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Research article

GROWTH AND SPORULATION OF NEMATOPHAGUS FUNGUS NEMATOCTONUS ROBUSTUS ON DIFFERENT MEDIA AT DIFFERENT TEMPERATURES

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ABSTRACT: Nematode trapping fungi captured nematode by forming different types of structure in a way to get supplement of nitrogen from the nematode, just like carnivorous plants. Fungal parasites of nematodes are broadly classified as either predaceous or endoparasitic. The genus *Nematoctonus* is unique in that some species are endoparasitic and some species are predaceous by established criteria. The generic diagnosis for *Nematoctonus* was given by Dreschsler that one assimilative hyphae which is hyaline, more or less branched, developing within minute living animals. Growth and sporulation of *N.robustus* was significantly varied with different temperature on corn meal agar medium. It is evident from results showing the effect of temperature on the biology of *N.robustus* that 20^0C was most optimum temperature for both growth and sporulation. With increase in the temperature there is increase in radial growth of this fungus but correspondingly there s decrease in sporulation.

Key words: *Nematoctonus*, CMA, endoparasitism, Temperatures, Media

INTRODUCTION

Nematode trapping fungi captured nematode by forming different types of structure in a way to get supplement of nitrogen from the nematode, just like carnivorous plants and derive nutrients by capturing and digesting small animal preys to supplement their photosynthesis energy with protein from captured insects. Fungal parasites of nematodes are broadly classified as either predaceous or endoparasitic. The genus *Nematoctonus* is unique in that some species are endoparasitic and some species are predaceous by established criteria. The generic diagnosis for *Nematoctonus* was given by Dreschsler that one assimilative hyphae which is hyaline, more or less branched, developing within minute living animals. Second one non assimilative hyphae which is developed outside of host animals mostly sparse often prostrate provided with clamp connections. However, developing some fungi as potential biopesticides is difficult because their mycelial growth on artificial media usually depends on the fungal species and on the components used in the culture media. The nutritional requirements for fungal growth vary among different biocontrol agents, for example, nematophagous fungi are greatly influenced by nutrients and culture conditions. Therefore, the objective of this experiment was to study physiological and
nutritional requirements of different isolates of nematophagus fungi in order to understand differences among the isolates for the proper mass production of this fungi.

MATERIALS AND METHODS

Collection of samples

Samples were collected from different selected site containing decaying wood and decomposed saw dust of mango (*Mangifera indica*) 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 fungi*:

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

Effect of temperature on the growth of *Nematoctonus robustus*:

Radial growth of *Nematoctonus robustus*was studied on corn meal agar medium at different temperature. The medium was prepared, autoclaved and poured into several sterilized 90 mm Petri dishes. 5 mm mycelia disc of 7 day old cultures was taken from the periphery and inoculated separately into Petri dishes. The inoculated Petri dishes were incubated at different temperatures: 20^{0}C, 25^{0}C, 30^{0}C & 35 ^{0}C. Three replications were maintained for each treatment. Radial growth of the fungus was measured daily until some petri dishes were fully covered by fungal growth or up to 10 days. The experiment was repeated two times and conducted in Complete Randomized design (CRD) and statistically analyzed.

Effect of different media on the growth of *Nematoctonus robustus*:

The radial growth of *Nematoctonus robustus* was studied on eight media: Corn meal agar media, Martin Agar medium, Beef extract Agar medium, Thornton agar medium, yeast extract agar medium ,Richard's Agar medium ,Czapek Dox Agar medium , Potato Dextrose Agar medium. Three petri dishes were used for each medium as replicates. All the eight media were prepared and sterilized at 15 lbs pressure for 20 minutes. 20 ml of each medium was poured into each of several petridishes. 5 mm fungal discs as inoculum were taken from the periphery of 13 day old cultures and inoculated separately into petri dishes containing different media. The inoculated petridishes were incubated at temperature *viz* 20^{0}C, 25^{0}C, 30^{0}C, and 35^{0}C. Radial growth of the fungus was measured daily upto ten days. The experiment was conducted in completely randomized design and statistically analyzed.

RESULTS & DISCUSSION

Effect of temperature on growth and sporulation of *Nematoctonus robustus* on CMA medium

Growth and sporulation of *N.robustus* was significantly varied with different temperature on corn meal agar medium. The radial growth rate was recorded maximum as 1.98 mm per day at 30^{0}C temperature while minimum growth rate per day was recorded about 1.35mm/ day at 20^{0}C. *Nematoctonusrobustus*was found to be slow growing fungus with maximum growth only about 30 mm after 10th day was while minimum growth was found 21 mm at 20^{0}C. In the plates after 10th day sporulation was found only at 20^{0}C. No sporulation was recorded at any other temperature during the experiment on the corn meal agar medium.

Effect of media on growth and sporulation of *Nematoctonus robustus*

The growth and sporulation of *N.robustus* were differed significantly based on different nutritional source. The growth of this fungus was highly favored by natural source of carbon and nitrogen other than the organic chemicals. The varying growth pattern and sporulation behaviour of *N.robustus* on different media of their nutritional requirement have been discussed below.

Corn Meal Agar Medium- Dullwhite colony with superficial mycelial growth and very few sporulation at lower temperature but significantly suppressed at higher temperature at about 25-35^{0}C. Spores are mostly cylindrical in shape and straight (about 80%) while about 20 % spores are slightly curve with average spore size about 10.66 × 4.1µm.

Potato Dextrose Agar Medium- Pure white colony with fluffy dense growth and mycelial accumulation due to extensive hyphal anastomosis and heavy sporulation at lower temperature but slightly reduced at higher temperature. Spores are mostly straight, cylindrical in shape, some are curved. The average size of spore at different temperatures on media range from is 8-9 × 4.1 µm and ratio of the occurrence of straight spore to curve spore is gradually increase from 85:15 at 20^{0}C to 90: 10 at 25, 30 and 35^{0}C respectively**.**

Thornton agar medium- Dull white colony with hairy mycelial growth with few sporulation at lower temperature 20^{0}C but significantly suppressed at higher temperature at about 25-35^{0}C. Spores are mostly (about 70%) straight and cylindrical in shape while about 30 % of spores are observed slightly curve, with average spore size of 12.71 × 4.1µm.

CzapekDox agar medium- Dull white colony with superficial hyaline mycelial growth with more sporulation at lower temperature but slightly decreased at higher temperature, i.e. 170.50 average number of spore per microscopic field at 20^0C followed by 128.93, 143.37 and 123.16 at 25^0C,30^0C and 35^0C respectively. Spores are mostly (about 80%) straight and cylindrical in shape while 20% spores are observed slightly curve. The average spore size is 8.61- 10.25 × 4.1µm at different temperature.

Beef extract agar medium- Dull white colony with superficial hyaline mycelial growth with more sporulation at 20^0C and slightly decreased with increase in temperature. Average number of spores per microscopic field is 175.73 at 20^0C followed by 158.73 at 25^0C and 139.47 at 30^0C and 120.56 at 35^0C. Spores are mostly (about 60%) straight and cylindrical shaped while 30-40% spores are curve with average spore size ranging from 8.02-10.81 × 4.1µm.

Richard agar medium- While colony with very sparse mycelia growth having more extensive sporulation at all the temperatures ranging from 111.04-148.53 average number of spores per microscopic field. Spore are mostly highly curved with the percentage occurrence of curved spore ranging from 70-80% while 20-30% spores are straight and cylindrical at different temperature.The spore size is varying from 9.02-12.71× 4.1µm

Martin agar medium- Small white colony with fluffy dense growth and mycelial accumulation due to extensive hyphal anastomosis. Among all the media tested, sporulation was recorded less at lower temperature which also reduces as the temperature increases. Spores are mostly straight and cylindrical in shape. Numbers of curved spores are less (20%) with average size ranging from 9.84-12.71 × 4.1µm.

Yeast extract mannitol agar medium- Poor colony growth and average colony diameter attaining about 7.9 to 8.2 mm at 10^{th} day after inoculation. No sporulation has been observed at all the range of temperature used for the experiment. *N.robustus* showed a higher degree of variation in radial growth on natural media like CMA, semi synthetic media like PDA and synthetic media containing amino acids like Thornton agar media. Other synthetic media like CzapekDox agar and Martin's agar medium containing various carbon and nitrogen sources also gives significant variation. It is appear that *N.robustus* has higher degree of preference for organic form of carbon and nitrogen sources and our study indicates its better growth and sporulation mainly on PDA and corn meal agar (CMA).

Table-1. Effect of different media on growth and sporulation of *Nematoctonus robusts* at temperature 20^0C

Nutritional sources (media)	Growth		Sporulation				
	Radial growth Rate (mm/ day)	Avg. growth at 10^{th} day (mm)	Avg. spore number/microscopic field (1017.2 µm^2)	Avg. size of spore(µm)		Shape of spore (percentage)	
				length	width	straight	curve
Cornmeal agar medium	1.35	21.0	37.23	10.66	4.1	80	20
PDA medium	1.87	18.7	318.30	9.02	4.1	85	15
Thornton agar medium	2.30	23.0	108.37	12.71	4.1	70	30
CzapekDox agar medium	1.87	19.7	170.50	10.25	4.1	80	20
Beef extract agar medium	1.50	15.0	175.73	10.81	4.1	70	30
Richard's agar medium	1.17	11.7	148.53	12.71	4.1	30	70
Martin agar medium	1.07	10.7	61.00	11.48	4.1	80	20
Yeast extract mannitol agar medium	0.82	8.2	-	-	-	-	-
CD (1%)	0.26	1.89	10.42				

(-) indicates no sporulation.

Similar kind of report was made by Young *et.al.* (1998) that the growth of *Arthobotrys oligospora* was best on corn meal agar. So it is indicate that natural sources of nitrogen and carbon are the best for the growth and sporulation of all kinds of nematophagous fungi. *Nematoctonus robustus* was found to utilize a wide range of carbon sources like dextrose, sucrose, maltose and mannitol etc.

Table-2. Effect of different media on growth and sporulation of *Nematoctonus robustus*at temperature 25^{0}C.

Nutritional sources (media)	Growth		Sporulation				
	Radial growth Rate (mm/ day)	Avg. Growth at 10th day(mm)	Avg. no of spore/microscopic field (1017.2µm^2)	Avg. size of spore(µm)		Shape of spore (percentage)	
				length	width	straight	curve
Corn meal agar medium	1.80	27.0	-	-	-	-	-
PDA medium	3.40	44.0	197.53	8.61	4.1	90	10
Thornton agar medium	3.63	45.3	-	-	-	-	-
CzapekDox agar medium	2.60	35.0	128.93	10.66	4.1	60	40
Beef agar extract	1.03	18.3	158.73	9.43	4.1	60	40
Richard's agar medium	1.30	13.0	142.83	10.25	4.1	20	80
Martin agar medium	1.27	18.7	35.23	12.71	4.1	80	20
Yeast extract mannitol agar	0.79	07.9	-	-	-	-	-
CD (1%)	0.32	3.21	12.03				

(-) indicates no sporulation.

Table-3. Effect of different media on growth and sporulation Ne*matoctonus robusts* at temperature 30^{0}C

Nutritional sources (media)	Growth		Sporulation				
	Radial growth rate (mm/ day)	Avg. growth at 10th day(mm)	Avg. no of spore/microscopic field (1017.2µm^2)	Avg. size of spore(µm)		Shape of spore (percentage)	
				length	width	Straight	curve
Corn meal agar medium	1.93	27.7	-	-	-	-	-
PDA medium	4.53	57.7	129.50	9.02	4.1	90	10
Thornton agar medium	3.33	45.7	-	-	-	-	-
CzapekDoxagar medium	3.20	43.0	123.37	8.61	4.1	70	30
Beef agar extract	1.43	25.3	139.47	9.02	4.1	40	60
Richard's agar medium	2.03	20.3	110.73	9.02	4.1	20	80
Martin agar medium	1.27	20.7	33.30	10.25	4.1	80	20
Yeast extract mannitol agar medium	0.80	8.0	-	-	-	-	-
CD (1%)	0.37	3.49	6.50				

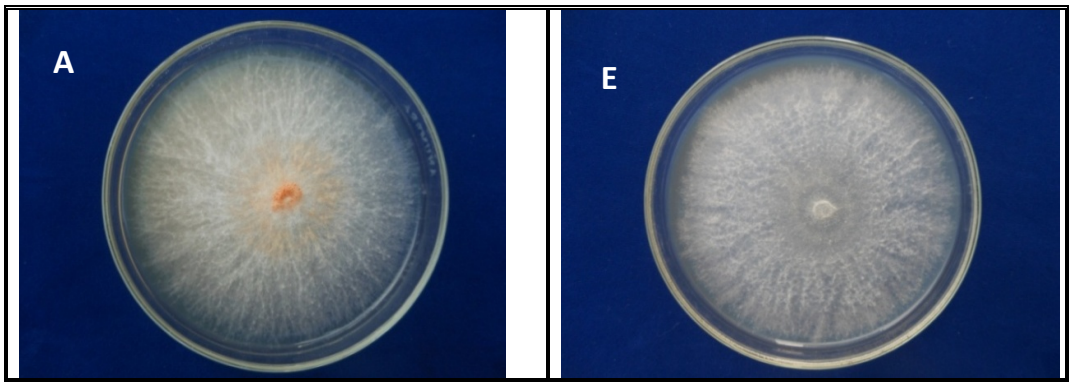
(-) indicates no sporulation

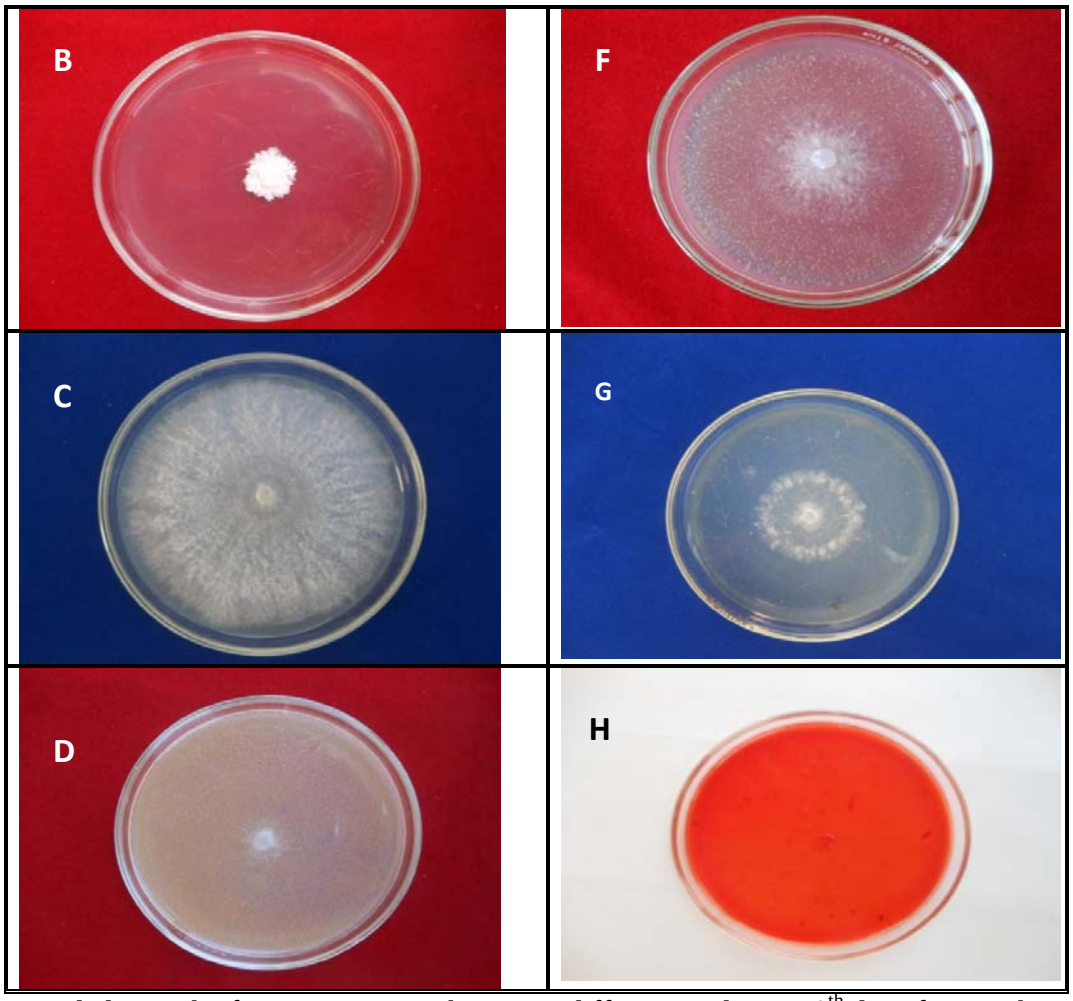
Among them dextrose was found to be most effective carbon source for growth and sporulation of *Nematoctonus* followed by sucrose. Although sucrose was reported as most effective carbon source for growth of *A.oligospora* (Lee *et.al*. 2004). Dextrose is a simple sugar (monosaccharide) where as sucrose is disaccharide containing two hexose. Other carbon sources like mannitol, sugar alcohol is also utilize by *Nematoctonus.* This indicate that *N.robustus* possess different enzymes like cellulose, invertase etc. which makes them a better utilizer of wide range of carbon sources and this favors them with a better competitive advantages in organic matter rich soil. *N.robustus* utilize several nitrogen sources like natural source of nitrogen like potato extract, maize extract and amino acids like aspergine, peptone, beef extract and yeast extract as well as inorganic sources of nitrogen like sodium nitrate, potassium nitrate etc . But their growth on media containing natural nitrogen source was found to be best in terms of their growth and sporulation. This also indicates the better competitive saprophytic ability of *Nematoctonus* to grow and colonize sources of natural organic substrates. It is evident from results showing the effect of temperature on the biology of *N.robustus* that 20^0C was most optimum temperature for both growth and sporulation. With increase in the temperature there is increase in radial growth of this fungus but correspondingly there s decrease in sporulation. Temperature at nearly 35^0C both growth as well as sporulation were hampered. This indicates a close association of this fungus with cold climatic region of world. That's why their predominant occurrence was mostly reported by Koziak*et.al.* (1986) from Coasta Rica region and by Thorn et al (1986) from West ontario of canada.

Table-4. Effect of different media on growth and sporulation of N*ematoctonus robusts* at temperature 35^0C.

Nutritional sources (media)	Growth		Sporulation				
	Radial growth Rate (mm/ day)	Avg. growth at 10^{th} day(mm)	Avg. no of spore/microscopic field (1017.2µm^2)	Avg. size of spore(µm)		Shape of spore (percentage)	
				length	width	Straight	curve
Corn meal agar medium	1.70	23.0	-	-	-	-	-
PDA medium	1.47	21.3	117.76	9.02	4.1	90	10
Thornton agar medium	1.30	21.0	-	-	-	-	-
CzapekDoxagar medium	1.40	14.3	123.16	9.02	4.1	70	30
Beef agar extract	1.00	16.0	120.56	9.02	4.1	40	60
Richard's agar medium	1.67	17.0	111.04	8.61	4.1	20	80
Martin agar medium	0.83	14.7	30.14	9.84	4.1	80	20
Yeast extract mannitol medium	0.81	8.1	-	-	-	-	-
CD (1%)	0.30	2.23	7.76				

(-) indicates no sporulation





Radial growth of *Nematoctonusrobustus* on different media on 10th day after Incubation

Fig-1: (A) Potato dextrose medium (B) Martin agar medium (C)Thornton's agar medium (D)Richard's agar medium (E)Maize agar medium(F) Czapek's agar medium (G)Beef extract agar medium (H)Yeast extract agar medium

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