J. Agric. Sci. Technol. (2008) Vol. 10: 165-171

Reproduction of the White Tip Nematode (*Aphelenchoides besseyi* Christie, 1942) in Different Monoxenic Cultures

S. Jamali[1], E. Pourjam[2]*, A. Alizadeh[2] and F. Alinia[3]

ABSTRACT

The reproductive range of the Iranian population of white tip nematode in rice, *Aphelenchoides besseyi,* was investigated *in vitro* to find out a suitable medium as well as a favorable fungal host for monoxenic culturing this nematode. Studies were carried out on rice seed associated fungi, pathogenic fungi and one mushroom (*Agaricus bisporus*) grown on three culture media, RPA (rice polish agar), OMA (oat meal agar) and PDA (potato dextrose agar). The nematode showed the greatest multiplication on *Fusarium verticillioides, F. proliferatum, Curvularia lunata* and *Magnaporthe salvini* in OMA and on *Alternaria alternata, Bipolaris oryzae* and *Pyricularia oryzae* in PDA. Among the fungi, tested *A. alternata, C. lunata, F. verticillioides, B. oryzae, M. salvini, F. proliferatum* and *P. oryzae* supported a high reproduction rate in the nematode in a descending rank. The nematode failed to multiply on *Aspergillus niger, Rhizoctonia solani* and *Agaricus bisporus* grown on any of the three media. The highest sex ratio (F:M) was achieved on OMA but the highest male percentage ratio was observed on PDA. The two pathogenic fungi, *B. oryzae* and *M. salvini* are reported as new fungal hosts for monoxenic culturing of this nematode.

Keywords: *Aphelenchoides besseyi*, Fungi, Host range, Monoxenic culture, Reproduction.

INTRODUCTION

Nematodes are important for the decomposition of and nutrient recycling in soil. Nematode species have a wide range of food preferences and knowledge of their feeding habits is essential to understand their biology and role in ecosystem processes. Fungus feeding nematodes rear on many different species of fungi, including saprophytic, pathogenic and mycorrhyzal fungi (Freckman and Caswell, 1985; Giannakis and Sanders, 1989; Ruess and Dighton, 1996). Culturing of nematodes on fungal species could provide a suitable means for obtaining enough pure population for further investigations.

Among the seed-borne pathogens of rice, *Aphelenchoides besseyi* Christie, 1942 is considered to be a major problem, causing white tip disease which is widely distributed throughout almost all the rice growing regions of the world and results in low yields and deteriorated seed quality (Rajan and Mathur, 1990). This nematode is not only a parasite of higher plants but it can also feed on fungi. Many fungal species can support the population growth of *A. besseyi in vitro*. The feeding and reproduction of this nematode on *Fusarium solani* (Huang *et al*., 1973), *Aureobasidium pullulans* (Huang *et al*., 1979), *Alternaria tenuis* (Todd and Atkins, 1958) and *Alternaria alternata* (Rajan *et al*., 1989) have already been reported. Rao

1. Department of Plant Pathology, College of Agriculture, Guilan University, Rasht, Islamic Republic of Iran.
2. Department of Plant Pathology, College of Agriculture, Tarbiat Modares University, P. O. Box 14115-336, Tehran, Islamic Republic of Iran.
3. Rice Research Institute, Rasht, Islamic Republic of Iran.
* Corresponding author, e-mail: pourjame@modares.ac.ir

[DOR: 20.1001.1.16807073.2008.10.2.3.7]

(1985) studied the population build up of white tip nematode in different monoxenic cultures and found that among various media examined, oat meal agar supported the highest population increase in the nematode. The population was highest on *Fusarium moniliforme*, followed by *Alternaria padwickii*, *Helminthosporium oryzae* and *Curvularia* sp., but the nematode failed to multiply on *Pyricularia oryzae*. In an other investigation, populations of *A. besseyi* from different hosts and various geographical areas were evaluated for their mode of reproduction and host range. It was determined that different populations exhibited parthenogenetic and amphimictic modes of reproduction (Gokte-Narkhedkar-Narkhedkar *et al*., 2001).

There is no information available on the reproduction of this nematode on RPA (rice polish agar) medium, a new substrate for monoxenic culturing; RPA contains rice polish as a natural substrate of the host in contrast to PDA (potato dextrose agar) and OMA (oat meal agar). Present attempt was made to study population build up of adults, juveniles and sex ratio of white tip nematode on different monoxenic cultures including rice seed associated fungi, pathogenic fungi and a mushroom, using OMA, RPA and PDA as media.

MATERIALS AND METHODS

Preparation of Fungal Isolates

Rice seeds were collected from rice fields in Guilan, Mazandaran and Golestan Provinces and were cultured on PDA medium. Five fungal species i.e. *Fusarium verticillioides*, *F. proliferatum*, *Alternaria alternata*, *Curvularia lunata* and *Aspergillus niger* were isolated and purified. The fungi were originally recovered from the same sites where the nematode was present. They were frequently found associated with paddy seeds sampled from different regions during cultural practices. In addition, four pathogenic fungi, *Bipolaris oryzae* (Brown Spot), *Magnaporthe salvini* (Stem Rot), *Pyricularia oryzae* (Blast) and *Rhizoctonia solani* (Sheath Blight) recovered from infected plants and one isolate of *Agaricus bisporus* obtained from the culture collections of the Plant Pathology Department of Tarbiat Modares University, were used in this study.

Preparation of Nematodes

Aphelenchoides besseyi was isolated from infected seeds collected from rice fields in Guilan Province, northern Iran. Nematodes were extracted following Coolen and D'Herde's method (Coolen and D'Herde, 1972), sterilized in a 1,000 ppm streptomycin sulfate solution for five minutes and then rinsed with sterilized water several times (Moore *et al*., 1985).

Experiments

Twenty pairs (female and male) of nematode were transferred to each Petri dish containing Purified fungus. The nematodes were released onto the Petri dish when 1/3 surface of the plate was colonized by the fungus mycelium. The plates were sealed with parafilm, kept in plastic bags and incubated at 28ºC in darkness (Rao, 1985). The nematodes were harvested four weeks after inoculation and the population recorded. For this purpose, each Petri dish lid was initially removed and the colony surface was thoroughly washed into a container then the medium was sliced and processed by a modified Baermann funnel technique (Hooper, 1990) for 48 hours. Total populations of nematodes in this water suspension were then counted in a counting dish under a stereomicroscope.

To examine the sex ratio of the nematode in the culture media, only the adults were counted and recorded. The multiplication factor of nematodes (Final nematode population/Initial inoculum level) was calculated for each host fungus.

[DOR: 20.1001.1.16807073.2008.10.2.3.7]

The experiment was conducted in a completely randomized design with two factors, three media and ten fungi, using four replicates and repeated twice. Transformed data on nematode numbers (mean of eight replicates derived from two experiments) were analyzed and the means were compared using Duncan's multiple range and LSD tests.

RESULTS

In total, ten different fungal species were tested for their capability to support the population build up of the fungal feeder nematode, *A. besseyi*, using three culture media. Four weeks after incubation, nematode mass cultures were established on *A. alternata, F. verticillioides, C. lunata, B. oryzae, M. salvini, F. proliferatum* and *P. oryzae*. Infrequent or no survival of nematodes was observed on *A. niger, A. bisporus* and *R. solani* (Table 2 and Figure 1). The highest reproduction rate was observed on *A. alternata* with 16,737 nematodes/plate. The lowest population was obtained from *P. oryzae* grown on OMA (27) and the remaining ranged from 11,137 to 274 nematodes/plate. Figure 1 shows the clusters of nematode rearing on *A. alternata* in the plate. The results indicated a varying degree of reproduction of *A. besseyi* rearing on different fungal species (Table 2). *A. alternata, C. lunata, F. verticillioides, B. oryzae, M. salvini, F. proliferatum* and *P. oryzae* had the highest average of nematode numbers in descending order (Table 2). A summary of mean comparison of nematode populations reared on various fungi and different culture media using Duncan's multiple range tests is presented in Table 2. Differences between

Table 1. Analysis of variance (ANOVA) of population growth of *Aphelenchoides besseyi*.

SOURCE	DF	SS	MS	F Value
Fungus	9	297005.73	33000.63	2629.7**
Medium	2	9421.74	4710.87	375.39**
Fungus × Medium	18	24504.78	1361.37	108.48**
Error	210	2635.32	12.54	
Total	239	333567.58		
C.V.= 9.19 %				

** indicating significant differences (P= 0.01).

Table 2. The ranges of population increase of *Aphelenchoides besseyi* reared on different fungal species grown in three culture media.

Fungal species	OMA	PDA	RPA	Total mean
Alternaria alternata	105.53 [a] a	129.37 a	92.08 a	109 a
Curvularia lunata	75.54 b	64.12 b	52.97 b	64.21 b
Fusarium verticillioides	104.7 a	46.97 d	36.57 c	62.75 b
Bipolaris oryzae	58.49 d	62.05 b	55.45 b	58.66 c
Magnaporthe salvini	66.52 c	54.12 c	51.36 b	57.33 c
F. proliferatum	34.69 e	27.77 e	11.29 d	24.58 d
Pyricularia oryzae	5.24 f	16.56 f	0.7 e	7.5 e
Agaricus bisporus	0.7 g	0.7 g	0.7 e	0.7 f
Aspergillus niger	0.7 g	0.7 g	0.7 e	0.7 f
Rhizoctonia solani	0.7 g	0.7 g	0.7 e	0.7 f
CD [b] to compare populations of the nematode at 1% level= 4.54				
CD to compare populations of the nematode at 5% level= 3.46				

[a] Data are transformed ($\sqrt{X+0.5}$) means of eight replicates.

Figures in each column with the same letter are not significantly different (P= 0.01).

[b] Critical difference between two populations of the nematodes.

[DOR: 20.1001.1.16807073.2008.10.2.3.7]

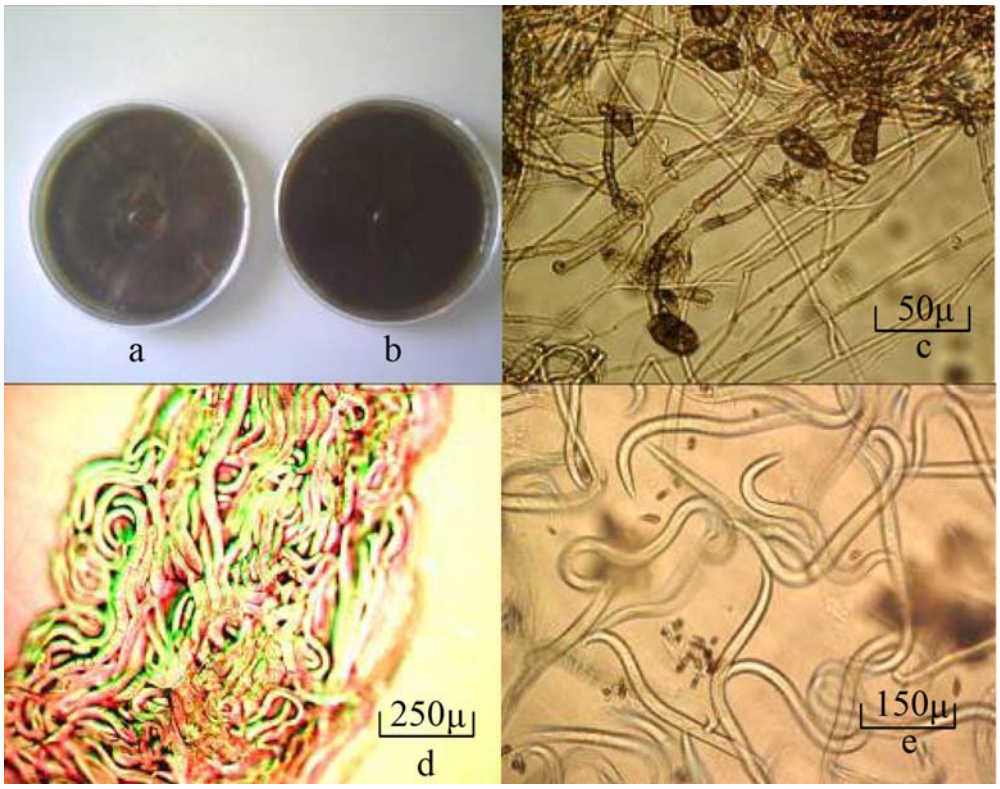

Figure 1. (a and b) *Alternaria alternata* plates without and with nematode (Darker); (c) Conidiophores and conidia of *A. alternate* and (d and e) *Aphelenchoides besseyi* growing on *A. alternata*.

the two nematode populations can also be compared with CD at both levels of probability (Table 2) and was found to be significant if was greater than CD in the LSD test. The results for the sex ratio of adults of *A. besseyi* reared on fungal hosts grown on RPA, OMA and PDA and the calculated multiplication factor are summarized in Table 3. According to the ANOVA test, there was significant difference in reproduction rate among the ten fungal species and three culture media. Furthermore, the results indicated a significant interaction between fungus and medium (Table 1).

The results revealed that the nematode showed greatest multiplication on *Fusarium verticillioides, F. proliferatum, Curvularia lunata* and *Magnaporthe salvini* in OMA and on Alternaria alternata, Bipolaris oryzae and Pyricularia oryzae in PDA. Among the culture media examined, the lowest population of adults and juveniles was observed on RPA (Table 2). *P. oryzae*, as an important pathogenic fungus of rice, exhibited variable results in supporting the nematode population on various media. While the nematode supported population growth on PDA, it was able to survive on OMA, as indicated by the presence of adults, but it failed to multiply on RPA.

The highest multiplication factor (418 times) was obtained on *A. alternata* grown in PDA and the lowest (0.7 time) on *P. oryzae* grown in OMA, respectively (Table 3). The female population in the harvested nematodes was highest on OMA, whereas the highest male ratio was observed on PDA (Table 3).

[DOR: 20.1001.1.16807073.2008.10.2.3.7]

Table 3. Sex ratio and multiplication factor of *Aphelenchoides besseyi* reared on different fungal species grown in three culture media.

Fungal species	PDA		OMA		RPA	
	Sex ratio (F:M)[a]	Multiplication factor	Sex ratio (F:M)	Multiplication factor	Sex ratio (F:M)	Multiplication factor
Alternaria alternata	4.1:1	418	8.5:1	278	7.1:1	212
Bipolaris oryzae	4.0:1	96	8.2:1	86	6.8:1	77
Curvularia lunata	4.4:1	103	8.6:1	143	7.0:1	70
Fusarium verticillioides	3.9:1	55	8.1:1	274	7.3:1	33
F. proliferatum	3.9:1	19	8.3:1	30	7.2:1	3
Magnaporthe salvini	4.4:1	73	8.3:1	11	7.0:1	66
Pyricularia oryzae	4.7:1	7	8.0:1	0.7	-[b]	-
Agaricus bisporus	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-	-

[a] F= Female, M= Male, [b] No multiplication and survival.

DISCUSSION

This study showed that the food source has an important impact on population growth in the nematode. The feeding preference of *A. besseyi* could be evaluated on the basis of the adult nematode population recovered from different fungal colonies. A clear influence of fungal species on the multiplication rate of *A. besseyi* was discernible (Table 2). *Alternaria alternata* was the most favorable fungus, supporting a higher nematode population. The results on population growth of the nematode reared on *A. alternata* grown on different media, revealed that PDA was a suitable substrate for the fungus. Multiplication of this nematode has been recorded on different fungi, but none has provided as high as a 418 times increase in the number of adults and juveniles as noted in the present investigation. In previous studies, the highest record of the population increase was 354 times (Rao, 1985). Therefore, this host (*A. alternata* on PDA) can be a good alternative for mass production of the nematode in the laboratory for biological, ecological, epidemiological and molecular studies. *Aureobasidium pullulans*, *A. tenuis*, *A. alternata* and *F. solani* have previously been used for mass production of the nematode (De Waele, 2002). In this study *Aspergillus niger*, *A. bisporus* and *R. solani* were found to be non-hosts for the nematode. It can be assumed that *A. niger,* despite being a cosmopolitan saprophytic fungus, plays no significant role on the reproduction and survival of this nematode in nature. On the other hand, *A. besseyi* is not considered to be a pest for *A. bisporus* in mushroom cultivations. The causal agent of blast disease, *P. oryzae*, supported a very low population, whereas multiplication of the nematode on the other fungi in this study was intermediate.

B. oryzae and *M. salvini* supported a relatively good multiplication of the *A. besseyi* population and are reported as new hosts. The three fungi species, *P. oryzae*, *B. oryzae* and *M. salvini* could be used to study the interaction between the nematode and rice pathogenic fungi. Differences between populations of the nematode on each fungus when compared on three culture media exhibited varying reproduction rates which were clearly influenced by the fungal cultures as well as by the type of culture media used. Similar findings were reported by Gokte-Narkhedkar-Narkhedkar and Mathur (1989). In another investigation using different culture media and fungal species, the

[DOR: 20.1001.1.16807073.2008.10.2.3.7]

highest multiplication rate of this nematode was achieved when *A. besseyi* was grown on 2% OMA and *F. moniliforme* (Rao, 1985). It seems that the effectiveness of a monoxenic host fungus on population increase is dependent on the type of culture medium used. In our studies, OMA was proven to be a better substrate for multiplication of the nematode on *F. verticillioides* than on RPA and PDA (Table 2). The results of our study are in agreement with those of Rao (1985) and Gokte-Narkhedkar-Narkhedkar and Mathur (1989).

In this work, the culture media showed definite effects on the sex ratio of the nematode as recorded on these fungal cultures. According to our data, the sex ratio ranges were highest on OMA (8-8.6) and lowest on PDA (3.9-4.7) (Table 3). The multiplication factor can also be used as a good index of potential reproduction of the nematode in different cultures (Table 3).

RPA medium as a substrate containing natural compounds of rice, showed a low multiplication rate and was a poor substrate for nematode reproduction. It may suggested that the influence of the fungi on the development and reproduction of nematodes appears to be more important than the type of media used (Tables 1 and 3). The nematode failed to multiply and survive on *P. oryzae* when grown on RPA, because this medium induced more sporulation in the fungus and it seems that the nematode prefers mycelia for feeding rather than conidia. Our findings proved the previous results (Rao, 1985; Gokte-Narkhedkar-Narkhedkar and Mathur, 1989; Rajan and Mathur, 1990; Gokte-Narkhedkar-Narkedkar *et al*., 2001) that the fungus species has an important function and must be taken into consideration in determining the reproduction rate of *A. besseyi*.

ACKNOWLEDGMENTS

The authors would like to thank the Rice Research Institute, Eng. Moosanejad for providing the necessary facilities and Dr. Mohammadi Goltapeh for providing mushroom isolate.

REFERENCES

1. Coolen, W. A. and D'Herde, C. J. 1972. *A Method for the Quantitative Extraction of Nematodes from Plant Tissue*. State Agriculture Research Centre, Ghent, Belgium, 77pp.
2. De Waele, D. 2002. Foliar Nematodes: *Aphelenchoides* Species. In: *Plant Resistance to Parasitic Nematodes*. Starr, J. L., Cook, R. and Bridge, J. (Eds). CAB International Publishing, UK, pp. 141-151.
3. Freckman, D. W. and Caswell, E. P. 1985. The Ecology of Nematodes in Agroecosystems. *Ann. Revi. of Phytopathol.*, **23**: 275-296.
4. Giannakis, N. and Sanders, F. E. 1989. Interactions between Mycophagous Nematodes, Mycorrhizal and Other Soil Fungi. *Agric. Ecosyst. Environ.*, **29**: 163-167.
5. Gokte-Narkhedkar-Narkhedkar, N. and Mathur, V. K. 1989. Reproduction and Development of *Aphelenchoides besseyi. Phytoparasitica*, **17**: 263-267.
6. Gokte-Narkhedkar-Narkhedkar, N., Mathur, V. K., Ramasundaram, P. and Sabesh, M. 2001. Reproductive Variations in *Aphelenchoides besseyi* Populations. *Indian J. Nematology*, **31**: 115-119.
7. Hooper, D. J. 1990. Extraction and Processing of Plant and Soil Nematodes. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. Luc, M., Sikora, R. A. and Bridge, J. (Eds). CAB International Publishing, UK, pp. 45-68.
8. Huang, C. S., Huang, S. P. and Chiang, Y. C. 1979. Mode of Reproduction and Sex Ratio of Rice White-tip Nematode, *Aphelenchoides besseyi*. *Nematologica*, **25**: 255-260.
9. Huang, C. S., Huang, S. P. and Lin, L. H. 1973. The Effect of Temperature on Development and Generation Periods of *Aphelenchoides besseyi*. *Nematologica*, **18**: 432-438.
10. Moore, J. C., Trofymow, J. A. and Morley, C. R. 1985. A Technique to Decontaminate Soil Microarthropods for Introduction to Gnotobiotic Systems. *Pedobiologia*, **28**: 185-190.
11. Rajan, A., L. and Mathur, V. K. 1990. Host Range and Morphological Studies on Four Isolates of *Aphelenchoides besseyi* Christie. *Indian J. Nematology*, **20**: 177-183.

[DOR: 20.1001.1.16807073.2008.10.2.3.7]

12. Rajan, A., L., Mathur, V. K. and Lal, A. 1989. Improved Culturing Technique for *Aphelenchoides besseyi* by Inducing Anhydrobiosis. *Indian J. Nematology*, **19**: 10-13.
13. Rao, J. 1985. Population Build-up of White-tip Nematode (*Aphelenchoides besseyi* Christie, 1942) in Different Monoxenic Cultures. *Oryzae*, **22**: 45-49.
14. Ruess, L. and Dighton, J. 1996. Cultural Studies on Soil Nematodes and Their Fungal Hosts. *Nematologica*, **42**: 330-346.
15. Todd, E. H. and Atkins, J. G. 1958. White-tip Disease of Rice. I. Symptoms, Laboratory Culture of Nematodes and Pathogenicity Test. *Phytopathology*, **48**: 632-637.

تکثیر نماتد نوک سفیدی برگ برنج (*Aphelenchoides besseyi* Christie, 1942) بر روی محیط های خالص

س. جمالی، ا. پورجم، ع. علیزاده و ف. علی نیا

چکیده

زادآوری جمعیت *Aphelenchoides besseyi* جداشده از شمال کشور، تحت شرایط آزمایشگاه بررسی شد و محیط کشت و قارچ مناسب برای تولید انبوه این نماتد مورد مطالعه قرار گرفت. مطالعه بر روی ده قارچ شامل قارچ‌های جدا شده از بذر، قارچ های مهم بیماریزا و یک گونه قارچ خوراکی (*Agaricus bisporus*) با استفاده از سه محیط کشت RPA، OMA و PDA صورت گرفت. بر اساس نتایج به دست آمده، محیط کشت OMA بهترین محیط برای تکثیر نماتد *A. besseyi* بر روی قارچ‌های *Curvularia lunata*, *Fusarium verticillioides*, *F. proliferatum* و *Magnaporthe salvini* می‌باشد. درحالیکه مناسبترین محیط برای تولید مثل این نماتد بر روی قارچ‌های *Alternaria alternata*, *Bipolaris oryzae* و *Pyricularia oryzae* محیط PDA است. در بین قارچ‌های مورد بررسی، بیشترین میزان تولید مثل *A. besseyi* به ترتیب بر روی *A. alternata*, *C. lunata*, *F. verticillioides*, *B. oryzae*, *M. salvini*, *F. proliferatum* و *P. oryzae* مشاهده شد. این نماتد برروی قارچ های *Aspergillus niger*, *Rhizoctonia solani* و *Agaricus bisporus* در تمامی محیط‌های کشت، فاقد قدرت تکثیر بود. بالاترین نسبت جنسی (ماده به نر)، متعلق به محیط OMA و بیشترین درصد نماتد نر بر روی محیط PDA مشاهده گردید. دو قارچ بیمارگر برنج، *B. oryzae* و *M. salvini* به عنوان میزبانهای قارچی جدید نماتد *A. besseyi* گزارش می‌شوند.

[DOR: 20.1001.1.16807073.2008.10.2.3.7]