KAVAKA 41: 96-102 (2013)

Present status of management of plant parasitic nematodes using Nematophagous fungi

N. G. Ravichandra[1] and Umarao[2]*
[1]Division of Nematology, Indian Agricultural Research Institute, New Delhi-110012
[2]*Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore
(Received on 23 November 2013; Accepted on 31 December 2013)

ABSTRACT

More than 200 known species of nematophagous fungi differ in their parasitic ability. Although many of the trap-forming and egg-parasitic fungi have been shown to survive in soil saprophytically, the endoparasites are mostly dependent on nematodes for nutrients. Molecular data have greatly improved the understanding of the function of the fungi in respect of their taxonomy, physiological/biochemical activity and ecology, including their function as biocontrol agents. Despite much research, the development of biological control agents for nematode management has proved difficult and although some commercial products have been developed, none is in widespread use. Research has concentrated mostly on the use of nematode-trapping fungi for the control of cyst and root-knot nematodes. *Paecilomyces lilacinus* and *Pochonia chlamydosporia* which attack nematode eggs have been extensively studied. Nematophagous fungi possessing the capacity to destroy or deleteriously affect nematodes vary considerably in both their biology and taxonomic relationships. They range from obligate, endoparasitic forms, many of which are zoosporic, to predacious trap-forming species and opportunistic fungi that colonize reproductive structures. They mostly belong to three phyla, Zygomycetes, Ascomycetes, and Basidiomycetes. Nematode-trapping Hyphomycetes (teleomorph: Orbiliaceae) contain the largest group of predaceous fungi. The systematic classification of nematode-trapping fungi is redefined based on phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA and â-tubulin genes. Major mechanisms of nematode management include mycoparasitism, antibiosis, competition, tolerance to biotic and abiotic stresses through enhanced root development, solubilization of inorganic plant nutrients and induced resistance.

Keywords: Parasitic nematodes, nematophagous, fungi

INTRODUCTION

Nematode management using fungal bioagents is an exciting and rapidly developing research area with implications for plant productivity, animal and human health and food production. This area includes a number of important disciplines, such as pathology, ecology, genetics, physiology, mass production, formulation and application strategies. The research, development and final commercialization of nematophagous fungal agents continue to confront a number of obstacles, ranging from elucidating important basic biological knowledge to socio-economic factors. Considerable advances have been made in separate areas but it is important to integrate and communicate these new findings.

The diversity in species of phytonematodes, their ubiquity and abundance in soils provide an opportunity for evolution of intimate and complex interaction among kinds of soil fungi. Nematophagous fungi are natural enemies of nematodes and play a vital role in the natural decline of nematode populations. Their potential for use as biological agents has remained uncertain for a long time, but recent advances in understanding the complex ecological factors that affect the behaviour of these natural enemies have cleared major doubts in the biomanagement of nematodes. Nematophagous fungi are micro fungi that can capture, kill and digest nematodes. They use special mycelial structures, the so-called traps, or spores to trap vermiform nematodes or hyphal tips to attack nematode eggs and cysts before penetration into the nematode cuticle, invasion and digestion (Butt et al., 2001). More

*Corresponding author. Email: umanema@gmail.com

than 200 known species of these potential fungi differ in their parasitic ability (Table 1). Although many of the trap-forming and egg-parasitic fungi have been shown to survive in soil saprophytically, the endoparasites are mostly more dependent on nematodes as nutrients. Molecular data have greatly improved our understanding of the function of the fungi in respect of their taxonomy, their physiological/biochemical activity and their ecology, including their function as biocontrol agents. The recognition of suppressive soils in which biotic factors prevent nematodes multiplying on susceptible crops has demonstrated that the biological control of nematodes has potential as a management strategy. Also, the environmental and health concerns over the use of some nematicides have led to increased interest in the development of strategies that integrate several control methods and reduce dependence on the use of chemicals. However, despite much research, the development of biological control agents for nematode control has proved difficult and although some commercial products have been developed, none is in widespread use. Research has concentrated on the use of nematode-trapping fungi for the control of cyst and root-knot nematodes, as these are the most economically important nematode pest species. However, these fungi proved difficult to manipulate and it was often not possible to ensure that they produced traps when the infective nematode juveniles were migrating towards roots.

Table 1: Major potential nematophagous fungi

Sl.No.	Nematophagous fungus	Behavioural Group	Infective Unit
1.	**Chytridiomycota:**	Endoparasite	Zoospore
	Catenaria anguillulae		
2.	**Oomycota:**		
	Nematophthora gynophila	Endoparasite	Zoospore
	Myzocytium humicola	Endoparasite	Adhesive zoospore cyst
3.	**Zygomycota:**		
	Stylopage and *Cystopage*	Predator	Adhesive hyphae
4.	**Mitosporic fungi:** Some of these have sexual stages in the Ascomycota (See Ascomycota below)		
	Arthrobotrys oligospora		
	Monacrosporium cionoparum	Predator	Adhesive nets
	Datylella brochopage	Predator	Adhesive branches
	Drechmeria coniospora	Predator	Constricting rings
	Hirsutella rhossiliensis	Endoparasite	Adhesive conidia
	Pochonia (Verticillium) chlamydosporium	Endoparasite	Adhesive conidia
	Dactylaria candida	Egg parasite	Hyphal invasion
		Predator	Adhesive knobs and non-constricting rings
5.	**Ascomycota:**		
	Atricordyceps (Sexual stage of *Harposporium oxycoracum*)	Endoparasite	Non-adhesive conidia
	Orbilia spp (Sexual stages of *Datylella*, *Arthrobotrys* and *Monacrosporium* spp.)		
6.	**Basidiomycota:**		
	Hohenbuehelia (gilled mushroom-the sexual stage of several *Nemactonus* species)	Predator	Adhesive conidia
	Pleurotus ostreatus (gilled mushroom)	Predator and toxin producer	Adhesive traps and toxic droplets

Two species of fungi, *Paecilomyces lilacinus* and *Pochonia chlamydosporia* (*Verticillium chlamydosporim)*, which attack nematode eggs, have been extensively studied for the control of several *Meloidogyne* species. Despite some early concern about human health risks associated with *P. lilacinus*, this fungus has been widely tested in the field and commercial products are available; presumably those isolates that have been collected from nematodes do not present a significant health risk. Inoculum produced on various grains has required large application rates and given variable results, and there is a need for improved formulations (Butt et al., 2001). Nematodes and several fungal antagonists interact in various ways and have been known to occur in agricultural soils. Nematode destroying fungi of diverse biology and affinities are ubiquitous in most soils an undoubtedly, in many instances, play a pivotal role in regulating nematode populations. The long convolution of fungi and nematodes within the restricted confines of the soil ecosystem results in widely differing biological interrelationships among various members of each group. Nematophagous fungi possessing the capacity to destroy or deleteriously affect nematodes vary considerably in both their biology and taxonomic relationships. They range from obligate, endoparasitic forms, many of which are zoosporic, to predacious trap-forming species and opportunistic fungi that colonize reproductive structures, like cysts and eggs.

ECOLOGY AND PHYSIOLOGY OF NEMATOPHAGOUS FUNGI

Fungi possess diverse strategies to obtain nutrients, as saprobes, pathogens (of plant and animal hosts), and symbionts (with other microbes, plants or animals). These strategies enable fungi to colonize a broad range of habitats on plants and animals (including humans), in agricultural and forest soils, and in aquatic environments. They can also be found in sites contaminated by heavy metals and grow on hypersaline microbial mats. They obtain nutrients in these diverse habitats by their highly variable structural and morphological forms, ranging from the unicellular yeast form to multicellular filamentous forms and a multitude of elaborate fruiting bodies.

Nematodes are among the most diverse and abundant groups of invertebrates and they have been found in the soil. Sandy biological crust, lead/zinc mines, saline and freshwater lakes and so on. The distribution of many soil nematode taxa is strongly influenced by factors, such as soil texture, soil temperature, and broad vegetation types. In soil, nematode individuals are found in the millions per m^2, and they exhibit a variety of feeding types and survival strategies. Many nematode species are parasites on plants or animals, some of them have become adapted to feeding on a range of plants found in their native habitat(s). Partly due to their limited dispersal abilities, relatively few species have been found to be obligate parasites with a narrow host range. However, they can cause huge losses in agricultural economy every year. Their agricultural significances have resulted in an increasing interest among scientists to use nematode-trapping fungi as biological control agents to control these parasitic nematodes. Among the multicellular fungi, there is a fascinating group that obtains their nutrients through predation and they include representatives from at least three phyla, Zygomycetes, Ascomycetes, and Basidiomycetes (Table 2).

Predaceous fungi are a special ecological group and include nematode-trapping fungi, endoparasitic fungi, opportunistic pathogenic fungi, and toxic fungi. Taxonomically, nematode-trapping Hyphomycetes (teleomorph: Orbiliaceae) contain the largest group of predaceous fungi. These fungi are predominantly soil-living organisms that have the ability to form infection structures, like adhesive hyphae, traps, and knobs, non-constricting rings and constricting rings to capture nematodes. The taxonomy and diversity of nematode-trapping fungi have been primarily studied using traditional morphological methods and rRNA sequences. These studies identified that the type of nematode-trapping devices was a highly reliable indicator about the evolutionary relationships among these species.

Isolates of *P. chlamydosporium*, even those collected from the same soil, differ greatly and must be carefully selected for introduction into soil as potential bioagents. Such tests enable many isolates to be eliminated before more time-consuming (and expensive) screens are conducted in nematode-infested soils (Kerry, 1998). Two species of fungi, *Paecilomyces lilacinus* and *P. chlamydosporium*, which attack nematode eggs, have been extensively studied for the control of several *Meloidogyne* species. Despite some early concern about human health risks associated with *P. lilacinus*, this fungus has been widely tested in the field and commercial products are available; presumably those isolates that have been collected from nematodes do not present a significant health risk. Of those that colonize nematode eggs, *P. chlamydosporium* is the most widely reported, but it is part of a complex of several closely related species, which include *P. chlamydosporia* var. *chlamydosporia, P. chlamydosporia,* var. *catenulatum, Verticillium suchlasporium*var. *suchlasporium, V. suchlasporium* var. *catenatum* and *Verticillium psalliotae.*

The efficiency of the fungus as a bioagent is affected by the susceptibility of the host plant, which influences the number of nematodes that invade the roots, the numbers becoming female and the size of the egg masses produced. Although much is known about the relationship between

Table 2 : Ecological characters of major nematophagous fungi

Group I		Group II	Group III	Group IV	Group V
Characters	Moderately fast growing, saprophytes, great predacious ability	Fast growing, good saprophytes, weak attractive, predacious ability	Moderately slow growing, weak saprophytes, moderate predacious ability	Slow growing, obligate parasites, great attractive ability	Slow growing, obligate parasites, great predacious ability
Example of nematophagous fungi	*Paecilomyces lilacinus, Dactlylella oviparasitica, Pochonia chlamydospor, Cladosporium herbarum*	*Arthrobotrys obligosporia*	*Dactylella candida*	*Drechemeria coniospora*	*Nematophthora gynophila*

specific root-knot nematode pest densities and yield loss, these relationships have rarely been considered in terms of their effects on the efficiency of fungal bioagents.

VARIABILITY IN NEMATOPHAGOUS FUNGI

As a result of environmental changes, temporary variations in the morphology of nematophagous fungi have been observed to which the fungi were subjected (Tarjan, 1961). Little work has been done on the activity of predacious fungi in soil under natural conditions. This is an important field of investigation in view of their possible utilization in the biological control of nematodes. Nematophagous fungi are poor competitors within the soil because the germlings that arise from their conidia are entirely dependent on captured nematodes for their development and subsequent survival. The majority of the fungi that produce traps do not do so when grown in pure culture and this is particularly true of those that produce 3-dimensional networks. Duddington (1955) found that a number of isolates of *Arthrobotrys robusta* and *Trichothecium cystosporium* showed a strong tendency to produce traps spontaneously in the absence of nematodes. *Trichothecium flagrans* lost the ability to produce traps, even in the presence of nematodes, within three months of its being isolated in pure culture.

The effect of environment on variability of *A. oligospora* indicated that the fungus, if heterocaryotic, had a nuclear ratio that was not easily altered. Anastomoses were observed to occur between the wild types of *A. oligo spora, A.* s*uperba* and *A. robusta.* The apparent ease with which anastomosis occurred between the three species suggested that they were probably strains of a single species. There were distinct differences between the protein patterns of the three species but the positions of between 25 and 43 per cent of their protein bands corresponded (Lobo, 1966). Traps were first produced by the mycelium that was 2 days old and the stimulus then spread throughout the colony along uninterrupted mycelial hyphae.

Low temperatures and pH values towards alkalinity enhance the trapping efficiency of *A. oligospora.* No traps were formed at 3OoC and 35 0 C. Weak Corn meal agar was found to be the best of 14 media tested for studies on the trapping activity of nematophagous fungi. Light affected trap production in *A. oligospora,* more traps being produced in the light than in the dark. No traps were produced on Potato dextrose agar, Yalt extract agar, r'lycophil agar, Chlamydospore agar, and Czapek Dox agar amended with yeast extract. Feder et al. (1960), from their work on *Dactylella doedycoides*, inferred that a fungus must be heterocaryotic to be able to produce its traps. Physiological age of a fungus culture affects its trapping efficiency, young networks being more sticky than older ones. Other factors influencing trap formations are temperature and type of medium a fungus is grown on (Tarjan, 1961).

TAXONOMY OF NEMATOPHAGOUS FUNGI

The majority of nematode-trapping fungi are Hyphomycetes, placed within the Orbiliales (Orbiliomycetes) based on morphological and/or molecular studies. Other examples include *Nematoctonus concurrens* Drechsler whose teleomorph belongs to the genus *Hohenbuehelia* (Basidiomycetes), which uses both adhesive traps and adhesive spores, while *Coprinus comatus* and *Pleurotus ostreatus* (Basidiomycetes) produce toxins from specialized hyphal stalks to immobilize and digest the nematode. Nematode-trapping fungi have been classified in a number of genera based on morphology of conidia (shape, septa and size) and conidiophores (branching, modifications of the apex). Traditional taxonomic concepts relied heavily on conidia and conidiophore morphology without taking in account the importance of trapping devices. This has led to a situation where species with diverse types of trapping devices have been assigned to one genus, while others with similar trapping devices can be found in different genera. A major prerequisite for fungal survival and activity in soil includes the diversity of trophic modes of nematophagous fungi (Xingzhong et.al., 2009).

The abundance and activity of *Hirsutella rhossiliensis* and *H. minnesotensis*, representatives of endoparasites and potential biocontrol agents against nematodes, are highly dependent on environmental factors. With molecular technology, traditional generic classification, generally based on the morphology of conidial characters, was challenged. Phylogenies based on rDNA sequences have indicated that trapping devices are more informative than other morphological characters in delimiting genera found that nematode-tapping fungi clustered into three lineages: species with constricting rings, species with various adhesive structures (net, hyphae, knobs and non-constricting rings) and species have no trapping devices. Based on results obtained from morphological and molecular characters, nematode-trapping fungi are classified into four genera: *Dactylellina,* characterized by stalked adhesive knobs including species characterized by non-constricting rings and stalked adhesive knobs; *Gamsylella,* characterized by adhesive branches and unstalked knobs; *Arthrobotrys,* characterized by adhesive networks; and *Drechslerella,* characterized by constricting rings.

Based on multigene data analyses, three genera, *Arthrobotrys*, *Dactylellina* and *Drechslerella*, are retained in the redefinition of the circumscription of three genera of nematode-trapping Orbiliaceous fungi. Species forming constricting rings (always with three cells) are placed in *Drechslerella. Arthrobotrys* is characterized by species forming adhesive networks. Taxa with unstalked adhesive knobs that grow out to form simple adhesive networks also are assigned to *Arthrobotrys*. Species that capture nematodes mainly by stalked adhesive knobs are placed in *Dactylellina*. Taxa with both nonconstricting rings and stalked adhesive knobs and taxa with unstalked adhesive knobs that grow out to form loops also are assigned to *Dactylellina.*

The systematic classification of nematode-trapping fungi is redefined based on phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA and â-tubulin genes (Yan Li et.al., 2001). *Arthrobotrys* is characterized by adhesive networks, *Dactylellina* by adhesive knobs, and *Drechslerella* by constricting-rings. Phylogenetic placement of taxa characterized by stalked adhesive knobs and non-constricting rings also is confirmed in *Dactylellina (Haard, 1968)*. Species that produce unstalked adhesive knobs that grow out to form loops are transferred from *Gamsylella* to *Dactylellina*, and those that produce unstalked adhesive knobs that grow out to form networks are transferred from *Gamsylella* to *Arthrobotrys. Gamsylella* as currently circumscribed cannot be treated as a valid genus. Predatory and non-predatory fungi appear to have been derived from non-predatory members of *Orbilia*. Based on their mode of action, nematophagous fungi may be classified into endoparasitic, predacious and opportunistic, which produce varied types of trapping devises like adhesive hyphae, non-modified, three dimensional adhesive nets, adhesive knobs, non-constricting rings and constricting rings.

Some promising fungal bio-agents: Include *Acrostalagmus, Harposporium* and *Meria (*Hyphomycetes) , *Catenaria, Myzocytium , Haptoglossa, Nematophthora* (Zoosporic forms), *Nematoctomus* (Basidiomycetes), *Pochonia chlamydosporia*:, *Hirsutella* sp.,*Paecilomyces lilacinus, Trichoderma viride.*

MECHANISMS OF NEMATODE MANAGEMENT:

i. **Parasitism:** *Trichoderma* is able to grow on the cyst surface and penetrate through the cyst wall and egg shell of cyst nematodes. The fungus has been reported to secrete many lytic enzymes. Chitinases of many *Trichoderma* spp. help parasitism of nematodes like *Meloidogyne* spp. (Lopez-Llorca et.al., 2008).

ii. **Antibiosis:** It produces antibiotics like trichodermin, dermadin, trichoviridin and sesquiterpene heptalic acid.

iii. **Competition:** Competition for space and/ or nutrients is an important mechanism of biocontrol. They have high rhizosphere competency and can easily colonize the roots. This may reduce the feeding sites for nematode.

iv. **Tolerance to biotic and abiotic stresses through enhanced root development:** The larger root system of plants colonized by *Trichoderma* could withstand the damaging effects of the nematodes more

v. **Solubilization of inorganic plant nutrients:** Enhanced nutrient uptake increases the tolerance of the host plant to pathogen attack.

vi. **Induced resistance:** Enzymes like peroxidase, polyphenoloxidase, phenylalanine ammonia lyase, catalase and chitinase are found to be induced in plants treated with *T.viride*.

5. **Endophytic fungi:** This group includes non-pathogenic and root infecting fungi and mycorrhiza. They are effective against endoparasitic migratory nematodes, may improve plant growth, able to be produced in mass, easy to formulate and used as seed /plant material treatment.

MOLECULAR ASPECTS OF NEMATOPHAGOUS FUNGI

Several reports are available on the molecular investigations carried out with regards to nematophagous fungi. Liamming Liang et.al., (2012) reported that cuticle-degrading proteases are involved in the breakdown of cuticle/eggshells of nematodes or insects, a hard physical barrier against fungal infections. Extraction and isoenzyme analysis of four isolates of *Arthrobotrys* including *A. musiformis*, *A. robusta* and *A. conoides* revealed that among the 14 enzymes studied by starch gel electrophoresis, using morpholine-citrate as gel/electrode buffer. Larriba et.al., (2012) noticed proteolytic enzymes such as the S8 proteases VCP1 and P32, secreted during the pathogenesis of nematode eggs and concluded that they are major virulence factors in *Paecilomyces lilacinus*. Fluorescent molecular probes were applied for detection of *Meloidogyne incognita* and *Pochonia chlamydosporia* var. *chlamydosporia* (Ciancio, 2005). A region in the *M. incognita* rDNA including ITS2 was selected for amplification and recognition with a real-time PCR assay, based on a combination of three specific motifs, each recognized by a specific fluorescent probe. The probes allowed recognition of single juveniles of *M. incognita* and of the mycelium- or soil-extracted fungal DNA.

Wei et al., (2009) isolated 137 fungal strains from females and eggs of *Meloidogyne* spp. and examined their *in vitro* protease production and chitinase activities. Kano et al., (2004) examined the morphology, physiology and molecular characteristics of *Monacrosporium megalosporum* . Yang Jinkui et al., (2006) were first to describe the purification and cloning of an infectious protease from a nematode-trapping fungus. The nematode-trapping device of this fungus is a three-dimensional network. The phylogenic relationship of the nucleotide sequence of the rDNA ITS region was close to those of *M. thaumasium* and *Geniculifera eudermata,* which also have nematode-trapping devices that are three-dimensional networks. The production of an extracellular serine protease by *Dactylella shizishanna* and its potential as a an extracellular alkaline serine protease (Ds1) was purified and characterized from the nematode-trapping fungus *Dactylaria shizishanna* using cation-exchange chromatography and hydrophobic interaction chromatography Wang et al., (2006).

FUTURE LINES OF INVESTIGATION

Although quite a good number of studies are carried out and already niche markets exist for these potential bioagents, considerable progress still needs to be made in the several areas including technical (production, formulation and application systems) ; agronomic (integration of bioagents into cropping systems) ; socio-economic (public perception, economic feasibility) ; political (improved registration procedures, improved extension services, support for small–medium-size enterprises and organic growers) ; bioactive compounds (bioactive compounds of bioagents sometimes referred to as toxins, are a major concern, because some people believe they are a health risk.

REFERENCES

Butt,T.M., Jackson, C. and Magan,N., 2001. Fungi as Biocontrol Agents- Progress, Problems and Potential. CABI, 389 pp

Ciancio, A., Loffredo, A.., Paradies, F., Turturo, C. and; Sialer, M. Finetti, 2005. Detection of *Meloidogyne incognita* and *Pochonia chlamydosporia* by fluorogenic molecular probes. *EPPO/OEPP Bull.,* 35 : 157-164

Duddington, C.L., 1955. Fungi that attack microscopic animals. *Bot. Rev,* **21:** 377-439.

Feder, W.A., Everard, C.O.R. and Duddington. C.L., 1960. Heterokaryotic nature of ring formation in the predaceous fungus *Dactylella doedycoides. Science,* **131**: 922-924.

Haard, K., 1968. Taxonomic studies on the genus *Arthrobotrys* Corda. *MycologIa*, **60:**1140–1159

Kano,Sanae., Almi Tadanori, Masumoto Seita, Kitamoto Yutaka and Morinaga Tsutomu, 2004. Physiology and Molecular Characteristics of a Pine Wilt Nematode-Trapping Fungus, *Monacrosporium megalosporum. Curr. Microbiol.,* 49: 158-164

Kerry, B.R. and Crump, D.H.,1998. The dynamics of the decline of the cereal cyst nematode, *Heterodera avenae*, in four soils under intensive cereal production. *Fundamental and Applied Nematology,* **21:** 617–625.

Larriba, Eduardo, Martín-Nieto, José, Lopez-Llorca and Luis Vicente, 2012. Gene cloning, molecular modeling, and phylogenetics of serine protease P32 and serine carboxypeptidase SCP1 from nematophagous fungi *Pochonia rubescens* and *Pochonia chlamydosporia. Canadian J. Microbiol.*, 58: 815-827

Liamming Liang, Zhaohui Meng, Fengping Ye, Jinkui Yang, Yuna Sun, Yu Guo, Qili Mi, Xiaowei Huang, Chenggang Zou, Zihe Rao, Zhiyong Lou and Ke-Qin Zhang, 2012. The crystal structures of two cuticle-degrading proteases from nematophagous fungi and their contribution to infection against nematodes. Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, 2 North Cuihu Road, Kunming 650091, China

Lobo, K.J., 1966. Studies on the variability and trapping activity of selected nematophagous fungi. Ph.D thesis submitted to the Department of Plant Pathology, McGill University, Montreal. 185 pp.

Lopez-Llorca, N.V., Macia-Vicente, J.G. and Jahnsson, H.B., 2008. Mode of action and interactions of nematophagous fungi. P.51-56. *In: Integrated management and biocontrol of vegetable and grain crops nematodes*, Ciancio,A. and Mukerji, K.G.(Edrs.).

Mankau, R., 1980. Biocontrol: Fungi as nematode control agents. *Ann. Rev. Phytopathol*., **28:** 418-435

Tarjan, A.C. 1961. Growth characteristics of *Dactylella drechsleri* n.sp., an adhesive-knobbed nematode-trapping Hyphomycete from F1orida. *Mycopatho1ogia*, **14:** 136-140.

Wang, R.B.,. Yang, J.K.., Lin, C., Zhang, Y. and Zhang, K.Q., 2006. Purification and characterization of an extracellular serine protease from the nematode-trapping fungus, *Dactylella shizishanna. Letters Appl. Microbiol.,* **42**: 589-594

Wei, Ben-Qiang, Xue, Qing-Yun, Wei, Li-Hui, Niu, Dong-Dong, Liu, Hong-Xia, Chen, Li-Feng and Guo, Jian-Hua, 2009. A novel screening strategy to identify biocontrol fungi using protease production or chitinase activity against root-knot nematodes-*Meloidogyne*. *Biocontrol sci.,& Tech.,* 19: 859-870

Xingzhong Liu, meichun Xiang and Yongsheng Che., 2009. The living strategy of nematophagous fungi. *Mycosci.,* **50**: 20-25

Yan Li, Kevin D. Hyde, Rajesj Jeewon, Lei Cai and Dhanasekharan Vijayakrishna, 2001. Phylogenetics and evolution of nematode-trapping fungi (Orbiliales) estimated from nuclear and protein coding genes. *Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming 650091, Yunnan Province, PR China*

Yang Jinkui; Zhang Ke-Qin and ; Wang Miao, 2006. Characterization of an extracellular protease and its cDNA from the nematode-trapping fungus *Monacrosporium microscaphoides. Canadian J. Microbiol*., 52: 130-139