bags were cased with a 3-4 cm deep layer of sterilized loamy soil that had been previously steam pasteurized at 65 °C for 4 hours. The pH of the casing soil was maintained at about 8.0 by adding $CaCO_3$ after pasteurization. After casing, the bags were kept in a cropping room, where the temperature was maintained at 30 ± 1 °C. A relative humidity of 80-90% coupled with 3 hours of light (200 to 500 Lux)/21 hours of darkness was required for successful cropping of this mushroom.

Nematode infestation time and inoculum

The polypropylene bags of one kg capacity, spawned separately with the spawn of the three test mushrooms were inoculated with 10, 100 or 1,000 individuals of each nematode per bag at the time of spawning. Non-inoculated bags were maintained as controls. Each treatment was replicated three times.

Observations on mycelial growth relative to the total external surface area of the bag were made 20 and 40 days after inoculation:

$$\% \text{ mycelial growth} = \frac{\text{Total area of mycelial growth (cm}^2\text{)}}{\text{Total surface area of the bag (cm}^2\text{)}} \times 100$$

Nematode multiplication rates were determined 40 days after inoculation. For this, final nematode population in each bag was assessed by processing 250 cm^3 of compost/substrate per bag using Cobb's decanting and sieving technique (Cobb, 1918) followed by Schindler's modification (Schindler, 1961).

Statistical analysis

Each treatment combination was replicated three

Table I. Environmental conditions maintained during the test of the mushrooms *Agaricus bisporus* and *A. bitorquis*.

Condition	Spawning period	Cropping period
A. bisporus		
Temperature:	24 ± 2 °C	17 ± 2 °C
Relative humidity:	85 – 95%	85 – 92%
Light:	Nil	Nil
Ventilation:	Minimum so that there was no fluctuation in temperature	Minimum so that there was no fluctuation in temperature
A. bitorquis		
Temperature:	28 ± 2 °C	23 ± 2 °C
Relative humidity:	85 – 95%	85 – 92%
Light:	Nil	Nil
Ventilation:	Minimum so that there was no fluctuation in temperature	Minimum so that there was no fluctuation in temperature

Table II. Mean effect of inoculum levels of *Aphelenchoides swarupi* and *Aphelenchus avenae* inoculated at spawning on mycelial growth of *A. bisporus*, *A. bitorquis* and *Calocybe indica* 20 days after inoculation at spawning time.

Nematode species	% mycelial growth						Mean	
	A. bisporus		*A. bitorquis*		*C. indica*			
A. swarupi	84.0	(71.3)	85.1	(72.7)	98.2	(83.6)	89.1	(75.9)
A. avenae	91.7	(76.0)	92.3	(76.5)	98.6	(84.2)	94.2	(78.9)
Mean	87.8	(73.6)	88.7	(74.6)	98.4	(83.9)		

$CD_{0.05}$:		
	Nematode	1.18
	Mushroom	1.44
	Nematode × Mushroom	2.04

Figures in parentheses are arc sine transformed values.

times according to a mixed factorial design in which the factors mushroom species, nematode species and nematode inoculum level were considered and all possible effects and interactions analysed.

RESULTS AND DISCUSSION

Effects on mycelial growth 20 days after inoculation

The data mean over nematode inoculum level is presented in Table II. The bags containing *C. indica* spawn exhibited maximum mean mycelial growth of 98.4%, significantly larger than that of *A. bitorquis* (88.7%) and *A. bisporus* (87.8%), which were not significantly different. The bags inoculated with *A. swarupi* showed mean mycelial growth of the three mushrooms (89.1%) significantly lower than that in bags inoculated with *A. avenae* (94.2%). The reduction in mycelial growth of *A. bisporus* and *A. bitorquis* was similar and the growth reduction in the bags inoculated with *A. avenae* was less than that in the bags inoculated with *A. swarupi*. The mycelial growth of *C. indica*, however, was affected very little by either nematode.

Mycelial growth decreased significantly with the increase in the population level of the nematodes (Table III), with a general average growth of the three mushrooms of 99.4% in the uninoculated controls, and in those infected by the two nematodes of 98.9, 93 and 75.2% in bags inoculated with 10, 100 and 1,000 nematodes, respectively. Although, visual inspection suggested the lowest inoculum level of both nematodes did not cause any obvious effect on mycelial growth, the effect was statistically significant with *A. swarupi* inoculations. At 100 and 1,000 nematodes per bag, mycelial growth was 91.7 and 66.1%, respectively, in the bags inoculated with *A. swarupi* and 94.4 and 84.3%, respectively, in the bags inoculated with *A. avenae*. Interestingly, all four of these growth values not only differed significantly among themselves but also from the non-inoculated controls. Similar mycelial growths of 99.0, 99.5 and 99.8% were recorded in the non-inoculated bags of *A. bisporus, A. bitorquis* and *C. indica,* respectively, but even the lowest inoculum level of ten individuals per bag affected mycelial growth adversely, and this effect was similar for all three mushroom species. At an inoculum level of 100 nematodes per bag, *A. bisporus* and *A. bitorquis* showed significant reductions in mycelial growth of 90.2 and 90.5%, respectively, compared to

Table III. Effect of different population levels of *A. swarupi* and *A. avenae* on mycelial growth of the mushrooms *A. bisporus, A. bitorquis* and *C. indica* 20 days after inoculation at spawning time.

Population level	% mycelial growth											
	Nematode species				Mean		Mushroom					
	A. swarupi		*A. avenae*				*A. bisporus*		*A. bitorquis*		*C. indica*	
10	98.8	(83.9)	98.9	(84.2)	98.9	(84.0)	98.7	(83.4)	98.7	(83.5)	99.3	(85.1)
100	91.7	(74.2)	94.4	(77.2)	93.1	(75.7)	90.2	(72.1)	90.5	(72.0)	98.5	(83.0)
1000	66.1	(56.5)	84.3	(68.0)	75.2	(62.3)	63.4	(53.2)	66.3	(55.0)	96.0	(78.6)
Control	99.7	(88.9)	99.2	(86.3)	99.4	(87.6)	99.0	(86.0)	99.5	(87.7)	99.8	(89.1)

$CD_{0.05}$:		
	Population level	1.66
	Population level × Nematode	2.35
	Population level × Mushroom	2.88

Figures in parentheses are arc sine transformed values.

the lowest inoculum level but although mycelial depletion in *C. indica* was significant it was not significantly different from that at the lowest inoculum level. The least mycelial growth of 63.4% was observed in *A. bisporus* inoculated with 1,000 nematodes per bag, closely followed by 66.3% in *A. bitorquis* at the same inoculum level. With *C. indica*, mycelial growth of 96% occurred even at the largest inoculum level.

Mycelial growth in the non-inoculated bags of all the mushrooms was 99-100% (Table IV). At the smallest inoculum of ten individuals a slight but significant reduction of mycelial growth was observed only in *A. bitorquis* and *C. indica* infested with *A. swarupi*. Mycelial growth of *A. bisporus* and *A. bitorquis* declined further with increasing population levels of the nematodes, and was of 50.5 and 52.3%, respectively, when they were inoculated with 1,000 individuals of *A. swarupi* per bag. The corresponding values in bags infected with 1,000 specimens of *A. avenae* were 76.3 and 80.3%, respectively, and not significantly different from one another. This clearly indicated that *A. bisporus* and *A. bitorquis* were equally affected by the presence of the nematodes, with *A. swarupi* being significantly more damaging to the mycelium than *A. avenae*. With *C. indica*, statistically similar mycelial growth was recorded when either nematode species was inoculated at 10 or 100 specimens per bag. At the largest inoculum level (1,000 specimens per bag), the mycelial growth of *C. indica* in the bags inoculated with *A. avenae* declined slightly but significantly (96.4%) compared with the 99% mycelial growth at the inoculum level of 100 individuals. When inoculated with *A. swarupi* at 100 and 1,000 individuals, the difference in mycelial growth was not significant, 97.9 and 95.6%, respectively.

The data indicate that *A. bisporus* and *A. bitorquis* are more susceptible than *C. indica* to both species of nematodes.

Effect on mycelial growth 40 days after inoculation

The suppressive effect of the nematodes on mycelial growth 40 days after inoculation was more severe than that observed after 20 days only for *A. bisporus* and *A. bitorquis*, and remained unchanged for *C. indica* (Table V). Mycelial growth of the three species of mushrooms 40 days after inoculation was of 42.7, 48.1 and 97.6% for *A. bisporus, A. bitorquis* and *C. indica*, respectively, with significant differences between the three. The effects of the two species of nematodes on mean mycelial

Table IV. Effect of different population levels of *A. swarupi* and *A. avenae* on mycelial growth of *A. bisporus*, *A. bitorquis* and *C. indica* 20 days after inoculation at spawning time.

Population level	% mycelial growth											
	A. bisporus				*A. bitorquis*				*C. indica*			
	A. swarupi		*A. avenae*		*A. swarupi*		*A. avenae*		*A. swarupi*		*A. avenae*	
10	98.8	(83.7)	98.5	(83.1)	98.5	(83.0)	98.8	(84.0)	99.2	(84.8)	99.3	(85.4)
100	87.6	(69.5)	92.9	(74.6)	89.7	(71.3)	91.2	(72.8)	97.9	(81.8)	99.0	(84.3)
1000	50.5	(45.3)	76.3	(61.1)	52.3	(46.3)	80.3	(63.7)	95.6	(78.0)	96.4	(79.1)
Control	99.0	(86.7)	99.0	(85.4)	100.0	(90.0)	99.0	(85.4)	100.0	(90.0)	99.7	(88.1)
$CD_{0.05}$:	Population level × Nematode × Mushroom						4.07					

Figures in parentheses are arc sine transformed values.

Table V. Effect of *A. swarupi* and *A. avenae* on mycelial growth of *A. bisporus*, *A. bitorquis* and *C. indica* 40 days after inoculation at spawning time.

Nematode species	% mycelial growth							
	Mushroom						Mean	
	A. bisporus		*A. bitorquis*		*C. indica*			
A. swarupi	34.9	(35.7)	43.6	(43.0)	96.8	(82.2)	58.5	(53.6)
A. avenae	50.5	(48.1)	52.5	(50.1)	98.4	(84.4)	67.2	(60.9)
Mean	42.7	(41.9)	48.1	(46.6)	97.6	(83.3)		

$CD_{0.05}$		
	Nematode	1.77
	Mushroom	2.16
	Nematode × Mushroom	3.06

Figures in parentheses are arc sine transformed values.

Table VI. Effect of different population levels of *A. swarupi* and *A. avenae* on mycelial growth of the mushrooms *A. bisporus*, *A. bitorquis* and *Calocybe indica* 40 days after inoculation at spawning time.

Population level	% mycelial growth					
	Nematode species		Mean	Mushroom		
	A. swarupi	*A. avenae*		*A. bisporus*	*A. bitorquis*	*C. indica*
10	57.6 (53.7)	69.4 (60.7)	63.5 (57.2)	39.4 (38.7)	51.6 (45.9)	99.5 (86.9)
100	43.1 (41.6)	54.9 (50.5)	49.0 (46.0)	22.0 (26.9)	28.8 (32.4)	96.1 (78.9)
1000	33.2 (29.9)	44.5 (43.5)	38.9 (36.7)	10.0 (14.8)	11.8 (17.9)	94.9 (77.3)
Control	99.9 (89.4)	99.8 (88.7)	99.8 (89.1)	99.5 (87.1)	100.0 (90.0)	100.0 (90.0)
$CD_{0.05}$	Population level			2.50		
	Population level × Nematode			3.54		
	Population level × Mushroom			4.33		

Figures in parentheses are arc sine transformed values.

growth were also significantly different, with *A. swarupi* more damaging (allowing 58.4% mean growth) than *A. avenae*, in which 67.1% mycelial spread was attained. Among the mushrooms, white button was highly susceptible to *A. swarupi* as only 34.9% of mycelium was retained in bags after 40 days of spawning, as against significantly more (43.6%) in Rodman's mushroom. Significantly greater mycelial retention (96.8%) was found in bags of *C. indica* inoculated with this nematode. However, *A. bisporus* and *A. bitorquis* were equally affected by *A. avenae*, showing mycelial growth of 50.5 and 52.5%, respectively. These values were not statistically different from each other. *Calocybe indica* suffered the least mycelial loss when inoculated with either nematode species, with 96.8 and 98.4% mycelial growth in the bags inoculated with *A. swarupi* and *A. avenae*, respectively, which were not significantly different.

Mycelial growth progressively declined with increase of the initial population of the nematodes, with 63.5, 49.0 and 38.9% mean growth attained at 10, 100 and 1,000 nematodes, respectively, as compared to 99.8% in the control (Table VI). The effects of the three inoculum levels differed significantly among themselves and from the control. *Aphelenchoides swarupi* caused significantly greater reduction of mycelial growth than *A. avenae* at all inoculum levels. While mycelial growth of *A. bisporus* and *A. bitorquis* was reduced greatly at all levels, the decline was not significant in *C. indica* at the lowest inoculum of ten nematodes. A slight but significant reduction of mycelial growth of *C. indica* did occur at initial populations of 100 nematodes and this reduction was similar to that at 1,000 nematodes per bag. The greatest damage (39.4, 22 and 10% mycelial growth at population levels of 10, 100 and 1,000 individuals per bag, respectively) was suffered by white button mushroom, followed by *A. bitorquis* (51.6, 28.8 and 11.8% mycelial growth, respectively). While the difference in mycelial depletion at initial levels of 10 and 100 individuals was not significant between *A. bisporus* and *A. bitorquis,* the two mushrooms suffered significantly different degrees of mycelial growth reduction at the largest inoculum level.

Forty days after inoculation (Table VII), white button mushrooms showed very little evidence of mycelial growth when inoculated with 1,000 individuals of *A. swarupi*. Only 1.7% mycelial growth was observed in these bags, which was significantly lower than the 5% mycelial growth attained in Rodman's mushroom at the same inoculum level. Even at the lowest initial inoculum of ten individuals of *A. swarupi*, mycelial growth of only 28.2% was recorded in *A. bisporus*, significantly lower than the 45.2% attained in *A. bitorquis*.

The mycelial depletion caused by *A. avenae,* though extensive, was less than that caused by *A. swarupi* at all levels. The *A. bisporus* bags treated with 10, 100 and 1,000 individuals of *A. avenae* showed mycelial growth of 50.7, 33.8 and 18.3%, respectively, and the corresponding growth in *A. bitorquis* for this nematode was 58.1, 33.5 and 18.6%. The figures showed that, while *A. swarupi* was more damaging to *A. bisporus* than to *A. bitorquis, A. avenae* depleted the mycelium of both species of mushrooms nearly similarly. In *C. indica,* neither nematode had a significant effect at the lowest population level of ten individuals. However, at initial inocula of 100 and 1,000 individuals/bag, the mycelial growth, although 93.1 and 97.4%, respectively, was significantly different from the 100% mycelial growth achieved in the control, but these differences would be of no practical significance in the cultivation of *C. indica.*

Nematode multiplication 40 days after inoculation

Similar mean nematode populations of 24.390×10^4 and 24.440×10^4 were recorded in *A. bisporus* and *A. bitorquis* (Table VIII). These means were significantly larger than the mean population of 0.003×10^4 recorded in *C. indica*. The mean population of 29.740×10^4 of *A. swarupi* was significantly larger than that of 2.818×10^4 of *A. avenae.*

Table VII. Effect of different population levels of *A. swarupi* and *A. avenae* on mycelial growth of *A. bisporus*, *A. bitorquis* and *C. indica* 40 days after inoculation at spawning time.

Population level	% mycelial growth					
	A. bisporus		*A. bitorquis*		*C. indica*	
	A. swarupi	*A. avenae*	*A. swarupi*	*A. avenae*	*A. swarupi*	*A. avenae*
10	28.2 (32.1)	50.7 (45.4)	45.2 (42.2)	58.1 (49.7)	99.5 (86.7)	99.5 (87.1)
100	10.2 (18.2)	33.8 (35.5)	24.2 (29.4)	33.5 (35.3)	94.8 (77.0)	97.4 (80.8)
1000	1.7 (4.3)	18.3 (25.3)	5.0 (10.5)	18.6 (25.4)	93.1 (75.0)	96.7 (79.7)
Control	99.7 (88.1)	99.3 (86.2)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
$CD_{0.05}$:	Treatment × Nematode × Mushroom			6.12		

Figures in parentheses are arc sine transformed values.

Table VIII. Population densities of the nematodes *A. swarupi* and *A. avenae* on the mushrooms *A. bisporus*, *A. bitorquis* and *Calocybe indica* 40 days after inoculation at spawning time.

Nematode species	Nematodes per bag (10^4×) in Mushroom						Mean	
	A. bisporus		*A. bitorquis*		*C. indica*			
A. swarupi	44.600	(1.291)	44.630	(1.303)	0.004	(0.002)	29.740	(0.866)
A. avenae	4.193	(0.588)	4.259	(0.596)	0.003	(0.001)	2.818	(0.395)
Mean	24.390	(0.940)	24.440	(0.950)	0.003	(0.002)		
$CD_{0.05}$:	Nematode				0.042			
	Mushroom				0.051			
	Nematode × Mushroom				0.073			

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

When nematode reproduction on the different mushrooms is considered, the populations of *A. swarupi* in *A. bisporus* and *A. bitorquis* (44.600×10^4 and 44.630×10^4, respectively) were similar but significantly larger than that of 0.004×10^4 recorded in *C. indica.*

The reproductive potential of *A. avenae* in all the test mushrooms was significantly lower than that of *A. swarupi* but populations of *A. avenae* in *A. bisporus* (4.193×10^4) and *A. bitorquis* (4.259×10^4) again were significantly larger than in *C. indica* (0.003×10^4).

The nematode population after 40 days of spawning time (Table IX) showed that the mean nematode populations increased significantly with the increase in the initial population levels of the nematodes. The initial populations of 10 and of 1000 nematodes increased to 11.430×10^4 and 33.290×10^4 individuals, respectively. Mean populations differed significantly from each other. *Aphelenchus swarupi* multiplied significantly more than *A. avenae* at all initial inocula. The difference was significant not only between nematodes but also all lev-

Table IX. Effect of different initial population levels of the nematodes *A. swarupi* and *A. avenae* on their final population densities 40 days after inoculation on the mushrooms *A. bisporus*, *A. bitorquis* and *Calocybe indica* at spawning time.

Population level	Nematodes per bag (10^4×) in					
	Nematode species		Mean	Mushroom		
	A. swarupi	*A. avenae*		*A. bisporus*	*A. bitorquis*	*C. indica*
10	20.600 (0.985)	2.260 (0.415)	11.430 (0.700)	16.780 (1.023)	17.500 (1.076)	0.000 (0.000)
100	37.390 (1.167)	3.414 (0.519)	20.400 (0.843)	30.620 (1.267)	30.580 (1.260)	0.003 (0.001)
1000	60.990 (1.310)	5.599 (0.646)	33.290 (0.978)	50.180 (1.468)	49.700 (1.462)	0.011 (0.005)
Control	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
$CD_{0.05}$:	Population level			0.060		
	Population level × Nematode			0.084		
	Population level × Mushroom			0.100		

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

Table X. Effect of different initial inoculum levels of *A. swarupi* and *A. avenae* on their final population densities 40 days after inoculation on *A. bisporus*, *A. bitorquis* and *C. indica* at spawning time.

Population level	Nematodes per bag (10^4×) in											
	A. bisporus				*A. bitorquis*				*C. indica*			
	A. swarupi		*A. avenae*		*A. swarupi*		*A. avenae*		*A. swarupi*		*A. avenae*	
10	30.490	(1.452)	3.083	(0.594)	31.310	(1.502)	3.695	(0.651)	0.000	(0.000)	0.000	(0.000)
100	56.040	(1.749)	5.195	(0.785)	56.110	(1.752)	5.044	(0.769)	0.002	(0.001)	0.004	(0.002)
1000	91.860	(1.964)	8.493	(0.971)	91.100	(1.960)	8.296	(0.964)	0.013	(0.006)	0.008	(0.004)
Control	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)
$CD_{0.05}$:	Treatment × Nematode × Mushroom						0.145					

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

els of treatment. Nematode populations were similar in *A. bisporus* and *A. bitorquis* at each level of inoculum and were significantly higher than the numbers attained in *C. indica*. The initial inoculum of ten individuals gave rise to means of 16.780×10^4 and 17.500×10^4 nematodes in *A. bisporus* and *A. bitorquis*, respectively. Interestingly, no nematodes could be recovered from *C. indica* bags at this level. However, at the highest inoculum level, the extremely low population of 0.011×10^4 nematodes was recovered from *C. indica* bags, in comparison to 50.180×10^4 and 49.700×10^4 individuals recovered from *A. bisporus* and *A. bitorquis*, respectively.

Nematode population densities recorded after 40 days of spawning (Table X) show nematode multiplication to be density dependent as the number of the nematodes increased progressively and significantly, but not linearly, in *A. bisporus* and *A. bitorquis*. Although the number of nematodes increased with the increase in initial inoculum in *C. indica* too, the increase was non-significant at all levels. *Aphelenchoides swarupi* achieved significantly larger populations than *A. avenae* at all inoculum levels. Population densities of *A. swarupi* in the two *Agaricus* species were statistically similar at every inoculum level. The same was observed with *A. avenae* but the final population densities of this nematode were smaller than those of *A. swarupi*, although similar to each other on white button and Rodman's mushrooms. For instance, at the inoculum level of 1,000 nematodes per bag, the final population on *A. bisporus was* 91.860 $\times 10^4$ individuals of *A. swarupi* and only 8.493×10^4 individuals of *A. avenae*. With *C. indica*, neither nematode could be recovered from the bags inoculated with ten individuals initially; nematodes of both species were retrieved from bags receiving initial inocula of 100 and 1,000 nematodes but their populations were significantly lower than those recovered from the former two mushrooms and not significantly different from each other.

After 40 days, multiplication rates of *Aphelenchoides swarupi* on *A. bisporus* were ×30,490, ×5604 and ×918.6 at initial inocula of 10, 100 and 1,000 individuals per bag, respectively (Table XI). Very similar reproduction rates were obtained for this nematode on *A. bitorquis.* Similar reproduction trends were observed for *A. avenae*, whose reproduction rates were ×3,083, ×519.5 and ×84.9 at initial inocula of 10, 100, and 1000 specimens per bag, respectively on *A. bisporus*, and virtually the same on *A. bitorquis*.

These results are in accordance with earlier research on the effects of myceliophagous nematodes on *A. bisporus* when inoculated at spawning time (Han *et al.,* 1974; Khanna, 1991, 1993). Measurable damage would occur only if the population density exceeded the crop's tolerance threshold (Seinhorst, 1960). Chandel (1982) studied the effect of different population levels of *A. sacchari* Hooper on *A. bisporus* and reported that the rate of multiplication was larger with smaller initial inocula and vice versa, thereby also indicating that the rate of multiplication was density dependent for myceliophagous nematodes. Initial fast growth of the nematode population followed by a later reduction has been reported for *Aphelenchoides composticola* Franklin (Arrold and Blake, 1968) and *A. sacchari* (Sharma *et al.*, 1981). Khanna and Sharma (1988) reported that, in ad-

Table XI. Multiplication rate of *A. swarupi* and *A. avenae* on *A. bisporus*, *A. bitorquis* and *C. indica* 40 days after inoculation at spawning time.

Population level	Multiplication rate (×) of					
	A. bisporus		*A. bitorquis*		*C. indica*	
	A. swarupi	*A. avenae*	*A. swarupi*	*A. avenae*	*A. swarupi*	*A. avenae*
10	30490.0	3083.0	31310.0	3695.0	0.0	0.0
100	5604.0	519.5	5611.0	504.4	0.2	0.4
1000	918.6	84.9	911.0	83.0	0.1	0.2
Control	0.0	0.0	0.0	0.0	0.0	0.0

dition to population level, the time of nematode inoculation also played a significant role on mycelial growth. *Aphelenchoides agarici* Seth *et* Sharma, at an initial inoculum of ten nematodes per 1,000 g compost at spawning time, caused mycelial depletion of 45% at 45 days after inoculation, which increased to 100% when 1,000 nematodes were inoculated. Inoculation of ten nematodes at casing depleted only 26.7% of mycelium after 40 days, but 50% at the initial inoculum of 1,000 individuals. The results of our study on *A. swarupi* and *A. avenae* agree with the above observations. One thousand individuals of *A. swarupi* inoculated at spawning restricted the mycelial growth of *A. bisporus* to 50.5% as against 98.8% when only ten individuals were inoculated. A similar trend on mycelial growth was observed with *A. avenae.* The effects of these nematodes were similar on *A. bitorquis*. However, *C. indica,* found to be susceptible in an earlier experiment (Kumar, 2007), showed mycelial growth of 75.6-99.3% 20 days after inoculation with different levels of *A. swarupi* or *A. avenae*. Arrold and Blake (1968) observed that the total sporophore yield of *A. bisporus* declined by 26% from the initial inoculum of one individual of *A. composticola* per 100 g compost, and by 46% when initial inoculum was increased to 50 per 100 g compost. Johnson (1957) reported that fifteen individuals of *Aphelenchoides* sp. per 1000 g compost inoculated at the time of spawning destroyed the mycelium completely by 4-5 weeks and that the initial inoculum of fifteen nematodes became 30,000,00 in 6 weeks. Khanna and Sharma (1989) studied the relative susceptibility of three commercially grown mushroom species, viz., *A. bisporus*, *A. bitorquis* and *Pleurotus sajor caju* Singer. to *Aphelenchoides agarici* Seth *et* Sharma. Only *P. sajor caju* showed resistance against this nematode. A similar study by Chandel and Sharma (1991) with *A. agarici* revealed that while the nematode was highly damaging for the mycelium of *A. bisporus* and *A. bitorquis*, it did not affect the mycelium of *P. sajor caju*.

The low final population densities of the nematodes found in *C. indica* after 40 days of spawning time inoculation, clearly indicates that no nematode multiplication occurred. Perhaps chemical composition or the high temperature requirement of *C. indica* during spawn run (30-35 °C) did not permit reproduction of *A. swarupi* and *A. avenae*. According to Khanna and Sharma (2001) there was no multiplication of *A. agarici* in button mushrooms beyond 30 °C. Thapa and Sharma (1987) reported high multiplication rates of *A. sacchari* and *Ditylenchus myceliophagus* Goodey on white button mushrooms between 22-23 °C and no multiplication beyond 30 °C.

In conclusion, it can be inferred that both *A. bisporus* and *A. bitorquis* are highly susceptible to *A. swarupi* and *A. avenae* as they are cultivated under conditions highly conducive to nematode multiplication. This synchronization of environmental requirements, similar for the nematode and the mushrooms, makes these mushrooms very susceptible to the nematodes. On the other hand, the specialty mushroom *C. indica*, though susceptible to *A. swarupi* and *A. avenae* (Kumar, 2007), requires a temperature during casing (30 ± 1 °C) that is not conducive to nematode infection and reproduction and hence suffered comparatively much less.

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