EFFECTS OF INOCULATION TIME ON DAMAGE POTENTIAL OF *APHELENCHOIDES SWARUPI* AND *APHELENCHUS AVENAE* TO RODMAN'S AGARICUS, *AGARICUS BITORQUIS*

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Summary. An experiment was conducted to assess the damage potential of *Aphelenchoides swarupi* and *Aphelenchus avenae* against the white button mushroom, *Agaricus bitorquis*, inoculated with 1000 individuals per bag of either test nematode. Half of the bags were inoculated at spawning time and half at casing time. Percentages of mycelial growth were recorded at casing time and pinhead formation stage. Treatmentwise sporophore production on a day to day basis was recorded for the entire cropping period so that weekly yield and flush gap period could be ascertained along with the total yield. The damage potential of *A. swarupi* and *A. avenae* was high when inoculated at spawning time as the nematodes restricted the mycelial growth significantly. Similar amounts of mycelial growth of 66% and 74.9% were recorded at casing time in the bags inoculated at spawning with *A. swarupi* and *A. avenae*, respectively, significantly less than the 98.5% mycelial growth in the control. However, the observations at pinhead stage revealed declines in mycelial growth, which was reduced to 39.8% and 49.1% in bags inoculated with *A. swarupi* and *A. avenae*, respectively. Mycelial growth in the bags inoculated at casing reached 52.9% at pinhead formation stage in bags inoculated with *A. swarupi*, compared to 59.1% in bags inoculated with *A. avenae*. Yield losses to the tune of 72% and 53%, respectively, were estimated in the bags inoculated with *A. swarupi* and *A. avenae* at spawning time. Percentage yield reduction was comparatively low when the nematodes were inoculated at casing and were 48.5 and 38.1% in the bags inoculated with *A. swarupi* and *A. avenae*, respectively. Thus, *A. swarupi* was more damaging than *A. avenae* and the cropping stage at which nematodes were inoculated affected the extent of damage caused to mushroom growth and production. Also, nematodes disrupted the mushroom flush pattern and reduced the cropping period.

Key words: Mushroom, mycelial growth, nematodes and yield loss.

Among the edible cultivated mushrooms, button mushrooms *Agaricus bisporus* (Lange) Imbach and *A. bitorquis* (Quél.) Sacc. have received the most attention by growers and consumers due to their impressive shape and mouth-watering taste. These are the main reasons why one third share of global mushroom production goes to *A. bitorquis* (Chang, 1996). In India, a major share (85%) of production is contributed by button mushrooms, though specialty mushrooms have considerable scope in the country (Tewari, 2005).

Agaricus spp. 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 manifold 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; Sandhu, 1995; Lewandowski *et al*., 1999; Khanna and Chandran, 2002; Khanna and Jandaik, 2002; Kumar, 2006). 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).

In India, where 90% of commercial production of mushroom comes from marginal farmers who grow this commodity in makeshift houses under unhygienic conditions, nematode incidence is extremely high. Losses to the mushroom crop due to myceliophagous nematodes have been reported to range from 26 to 75% depending on nematode initial population and species (Arrold and Blake, 1968).

Among myceliophagous nematodes, those of the order Aphelenchida contribute most of the species, with four myceliophagous genera, twenty species of which have been associated with mushroom cultivation (Seth and Sharma, 1986; Bajaj and Walia, 1999; Kumar *et al*., 2007a). Therefore, an investigation was undertaken to estimate the losses of white button mushroom, *Agaricus bitorquis*, as affected by *Aphelenchoides swarupi* Seth *et* Sharma and *Aphelenchus avenae* Bastian when inoculated at different stages of cropping.

MATERIALS AND METHODS

Preparation of the material

Isolation and raising of pure cultures of test myceliophagous nematode species. Mushroom substrate samples were collected from different mushroom units of Himachal Pradesh. Nematode suspensions having *Aphelenchoides swarupi* and/or *Aphelenchus avenae* in abundance

were chosen. Individuals of both nematode species, preferably gravid females, were hand picked separately under a stereoscopic microscope and surface-sterilized with mercuric chloride (0.1%) for 5-10 seconds followed by 2-3 washings in distilled water to remove residues of the chemical. Sterilized specimens were added singly onto Petri plates already inoculated with fully grown *A. bisporus* mycelium on malt extract medium. The plates showing nematode multiplication had pure cultures of respective nematodes and were selected and maintained for use in the 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 carefully from the culture with a cork borer and one piece was placed separately at the centres of plates containing solidified malt extract medium (malt extract 25 g + Agar-2 20 g + distilled water to make final volume up to 1000 ml) under aseptic condition. Plates inoculated with mycelium were incubated at 25 ± 2 °C for 14 days to allow complete spread of the mycelium before nematodes were added.

Substrate preparation and production of mushroom. Compost required for the experiments with *A. bitorquis* was obtained from the Department of Horticulture, United Nation Development Project (UNDP), Chambaghat, Solan. The composition of the compost was wheat straw 1000 kg, poultry manure 400 kg, wheat bran 100 kg, urea 14.5 kg, gypsum 30 kg and lindane dust 800 g. Spawn (S-11) supplied by Spawn Laboratory, Chambaghat, Solan (HP) was used in the experiment. Pasteurized compost containing 68-70% moisture and of pH 7.2 was spawned at 0.5% on a fresh weight basis and 10 kg were poured into each of 20 propylene bags. After the addition of spawn, the contents (compost and spawn) of the bags were thoroughly mixed. The material was gently hand pressed after spawning to consolidate it. Finally, the bags were covered with newspaper sheets dipped in 2% formalin (to avoid aerial contamination) and transferred to a spawn run room at 28 ± 2 °C and 85-95% relative humidity. When the mycelium had fully spread on the compost after 25 days of spawning, the newspaper was removed from the top of the bag and casing soil (spent compost and farm yard manure in equal proportion) was spread over the colonised compost as a 4 cm deep layer. During the cropping period, 23 ± 2 °C temperature and 85-92% relative humidity with minimum ventilation were maintained.

Nematode inoculation. The damage potential of *A. swarupi* and *A. avenae* was tested by inoculating either test nematode species at 1000 specimens/bag at spawning time or casing time. Uninoculated bags were maintained as controls.

Observations

Mycelial growth at casing time and pinhead stage. Percentages of mycelial growth were recorded at casing time and pinhead formation and compared with the mycelial spread in the uninoculated control bags, using the formula:

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

[Total surface area (cm^2) = 2π r(r+h)], where r = radius of bag and h = height of the bag]

Determination of the nematode population. Nematode population per 250 cm^3 of compost substrate from each bag was estimated at three stages, viz., casing time, pinhead stage and at the end of cropping. Samples were processed by Cobb's decanting and sieving technique (Cobb, 1918) followed by Schindler's modification (Schindler, 1961).

Flush pattern and total sporophore yield. Sporophore yield on a day to day basis was recorded for each replicate of all the treatments and the uninoculated control from the first day of button appearance to the end of cropping. Since the buttons appeared in distinct successions of flushes at intervals of 7 to 8 days in the control, weekly yields per treatment were derived by pooling data to give an idea of the yield per flush. Data on yield per flush for each treatment were then pooled to estimate total sporophore yields. The flush gap period of one replicate per treatment was also recorded.

Table I. Effect of *Aphelenchoides swarupi* and *Aphelenchus avenae* inoculated at different cropping stages on mycelial growth of the mushroom *Agaricus bitorquis*.

Treatment	% mycelial growth at	
	Casing time	Pin head formation
Spawning time inoculation of *A. swarupi*	66.1 (54.37)	39.9 (39.13)
Casing time inoculation of *A. swarupi*	98.5 (84.25)	52.9 (46.65)
Spawning time inoculation of *A. avenae*	75.0 (59.97)	49.2 (44.51)
Casing time inoculation of *A. avenae*	98.8 (84.50)	59.2 (50.28)
Uninoculated control	98.5 (85.09)	100.0 (90.00)
$CD_{0.05}$	5.75	2.84

Figures in parentheses are arc sine transformed values

Visual symptoms on sporophores. Special attention was paid to the observation of visual symptoms, if any, in and on compost and sporophores. The appearance of sporophores growing in infested bags was assessed carefully.

Statistical analysis. Each treatment was replicated four times according to a completely randomized design. All data were statistically analysed to calculate the critical difference (CD) at $P \leq 0.05$

RESULTS

Effect on mycelial growth. Significantly different mycelial growths of 66 and 74.9% (with reference to growth in the control at pinhead time) were observed at casing time in the bags inoculated with *A. swarupi* and *A. avenae* at spawning time, respectively, figures that were significantly less than the 98.5% mycelial growth in the uninoculated control (Table I). However, at the pinhead stage the mycelial growth had declined to 39.8% and 49.1% in bags inoculated with *A. swarupi* or *A. avenae*, respectively, figures that were significantly different from each other. In the bags inoculated at casing, mycelial growth at pinhead formation stage had reached 52.9% in bags inoculated with *A. swarupi* compared to 59.1% in bags inoculated with *A. avenae*. Interestingly, the per cent mycelial growth at this stage was similar in bags inoculated with *A. swarupi* at casing to that in bags inoculated with *A. avenae* at spawning. This indicates that *A. swarupi* is more damaging than *A. avenae* to mycelium of *A. bitorquis.*

Effect on total sporophore yield. Total sporophore yields of *A. bitorquis* (Table II) revealed significant decreases of button production in all inoculation treatments as compared to the control. The lowest sporophore yield of 522.5 g per bag as compared to 1865.0 g in the control was recorded when *A. swarupi* was inoculated at spawning. A significantly higher yield of 876.2 g was obtained from the bags inoculated with *A. avenae* at spawning. Inoculation of *A. swarupi* and *A. avenae* at casing gave 960 and 1155 g sporophores per bag, respectively, values that were significantly different from each other. Overall yield losses of 72 and 53% were estimated in the bags inoculated with *A. swarupi* and *A. avenae*, respectively, at spawning. Yield reduction was comparatively low in the bags inoculated at casing time, which showed losses of 48.5 and 38.1% in treatments with *A. swarupi* and *A. avenae* respectively.

Sporophore yields of *A. bitorquis* were badly affected by both nematodes, with greater damage caused by *A. swarupi* than by *A. avenae*. Yield losses reached 72% with inoculation at spawning time of *A. swarupi* and was significantly larger than that incurred in bags inoculated with *A. avenae* at the same time (53%). A similar difference in button production was recorded when these nematodes were inoculated at casing but the actu-

Table II. Effect of *A. swarupi* and *A. avenae* inoculated at spawning or casing time on weekly and total sporophore yield of *A. bitorquis*.

Treatment	Weekly yield per bag (g)							Total yield per bag (g)	Yield loss (%)
	1st	2nd	3rd	4th	5th	6th	7th		
Spawning time inoculation of *A. swarupi*	157.5	160.0	95.0	70.0	40.0	0.0	0.0	522.5	72.0
Casing time inoculation of *A. swarupi*	200.0	200.0	185.0	172.5	137.5	65.0	0.0	960.0	48.5
Spawning time inoculation of *A. avenae*	197.5	202.5	192.5	113.7	65.0	105.0	0.0	876.2	53.0
Casing time inoculation of *A. avenae*	237.5	272.5	177.5	216.2	138.7	112.5	0.0	1155.0	38.1
Uninoculated control	287.5	465.0	290.0	397.5	200.0	170.0	55.0	1865.0	-
$CD_{0.05}$	44.4	52.2	38.5	34.2	37.5	47.9	6.2	115.3	

al losses were much smaller when inoculation was at this later stage.

Effect on flush pattern. In *A. bitorquis*, sporophores normally emerge at a gap of 7-8 days. However, the bags inoculated with the nematodes showed a disruption to the normal flush pattern (Table II).

A normal pattern of low and high yield flushes in succession was recorded up to at least the fifth week in the uninoculated control. Bags inoculated at spawning time with *A. avenae* produced almost similar yields in the first and second weeks but yield declined progressively thereafter. Bags inoculated at casing time with *A. avenae* showed the pattern of low and high yields in succession, though with much lower peaks than the control, until the fourth week; thereafter there was a progressive decline and cropping ceased in the seventh week. The shortest crop duration was in bags inoculated with *A. swarupi* at spawning, where no sporophore production occurred beyond the fifth week. However, cropping continued until the sixth week in all other nematode inoculated treatments.

Effect on nematode multiplication. The data regarding multiplication of the nematodes in relation to the initial inoculum as recorded at casing time, pinhead formation stage and crop termination are in Table III.

When inoculated at spawning time, nematodes multiplied manifold as they fed upon the developing mycelium and *A. swarupi* reached 21,200 specimens per 250 cm^3 compost as compared to 3,980 *A. avenae* individuals at casing. The nematode populations increased tremendously thereafter and reached 583,000 and 21,800, respectively, at pinhead formation stage. Nematodes inoculated onto fully developed mycelium at casing multiplied at a much faster rate due to the abundant availability of mycelium and reached 41,750 *A. swarupi* and 6,770 *A. avenae* individuals at pinhead formation stage. The greatest number of nematodes was recorded at pinhead formation stage in the bags inoculated at spawning time with *A. swarupi* followed by the same species inoculated at casing, with corresponding multiplication rates of ×23320 and ×1670. The multiplication potential of *A. avenae* was much lower, as it had reproduction rates of ×872 and ×270.8 in the bags inoculated at spawning and casing time, respectively. Multiplication rates of both nematodes were higher between casing and pinhead formation stage as compared to the period between spawning and casing.

At crop termination, a decline in nematode count was observed in the bags inoculated at spawning. Only 52,950 individuals of *A. swarupi* per 250 cm^3 compost were recovered at crop termination as compared to 583,000 retrieved at pinhead formation. The same was true of *A. avenae*, in which a multiplication rate of ×410 was observed at crop termination as opposed to ×872 at

Table III. Multiplication potential of *A. swarupi* and *A. avenae* on *A. bitorquis*.

Treatment	Nematode population (× 10^4) per 250 cm^3 of compost at					
	Casing time		Pinhead formation		Crop termination	
	Population	Multiplication rate[1]	Population	Multiplication rate[1]	Population	Multiplication rate[1]
Spawning time inoculation of *A. swarupi*	2.120 (1.756)*	848	58.30 (50.22)**	23320	5.295 (2.373)*	2118
Casing time inoculation of *A. swarupi*	0.000 (1.000)	-	4.175 (11.53)	1670	15.000 (3.969)	6000
Spawning time inoculation of *A. avenae*	0.398 (1.181)	159.2	2.180 (8.368)	872	1.025 (1.416)	410
Casing time inoculation of *A. avenae*	0.000 (1.000)	-	0.677 (4.665)	270.8	0.683 (1.289)	273.2
Uninoculated control	0.000 (1.000)	-	0.000 (0.000)	-	0.000 (1.000)	-
$CD_{0.05}$	0.157	-	10.569	-	0.758	-

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

**Figures in parentheses are arc sine transformed values.

[1]Multiplication rate = observed nematode populations divided by initial nematode population (25 individuals)/250 cm^3 soi

Table IV. Correlations between sporophore yield, mycelial growth, and nematode population in *A. bitorquis*.

	Yield	Mycelial growth at casing	Mycelial growth at pinhead	Nematode population at casing	Nematode population at pinhead
Yield	1.0000				
Mycelial growth at casing	0.7228*	1.0000			
Mycelial growth at pinhead	0.9753*	0.6163*	1.0000		
Nematode population at casing	-0.6384*	-0.8178*	-0.5156*	1.0000	
Nematode population at pinhead	-0.6028*	-0.7123*	-0.4702*	0.9582*	1.0000

* Significant at 5% level of confidence.

pinhead formation. The bags inoculated at casing showed a progressive increase in nematode populations and maximum densities of 15,000,000 individuals of *A.* swarupi and 102,500 of *A. avenae* per 250 cm^3 compost were recorded at crop termination.

Correlation between nematode population, mycelial growth and sporophore yield. Highly positive correlations between mycelial growth at casing and pinhead formation with sporophore yield were recorded. Both of these factors were negatively correlated with nematode populations, meaning that the higher the nematode population the lower would be the mycelial growth and sporophore yield (Table IV).

Effect on flush gap period. The information gathered on a day to day basis of sporophore production, relating to flush gap periods, is summarised in Table V. In commercial practice, fruiting bodies of *Agaricus* spp. appear in distinct flushes of low and high yields at intervals of 6-12 days (Flegg *et al.,* 1985). A normal flush gap period of 7-8 days was observed up to the middle of the sixth week in the uninoculated control bags of *A. bitorquis*. Button production was delayed by four days when *A. swarupi* was inoculated at spawning and ever decreasing flush gap periods of six, five, four and four days were recorded over the next 20 days. Thereafter, the sporophores appeared at intervals of three and two days in the fourth week and cropping ceased completely by the fifth week. The bags inoculated with *A. swarupi* at casing showed a delay of two days in crop initiation as compared to the control. Sporophores then appeared at flush gap periods of six, five, four and four days up to the third week. Thereafter, the gap was further reduced to 2-3 days up to the first day of the sixth week, when cropping ceased. Sporophores emerged late by three days in bags inoculated with *A. avenae* at spawning and a normal flush gap of seven days was recorded at first, up to the second week. From then, the buttons appeared at intervals of five days into the third week, after which there was a further decline in flush gap period with the sporophores appearing at intervals of 2-4 days until the termination of the crop in the middle of the sixth week. The least disturbance in the flush gap period was observed in bags inoculated with *A. avenae* at casing time, in which buttons appeared at gaps of 6-8 days up to the fourth week and then of 3-5 days until the termination of the crop.

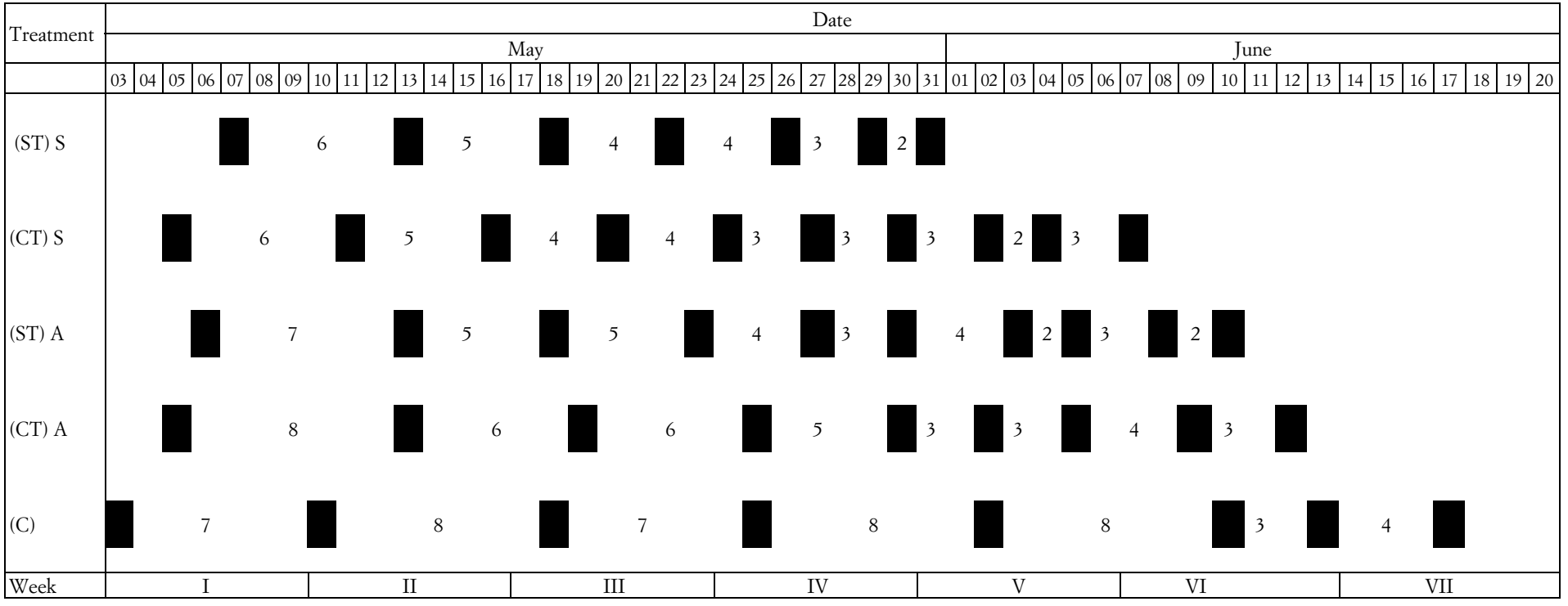
The presence of nematodes not only disturbed the unique flush pattern of this mushroom but also reduced the total duration of cropping, eventually resulting in lower productivity. Whereas sporophore production continued until the seventh week in nematode-free control bags, the cropping period was reduced drastically to 25 days in the bags inoculated with *A. swarupi* at spawning.

DISCUSSION

The damage potential of *A. swarupi* and *A. avenae* to *A. bitorquis* was high. When inoculated at spawning time, and observed at casing, the nematodes inhibited mycelial growth significantly as compared to the control. Khanna and Kumar (2005) reported that *Aphelenchoides agarici* Seth *et* Sharma and *A. composticola* Franklin depleted more mycelium when inoculated at spawning than casing. Relative to mycelial spread in the control at pinhead formation (designated as 100%), mycelium spread was depleted in all the bags receiving nematodes. Per cent mycelial growth was less in the bags inoculated at spawning time than in those inoculated at casing time and in the bags inoculated with *Aphelenchoides swarupi* than those inoculated with *Aphelencus avenae*. These results are in accordance with earlier findings by Kumar *et al.* (2007a, 2007b).

Chandel and Sharma (1991) reported almost similar damage to the mycelium of *A. bisporus* and *A. bitorquis* by *Aphelenchoides sacchari*. Previous studies reported yield losses of 89, 95 and 27% with inoculation at spawning time of *Ditylenchus myceliophagus* Goodey, *Aphelencoides composticola* and *Aphelenchus avenae*, respectively, and of 40, 64 and 21.4% with inoculation at casing time, thus emphasizing the importance of the in-

Table V. Effect of *A. swarupi* and *A. avenae* on flush gap period of sporophore emergence in *A. bitorquis*.



Flush

Numbers between flushes represents flush gap period in days

ST = Spawning time inoculation

CT = Casing time inoculation

S = *A. swarupi*

A = *A. avenae*

C = Uninoculated control

oculation time on the extent of yield losses (Khanna, 1991, 1993; Khanna and Jandaik, 2002). The observations recorded with *A. avenae* in these investigations agree with findings of Hooper (1962) and Gitanjali and Nandal (2000), who reported yield losses due to this nematode, but not with the findings of McLeod (1968), who reported that although *A. avenae* multiplied on spawned compost it did not affect the yield or cropping duration. Khanna and Jandaik (2002) reported 6.8-26.5 % losses due to *A. avenae* when different numbers were inoculated at spawning time.

Reports of delay in button appearance and disruption in flush pattern due to the saprophagous nematodes *Caenorhabditis elegans* Maupus and *Panagrolaimus fuchsi* Ruhm have been published (Grewal, 1991; Grewal and Richardson, 1991; Chandran, 2000; Khanna and Kumar, 2005). However, the disturbances in cropping pattern and reductions in flush gap period due to myceliophagous nematodes that we found are reported for the first time.

Both *A. swarupi* and *A. avenae* multiplied freely on the freshly growing mycelium of *A. bitorquis* until mycelium development peaked, and multiplication was greater for the former than for the latter. Nematode population declined thereafter and smaller population growth rates were recorded at crop termination for both species of nematodes. This reduction in population was apparently due to the lack of food as most of the mycelium had been exhausted by this time. Similar trends of increase in nematode population, reaching a maximum during the early cropping stage, followed by sudden decline by the end of the crop have been reported earlier for *Aphelenchoides agarici* and *A. composticola* infesting *Agarici bisporus* (Khanna and Sharma, 1988; Khanna, 1991).

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