DAMAGE POTENTIAL OF *APHELENCHOIDES SWARUPI* AND *APHELENCHUS AVENAE* IN MILKY MUSHROOM, *CALOCYBE INDICA*

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Summary. The damage potential of the nematodes *Aphelenchoides swarupi* and *Aphelenchus avenae*, at 1000 individuals per 10 kg of compost, in milky mushroom (*Calocybe indica*) was estimated by inoculation of the nematodes at the times of spawning and casing. Nematodes inoculated at spawning did not cause significant mycelial depletion in *C. indica.* Feeding by nematodes occurred only after casing and, thus, significant mycelial depletion was observed at pinhead. Total production showed no significant differences among the nematode inocula irrespective of inoculation stage and nematode species involved. *Aphelenchoides swarupi* caused sporophore losses of 15.7 and 13.0% when inoculated at spawning and casing times, respectively. Corresponding losses by *A. avenae* were 11.0 and 11.6%. The flush pattern in *C. indica* inoculated with the nematodes showed no disturbance in weekly yield until the fourth week but production of fruiting bodies declined continuously thereafter up to the seventh week. Neither species of nematode, when inoculated at spawning, showed multiplication until casing. The greatest multiplication rate of 319× was recorded for *A. swarupi* inoculated at spawning.

Keywords: Inoculation level, inoculation time, mushroom nematodes, nematode reproduction, yield loss.

Milky mushroom, *Calocybe indica* Purkayastha *et* Chandra, is fast gaining acceptance as a speciality mushroom in India because of its drying and rehydration capability and milky white colour (Bhardwaj, 2004). This mushroom species is observed to be highly susceptible to various pests and pathogens, which may gain entry right from mixing of the substrate ingredients onward. Various nematode, insect and mite pests cause significant damage to mushrooms, particularly under unhygienic conditions (Lewandowski *et al.*, 1999; Khanna and Chandran, 2002; Khanna and Jandaik, 2002; Kumar *et al.*, 2008, 2008a).

At present, no information is available on the extent of damage caused by myceliophagous nematodes to milky mushroom. Therefore, investigations were undertaken to assess the effect of the two most frequently encountered pathogenic aphelenchid nematodes, *Aphelenchoides swarupi* Seth *et* Sharma and *Aphelenchus avenae* Bastian, inoculated at two different times, on the extent of damage and quality of the yield of *C. indica* and on the reproduction of the nematodes.

MATERIALS AND METHODS

Isolation and culturing of the nematodes

The populations of *A. swarupi* and *A. avenae* were isolated from samples of mushroom substrate 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% for 0.5-1 minute, followed by two or three washings in distilled water to avoid toxicity. Gravid females of the nematodes were inoculated singly onto Petri plates (9 cm diameter) of malt extract agar medium already impregnated with fully grown mycelium of *Agaricus bisporus* (Lange) Imbach. The nematode cultures thus raised were maintained, multiplied on the same mycelium at 25 ± 1 °C and used in the experiments. All motile stages of the nematodes were extracted by removing the upper layer of the medium in clean water and inoculated instantly.

The pure culture of *A. bisporus* had been obtained from the National Research Centre for Mushrooms, Chambaghat, Solan, India. Small uniform pieces of the mycelium culture were cut with a cork borer and one piece was placed, under aseptic conditions, at the centre of each Petri plate (9 cm diameter) containing solidified malt extract agar. After inoculation, the Petri plates containing *A. bisporus* were incubated at 25 ± 1 °C for 14 days to allow complete spread of the mycelium.

Preparation of substrate and spawning

Wheat straw, chopped into small pieces, and wheat bran at 5% (w/w) of straw and soaked separately in clean tap water for 10-12 h were combined. After draining out excess water from the bran, the substrates were mixed and sterilized at 1.5 kg/cm^2 for one hour in a steam sterilizer. The sterilized substrate was spawned with 4% (wet weight basis) of wheat grain spawn of *C. indica* and 10 kg amounts were transferred to polypropylene bags. The bags were kept in a spawn running chamber at 30-35 °C. When the substrate was completely colonized with the mycelium of *C. indica*,

the bags were cased with a 3-4 cm thick layer of sterilized loam soil. The casing material had been steam pasteurized at 65 °C for 4 hours before use. The pH was maintained around 8.0 by adding $CaCO_3$ after pasteurization. After casing, the bags were kept in the cropping room at 30 ± 1 °C, 80-90% RH, with 3 h per 24 h of light, as per the cropping requirement of this mushroom. To maintain the humidity, water was sprayed two or three times per day on the walls and floors of the growing chamber.

Nematode inoculation

The damage potential of *A. swarupi* and *A. avenae* was tested by inoculating them separately at 1000 nematodes/bag (a random mix of all stages) at spawning or casing time. Uninoculated bags were maintained as controls. Each treatment was replicated four times. The experiment was laid out in a Completely Randomized Design (CRD).

Observations

Mycelial growth. Per cent mycelial growth at casing time and pinhead formation were recorded in all the treatments and compared with the mycelial spread in uninoculated control bags, using the formula below to calculate per cent mycelial growth:

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

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

Nematode population. The nematode population per 200 cm^3 of compost/substrate from all the bags inoculated with the nematodes was estimated at three stages, viz., casing time, pinhead stage and at the end of cropping. Compost/substrate 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 bag from the first day of button appearance to the end of cropping. Since the buttons appeared in a distinct succession of flushes at intervals of 7 to 8 days in the control, yields per treatment were pooled at weekly intervals 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.

Flush gap period. The flush gap period of one replicate of each treatment was recorded.

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

Statistical analysis. All data were statistically analysed to calculate the critical difference (CD) at P ≤0.05.

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

Treatment	Per cent mycelial growth at	
	Casing time	Pinhead formation
Inoculation of *A. swarupi* at spawning	94.9 (77.6)	87.4 (69.3)
Inoculation of *A. swarupi* at casing	97.8 (83.9)	90.2 (71.7)
Inoculation of *A. avenae* at spawning	95.7 (78.6)	88.4 (70.2)
Inoculation of *A. avenae* at casing	99.0 (85.9)	90.4 (71.9)
Uninoculated control	98.5 (85.1)	100.0 (90.0)
$CD_{0.05}$	(NS)	(2.3)

Figures in parentheses are arc sine transformed values.

Table II. Effect of *A. swarupi* and *A. avenae* on weekly and total sporophore yield of *C. indica* (spawning and casing time inoculation).

Treatment	Weekly yield (g)							Total yield (g)	Loss (%)
	1st	2nd	3rd	4th	5th	6th	7th		
Inoculation of *A. swarupi* at spawning	316.2	462.5	267.5	346.2	160.0	40.0	5.0	1597.4	15.7
Inoculation of *A. swarupi* at casing	280.0	465.0	255.0	406.3	180.0	47.5	15.0	1648.8	13.0
Inoculation of *A. avenae* at spawning	315.0	478.7	265.0	351.2	177.5	75.0	25.0	1687.4	11.0
Inoculation of *A. avenae* at casing	282.5	473.7	250.0	410.0	157.5	72.5	28.8	1675.0	11.6
Uninoculated control	297.5	468.8	257.5	417.5	228.7	157.5	67.5	1895.0	-
$CD_{0.05}$	NS	NS	NS	NS	46.9	35.4	24.1	111.9	

Table III. Multiplication of *A. swarupi* and *A. avenae* on *C. indica*.

Treatment	Nematode population ($\times 10^3$) per 200 cm^3 of compost at					
	Casing time		Pinhead formation		Crop termination	
	Population	Multiplication rate (×)	Population	Multiplication rate (×)	Population	Multiplication rate (×)
Inoculation of *A. swarupi* at spawning	0.081 (1.039)	4.05	0.885 (1.372)	44.25	6.380 (2.705)	319.0
Inoculation of *A. swarupi* at casing	0.000 (1.000)	-	0.320 (1.148)	16.0	2.655 (1.886)	132.75
Inoculation of *A. avenae* at spawning	0.024 (1.012)	1.20	0.293 (1.132)	14.65	0.845 (1.357)	42.25
Inoculation of *A. avenae* at casing	0.000 (1.000)	-	0.095 (1.046)	4.75	0.338 (1.156)	16.9
Uninoculated control	0.000 (1.000)	-	0.000 (1.000)	-	0.000 (1.000)	-
$CD_{0.05}$	0.009		0.098	-	0.316	-

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

RESULTS

Effect on mycelial growth. Periodical observations (Table I) revealed non-significant differences in per cent mycelial growth in inoculated and uninoculated bags at casing time, indicating that no damage by the nematodes had occurred up to this time in the bags inoculated with either species of nematode at spawning. However, a significant decline in per cent mycelial growth was recorded in all the inoculated bags at pinhead formation in comparison to the uninoculated control, which showed 100% mycelial impregnation. Per cent mycelial growth figures of 87.4 and 88.4 recorded in the bags inoculated with *A. swarupi* and *A. avenae,* respectively, at spawning, were similar. The bags inoculated with *A. swarupi* and *A. avenae* at casing exhibited mycelial growths of 90.2 and 90.4%, respectively, which were similar but significantly greater than the mycelial growth attained in the bags inoculated at spawning.

Effect on total sporophore yield. Data on weekly and total sporophore yields in the presence of the test nematodes produced some interesting results (Table II). Although a significant decline in sporophore yields was recorded in the bags inoculated with nematodes as compared to the control, total production showed non-significant variations among the treatments receiving nematode inocula irrespective of inoculation stage and nematode species involved. The total sporophore yields of 1597.4, 1648.8, 1687.4 and 1675.0 g attained in bags inoculated with *A. swarupi* at spawning and casing and *A. avenae* at spawning and casing, respectively, were statistically similar. Also, non-significant differences in weekly sporophore yields up to the fourth week were recorded among the inoculated treatments. The sporophore yields in the fifth, sixth and seventh weeks, in nematode inoculated treatments, differed from yields from uninoculated bags, but the differences were not significant among the inoculated treatments.

Aphelenchoides swarupi caused sporophore yield losses of 15.7 and 13.0% when inoculated at spawning and casing time, respectively. The corresponding losses caused by *A. avenae* were 11.0 and 11.6%.

Effect on flush pattern. Calocybe indica (Table II) showed no disturbance in weekly yield pattern until the fourth week after crop initiation in any of the treatments when compared with the control. Sporophores appeared in successions of low and high yields in all the treatments, including the control, with spectacular overlapping peaks, which meant that there were no difference in their yields until the fifth week. Thereafter, production of fruiting bodies declined continuously up to the seventh week in all the treatments, including the control, but this decline was greater in nematode inoculated bags compared to uninoculated bags. The yield declines observed among the inoculated treatments were similar for the total crop duration.

Table IV. Correlations between sporophore yield, mycelial growth, and nematode population in *C. indica*.

	Yield	Mycelial growth at casing	Mycelial growth at pinhead	Nematode population at casing	Nematode population at pinhead
Yield	1.0000				
Mycelial growth at casing	0.4688*	1.0000			
Mycelial growth at pinhead	0.5702*	0.3733	1.0000		
Nematode population at casing	-0.9235*	-0.5113*	-0.4849*	1.0000	
Nematode population at pinhead	-0.8836*	-0.5195*	-0.6042*	0.8709*	1.0000

*Significant at 5% level of confidence

Effect on nematode multiplication. The data regarding the multiplication of *A. swarupi* and *A. avenae* that were recorded at casing, pinhead formation and the end of cropping are in Table III. Extremely low populations of *A. swarupi* and *A. avenae* were observed in bags inoculated at spawning. Nematode multiplication picked up thereafter and reached the respective levels of 44.3× and 14.7× at pinhead formation stage. At this stage, the largest population was recorded in bags inoculated with *A. swarupi* at spawning (885 specimens/200 cm^3 compost) and the smallest in bags inoculated with *A. avenae* at casing (95 specimens/200 cm^3 compost), and these were significantly different from each other. *Aphelenchus avenae* multiplied comparatively slowly and totals of 0.845×10^3 and 0.338×10^3 nematodes/200 cm^3 compost were recovered from spawning and casing time inoculations, respectively, at crop termination. The greatest multiplication rate of 319× by crop termination was recorded with *A. swarupi* inoculated at spawning time. The multiplication rate of *A. avenae* was appreciably lower, reaching only 42.3× at crop termination.

Correlation between nematode population, mycelial growth and sporophore yield. Correlation analysis showed a positive relationship between mycelial growth and sporophore yields in milky mushroom irrespective of the stage of mycelial growth (Table IV). Inverse relationship were found between nematode population and mycelial growth, and nematode population and sporophore yields.

Effect on flush gap period. A normal flush gap period in *C. indica* was found to be of 6-9 days up to the sixth week in uninoculated bags (Table V). The presence of *A. swarupi* did not hamper the flush gap appreciably as fruiting bodies appeared at intervals of 5-8 days up to the fifth week. The interval was reduced to 3-4 days thereafter, until the termination of the experiment in the seventh week. Sporophores appeared later by two days in both the treatments with *A. swarupi* as compared to control and the cropping ceased three days earlier. This represented a shortening of the total cropping duration by five days. A flush gap of 6-9 days was maintained in the bags inoculated with *A. avenae* up to the fifth week irrespective of the time of inoculation. This gap period was similar to that of the uninoculated control. Fruiting bodies emerged at gaps of 3-5 days after the fifth week. The cropping duration was shortened by 3-4 days in treatments with *A. avenae*.

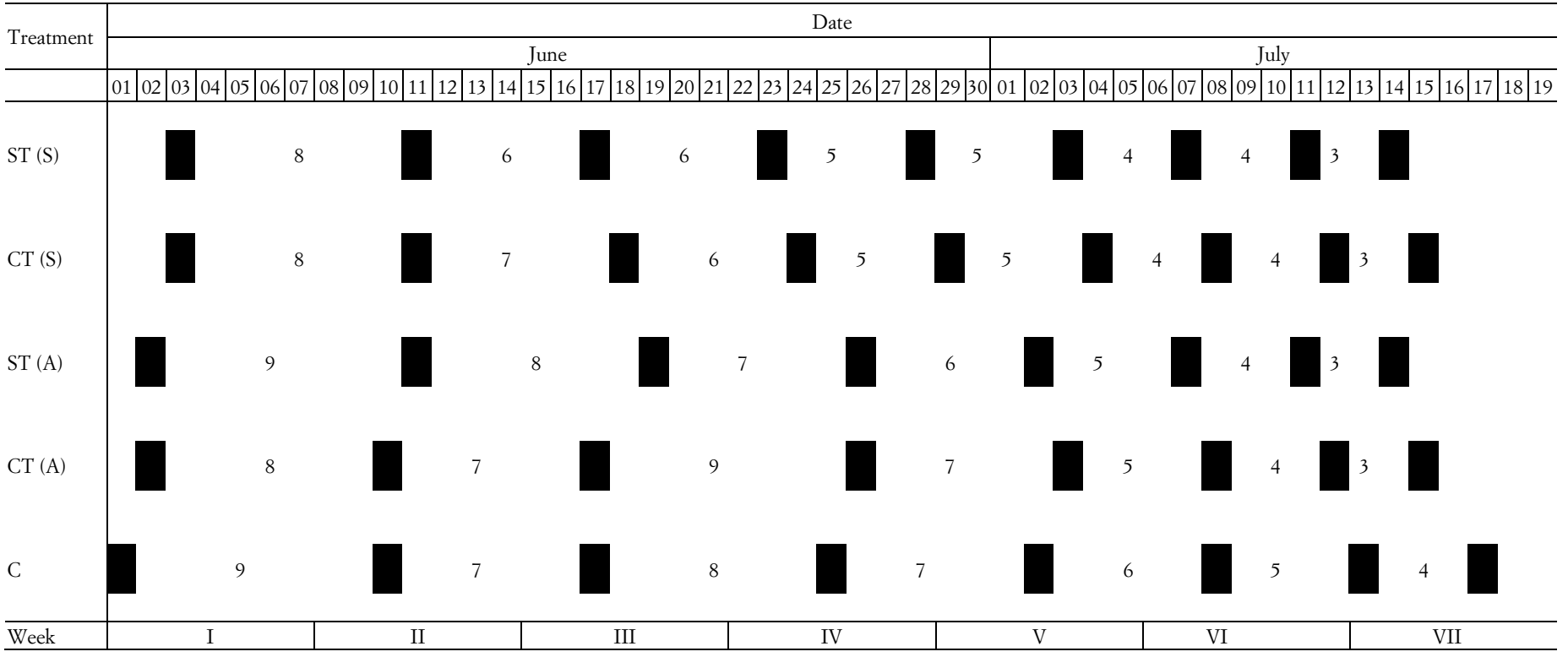
Visual symptoms on sporophores. Besides quantitative losses, these nematodes also caused qualitative deterioration in the infested mushrooms. The surface of heavily infested compost sank and became soggy. The infested pinheads turned brownish and water soaked. Such pinheads dried prematurely and fruiting bodies never emerged out of them. Their gills and stipes showed browning and were not fit for consumption. These sporophores were prone to attack by secondary fungal pathogens and succumbed quickly. When such symptomatic fruiting bodies were cut open and washed to flush out any nematodes that were present, nematodes were observed.

DISCUSSION

This is the first investigation of the damage potential of nematodes against milky mushroom, *C. indica*. These results find considerable support from earlier findings in which less mycelial depletion was observed in *C. indica* when the nematode species under investigation were inoculated at spawning rather than casing time. However, in *A. bisporus*, both of the nematode species tested here, when inoculated at spawning time, caused more mycelial depletion (Kumar, 2006; Kumar *et al*., 2007).

The yield losses caused by both species of nematodes in *C. indica* in our tests were similar irrespective of the time of inoculation, the per cent yield losses being in the range 11.0-15.7 when the nematodes were inoculated at spawning or casing time. Per cent yield losses in *A. bisporus* were greater and ranged from 42.6 to 80.3 at similar inoculation times (Kumar, 2006). Previous studies on yield losses recorded declines in sporophore production of *A. bisporus* of 89.0, 95.0 and 27.0% with spawning time inoculations of *Ditylenchus myceliophagus*, *Aphelenchoides composticola* and *A. avenae*, respectively, as compared to the corresponding losses of 40.0, 64.0 and 21.4% for casing time inoculation, thus highlighting the

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



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			

role of time of inoculation (Khanna, 1991, 1993; Khanna and Jandaik, 2002). The observations recorded on *A. avenae* during the present investigations were in accordance with observations of Hooper (1962) and Gitanjali and Nandal (2001), who reported yield losses due to this nematodes, but at variance to those 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 in *A. bisporus* due to *A. avenae* with inoculations at different spawning times.

A slight reduction in the total cropping period of *C. indica* was recorded in nematode-treated bags as compared to the control in our experiment. Reports of delay in sporophore appearance and disruption of the flush pattern due to saprophagous nematodes like *Caenorhabditis elegans* and *Panagrolaimus fuchsi* have been published earlier (Grewal, 1991; Grewal and Richardson, 1991; Chandran, 2000; Khanna and Kumar, 2005; Kumar, 2006). Our observations provided evidence of *C. indica* being susceptible to both species of nematodes tested, the level of susceptibility being the same for both. However, the high temperature requirement of this mushroom saved it from nematode damage to a great extent. *Calocybe indica* requires a spawning time temperature of 33±1 °C and cropping temperature of 30±1 °C. Such a high spawning time temperature is not conducive to nematode reproduction despite the availability of ample food in the form of mycelium. The high temperature during the cropping phase allowed limited multiplication of the aphelenchids, which have optimum development temperatures between 23 and 28 °C and which do not normally reproduce at temperatures above 30 °C (Cayrol, 1967; Khanna and Sharma, 1989; Tan *et al.,* 1992).

The information we have provided showed that nematodes inoculated at spawning did not cause any significant mycelial depletion in *C. indica*. Feeding by nematodes occurred only after casing and, thus, significant mycelial depletion was observed at pinhead formation.

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