INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-5, Issue-2, April-June-2014 Coden : IJABPT Copyrights@2014

ISSN : 0976-4550

Received: 03rd Jan-2014 *Revised: 15th Feb-2014* *Accepted: 20th Feb 2014*

Research article

MORPHOLOGICAL CHARACTERIZATION AND MASS PRODUCTION OF NEMATOPHAGOUS FUNGUS NEMATOCTONUS ROBUSTUS

Ramesh babu S, Anirudh and R.K. Singh

Agricultural Research station, Lam, Guntur. A.P, ramesh.surapu@yahoo.com

ABSTRACT: The plant parasitic nematodes infect the root tissues of the plant causing root galls that lead to reduced water and mineral uptake in the plant root system. Nematophagous fungus are used as biocontrol for the nematodes. Among those *Nematoctonus* are one of the species used as bioagent. *Nematoctonus* species produces an extensive mycelium and capture many nematodes with hour glass shaped adhesive knobs on the hyphae. Nematodes become attached to these adhesive knobs and the cuticle of nematode is penetrated by the infective hyphae. This isolate of *Nematoctonus robustus* is characterized by hyaline mycelium, dikaryotic in nature containing genetically two different nuclei in each cell, having distinct clamp connection. The fungus has better colonizing ability on natural solid substrates like wheat straw and rice straw. It also show good ability to colonize on different cereal grains and various other waste products like coconut coir and FYM etc. This species is one of the best used for mass production and effective for control of plant parasitic nematodes.

Key words: *Nematoctonus*, CMA, endoparasitism, Mass production

INTRODUCTION

Nematodes are microscopic multicellular roundworms that inhabit marine, freshwater and terrestrial environments. Some are beneficial soil microorganisms that play an important role in essential soil processes while others cause plant diseases (Dufour*et al*., 2003). The plant parasitic nematodes infect the root tissues of the plant causing root galls that lead to reduced water and mineral uptake in the plant root system. *Nematoctonus* species produces an extensive mycelium and capture many nematodes with hour glass shaped adhesive knobs on the hyphae. Nematodes become attached to these adhesive knobs and the cuticle of nematode is penetrated by the infective hyphae. Once inside the host, assimilative hyphae form to digest and absorb the contents of the nematode. The absorbed nutrients are used to produce additional fertile hyphae with adhesive knobs and conidia (Drechsler 1946, Thorn and Barron 1986). Among *Nematoctonus* species *Nematoctonusrobustus* was the most frequent species found in two samples and characterized by cylindrical and curved conidia and predominantly intercalary predatory hour glass cells surrounded by an adhesive mucoid drop. Guima and Cooke (1971) named as nematotoxin. *Nematoctonusrobustus* killed nematode by production of toxin. This toxin production activity is one of the advantage of using this fungus for possible biological control agent. The genus*Nematoctonus* was erected first time by Drechsler in1941 distinguishing it from other nematode destroying fungi by hyphae with clamp connections, a characteristic feature of the basidiomycotina. The genus *Nematoctonus* is unique in that some species are endoparasitic and some species are nematode trapping fungi by established criteria. Economically viable methods for the development of fungal material production in the laboratory are necessary and an important step in enabling the commercial production of nematophagous fungi. This work aimed at evaluating the mycelia mass production of N. robustus in different substrates nematophagous

MATERIALS AND METHODS

Collection of samples

Samples were collected from different selected site containing decaying wood and decomposed saw dust of mango (Mangiferaindica) tree, decompost leaves of other tree and rhizospheric soils of cultivated fields from BHU campus, Varanasi, Uttar Pradesh. Three samples of 50 g from each sites were collected in separate polythene bags, which were double-sealed to prevent evaporation and brought to the laboratory and mixed well to a composite of 150 g and from theses composite samples 1 g have been taken for the isolation of nematophagous fungi .

Observation of nematophagous fungi

For the isolation of nematophagous fungi, the method described by Duddington (1955) have been followed. One gram of sample was sprinkled over the **surface** of sterile petri dishes already poured with sterilized water agar medium. Population of pure culture of saprophytic nematode was added as bait in all of these plates. Three replication of each sample were maintained and incubated at room temperature (25-30^{o}C) for 15 days. The Petri dishes were observed regularly to get the colonies of nematophagous fungi under stereoscopic binocular microscope as well as light microscope. The fungus were identified and recorded on the basis of measurement of spore size and shape, formation of trapping structure, clamp formation etc. on the bases of key given by Cooke and Godfrey (1964), Thorn and Barron (1986), Koziak*et.al*. (2007).

Isolation, Purification and maintenance of culture:

Pure culture of fungus was made by single spore isolation technique described by Tuite (1969). Conidia was picked with the help of sterilized fine needle and dragged lightly across in petri dishes containing water agar medium. Well separated spores were located under stereoscopic microscope (100X). A disc of agar containing a single spore was cut and transferred into a petri dish containing maize meal agar medium. The single spore inoculated Petri dishes were incubated at 25±1^{o} C for growth and sporulation. After 7 days of inoculation, the culture was transferred from its peripheral growth to aseptically new petridishes containing maize meal agar medium at 25±1^{o} C. Pure cultures of the fungus was maintained by regular subculturing at the interval of 20 days.

Identification of *nematoctonus f*ungi

For identification of *Nematoctonusrobustus*, spore size, shape, clamp formation were measured and compared with the original description given by Thorn and Barron 1986, Koziak*et.al*. (2007).

Morphological variation in *Nematoctonusrobustus:*

For morphological variation, culture of *Nematoctonusrobustus*was grown in maize meal agar medium for growth and sporulation. Slides were made in sterilized distilled water from seven day old culture and size of conidia, was measured under under a research microscope. For minimum 100 conidia was measured per microscopic field (1017.2µm^{2}) to study of variability in morphology and sporulation. Photographs of conidia, hour-glass formation and clamp formation were taken in Nikon Photomicrograph Microscope.

Collection and maintenance of plant parasitic nematode (*Meloidogynegraminicola)* and saprophytic nematodes

Population of second stage juveniles of *Meloidogynegraminicola* was obtained from microplot regularly maintained on Rice-Wheat plant in the wire net house of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Bananas Hindu University. Sufficient number of egg masses of this nematode were picked from infected roots of rice plants and collected separately in a cavity blocks. The cavity blocks containing egg masses were incubated for 4-5 days at 25-30^{0} C for hatching to get required population of 2nd stage juvenile of *M. graminicola*. Pure cultures of saprophytic nematode was prepared by picking the saprophytic nematodes from the plates having one gram soil sample in already poured water agar medium by sterilized needle. Saprophytic nematode multiplied and maintained for future use as a bait or experimental purpose.

Mass culture of *Nematoctonusrobustus* on different substrates:

Several substrates such as Farm Yard Manure (FYM), Grains of Wheat (*Triticumaestivum*L.), Maize (*Zea mays* L.), Sorghum (*Sorghum bicolor* L.) and straw of wheat (*Triticumaestivum*L.) and rice (*Oryza sativa*) were taken separately in 250 ml conical flask for growing the mass culture of *Nematoctonusrobustus*. Straw were made 1-2 cm length powdered in warring blender before filling into the conical flasks. Amount of substrate and water were used as given below:

FYM

Farm Yard Manure (FYM) 10 g + 10 ml water

GRAINS

Wheat grain 20 g + 35 ml water
Maize grain 20-g + 35ml water
Sorghum grain 20 g + 35 ml water

STRAW

Wheat straw 5 g + 40 ml water
Rice straw 5 g + 40 ml water
Coconut coir 5 g + 40 ml water

Each substrate was taken into several 250 ml conical flasks and moistened with desired amount of water as mentioned above. The flasks were plugged with cotton and sterilized at 15 psi for 20 minutes. A 5 mm fungal disc was cut from the periphery of seven day old culture by a sterilized cork borer and inoculated in the centre of a substrate contained in flasks with the help of sterilized inoculating needle. One fungal disc was inoculated into each flask. Four replications were maintained of each treatment. The inoculated flasks were incubated at 25 ± 1^0C. Visual ratings were made to assess the growth of *Nematoctonusrobustus* after 15 days of inoculation.

RESULTS & DISCUSSION

Collection of samples for isolation of predacious fungi:

Samples containing decaying wood, decomposed saw dust from different mango plants, decomposed leaf with soil from other different trees and rhizospheric soil samples of different crops have been collected to isolate different predacious fungi. The predominant occurrence of *Arthobotrys species* have mostly been recorded from rhizospheric soil and decomposed leaf samples. The occurrence of *Monacrosporium* from rhizospheric soils of agricultural crops and that of *Harposporium* species and *Dactylariabrocophaga* have been recorded from decomposed leaf and saw dust samples. The occurrence of *Nematoctonus* species was mostly associated with the sample containing as decaying wood and decomposed saw dust. Thus it shows a close association of different predacious fungi with types of sampling materials and with sites of collection.

Morphological characterization of *Nematoctonusrobustus*

This isolate of *Nematoctonusrobustus* is characterized by hyaline mycelium, dikaryotic in nature containing genetically two different nuclei in each cell, having distinct clamp connection diameter about 2.0-2.4 µm and placed at a distance of about 9.1-10 µm to 13-15µm throughout the mycelium confirming its taxonomic association with the kingdom basidiomycota. It sporulate extensively with small cylindrical to straight and slightly curved to kidney shaped, sausage shaped conidia (10.72 µm × 4.1µm), solitary in nature produced on a short and simple conidiophore on hyphae. Conidia germinate simple from single or both end with long germ tube and produce a swelling followed by an hour glass cell (trapping device) surrounded by a mucoid drop terminally under nematode induced condition. Mycelium also bears large predatory hour glass, sized about 2.5-3.0 µm × 1.5-1.6 µm surrounded by a large adhesive mucoid drop during interaction with nematodes. A colony of *Nematoctonus* consists of assimilative hyphae may be relatively consistent with in a species.

Morphology and biology of fungi

As per the key of classification of *Nematoctonus* given by Thorn and Barron (1986), this isolates of *Nematoctonus* is identified as *Nematoctonusrobustus.*. This species of *Nematoctonus* produces hour glass attached to mouth part of nematode and grow their assimilative hyphae inside the nematode body and extensively sporulating hyphae outside that confirms its predatory nature rather than endoparasitism. *Nematoctonusrobustus* was characterized by Koziak*et al* (1986) with the presence of simple, conidial pegs with kidney shaped, sausage shaped, or straight and cylindrical conidia , size about 6.4-12.0 µm × 2.4-4.0 µm and predatory adhesive knobs predominantly intercalary and occasionally terminal hour glass cells, 5.6-8.8 µm long × 2.4-3.2 µm wide at base, surrounded by an adhesive mucoid drop 5-10 µm in diameter and conidia usually not germinating on water agar with nematodes and absence of aleuriospores. Our isolates also show similar morphological properties.

Growth on different substrates. Mass culture preparation of *N.robustus* on solid based substrates indicates its easiest and effective mass multiplication. The fungus has better colonizing ability on natural solid substrates like wheat straw and rice straw. It also show good ability to colonize on different cereal grains and various other waste products like coconut coir and FYM etc. This was found to be easiest and economic technology for large scale production of *Nematoctonus* and its easy applicability in farmers field and easy acceptability to farmer community in near future. A similar technology for large scale mass multiplication of *A.oligospora* have already been developed by Mastskievich (1919) on different solid base substrates like top manure, saw dust manure and other subustrates.

Growth of nematoctonusrobustus on different substrates

On the basis of visual ratings, very good (excellent) growth of *N.robustus* was recorded on wheat straw and rice straw. Whereas among grain wheat grain, maize grain and sorghum grain good growth of this fungus was observed. In coconut coir and farm yard manure the fair growth of fungus was observed.

Coden : IJABPT, Copyrights@2014, ISSN : 0976-4550

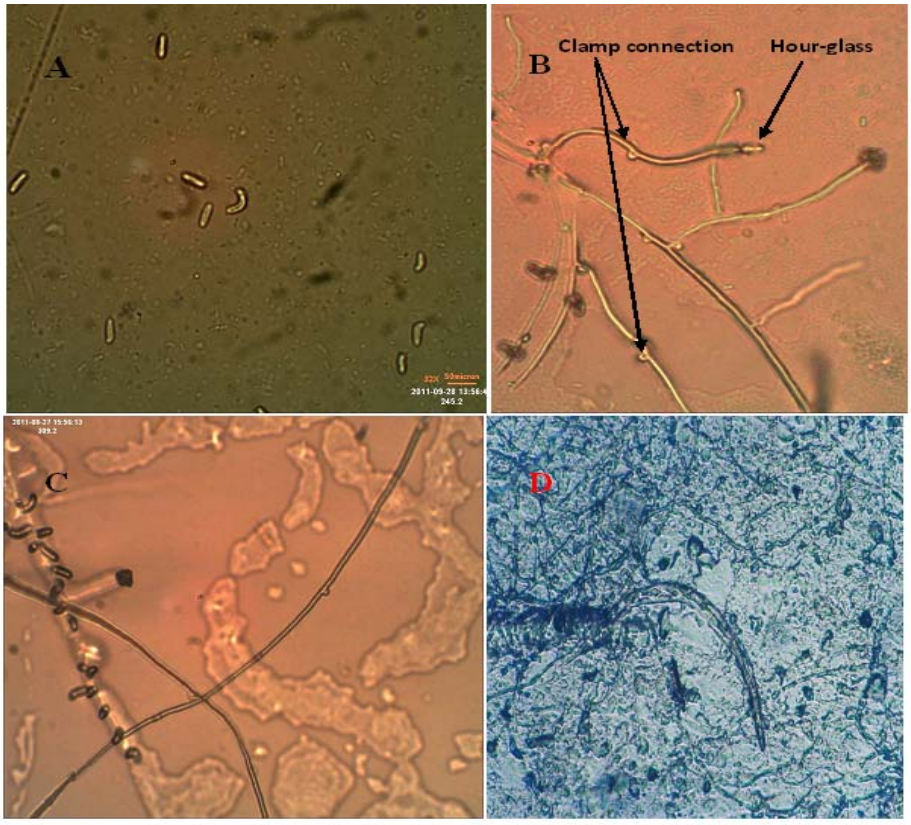

Fig-1: (A) Conidia of the *Nematoctonusrobustus*(B) Hour-glass formation of *Nematoctonusrobustus*(C) Clamp connection of *Nematoctonusrobustus* (D) Trapping of nematodes by *Nematoctonusrobustus*

Fig-2: (A)Mass culture on Sorghum grains (B) Mass culture on Maize grain(C) Mass culture on Rice straw (D) Mass culture on Wheat straw(E)Mass culture on Coconut coir

Table-1: Biomass growth of *Nematoctonusrobustus* in different solid substrate.

Type of substrate	Visual appearance
Wheat straw	+++
Rice straw	+++
Sorghum grain	++
Wheat grain	++
Maize grain	++
Coconut coir	+
Farm yard manure	+

Notes: **Very good (+++), Good (++), Fair (+)**

REFERENCES

Ashour -E.H.; Mostafa,-F.A.M. (1999). Effect of pollution with certain heavy metal on the growth of the Nematophagous fungus, *Arthrobotrysoligospora*, trap formation, root-knot nematode infection and enzymes production. Pakistan-Journal-of-Biological-Sciences 2(2) p. 515-522.

Alger, M.E, (1980). Nematophagous fungi of king's county, Nova scotia. M.Sc thesis, Acadia University, Wolfville.

Barron, G. L. and Dierkes, Y. (1977). Nematophagous fungi: *Hohenbuehelia*, the perfect state of *Nematochtonus*. Can. J. Bot., 55: 3054-3062.

Caryol, J. C. (1983). Biological control of Meloidogyne by Arthobotrysirregularis. Revue de nematologica, 6: 265-274

Cooke, R.C. and Pramer, D. (1968). *Monacrosporiumrutgeriensis*sp. N, a nematode trapping Hyphomycete. Phytopath, 58: 544-545.

Drechsler, C. (1941) Somehyphomycetes parasitic on free living terrricolous nematodes. Phytopathology, 31: 773-781.

Duddington, C. L. (1940). Predaceous fungi. Nature,145: 150-151.

Grewal, P. S. and Sahi, H. S. (1988). A new and cheaper technique for rapid multiplication of Arthobotrysconoides and its potential as a bionematicides in mushroom culture. Current science, 57: (1) 44-46.

Jansson, H. B., Jeyaprakash, A. and Zuckerman, B. N. (1985). Differential adhesion and infection of nematode by the endoparasitic fungus *Meriaconiospora* (Deuteromycetes). Applied and Environmental Microbiology, 49(3): 552-555

Jones, (1964). Nematoctonus robustus sp. Nov. Trans. Br. Mycol.soc.47 (1), 57-60.

Koziak, A. T. E. Cheng KeiChin Thorn, R. G. (2007). Phylogenetic analyses of Botany.85: 8, 762-773.